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INVISIBLE DESIGNERS: BRAIN EVOLUTION THROUGH THE LENS OF PARASITE MANIPULATION

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ABSTRACT

The ability of parasites to manipulate host behavior to their advantage has been studied extensively, but the impact of parasite manipulation on the evolution of neural and endocrine mechanisms has remained virtually unexplored. If selection for countermeasures has shaped the evolution of nervous systems, many aspects of neural functioning are likely to remain poorly understood until parasites—the brain's invisible designers—are included in the picture. This article offers the first systematic discussion of brain evolution in light of parasite manipulation. After reviewing the strategies and mechanisms employed by parasites, the paper presents a taxonomy of host countermeasures with four main categories, namely: restrict access to the brain; increase the costs of manipulation; increase the complexity of signals; and increase robustness. For each category, possible examples of countermeasures are explored, and the likely evolutionary responses by parasites are considered. The article then discusses the metabolic, computational, and ecological constraints that limit the evolution of countermeasures. The final sections offer suggestions for future research and consider some implications for basic neuroscience and psychopharmacology. The paper aims to present a novel perspective on brain evolution, chart a provisional way forward, and stimulate research across the relevant disciplines.

INTRODUCTION

THE ability of some parasites to manipulate their hosts' behavior is a growing topic in biology (Hughes et al. 2012; Adamo 2013; Mehlhorn 2015a; Poulin and Maure 2015; Heil 2016), and has become a staple

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of popular science (McAuliffe 2016; Simon 2018). From "zombie ants" to "kamikaze crickets" and "mind-controlling wasps," the parade of behavior-altering parasites and their victims offers riveting examples of how nervous systems can be hijacked to serve the interests of hostile organisms. Despite its sensational qualities, behavioral manipulation is not a rarity in nature: the list of manipulating organisms is long and includes viruses, bacteria, protozoa, fungi, helminths (parasitic worms), and insects such as wasps and flies (Hughes et al. 2012; Mehlhorn 2015a; Poulin and Maure 2015). Just as important, behavior-altering strategies have a remarkably deep evolutionary history. Parasites have attempted to control their hosts' behavior for hundreds of millions of years (Adamo 2013). Host manipulation has evolved independently at least 20 times; fossilized ants show that present-day manipulation strategies by fungi and helminths were already well established around 30–50 million years ago, suggesting that they originated much earlier (see Poulin 2010; Hughes 2014).

Research on behavioral manipulation has focused almost exclusively on the evolution of parasites and their strategies. This includes the specific biochemical mechanisms employed by parasites (e.g., Adamo 2012, 2013; Perrot-Minnot and Cézilly 2013; Herbison 2017; Libersat et al. 2018), the evolutionary trajectories that lead to manipulation (e.g., Poulin 2010; Thomas et al. 2012; Loreto et al. 2018), and the corresponding tradeoffs (e.g., Poulin et al. 2005; Roitberg 2012). What has been almost entirely neglected is the effect of parasites on the evolution of their hosts' nervous and endocrine systems. Millions of years of attacks by manipulating organisms must have exerted a powerful selective pressure on brain evolution in animals. If so, present-day nervous systems should embody a variety of countermeasures to manipulation accumulated through a long coevolutionary history—possibly reaching all the way down to some of the most basic, ubiquitous features of neural functioning.

This crucial observation was made by Read and Braithwaite (2012) in an afterword to a book chapter, but—to my knowledge—has not been followed up in the literature until now. It is worth quoting some passages: "There are two ways hosts can protect themselves from behavior attack. One way is to kill or incapacitate the causal pathogen. The other way is to counter the manipulation itself, either by making behavior control systems less vulnerable to attack, or by recalibrating things to accommodate the manipulation. Immunologists study the first kind of defense; next to nothing is known about the other kind [. . .]. How much of our neural complexity is a necessary defense against manipulative invaders? How much of the enormous redundancy is to provide system level functionality if part of the system is attacked? How much of the complex process of wiring a brain during development is to prevent pathogen re-wiring?" (Read and Braithwaite 2012:195). The authors predicted that these questions would soon become central to behavioral biology and neuroscience. Instead, parasites have continued to claim the spotlight, and the fascinating issue of how the brain protects itself from manipulation has been left unaddressed.

Overview

In this paper, I begin to systematically explore the question of how parasite manipulation may have shaped the evolution of brain mechanisms. I start by reviewing the strategies employed by parasites that target the central nervous system and related endocrine pathways. I then present a taxonomy of possible countermeasures to manipulation, and consider the likely evolutionary responses by parasites (Table 1). A broad range of potential countermeasures are discussed, from the more plausible (e.g., increasing the complexity and metabolic costs of molecular signals) to the more speculative (e.g., employing individualized "signatures" to protect signaling pathways from eavesdropping and intrusion). For each hypothetical strategy, I consider what neural and endocrine mechanisms might implement it in the real world and look for possible examples in the literature. This section has a deliberate exploratory character: the goal is not to demonstrate that a given mechanism works—fully or in part—as a countermeasure to manipulation, but to

TABLE 1
A taxonomy of countermeasures against behavioral manipulation

Host countermeasures	Possible examples	Possible responses by parasites
Restrict access to the brain	Blood-brain barrier as physical defense	Cross/bypass the barrier
	Blood-brain barrier as chemical defense	Target weak spots in the barrier
	Loss of molecular entry points	Target influx/efflux mechanisms
	Decoy molecules	Find new molecular entry points
		Avoid decoy molecules
Increase manipulation costs	Metabolically costly signals	Cost-sharing
	Toxic signaling molecules (dopamine, nitric oxide, antimicrobial peptides)	Indirect attacks, genomic manipulation
		Detoxification, antimicrobial resistence
Increase signal complexity	Diversification of signals/receptors	Indirect attacks, genomic manipulation
	Co-transmission	Increase manipulation complexity
	Convergent signaling	
	Pulsatile signaling	
	Individualized signatures	
Increase robustness	Passive: redundancy, modularity	Target core regulatory processes ("knots")
	Reactive: negative feedback, specialized detection/interference mechanisms	Target vulnerable feedback loops
	Proactive: immune-activated countermeasures, preemptive compensation	Target/escape detection mechanisms
		Target/escape immune responses
		Increase manipulation strength

Each type of countermeasure is followed by possible examples of neurobiological mechanisms and evolutionary responses by parasites.

single out promising candidates for further investigation. Next, I discuss the constraints that limit the evolution of countermeasures in the hosts. I suggest that metabolic, computational, and ecological considerations contribute to explain why complex "manipulation syndromes" are mostly observed in small animals such as ants and snails, and why there are no established examples of adaptive behavior manipulation in our species.

