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Sickness and the Social Brain: How the Immune System Regulates Behavior across Species

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Abstract
Many instances of sickness critically involve the immune system. The immune system talks to the brain in a bidirectional loop. This discourse affords the immune system immense control, such that it can influence behavior and optimize recovery from illness. These behavioral responses to infection are called sickness behaviors and can manifest in many ways, including changes in mood, motivation, or energy. Fascinatingly, most of these changes are conserved across species, and most organisms demonstrate some form of sickness behaviors. One of the most interesting sickness behaviors, and not immediately obvious, is altered sociability. Here, we discuss how the immune system impacts social behavior, by examining the brain regions and immune mediators involved in this process. We first outline how social behavior changes in response to infection in various species. Next, we explore which brain regions control social behavior and their evolutionary origins. Finally, we describe which immune mediators establish the link between illness and social behavior, in the context of both normal development and infection. Overall, we hope to make clear the striking similarities between the mechanisms that facilitate changes in sociability in derived and ancestral vertebrate, as well as invertebrate, species.

Introduction: The Interplay between Infection, Sickness, and Behavior

When an individual is infected with a pathogen (disease-causing agent), the body mounts a defense along multiple dimensions to most effectively deal with the threat. In addition to immune responses such as antibody production, inflammation, and immune cell trafficking, many organisms have evolved behavioral reactions, termed "sickness behaviors" [Hart, 1988; Dantzer et al., 2008]. Sickness behaviors are a coordinated set of adaptive, behavioral responses to an infection that help the individual fight the pathogen. These can manifest in a variety of ways, depending on environmental context, species, biological sex, and type of infection. Regardless, due to their ability to confer selective advantage and survival in many circumstances, their presence has persisted in many organisms across evolution. One of the more nuanced sickness behaviors is a change in social motivation

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[Eisenberger et al., 2017]. This change serves multiple purposes for individual, and group, survival. On the one hand, avoiding conspecifics when sick can prevent the spread of illness within the group, and allow for the reallocation of energetic resources toward immune defense [Eisenberger et al., 2010]. On the other hand, in some contexts, approaching a supportive social figure can mean the individual may receive relief, in the form of food, warmth, emotional support, or protection [Inagaki et al., 2015]. All of which can aid in the battle and make it more likely that the sick individual survives. These examples demonstrate the importance of the regulation of social behavior in the context of infection.

In this review, we reflect on the crosstalk between the immune system and social behavior, with a special focus on how these systems have coevolved. We will discuss in detail (1) what is known about sickness-induced social changes across species, (2) the brain regions and immune players involved in mediating these interactions, as well as (3) the intersection between sickness, the social brain, and neuroimmune development. Of note, while many social behaviors (and the systems that control them) are different between males and females, due to the inherent complexity of trying to compare these factors across species, those differences will not be the focus here. For a review including sex differences in the immune system and social brain, see Smith and Bilbo [2021]. We hope that in detailing the evolutionary conservation of these systems and sickness behaviors, we can provide a more detailed look into the role that these processes play in survival, but also their importance in normal development.

Sickness Behaviors across Species

In a set of seminal studies in the 1970s, Matthew Kluger's group demonstrated the importance of behavioral fever in the context of infection. Using desert iguanas (*Dipsosaurus dorsalis*) as a model system, Bernheim and Kluger [1976] showed that injection of a live pathogenic bacteria (*Aeromonas hydrophila*) induces fever, and prevention of this fever via a nontoxic dose of sodium salicylate increases risk of fatality to the infection. Additionally, since lizards are ectotherms, and have no internal machinery to produce heat, it was demonstrated that these animals use their environment to generate this lifesaving fever. Indeed, Vaughn et al. [1974] showed that when experiencing a bacterial infection, desert iguanas move to warmer regions in their environment to increase their body temperature. This behavioral response is not restricted to ectotherms, however, as it is also observed in experimentally infected mammals, such as rats, mice, and rabbits [Satinoff et al., 1976; Akins et al., 1991; Almeida et al., 2006]. Since the behavioral fever response is similar in lizards and mammals, the authors suggest that the infection is likely acting on the hypothalamus, a temperature control node in the central nervous system that is conserved between reptiles and mammals [Kluger et al., 1973; Vaughn et al., 1974]. These were some of the first experiments to describe how the host immune system can respond to infection by altering brain function, thereby changing behavior in an adaptable way [Evans et al., 2015].

A wide array of behavioral responses to sickness have been extensively detailed in the rodent literature since the 1980s [Hart, 1988; Dantzer and Kelley, 1989; Dantzer et al., 2008], and have since been described in many vertebrate species, including humans [Inagaki et al., 2015; Muscatell et al., 2016]. In most instances, these behaviors comprise more than just moving to a warmer environment to induce fever. They include a wide array of mood and motivational changes, including fatigue, decreased appetite, depressed mood, social withdrawal, and others. While sickness behaviors have historically been studied in the context of vertebrate physiology, more work has been done to identify their presence and importance in invertebrates. As mentioned previously, these behaviors confer advantage for survival, and thus have evolved to be present in many species, including field crickets [Jacot et al., 2004; Kelly and Leroux, 2020], honeybees [Kazlauskas et al., 2016], caterpillars [Adamo et al., 2007], fruit flies [Vale and Jardine, 2015, 2017; Surendran et al., 2017], *Caenorhabditis elegans* [Singh and Aballay, 2019], and many others.