In the following section I advance suggestions for research on this topic. No doubt, identifying host countermeasures in the intricate workings of the brain is a formidable task. Some antiparasite adaptations may have originally evolved with different functions; others may have been recruited and exapted in the service of different goals. Others still may have become useless over time—either because they were successfully thwarted by parasites, or because they proved so effective that parasites were forced to take a different evolutionary route. Such "frozen countermeasures" can persist indefinitely if they have become embedded into basic neural processes. Mapping what Read and Braithwaite called the "ghost of manipulations past" (Read and Braithwaite 2012:195) is going to require a great deal of reverse engineering, modeling, and comparative analysis. Finally, I consider some implications for basic neuroscience and psychopharmacology. My goal in this paper is not to provide definitive answers but to open up a new area of research, chart a provisional way forward, and stimulate responses from researchers across the relevant disciplines.

Hijacking the Brain: Parasite Manipulation Strategies

Controlling behavior requires the coordinated action of multiple systems, including neural and hormonal mechanisms but also sensory and motor organs. Each node in the control network is also a potential target

for manipulation. Since the topic of this paper is brain evolution, I restrict my focus to the central nervous system (CNS) and the major endocrine pathways that relay signals between the brain and the rest of the body. I do not discuss the strategies of parasites that take direct control of the host's muscles (as it has been suggested in the case of the ant-infecting fungus *Ophiocordyceps unilateralis*; Fredericksen et al. 2017); target sensory receptors; and indirectly affect behavior by depleting energetic reserves, castrating/sterilizing the host, or altering sex determination (see Adamo 2012; Lafferty and Shaw 2013). Also excluded is manipulation through sensory cues and signals, as practiced by brood parasites in birds (e.g., cuckoos; Langmore and Spottiswoode 2012) and social parasites in insects (e.g., beetles that parasitize ant colonies; Grüter et al. 2018). Before addressing specific mechanisms, however, it is useful to briefly consider the functions of manipulation from the perspective of parasites. When they hijack the host's nervous system, what kinds of behaviors are they attempting to produce, and why?

FUNCTIONS OF BEHAVIOR MANIPULATION

Transmission

The first and most frequently studied function of manipulation is to increase the probability of transmitting the parasite. This can take a number of distinct forms depending on the parasite's life cycle and transmission route. In *trophic* transmission, an intermediate host is manipulated to make it more susceptible to predation by the definitive host (Lafferty 1999). To this end, a parasite may reduce the host's avoidance and antipredator behaviors, or even replace avoidance with attraction. A classic example is protozoan *Toxoplasma gondii*: infected rats lose their innate aversion to the odor of cats—the definitive host—and may even become attracted to it (this phenomenon has been dubbed "feline attraction"; Berdoy et al. 2000; Ingram et al. 2013; Kaushik et al. 2014). Alternatively or in addition, parasites may induce behaviors that actively lure predators toward the host. Gammarid crustaceans infected by spiny-headed worms become attracted by light (positive phototaxis), and when the water is disturbed they do not respond by escaping; instead, they swim to the surface and cling to a solid object, thus becoming easy prey for birds. When California killifish are infected by the fluke *Euhaplorchis californiensis*, they begin to display atypical swimming behaviors—such as jerky, conspicuous movements and sudden swims to the surface—that attract bird predators (Adamo 2002, 2013; Lafferty and Shaw 2013). The abdomen of tropical ants infected by the roundworm *Myrmeconema neotropicum* turns from black to bright red; the ants then climb into patches of red berries and raise their abdomen to mimic a fruit, thus attracting the frugivorous birds that serve as the parasite's final host (Yanoviak et al. 2008; for more examples see Poulin 2010; Lafferty and Shaw 2013; Mehlhorn 2015b).

In addition to trophic transmission, parasites can move between hosts through skin contact, bodily fluids (e.g., saliva, blood), or excretions (e.g., vomit, feces), and indirectly through animal vectors (e.g., mosquitoes). For vector-borne parasites, a way to facilitate transmission is to alter the behavior of the vector so it will visit more hosts in the same amount of time (e.g., a mosquito may suck blood from more individuals, spending a shorter time on each). This is what happens to mosquitoes infected with malaria *Plasmodium*, although it is still unclear whether the behavioral change is a targeted manipulation by the parasite or a compensatory response by the host (Cator et al. 2013; Heil 2016). Rabies viruses offer a remarkable example of facilitation in a directly transmitted parasite. In the acute phase, the symptoms of rabies combine increased production of infected saliva, aversion to water (which further concentrates the saliva), and unpredictable bouts of aggressive biting that transmit the virus to the victims (Hemachudha et al. 2013; Jackson 2013). In principle, sexually transmitted parasites can spread more effectively by manipulating aspects of the host's sexual behavior, including frequency of mating, mate choice selectivity, attractiveness to potential mates, and specific copulatory behaviors. Although there have been initial

reports of manipulation by sexually transmitted parasites, the evidence is still scant and the topic remains understudied (see Adamo 2014; Heil 2016).