Social Changes in Sickness Behaviors

One of the most intriguing changes that happens in response to infection is altered sociability. Sickness-induced changes in social behavior in humans (and other species) can often be bimodal. Sometimes, it is more advantageous to isolate from others to prevent spread of an infection [Kavaliers and Choleris, 2018; Smith and Bilbo, 2021], while in other contexts, seeking out social inclusion/support could assist with recovery. Many studies using human participants have shown that experimental exposure to the endotoxin lipopolysaccharide (LPS) in a laboratory setting elicits increased feelings of loneliness, social disconnection, and social sensitivity compared to

control-treated individuals [Moieni et al., 2015a, b; Eisenberger et al., 2017]. Conversely, Inagaki et al. [2015] found that treatment with LPS causes participants to report a greater desire for social connectedness, specifically toward a support figure. In line with this, positive social feedback from an unfamiliar peer is rated as more rewarding if participants were given LPS, compared to control [Muscatell et al., 2016]. It is likely to be the case that shifts in social behavior in both directions are critical for the optimal response to sickness, allowing for integration of stimuli that signal which social interactions in the environment should be avoided, and which may provide support and care.

In addition to humans, changes in social motivation in response to sickness have been described in many other animals. Social vocalizations in passerine birds [Garamszegi et al., 2004], field crickets [Jacot et al., 2004; Kelly and Leroux, 2020], and vampire bats [Stockmaier et al., 2020] have all been studied in the context of immune challenge. In all cases, infection causes a decrease in social contacts, which can be interpreted as social withdrawal. Recent work has shown that viral infection in honeybees causes social withdrawal, a behavior that is adaptive likely because it prevents spread of the virus amongst the colony [Geffre et al., 2020]. Additionally, LPS administration in wild barn mice decreases conspecific interactions and overall social connectedness [Lopes et al., 2016]. In adult laboratory rats and mice, many groups have shown that social interaction robustly decreases following LPS injection [Bluthé et al., 2000; Dantzer et al., 2008]. Sickness-related social behaviors in monkeys have also been studied in the context of naturally occurring infection. For example, researchers have shown that red colobus monkeys infected with ringworm are lethargic, groom less, and copulate less [Ghai et al., 2015], and that deworming vervet monkeys of gastrointestinal parasites significantly increases their propensity for social interaction [Chapman et al., 2016]. In zebrafish, infection with LPS decreases swimming and drastically reduces social preference [Kirsten et al., 2018; Petitjean et al., 2021]. Finally, in a series of studies by Schall and colleagues, it was demonstrated that malaria infection in western fence lizards decreases aggression, social displays, and social dominance [Schall and Dearing, 1987; Schall and Houle, 1992; Dunlap and Schall, 1995]. Taken together, there are numerous examples providing evidence for the conservation of sickness-induced social change across species.

Interestingly, while social withdrawal is the most common response in animals other than humans, the bimodal effects of immune stimulation on social behavior have been observed as well. Often, the direction of social change is dependent on other contextual factors, including environment and/or biological sex. For instance, LPS increases preference for a familiar partner in female, but not male, prairie voles [Bilbo et al., 1999]. Furthermore, immune challenge in rats decreases social initiations but increases huddling behaviors [Yee and Prendergast, 2010]. In male and female rhesus macaques, both low and high doses of LPS strikingly increase, rather than decrease, social affiliation [Willette et al., 2007]. In support of this, Carlezon et al. [2019] found that immune activation with LPS in the early postnatal period significantly increases social ultrasonic vocalizations in standard lab mice. It is important to note here that in addition to changes in the social behavior of the infected individual, infection can also influence how other, noninfected individuals in the environment interact with the infected. There are numerous examples of "social distancing" in the animal world, facilitated by aversive sickness-induced sensory cues [Hart and Hart, 2021; Stockmaier et al., 2021]. Wild mandrills groom parasite-infected mandrills less, and avoid compounds in their fecal material [Poirotte et al., 2017]. Humans injected with LPS have a significantly more aversive body odor than control participants [Olsson et al., 2014]. Finally, mice injected with LPS release sickness-related compounds in their urine, which causes avoidance behaviors in noninfected mice [Boillat et al., 2015]. Interestingly, it has been shown that LPS injection induces the release of these aversive odor cues in adult, but not prepubertal, male rats, suggesting that these sickness-induced social cues are regulated differently based on age [Arakawa et al., 2009]. This leads to conspecific avoidance of sick adults, but not sick juveniles [Rieger et al., 2022]. These results suggest that the effects of immune stimulation on sociability are very dependent on age, biological sex, and social context. Notably, some of the studies described here were conducted on animals in the juvenile period, as this is the time of highest sociability in these animals. It is likely the case that many of the behavioral changes described vary based on age; however, these details are beyond the scope of the current review. Additionally, it is worth mentioning that most of the studies described in this section use LPS (a bacterial mimetic) rather than a naturally occurring infectious agent. While this provides many experimental advantages, the question remains whether these responses are generalized across different types of infection, or a specific effect of LPS.