Movement

Another common goal of manipulation is to make the host move to a different habitat, one that is favorable to the parasite or its offspring. Typically, the new habitat is used for sexual reproduction or the release of infective propagules (e.g., spores or cysts; see Poulin 2010; Moore 2013; Mehlhorn 2015b). Grasshoppers and crickets infected by horsehair worms begin to seek out water and eventually jump into it, releasing the adult parasite—which can then complete its reproductive cycle—and usually dying in the process (Poulin 2010; Mehlhorn 2015b). Bees infected by larvae of the parasitoid fly *Apocephalus borealis* fly far away from their hive, die, and release the larvae as these are ready to pupate (Core et al. 2012).

Protection

Less intuitively, parasites may also use the host as a "bodyguard" to protect their offspring during critical developmental stages. This is most commonly seen in parasitoid insects. For example, braconid wasp larvae grow inside caterpillars, feeding on the host's tissues. After the larvae exit to pupate, the dying caterpillar remains coiled on the cocoons that contain the pupae and begins to perform violent head-thrashing movements. The movements keep away potential predators and increase the survival of the pupae. Other wasps parasitize spiders, and manipulate them into weaving special "cocoon webs" to protect the developing pupae (Gonzaga et al. 2017; these and other examples of bodyguard manipulation are reviewed in Maure et al. 2013).

conditionally helpful parasites

The strategies that promote the fitness of parasites generally reduce that of the hosts—not infrequently to the point of killing them. Still, manipulation may have a silver lining if the parasite's strategy turns out to benefit the host under particular conditions. For example, trophic parasites should be selected to *enhance* the intermediate host's antipredator behaviors (and hence survival) until they are ready to be transmitted to the definitive host (Parker et al. 2009). There is evidence that, in the early phase of the infection, spiny-headed worms protect their gammarid hosts by making them less susceptible to predation (Dianne et al. 2011). Other conditionally helpful parasites may increase their host's mating success or help prevent infections by other and potentially more harmful parasites (see Fellous and Salvaudon 2009; Weinersmith and Earley 2016). The net effect on the host's fitness is still likely to be negative in most cases, but may turn positive if ecological conditions put a premium on the specific phenotype induced by the parasite. For example, heightened antipredator behaviors may provide a net benefit if predation risk is especially severe; enhanced immunity from other parasites may be a crucial advantage under high pathogen threat (see Weinersmith and Earley 2016).

mechanisms of behavior manipulation

The most obvious target of behavior manipulation is an animal's brain. Parasites can penetrate inside the brain and attack it from within or secrete neuroactive compounds that will reach the brain through circulation. In both cases, they need to get past the blood-brain interface and its defenses. Alternatively, a parasite may take an indirect route and target endocrine organs such as the thyroid, gonads, or various components of the immune system. The hormones produced by these organs modulate brain function and can powerfully affect behavior. The same applies to the cytokines secreted by immune cells such as macrophages and lymphocytes. Again, the parasite may lodge itself inside an organ or manipulate it from the outside. Endocrine systems are not just a potential target for hijacking: parasites can eavesdrop on the host's hormonal signals to gain precious information about the state of the organism and respond adaptively. For example, enteric pathogens such as *Escheri-*

chia coli and *Salmonella enterica* can sense increases in stress-related catecholamines (such as epinephrine and norepinephrine), and respond by accelerating growth and expressing virulence factors (Roshchina 2010; Stevens 2010; Neuman et al. 2015). Likewise, many sexually transmitted microbes regulate their growth in response to sex hormone levels (Jahoor et al. 2010). In addition to hormones, peripheral nerves that relay information to the CNS are vulnerable pathways that can be exploited to indirectly modulate brain function. Even more circuitously, some parasites may affect the host's behavior by manipulating its microbiota—including mutualists and commensal microorganisms (e.g., gut microbes) that may enjoy privileged channels of influence and communication with the host (Dheilly et al. 2015; more on this below).

When a parasite gains access to the brain or another endocrine organ, the simplest way to affect the host's behavior is to physically destroy part of the organ's tissue (Lafferty and Shaw 2013). Although this strategy can produce gross behavioral alterations, it carries a high risk of prematurely killing the host and is not well suited to yield subtle or coordinated changes. Unsurprisingly, then, parasites have evolved a striking variety of biochemical means of manipulation. For ease of presentation, these mechanisms can be grouped into three overlapping categories: *immunological*, *neuropharmacological*, and *genomic/proteomic* (Adamo 2013; Herbison 2017).

The immunological route is indirect but potentially very effective. The immune system enjoys extensive crosstalk with the brain through cytokines and autonomic nerves, and powerfully modulates behavior in response to infections—by affecting the animal's overall activity levels, sleeping patterns, feeding and food preferences, as well as a broad range of social behaviors (including mating; see Adamo 2014). By triggering or suppressing specific immune responses, parasites can exploit the associated behavioral changes to their advantage. Since the immune system is designed to detect and react to parasites *and* to modulate a range of neural and behavioral processes in the host, one could say that immune pathways are preadapted for manipulation (Adamo 2013). At the same time, immune reactions normally function to benefit the host; this makes it hard to conclusively demonstrate that a given behavioral symptom is in the interest of the parasite (Herbison 2017). Most of the putative examples come from invertebrates. When it infects its snail host, the fluke *Trichobilharzia ocellata* induces secretion of a cytokine that suppresses egg laying; the energy diverted from reproduction can then be used to support the parasite's growth (de Jong-Brink et al. 2001). A few hours before emerging from caterpillars, the larvae of parasitoid wasps secrete compounds that raise octopamine levels and suppress their host's feeding and locomotion; although the mechanism is not fully understood, there are reasons to think that immunological manipulation is involved (Adamo 2005, 2013; but see Herbison 2017 for a skeptical perspective). In gammarids infected by various species of helminths, the changes in serotonergic transmission induced by the parasites (see below) seem to be partly mediated by neuroinflammation, through the release of cytokines and nitric oxide (Helluy 2013). Abundant cytokines and nitric oxide are also released when toxoplasma cysts infect the brain of the host; but as in most other vertebrate examples, it is unclear whether the immune reactions triggered by the parasite are part of an adaptive manipulation strategy (see Herbison 2017).

When parasites secrete substances that directly affect the nervous system, they are said to engage in neuropharmacological manipulation (Adamo 2012, 2013). The typical targets are neurotransmitters and neuromodulators—most notably serotonin, dopamine, octopamine (an invertebrate analogue of norepinephrine), opioids, vasopressin, and nitric oxide (Perrot-Minnot and Cézilly 2013). Note that the standard distinction between neurotransmitters and neuromodulators is only heuristic: "classic" neurotransmitters such as dopamine and serotonin can also diffuse out of the synapse and exert modulatory effects (see Gutiérrez 2009; Leng 2018). Neuroactive hormones (e.g., sex and stress hormones) can also be used to influence the host's behavior. By directly modulating brain function, parasites can potentially achieve

highly specific changes and bring about novel behavioral patterns (in contrast, immunological manipulations can only exploit the host's evolved responses to infection). Neurotransmitters and hormones are highly conserved across species; their phylogenetic stability facilitates the evolution of specialized mechanisms of biochemical offense. Unsurprisingly, neuropharmacological manipulation is very common and has been documented in dozens of species (see Perrot-Minnot and Cézilly 2013).

Sometimes, alteration of a single key neurotransmitter triggers an entire "syndrome" of coordinated behaviors that can be exploited by the parasite. By increasing the synthesis of serotonin, helminths that parasitize gammarids produce a suite of behavioral changes that include reduced activity levels, positive phototaxis, and impaired escape behaviors (Perrot-Minnot et al. 2014). In other cases, the parasite secretes a mixture of molecules that target multiple biochemical systems at once. The best-known example is that of parasitoid jewel wasps, which use cockroaches as food supply for the developing larvae. These wasps inject a venomous "cocktail" directly into the brains of the cockroaches (ganglia). After the injection, the cockroach engages in vigorous self-cleaning for about 30 minutes, while the wasp leaves in search of a suitable nest. When the wasp returns, grooming has given way to a lethargic state; the cockroach lets the wasp bite off its antennae, obediently follows it to the nest, and remains immobile as the larvae are deposed in its body. To achieve this manipulation feat, jewel wasps rely on a mixture of molecules that includes dopamine, octopamine receptor antagonists, and opioid agonists (Libersat and Gal 2014). Dopamine is also one of the main biochemical targets of toxoplasma. As is common in microbial parasites, toxoplasma cysts do not directly secrete dopamine into the host's brain; instead, they release a key enzyme in the synthesis of dopamine (tyrosine hydroxylase) to increase its production by infected neurons (Gaskell et al. 2009; Adamo 2012, 2013). In male rodents, toxoplasma also invades the testes, where it stimulates the production of testosterone. Elevated testosterone increases vasopressin expression in the medial amygdala, a neurochemical alteration that seems to play a key role in the onset of "feline attraction" (Adamo 2013; Vyas 2015a,b; Heil 2016). This is another example of how behavioral manipulation may require the coordinated exploitation of multiple neurobiological systems.

To survive and reproduce within their hosts, many parasites rely on mechanisms that modify gene expression and protein synthesis in the host's cells. For example, viruses may induce the hosts to synthesize viral proteins by supplying strands of messenger RNA or directly insert copies of their genes into the host's DNA (retroviruses). Both protozoan and helminth parasites release vesicles containing small noncoding RNAs, which can regulate gene expression by host cells in myriad ways (e.g., Buck et al. 2014; Cheeseman and Weitzman 2015; Linhares-Lacerda and Morrot 2016; Bayer-Santos et al. 2017). All of these genomic/proteomic means of manipulation can be used to influence behavior. Caterpillars infected by the virus *Lymantria dispar* nucleopolyhedrovirus climb to elevated positions before dying and rupturing, thus spreading large amounts of infectious particles on the ground. To produce this behavior, the virus induces the host to synthesize a virally encoded enzyme; in turn, the enzyme inactivates a key hormone that regulates circadian cycles of climbing and descending (Hoover et al. 2011). Crickets and grasshoppers infected by hairworms show similar profiles of altered protein expression in the CNS, suggesting some kind of coordinated genomic/proteomic manipulation (Biron et al. 2005, 2006; Herbison 2017).

Clearly, the distinction between genomic/proteomic mechanisms and the other categories reviewed in this section is a fuzzy one: in many cases, parasites target gene expression to manipulate the host's immune system or modulate the activity of neurotransmitters, neuromodulators, and neuroactive hormones. Conversely, changes in immune and neurochemical profiles may lead to alterations in gene expression. For example, there is evidence that elevated testosterone in rats infected by toxoplasma triggers epigenetic modifications in the vasopressin gene, leading to increased expression of this neuromod-

ulator in the medial amygdala (Vyas 2015a,b; Herbison 2017).

Viruses possess sophisticated abilities of genomic manipulation, and some macroparasites (e.g., wasps) have evolved to exploit viruses as intermediate vectors to alter their host's behavior. The relationship is symbiotic: the virus provides the biochemical tools for manipulation, while at the same time it employs the macroparasite as a delivery device (Dheilly et al. 2015; Herbison 2017). The best-studied example is that of parasitoid wasps that use lady beetles as bodyguards to incubate their eggs. When the developed larva exits the abdomen to spin a cocoon and pupate, the lady beetle enters a phase of paralysis with tremors—a phase that lasts until the adult wasp emerges from the cocoon. The cause of the paralysis is not a compound secreted by the larva, but a symbiotic virus (*Dinocampus coccinellae* paralysis virus) injected by the wasp along with the eggs. The virus infects the lady beetle's CNS and triggers paralysis at the appropriate time; intriguingly, the mechanism used to produce the paralysis seems to exploit the host's own immune response, making this an instance of immunological manipulation by proxy (Dheilly et al. 2015).