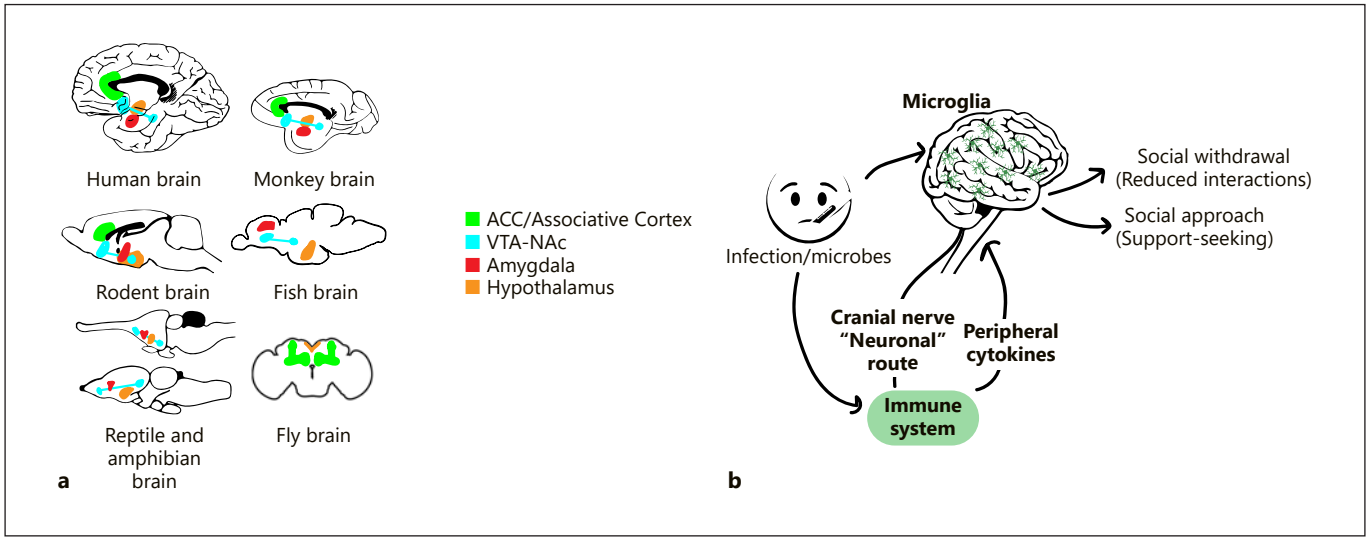

Fig. 1. a Some of the most common and conserved nodes of the social behavior brain network. Hypothalamus is present in every species' brain pictured. Mesolimbic dopamine system (VTA-NAc) and amygdala are present in all but fly brain. ACC/associative cortex is present in mammals, and an analogue – the mushroom body, is present in flies. **b** The pathways which translate sickness into changes in sociability. When infected, the pathogen often directly activates the immune system, which can then talk to the brain through either the "neural" route vial the cranial nerves, or via peripheral cytokines that stimulate immune cells in circumventricular organs, e.g., microglia (brain resident macrophages). Sometimes, the pathogen can cross the blood-brain barrier and directly activate microglia. These effectors modulate neural activity in socially relevant brain regions (**a**) to elicit changes in social behavior.

Neural Correlates of Social Behavior

Social behaviors, and their representations in the brain, are complex and multifaceted. They involve the integration and discrimination of sensory cues, reward and motivation signals, control of movement, and higher-order decision-making. Thus, there are many brain regions that are involved in the species-typical expression of social behaviors (Fig. 1a). Some work has been done to identify a core "social decision-making network" in vertebrate animals that includes areas such as the amygdala, prefrontal cortex (PFC), mesolimbic reward system, and hypothalamus [O'Connell and Hofmann, 2012]. It is known that the amygdala, PFC, and the accompanying anterior cingulate cortex (ACC) are all critical for multisensory integration and decision-making using social cues [Ghazanfar and Schroeder, 2006; Martínez-Sanchis, 2014; Martin-Cortecero and Nuñez, 2016; Van der Stoep et al., 2019]. In fact, the ACC and amygdala work together to inform social decisions in primates [Dal Monte et al., 2020]. As part of the "associative" network in the brain, the ACC is critical for social communication and cognition across species [Chang et al., 2013; Bicks et al., 2015; Apps et al., 2016; Block et al., 2020]. Further, dysfunction in the ACC is causal to social deficits present in a widely used genetic mouse model of autism spectrum disorder [Guo et al., 2019].

The hypothalamus, a midbrain region that controls a wide range of homeostatic functions, possesses various subregions that are involved in mediating different types of social behaviors. For example, the ventromedial hypothalamus (VMH) is critical for adult mating and aggressive behaviors [Remedios et al., 2017; Chen and Hong, 2018], while the medial pre-optic area (mPOA) and paraventricular nucleus (PVN) control sex-specific social motivation and parenting behaviors [McHenry et al., 2017; Resendez et al., 2020]. Recently, it was shown that a circuit between the amygdala and hypothalamus mediates social reward, in part by engaging dopamine release in the nucleus accumbens (NAc) in mice [Hu et al., 2021]. The mesolimbic reward system consists of the NAc, dorsal striatum, and ventral tegmental area (VTA). Here, dopamine encodes the "wanting" or motivational drive for a social stimulus, and endogenous opioids facilitate the "liking" or pleasurable aspects that come with receiving that stimulus [Loseth et al., 2014; Berridge and Robinson, 2016]. In the social context, dopaminergic projections from the VTA to the NAc can modulate social approach and avoidance [Dölen et al., 2013; Kohls et al., 2013; Ghosal et al., 2019] and pharmacological increase of dopa-

mine in the nucleus accumbens causes increased social play behavior in adolescent male rats [Manduca et al., 2016]. Recent work has highlighted the potential role for the dopamine system as the mediator between infection and sociability (see comprehensive review in Kopec et al. [2019]). Due to their critical contributions to sociability, the ACC, amygdala, mesolimbic reward system, and hypothalamus are all likely involved in the neuroimmune control of social behavior (Fig. 1a).

One remaining question is: how are peripheral sickness signals translated to social regions in the brain? One route, is via the cranial nerves, which include the glossopharyngeal, hypoglossal, and vagus nerves [Romeo et al., 2001; Dantzer et al., 2008]. Cytokines produced at the site of the infection activate primary afferent nerves in the periphery, which send this information up these nerves and into the brain. These nerve pathways link crucial components of the social reward and motivation network in the vertebrate brain, including the amygdala, striatum, and frontal cortex [Hachem et al., 2018]. Recently, it has been demonstrated that stimulation of the vagus nerve can directly influence dopamine release into the nucleus accumbens, and increase reward-seeking behaviors in mice [Han et al., 2018]. In addition to this neuronal route through the sympathetic nervous system, peripheral infection can influence the brain and behavior through immune effectors in both the periphery and the brain. Cytokines produced by macrophage-like cells in the circumventricular organs diffuse into the brain parenchyma, and can activate microglia (the brain-resident macrophages). We will discuss in more detail cytokines, microglia, and their effects in different brain regions in the following sections. It is likely that many neuronal and cytokine pathways co-occur in many types of infection (Fig. 1b).

Evolutionary Conservation of Social Reward Systems

The social behavior network is remarkably conserved across vertebrate species [Goodson, 2005]. In a meta-analysis conducted by O'Connell and Hofmann [2012], the authors demonstrate significant conservation of brain region and gene expression across 88 species representing five separate vertebrate lineages. Among the brain regions analyzed were the VTA-NAc, amygdala, and mPOA of the hypothalamus. In addition to neuroanatomical conservation, expression of the receptors that mediate social behavior, including the dopamine 1 receptor, showed little variation between the species assessed. Interestingly, however, there was significantly less conservation of the site of ligand production, and the ligands themselves, for those receptors. The authors suggest that the high conservation of receptor distributions may be explained by the need for animals of all types to respond to salient social stimuli, while differences in the local production of ligands could be selected for by lineage differences in life history and ecology. These require differential weighting of various social modalities, e.g., differences in predominantly used sensory inputs. The structure and function of the nonapeptide systems, which consist of mesotocin (MT) and arginine vasotocin (VT) in birds, and the homologous oxytocin (OT) and vasopressin (VP) in mammals, are strikingly conserved across birds, fish, and mammals [Goodson et al., 2012a]. Oxytocin and vasopressin are neuropeptides released by the hypothalamus that influence social behaviors in many species, from insects to humans [Insel and Young, 2000; Donaldson and Young, 2008; Goodson et al., 2009, 2012b]. Additionally, in fish, there is work showing the conservation of the dopaminergic reward system between lampreys and mammals [Pérez-Fernández et al., 2014], although most focus to date has been on the role of dopamine in movement, not reward [Ryczko et al., 2016] (for a comprehensive review of the dopamine system in chordates, see Yamamoto and Vernier [2011]). Zebrafish have brain region analogues to many of the core social behavior nodes, including the amygdala, hypothalamus, and VTA-NAc [Geng and Peterson, 2019]. Although it is true that there are many complex neurotransmitter and neuropeptide systems that contribute to sociability in the social behavior network, here, and for the rest of this review, we focus on dopamine as it is the most well-described mediator between sickness and social behavior [Kopec et al., 2019]. All told, there is significant evidence that the midbrain structures important for social behavior, including the amygdala, hypothalamus, and mesolimbic dopamine system, are present and functionally relevant in many vertebrate and invertebrate species.