MANIPULATION BY MUTUALISTS AND COMMENSALS

To end this section, it is worth considering the possibility that the host's behavior may be manipulated not just by parasites but also by mutualists and commensals, notably the gut microbiota. Clearly, gut microbes have the biochemical potential for manipulation and a privileged relationship with the host. Some of them can produce neurotransmitters such as γ-aminobutyric acid (GABA), acetylcholine, epinephrine, norepinephrine, dopamine, and serotonin; and may be able to modulate the permeability of the blood-brain barrier (BBB) by secreting specific proteins, thus increasing the penetration of neuroactive substances into the CNS (Cryan and Dinan 2012; Sherwin et al. 2016). Like enteric pathogens, they can sense and respond to fluctuations in the host's catecholamines (Rumbaugh 2007). Changes in the gut microbiota are also associated with levels of hunger- and metabolism-related hormones such as leptin, ghrelin, and neuropeptide Y. Besides releasing hormonal signals and toxins, microorganisms that colonize the gut of vertebrates may stimulate the vagus nerve (for example, with adrenergic chemicals) to deliver signals directly to the brain (Cryan and Dinan 2012; Alcock et al. 2014; Sherwin et al. 2016).

Based on these data and studies showing associations between the microbiota and various aspects of behavior, some authors have suggested that gut microbes manipulate the host for their own benefit. There is some experimental evidence that the composition of the gut microbiota modulates food choice in the fruit fly *Drosophila melanogaster*, in ways that favor the dominant species of bacteria (e.g., by inducing aversion to protein or attraction to carbohydrates; Wong et al. 2017; Yuval 2017). Alcock et al. (2014) considered the possibility that similar scenarios may play out in humans, and speculated that gut microbes may affect food preferences, patterns of hunger and satiety, and even obesity risk. To illustrate, one of their predictions is that increased microbial diversity should limit the ability of individual species to manipulate the host, leading to fewer cravings and more satiety.

Despite these promising leads, the evidence that the microbiota has consistent effects on behavior is still relatively weak. Establishing the causal direction of correlations is challenging, and there are justified concerns with the replicability of findings in this area (Forsythe et al. 2016; Hooks et al. 2018). What is more, evolutionary scenarios involving gut microbes are not a straightforward extension of those involving exploitative parasites, and face additional theoretical problems (Johnson and Foster 2018). The gut microbiota comprises a large number of species and strains that compete with one another for space and resources. If one of them were to evolve the ability to manipulate the host's behavior, it would have to pay the full cost of manipulation but share the benefits with its nonmanipulative counterparts, thus granting them a competitive advantage. Unless special conditions apply, selection should

limit the evolution of this kind of strategy; and, if this is the case, the behavioral effects of the microbiota might be best explained as byproducts of local competition for growth (for details see Johnson and Foster 2018). In summary, manipulation by mutualists and commensals is an intriguing possibility, but the underlying logic is still poorly understood and the phenomenon has yet to be convincingly demonstrated across species.

Securing the Brain: Possible Host Countermeasures

restrict access to the brain

The Blood-Brain Barrier as a Physical Defense

A first line of defense against behavior-altering parasites is to keep them out of the CNS. Naturally, the benefits of restricting access to the brain also apply to pathogens that do not adaptively manipulate behavior, but may damage the brain and produce detrimental neurological symptoms. In vertebrates and some invertebrates (including crustaceans and insects), the BBB provides an important layer of physical security (Daneman and Prat 2015; see Abbott 1992). Parasites have a number of options: they can manufacture behavior-altering substances and release them in the blood; invade other endocrine organs and manipulate hormone secretion; take the immunological route by activating specific immune responses; or cross the barrier to reach the brain.

Many parasites—from viruses and bacteria to helminths—have evolved the ability to penetrate or bypass the BBB (see Feustel et al. 2012; Masocha and Kristensson 2012). An interesting case study is the protozoan *Trypanosoma brucei*, the agent of sleeping sickness (Lundkvist et al. 2004; Mogk et al. 2017). There is still considerable uncertainty on how this parasite manages to enter the CNS (Kennedy and Rodgers 2019). Trypanosoma stimulates the release of various cytokines by lymphocytes and neurons; the same cytokines (including IFN-γ) facilitate the passage of immune cells from the blood to the brain, a mechanism that may be exploited by the parasite to cross the barrier (Grab and Kennedy 2008; Rodgers 2010; Kennedy and Rodgers 2019; Masocha and Kristensson 2019). Other data suggest that this parasite may bypass the BBB entirely, and penetrate through the blood-cerebrospinal fluid barrier in the choroid plexus—a site of intense cellular trafficking where leukocytes enter the CNS—and/or in the circumventricular organs (Mogk et al. 2017; Kennedy and Rodgers 2019). This illustrates the principle that parasites should evolve to target the inevitable "weak spots" in the barrier, which include passages for cranial nerves (e.g., the olfactory nerve; Masocha and Kristensson 2012) and several regions of relatively high permeability (e.g., around the pineal gland and the median eminence of the hypothalamus; Daneman and Prat 2015).

The Blood-Brain Barrier as a Chemical Defense

Another key function of the BBB is to regulate the flux of neurotransmitters/neuromodulators, hormones, and other molecules from the blood to the brain and vice versa. The brief summary that follows is based on studies of mammals such as rats, dogs, and humans, even though several BBB mechanisms are surprisingly conserved in both vertebrates and invertebrates (see DeSalvo et al. 2014; Hindle and Bainton 2014; Hindle et al. 2017; Saili et al. 2017). For some molecules such as serotonin and steroid hormones, the flux is bidirectional; other molecules only flow from the blood to the brain (influx) as, for example, insulin and thyroid hormone. The molecules that are only transported *out* of the brain (efflux) include GABA, glutamate, norepinephrine, dopamine, and the dopamine metabolite homovanillic acid (HVA). There are also active transporters that expel a variety of toxins and other foreign substances (xenobiotics), as well as metabolizing enzymes that inactivate them (Ohtsuki 2004; Banks 2012; Zhao et al. 2015; Saili et al. 2017). By clearing neuroactive molecules such as dopamine and GABA, the BBB probably contributes to regulate neurotransmission (Ohtsuki 2004). At the same time, the constant efflux of neurotransmitters and xenobiotics protects the brain from neurophar-

macological manipulation by compounds released in the blood, either by parasites lodged outside the CNS or by gut microbes and other mutualists/commensals. Recent experimental work shows that it is possible to chemically alter behavior without crossing the barrier, by modulating the activity of efflux transporters (Hindle et al. 2017). Although there is considerable literature on how parasites manage to cross the barrier to get *inside* the brain (e.g., Masocha and Kristensson 2012; Mogk et al. 2017), the question of how manipulation from the *outside* may have contributed to shape the biochemical functions of the barrier has received little if any attention.

Loss of Molecular Entry Points

To recognize and invade the host cells, many parasites make use of surface molecules. In particular, the layer of glycans (polysaccharides) that envelopes cell membranes is a common target for attack (see Gagneux et al. 2017). Parasites can mimic the composition of host glycans to escape immune detection, synthesize toxins that bind to specific glycans expressed by the host, or exploit glycans as attachment and entry points into the host cells. Conflict with parasites may explain why glycans tend to evolve rapidly, and often through loss of function—since loss of a particular glycan may eliminate a point of pathogen entry or pathogen action (Springer and Gagneux 2013; Schnaar et al. 2014). An especially intriguing case is that of *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc), two sialic acids that cap membrane glycans in vertebrates. Neu5Ac is converted into Neu5Gc by a specific enzyme, and the two are expressed in variable proportions across different species and tissues. The ability to synthesize Neu5Gc has been lost independently by humans, several other mammals, birds, and platypuses (Springer and Gagneux 2013; Gagneux et al. 2017); but even vertebrates that express high levels of Neu5Gc in bodily tissues show extremely low amounts of this molecule in the brain. There is recent evidence that suppression of Neu5Gc protects the brain from bacterial toxins that use it as a binding site (Naito-Matsui et al. 2017). To my knowledge, the potential implications of parasite-driven glycan evolution for brain physiology and function have yet to be explored.

Decoy Molecules

To prevent parasites from infecting their cells, hosts can evolve decoy surface molecules—molecules that mimic those exploited by parasites, but fail to perform the same function or even trigger a defensive response when bound. This is one of the many forms of "molecular deception" that may take place between parasites and hosts (Massey and Mishra 2018). A well-studied example of decoy molecules in humans is carcinoembryonic antigen-related cell adhesion 3 (CEACAM3), which mimics the cell adhesion molecule and immune inhibitory receptor CEACAM1 (Zimmermann 2019). Several bacterial and fungal pathogens bind CEACAM1 in order to attach to host cells, penetrate them, and/or modulate innate immune responses. Granulocytes express CEACAM3, which has a similar structure but induces phagocytosis of the parasites that bind it. In the resulting coevolutionary race, CEACAM1 is selected to avoid binding to parasites, parasites are selected to selectively bind CEACAM1 while avoiding CEACAM3, and CEACAM3 is selected to counteract the resulting binding loss (Zimmermann 2019). Unsurprisingly, CEACAM3 is one of the fastest-evolving genes in the human genome; moreover, CEACAM3 homologues from various primate species bind preferentially to host-specific parasites (Adrian et al. 2019). The case of CEACAM3 illustrates the logic of this countermeasure, and raises the question of whether the surface molecules expressed by cells in the CNS include decoys specialized against brain-targeting parasites. A possible example is the neural cell adhesion molecule NCAM-120, which may function as a decoy receptor for the rabies virus (see Hotta et al. 2007).

INCREASE THE COSTS OF MANIPULATION

Metabolically Costly Signals

In principle, the most direct way to control the host's behavior is to hijack signaling pathways by directly releasing neuroactive

substances such as neurotransmitters, neuromodulators, and hormones. In some cases, entire suites of coordinated behaviors can be evoked by raising the levels of key neurotransmitters such as serotonin and dopamine, either in the brain as a whole or in strategically picked regions. To counteract this type of attack, the host can raise the metabolic cost for the parasite, by increasing the amount of substance required to produce the same behavioral effect. In principle, this can be achieved by: lowering the concentration of receptors; expressing receptors with lower binding affinity to the signaling molecule; altering the signaling pathway downstream of receptors to make it less sensitive (that is, requiring the activation of more receptors for the same effect); and metabolizing or clearing the signaling molecule at a faster rate. Note that—besides their other potential side effects—these countermeasures are also expensive for the host, as they increase the cost of internal signaling. However, the host usually enjoys the advantage of a larger size and higher metabolic capacity, and the additional costs may be minimal or even negligible from the host's perspective. If the marginal fitness costs of synthesizing larger quantities of neuroactive substances are much steeper for the parasite, the host can effectively defend itself against direct hijacking.

In response to escalating costs, parasites have two main options. First, they can infect the host in larger numbers to share the additional metabolic costs (Weinersmith et al. 2014; Gopko et al. 2017). Second, they can evolve indirect manipulation mechanisms that act upstream or downstream of the expensive signal. And, indeed, this is what happens in the majority of known cases. Instead of directly synthesizing neuroactive molecules, parasites tend to upregulate the synthesis of endogenous substances by the host; an example is toxoplasma, which increases the host's production of dopamine and testosterone. Another strategy is to interfere with metabolism and clearance mechanisms; for instance, wasp larvae raise octopamine levels in parasitized caterpillars by secreting substances that retard the breakdown of this hormone (see Adamo 2012, 2013). Parasites may also alter the expression of the host's receptors, or target downstream nodes in the signaling pathway—including second messengers that transduce and amplify the signals relayed by receptors (Adamo 2013; Heil 2016; Herbison 2017). Indirect forms of neuropharmacological manipulation are the norm, but there are exceptions: one is the venom of jewel wasps, which contains significant amounts of dopamine and/or dopamine-like substances (Libersat and Gal 2014; Herbison 2017). The exception is telling because the size disparity between wasps and cockroaches is minimal; this makes it metabolically feasible for the wasp to synthesize large quantities of neuroactive compounds. It is also relevant that the wasp injects the venom directly into the cockroach's brain, literally breaking through the BBB and its chemical defenses.