While considerably less is known about brain circuits in ancestral vertebrates and invertebrates, there is still a fair amount of evidence that the core "social behavior" brain regions are conserved and contribute to social motivation and reward. The hypothalamus, considered to be an "ancient" region of the mammalian brain, has correlates that predate vertebrate origin. In an integrated transcriptome study, Lemaire et al. [2021], identified nuclei in the brain of the *Ciona* tadpole that closely resemble those of hypothalamic nuclei in the rodent and human brain. In the amphibious African clawed frog (*Xenopus laevis*), the amygdala is responsible for initiating socially

appropriate vocalizations [Hall et al., 2013]. In fruit flies (*Drosophila melanogaster*) and other insects, the mushroom body, an associative cortex similar to the ACC in mammals, is influenced by social experience and promotes social attraction [Sun et al., 2020]. Dopamine as a neurotransmitter is crucial for the regulation of social behaviors among many insect species. Dopamine has an effect on learned, nonsocial reward in flies [Riemensperger et al., 2011], and dopaminergic neurons also control aggression in male flies, a type of social interaction [Alekseyenko et al., 2013]. Additionally, increased dopamine signaling in flies causes an increase in male-male courtship behaviors, an interesting phenomenon that is also replicated by targeting the mushroom body with the female *transformer* gene [Ferveur et al., 1995; Liu et al., 2008]. Dopamine in red harvester ants also facilitates foraging – a coordinated social behavior that is specific to eusocial species [Friedman et al., 2018]. Recently, new tools have facilitated electrophysiological recordings of the mushroom body in freely moving honeybees while they participate in innate social behaviors [Duer et al., 2015; Geffre et al., 2020; Paffhausen et al., 2020]. More work in this area will be essential to understanding the similarities and differences between vertebrate and invertebrate social behaviors, and the brain regions and neurotransmitters that modulate them.

Immune and Microbial Mediators That Exert Their Effects on Social Brain Systems and Behavior

As described earlier, cytokines play a critical role in the induction of sickness behaviors and can activate microglia and neurons that control these behaviors [Dantzer, 2009]. For examples of the many changes in cytokine levels in response to infection, see the "Molecular signature" column in Table 1. Over 20 years ago, Song et al. [1999] demonstrated that systemic administration of pro-inflammatory cytokines interleukin-2 (IL-2) and interleukin-6 (IL-6) drastically reduce dopamine release into the nucleus accumbens. Additionally, Bluthé et al. [2000] demonstrated that genetic knock-out of pro-inflammatory cytokine IL-6 in rats prevents LPS-induced deficits in social exploration, and injection of anti-inflammatory interleukin-4 (IL-4) or other anti-inflammatory drugs have similar effects [Fishkin and Winslow, 1997; Bluthé et al., 2002]. Since these initial experiments, it has become increasingly recognized that cytokines have a profound effect on the central nervous system. For example, they can directly impact neurons, influencing the metabolism of neurotransmitters as well as changing neuronal excitability in specific brain regions [Miller et al., 2013; Treadway et al., 2019]. Alternatively, cytokines can influence microglia and astrocytes, which in turn use cytokines and other signals to regulate neural activity [Béchade et al., 2013; Haydon and Nedergaard, 2015; Badimon et al., 2020]. For these reasons, many studies have linked elevated pro-inflammatory cytokines to changes in brain function and social behavior following an immune challenge.

There is a wealth of evidence that many socially relevant brain regions are affected by cytokines and inflammation. Both the ACC and ventral striatum have been shown to facilitate inflammation-induced social changes in human participants [Inagaki et al., 2015; Muscatell et al., 2016]. Typhoid vaccine injection in humans produces elevated levels of IL-6 in the blood, which correlate with brain activation in the ACC, amygdala, NAc, and other regions [Harrison et al., 2009]. Following exposure to endotoxin (LPS), IL-6 and tumor necrosis factor α (TNFα) levels positively correlate with fMRI activation in the amygdala [Inagaki et al., 2012] and ventral striatum [Inagaki et al., 2015] in humans. In mice, it has been shown by multiple groups that LPS robustly increases pro-inflammatory cytokine production and neural activation in the hypothalamus, while decreasing dopamine [Frenois et al., 2007; Araki et al., 2016; Burfeind et al., 2016, 2018; Zenz et al., 2019; Veit et al., 2021]. Additionally, administration of LPS causes upregulation of the pro-inflammatory cytokines interleukin-1β (IL-1β), TNFα, and IL-6 in the PFC [Audet et al., 2011] and causes degradation of dopamine and serotonin in the nucleus accumbens [Dölen et al., 2013; van Heesch et al., 2014; Korte-Bouws et al., 2018]. In the amygdala, treatment with LPS in mice induces microglial activation, increases gene expression of pro-inflammatory cytokines, and causes neuronal hyperexcitability [Zheng et al., 2021]. Intriguingly, blocking cytokine signaling is often sufficient to prevent sickness-induced social changes. For example, administration of an interleukin-1 (IL-1) receptor antagonist can rescue the effects of LPS on social behavior and amygdala activation in adult male rats [Kent et al., 1992; Bluthé et al., 1994]. In 2016, Filiano et al. described the first experiments that elucidated the unexpected role of interferon-γ (IFN-γ, a T cell-derived cytokine) in regulating social behavior. They showed that SCID mice (mice lacking an adaptive immune system) show no social preference, while SCID mice repopulated with lymphocytes demonstrate a normal social preference [Filiano et al., 2016]. The effects of some cytokines are mixed, however. Injection with interleukin-4 (IL-4), an anti-inflammatory cytokine, actually