Several authors have pointed out that, in all likelihood, indirect manipulation strategies have evolved to avoid the cost of synthesizing signaling molecules (e.g., Adamo 2012, 2013; Heil 2016). What has not been discussed is the possibility that the physiological parameters that determine the cost of signaling (e.g., receptor density/sensitivity, clearance/breakdown rates, transduction mechanisms) have themselves evolved so as to prevent more direct forms of manipulation. Since present-day instances of manipulation are mostly of the indirect kind, selection to increase the costs of signaling would have peaked a long time ago, possibly in the early stages of brain evolution. Assuming that cost-increasing countermeasures evolved as hypothesized here, are they still relevant to understanding brain function in living organisms? Paradoxically, if those countermeasures were so effective that they forced most parasites to adopt indirect strategies, they would have rendered themselves obsolete, eventually becoming a net cost without any countervailing benefits. If so, they may have been selected out owing to the relentless pressure for efficiency (Sterling and Laughlin 2015). This fate is most likely for easily reversible parameters such as receptor density. On the other hand, some costly mechanisms that originally evolved as countermeasures may have become so entrenched that they are hard or impossible to eliminate by incremental changes. At present, these scenarios remain speculative but suggest a novel, in-

triguing perspective on the economy of neural and hormonal signaling.

Toxic Signaling Molecules

Increasing the energetic demands of signaling is not the only way to impose costs on parasites. An even more direct strategy would be to employ toxic molecules as chemical signals. In principle, this countermeasure achieves two goals at once: it turns the brain into an inhospitable environment for manipulative parasites, and forces them to synthesize dangerous substances that may hinder their survival and reproduction. If the fitness costs are sufficiently large, toxic signals can work as deterrents against direct hijacking.

Among classic neurotransmitters, dopamine is the most likely candidate as a toxic signal. Dopamine molecules are unstable and tend to oxidate spontaneously, yielding highly reactive quinones that can damage proteins, DNA, and other macromolecules. Moreover, dopamine metabolism produces reactive oxygen species (ROS) and induces oxidative stress in dopaminergic neurons (Stokes et al. 1999). In total, synthesizing and storing dopamine is hazardous, but even breaking it down involves a potential risk of damage. Indeed, some species of green algae exploit the toxicity of dopamine to repel marine herbivores and avoid being eaten by them (Van Alstyne et al. 2006). Serotonin is often used by yeast and bacteria as a communication and synchronization signal, but only a few species of microbes (including some gut symbionts) have the ability to synthesize or metabolize dopamine (Roshchina 2010).

Nitric oxide is a versatile molecule with multiple physiological roles, including immunity (particularly inflammation) and vasodilation. It is employed as a neuromodulator in the CNS, where it contributes to regulate neural plasticity, sleep, feeding, thermoregulation, and reproduction (Calabrese et al. 2007; Garthwaite 2008). A chemically unstable free radical, nitric oxide has the potential to cause molecular damage and interfere with cellular respiration. Unsurprisingly, it has antimicrobial properties; and in the presence of ROS, it forms a number of highly toxic compounds, including reactive nitrogen species (RNS; Guix et al. 2005; Moncada and Bolaños 2006; Calabrese et al. 2007). RNS have a range of powerful antimicrobial effects, both alone and in synergy with ROS (Fang 2004; Jones et al. 2010). To complicate the story, many bacteria synthesize nitric oxide endogenously as a *defense* against antibiotics. The protective qualities of nitric oxide—a molecule that "seems to be playing for both teams" (Patel and Crane 2010: 235)—stem from its ability to directly detoxify certain antibiotic compounds, suppress the ROS-producing reactions that mediate antibiotic toxicity, and/or activate the expression of antioxidant enzymes (Gusarov et al. 2009; Patel and Crane 2010).

Of course, the use of dopamine and nitric oxide as signaling molecules is very ancient, and the same applies to its possible history as a host countermeasure. Still, it is noteworthy that so many of the behaviors that parasites attempt to manipulate are mediated or regulated by these molecules (see Adamo 2013; Perrot-Minnot and Cézilly 2013; Mehlhorn 2015b). The synthesis of dopamine in the brain is highly localized, which may contribute to make this neurotransmitter a promising manipulation target. At the same time, the restricted distribution of dopaminergic neurons may itself be linked to the toxicity of dopamine. There is evidence that several enzymes involved in the synthesis and metabolism of dopamine, nitric oxide, and other neurotransmitters in animals originate from bacteria, and were acquired through horizontal gene transfer (Iyer et al. 2004). Animals have a long history of co-opting microbes (and/or their genes) to combat other microbial species (see Thompson 2013:Chapters 5–6). It would be interesting to know if the original functions of dopamine in bacteria included chemical defense and/or offense against other microbes.

The scope for toxic signals becomes even broader if one considers the rich variety of modulatory peptides. Many neuropeptides with modulatory and endocrine functions have a chemical structure very similar to that of antimicrobial peptides involved in immune defense (Brogden et al. 2005). More directly, there is experimental evidence that several neuropeptides that are also involved

in immunity—including substance P, neuropeptide Y, and neurokinin A—have antimicrobial properties against bacteria, yeast, and protozoa (El Karim et al. 2008; Augustyniak et al. 2012). The standard interpretation of these findings is that, on top of their signaling functions, neuropeptides contribute to protect the brain from pathogens (e.g., El Karim et al. 2008). The complementary hypothesis I am suggesting is that these peptides may have been adopted as signaling molecules in the CNS precisely *because* of their antimicrobial effects, as a countermeasure against neuropharmacological hijacking by parasites. This possibility is broadly consistent with phylogenetic analyses suggesting that, across mammalian evolution, molecules with immune-specific functions have been gradually recruited for expression in the nervous system (Castillo-Morales et al. 2014).