Table 1. Cytokine changes in response to peripheral immune stimulation or infection in diverse species

Species	Infection type (dose)	Behavioral effect	Molecular signature	Citation
Human	LPS (0.8 ng/kg)	Social disconnection, greater desire for support figure	Increased IL-6, TNFα	[Inagaki et al., 2012, 2015; Muscatell et al., 2016]
			Enhanced neural sensitivity to social stimuli	
Mouse	LPS (10 μg or 330 μg/kg)	Decrease in social exploration/ preference	Increased TNFα, IL-1β, and IL-6	[Bluthé et al., 1994; Audet et al., 2011; Smith et al., 2020]
	TNFα (2.5 μg)			
	IL-1β (50 ng)			
Rat	LPS (5 mg/kg or 150 μg/kg)	Decrease social initiations, increased time spent huddling	Increased IL-1β in microglia	[Buttini and Boddeke, 1995; Yee and Prendergast, 2010]
Boars	Osteochondrosis (natural)	Increased social contact	IL1-rα and IL-12	[Munsterhjelm et al., 2017]
Pigs	LPS (1.2 μg/kg)	Increased social ear manipulation	No change in IFN-y, TNFα, or IL-18	[Veit et al., 2021]
			Decreased hypothalamic dopamine and serotonin	
Fish	LPS (45/90/180 μg)	Decreased social investigation/ contact	Increased TNFα, IL-1β, IL-6	[Kirsten et al., 2018; Petitjean et al., 2021]
	Aeromonas hydrophila bacterin (50 μL of 4×10^5)			
Reptiles	IL-1β (10 ng/g)	Decrease in locomotor activity	Unknown	[Dunlap and Schall, 1995; Dunlap and Church, 1996]
	Malaria parasite (natural)	Decreased male courtship behaviors		
Amphibians	LPS (2 μg/g)	Decreased feeding and movement (social not assessed)	Increased expression of many cytokine-related genes	[Llewellyn et al., 2011; Gardner et al., 2018]
Xenopus	LPS (20 or 200 μg)	Unknown	Increased IL-1β	[Zou et al., 2000]
Rhesus macaques	LPS (4 or 40 ng/kg)	Increased social contact	Leukocyte, neutrophilic, IL-6, and cortisol changes	[Willette et al., 2007]
Honeybees	LPS (4 μg per bee)	Decreased antennal contacts	Unknown	[Kazlauskas et al., 2016]
Birds	Sheep red blood cells (5×107 SRBCs)	Decreased social song production	Decreased testosterone	[Garamszegi et al., 2004]
Field crickets	LPS (10 mg/g)	Decreased social calling rate	Unknown	[Jacot et al., 2004]
Vampire bats	LPS (5 mg/kg)	Decreased social contact calls	Unknown	[Stockmaier et al., 2020]

elicits social changes on its own, but ameliorates social deficits when co-administered with LPS [Bluthé et al., 2002], suggesting a complex role for some anti-inflammatory cytokines in social behavior. In all, these data suggest that cytokines can causally modulate social behavior following infection by acting on social brain networks.

Microglia play a starring role in translating peripheral immune activation to brain and behavior. As the resident macrophages in the brain, they express and respond to cytokines, maintain the local environment, and can directly and indirectly modulate neuronal activity. Thus, these little cells are uniquely suited to have a big impact on brain function during illness. Indeed, it has been shown many times that microglia release IL-1β, TNFα, and IL-6 in response to peripheral LPS injection [van Dam et al., 1992; Buttini and Boddeke, 1995; Smith et al., 2020], an effect ameliorated by anti-inflammatory interleukin-10 (IL-10) [Heyen et al., 2000]. Furthermore, microglia have altered morphology in the NAc following LPS exposure [Siemsen et al., 2020]. Given that it is un-

likely for LPS to cross the blood-brain barrier, it has been argued that this cytokine release by microglia could be one major way that experimental, non-central nervous system (CNS) infection impacts social behavior. Microglial function and social behavior are related, as a variety of disruptions to microglia function influence social behavior. Recently, Lowery et al. [2021] showed that loss of P2YR12 in microglia causes decreased following and olfactory investigation of a novel social stimulus [Lowery et al., 2021]. Further, elevated protein synthesis in microglia and deficient microglia-neuron signaling both decrease social investigation [Zhan et al., 2014; Xu et al., 2020]. Finally, chronic activation of microglia using DREADDS (designer receptors exclusively activated by designer drugs) prevents LPS-induced decreases in sociability, implicating a causal role for microglia in immune activation and social behavior [Binning et al., 2020]. These findings elegantly demonstrate that microglia, social behavior, and immune activation are intricately linked.

Immune Mediators of Social Behavior across Species

Cytokine responses to pathogen exposure exist in a wide array of vertebrates that display sickness behaviors. Sickness, cytokines, and social behavior are strongly correlated in boars [Munsterhjelm et al., 2017], rhesus macaques [Willette et al., 2007], humans [Muscatell et al., 2016], zebrafish [Kirsten et al., 2018], rats [Buttini and Boddeke, 1995; Yee and Prendergast, 2010], mice [Smith et al., 2020], and pigs [Veit et al., 2021]. For a detailed list, see Table 1. While the effects of sickness on social behaviors in amphibians and reptiles is understudied, cytokines are known to mediate behavioral fever in lizards and amphibians [Dunlap and Church, 1996], and LPS injection induces sickness behaviors in toads [Llewellyn et al., 2011], an effect likely mediated by pro-inflammatory cytokines [Gardner et al., 2018]. Little is also known about the role of invertebrate cytokine networks in mediating social behaviors. Data suggest that invertebrate cytokine-like molecules and vertebrate factors do not have the same evolutionary origin, suggesting there may be different mechanisms by which the brain is influenced by the immune system in invertebrates [Beschin et al., 2001]. Nonetheless, there may still be some role for invertebrate cytokine signaling in sociability. Using publicly available transcriptomes from socially enriched mice, rats, fish, and flies, Filiano et al. [2016] demonstrate a strong conservation of the IFN-γ gene signature in more social organisms, suggesting an evolutionarily conserved role for adaptive immune signals in regulating social behavior [Filiano et al., 2016]. Nonetheless, while infection and social behavior do seem to be linked in invertebrate species, the molecular mechanisms linking the two have been woefully understudied.

While there is still much to discover about the diverse functions of microglia, there is considerable evidence of their presence in many species. In a study across 33 different mammalian species, Santos et al. [2020] found little variation in overall microglial densities and microglia-neuron ratio, implying the preserved importance of these cells [Santos et al., 2020]. With the advent of single-cell transcriptomic profiling, researchers have recently begun to disentangle the conserved and divergent transcriptional programs for microglia. Analyzing the gene expression profile of microglia in humans, macaques, marmosets, rats, mice, hamsters, chickens, and zebrafish demonstrates remarkable conservation of homeostatic marker genes, including Aif1 (IBA1) and P2YR12 [Geirsdottir et al., 2019]. Among ancestral vertebrates, reptiles have microglia [Castellano et al., 1991; Tosches et al., 2018], but they have been studied only in the context of nerve regeneration [Nacher et al., 1999]. Microglia are present and have been studied extensively in zebrafish, making them a powerful model organism for studying conserved vertebrate neuroimmune mechanisms. Microglia in zebrafish respond to infection and brain injury in ways similar to human microglia [Peri and Nüsslein-Volhard, 2008; Var and Byrd-Jacobs, 2019, 2020] and even establish regional heterogeneity in the brain similar to mammalian microglia [Wu et al., 2020].

Most of what is known about microglia in invertebrates comes from studies in annelids, most notably the medicinal leech (*Hirudo medicinalis*) [Del Río-Hortega, 1920]. Work more recently has demonstrated that microglia in the leech express markers similar to those identified in mammalian microglia, suggesting that some core features of microglial identity emerged early in evolution [Drago et al., 2014; Sharma et al., 2021]. Additionally, microglia in the freshwater snail proliferate in response to trauma, a similar response to what is observed in mammalian microglia [Peruzzi and Sonetti, 2004]. Among the nematodes, *C. elegans* have glia, and cells similar to microglia [Singhvi and Shaham, 2019]. For a detailed, comparative review of microglia across species, including invertebrates, see Sharma et al. [2021]. Very little is known about the impact of infection on microglia in invertebrates, reptiles, or amphibians, however.

Immune Mediators of Neurodevelopment

Adaptive Roles

In addition to being responsible for translating sickness signals to adaptive "sickness behaviors," microglia and the immune system are necessary for the healthy development of social behavior circuits in the brain. As mentioned above, genetic manipulations that result in elevated protein synthesis in microglia or impair microglia-neuron signaling both decrease social investigation [Zhan et al., 2014; Xu et al., 2020]. Further, Nelson and Lenz [2017] have demonstrated that depletion of microglia early in life in rats decreases neonatal ultrasonic vocalizations, juvenile social play behavior, and male adult sex behaviors [VanRyzin et al., 2016; Nelson and Lenz, 2017]. Even more recently, VanRyzin et al. [2019] showed that microglial engulfment of newborn cells in the developing amygdala of male rats programs juvenile social play [VanRyzin et al., 2019]. Excitingly, Kopec et al. [2018] highlighted one important mechanism by which microglia control the development of circuits mediating social play behavior in male rats. Kopec et al. [2018] showed that dopamine 1 receptor (D1r) density in the NAc is correlated with complement-dependent microglial phagocytosis, and that blocking microglial phagocytosis at postnatal day 30 (P30) prevents the appropriate developmental decline in social play behavior by P38 [Kopec et al., 2018]. Notably, this effect was only present in males, once again suggesting that there are sex-specific differences in the immune influences on social behavior.

Perhaps not so surprisingly, little is known if/how the immune system in species other than rodents contributes to social neural wiring during development. Due to the scarcity of tools in these organisms, this is a challenging question to answer [Sharma et al., 2021]. It is likely the case that microglia acquired these specializations in parallel with the evolution of more complex brains in derived organisms, but detailed studies on this hypothesis have yet to be done. There is now some exciting, yet limited, evidence that microglia refine axons via trogocytosis in the developing frog [Lim and Ruthazer, 2021]. Now, with the recent discovery of distinct, socially relevant brain regions in invertebrates [Paffhausen et al., 2020; Sun et al., 2020], more work is needed to elucidate the critical periods in development of these regions, as well as the potential role of microglia in this development.

Maladaptive Roles – When Things Go Wrong

Involvement of the immune system (including microglia) in the brain's response to sickness outside of developmental windows is adaptive. As described in previous sections, the immune system can efficiently and effectively influence brain and behavior to prevent the spread of illness and optimize recovery. However, when a challenge to the immune system occurs during critical windows of developmental plasticity, this can have devastating consequences. There is a wealth of evidence that immune activation early in life impacts social brain development and long-term social behavior [Bilbo and Schwarz, 2009; Smith et al., 2020]. In a maternal immune activation model, male offspring born to mothers injected with the viral mimetic Poly:IC exhibit fewer social ultrasonic vocalizations in early life and show a decrease in social preference in adulthood [Malkova et al., 2012]. Interestingly, this effect is mediated by the pro-inflammatory cytokine Interleukin-17A (IL-17a), as blocking this cytokine in the mother prevents the effects of Poly:IC, demonstrating that activating (and inhibiting) the immune system during development influences later-life social behavior [Choi et al., 2016]. This line of work suggests a relationship between the prenatal immune environment and later life manifestations of social behaviors, especially in males.

Other mechanisms of immune activation during other critical developmental windows have also been shown to exert far-reaching effects on social behavior. For instance, maternal high fat diet, a condition known to increase brain inflammation and impact microglia in developing embryos [Bilbo and Tsang, 2010; Bolton and Bilbo, 2014; Liu et al., 2021], results in decreased sociability in male offspring [Buffington et al., 2016]. Furthermore, exposure to LPS during postnatal development in mice at postnatal day 4 (P4) causes female-specific deficits in social exploration and preference [Smith et al., 2020]. Interestingly, this effect was *not* dependent on microglial myeloid differentiation protein 88 (MyD88) signaling, suggesting there are other immune effectors in females that translate infection to changes in social behavior. Finally, prenatal exposure to Poly:IC followed by subsequent exposure to LPS on P9 induces social, immune, and microglial changes in both sexes, with more pronounced behavioral and cytokine changes in male mice compared to females [Carlezon et al., 2019]. Taken together, these data represent the beginning of an innovative avenue for research into the long-term consequences of immune/brain crosstalk, with a special emphasis on the development of social behavior.

Conclusions – Importance for Studying Immune-Social Brain Crosstalk

We experience a never-ending "evolutionary arms race" against pathogens. With the continual onslaught from pathogens, our immune system also continually adapts to use new tools to mount a sufficient defense. For many years, the brain was considered a site of "immune privilege," mainly due to the blood-brain barrier and its ability to protect itself in the case of infection. While we now know more about the crosstalk between the immune system and the brain, there is still much to learn, specifically in the context of sex, age, and species differences. It is clear that infection and the ensuing immune reaction can have a profound effect on a variety of social behaviors across multiple species. However, the exact mechanisms by which this occurs, and if they differ between males and females, have not been fully explored. Additionally, it is evident that young versus old rodents express sickness-induced social changes differently, but is this consistent across species? How does ageing of the brain (and immune system) affect this crosstalk? Moving forward, more work linking microglia and immune effectors as key mediators in social behavior during sickness and development will help to elucidate the biology dictating this complex process. Developing a greater understanding of how infection can impact the brain will provide valuable insight about the molecular mechanisms by which different systems in the brain govern complex behaviors.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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