increase the complexity of signals

From a computational perspective, neuroactive substances function as internal signals that transmit information between neurons, between different networks within the brain, and between the brain and other organs and tissues. Parasites can hijack a signaling pathway by producing new signals or corrupting existing ones; in either case, they need to "break" the code employed by the host. The same applies to parasites that eavesdrop on the host's chemical signals. Since complex communication codes are harder to mimic and subvert, the host can increase the complexity of signals as a countermeasure against manipulation—a strategy that has been termed *defensive complexity* by Chastain et al. (2012). Using evolutionary modeling these authors showed that, as internal signals become more elaborate, the time required to break the code and evolve effective manipulation strategies tends to increase steeply—in certain cases, exponentially so. This means that the host can potentially gain the upper hand in the conflict, forcing the parasite to resort to other means of manipulation. Although Chastain et al. (2012) developed their model to explain the evolution of signaling within the immune system, the exact same logic applies to neural transmission and hormonal pathways.

With the evolution of complex codes, basic neuropharmacological manipulations that increase or decrease the concentration of a single neuroactive molecule should become ineffective. Injecting serotonin in gammarid crustaceans is sufficient to reproduce the full syndrome caused by helminth infection (Perrot-Minnot et al. 2014); but increasing the availability of dopamine in the brain of rodents fails to evoke the "feline attraction" induced by toxoplasma—if anything, it seems to prompt avoidance behaviors rather than approach (Eskow Jaunarajs et al. 2011; see Adamo 2012, 2013). Even though toxoplasma's mechanism of action is still incompletely understood, it is obviously more complex than simply boosting dopaminergic transmission in the host. For example, this parasite may rely on the joint action of dopamine and other molecules (such as testosterone and vasopressin) and/or the patterned release of dopamine-increasing enzymes.

Naturally, complexity does not come for free. To begin, the host faces the metabolic expense of producing the signal and maintaining the additional biochemical machinery, such as receptors and enzymes. As noted earlier, metabolic costs are likely to be disproportionately more severe for parasites, which tend to be much smaller than their hosts and have vastly lower energetic resources (see Lafferty and Kuris 2002). Even more importantly, increasing the complexity of a system to prevent a certain type of perturbation tends to create new points of fragility. For example, each additional component (e.g., a receptor or neuromodulator) creates new opportunities for failure and malfunction, and opens up new windows of vulnerability (e.g., deleterious mutations, interference with other pathways). Potentially complex interactions among multiple components can amplify local failures into catastrophic events; and although the system may be rendered more secure against direct hijacking, it may also become more vulnerable to other forms of attack (e.g., genomic manipulation). Such *robustness-fragility tradeoffs* are pervasive in biological systems, and tend to drive up the complexity of organisms over

evolutionary time (Kitano 2004, 2007; Alderson and Doyle 2010; see Del Giudice and Crespi 2018).

Diversification, Co-transmission, and Convergent Signaling

One way to increase the complexity of chemical signaling is to expand the set of molecules used for transmission and the receptors that bind to those molecules. The variety of neuromodulators in animals is staggering; most notably, there are more than 100 distinct neuropeptides and several hundred neuropeptide receptors (Hökfelt 2009; Jékely et al. 2018). Even simple physiological mechanisms may be modulated by dozens of molecules, with partially overlapping functions and the potential for complex interactions. A classic example is the pyloric component of the crustacean stomatogastric system, which is regulated by over 30 neuromodulators despite its apparently straightforward pumping/filtering function (Selverston 2007; see also Nusbaum et al. 2017). This abundance of signals may provide robustness through redundancy, or allow for precise fine-tuning of the control system; the possibility that it partly reflects defensive complexity against parasites has yet to be explored. Footprints of defensive complexity might also be found in the evolution of receptors for neurotransmitters and neuropeptides, which have an intricate phylogenetic history of duplication, diversification, convergence, and loss of function (see Yamamoto and Vernier 2011; Katz and Lillvis 2014).

The availability of multiple neuromodulators makes it possible to increase complexity by signaling with *combinations* of molecules. This principle is well illustrated by *co-transmission* (or *co-release*), whereby a single neuron releases more than one neuroactive molecule. Far from being a rarity, co-transmission has turned out to be widespread in nervous systems (Gutiérrez 2009; Nusbaum et al. 2017). Most commonly, monoaminergic neurons (which release dopamine, serotonin, epinephrine, or norepinephrine) also release excitatory/inhibitory neurotransmitters (glutamate, GABA) or modulatory neuropeptides such as cannabinoids and substance P (Gutiérrez 2009; Hökfelt 2009; Trudeau et al. 2009; Tritsch et al. 2016). Since different molecules have different timescales of action, co-transmission can be employed to regulate neural activity (and behavior) in remarkably sophisticated ways. For example, the fast excitatory action of glutamate may support short-term learning, whereas the slow inhibitory action of serotonin may sustain motivation over the long term (Fischer et al. 2015). In the roundworm *Caenorhabditis elegans*, glutamate promotes food search, while the co-released peptide NLP-1 initiates a negative feedback response that eventually terminates the foraging behavior (Nusbaum et al. 2017).

The possible functions of co-transmission include more accurate modulation of the postsynaptic response; more precise timing of action; increased flexibility through state-dependent patterns with opposite or complementary effects; and the opportunity for a neuron to target different signals to different neurons within a complex circuit (see Tritsch et al. 2016; Nusbaum et al. 2017). However, it is also the case that combined signals are harder to mimic, making the system less vulnerable to hijacking. There are well-studied cases in which the combined action of two or more molecules is required to fully express a particular behavior: for example, egg laying in *C. elegans* is jointly controlled by serotonin and the neuropeptide NLP-3 (Brewer et al. 2018). To take control of egg laying, a hypothetical parasite would have to secrete the correct combination of molecules, possibly in a specific concentration and temporal pattern. In the sea slug *Aplysia*, the cholinergic neurons that control feeding co-release two different peptides with synergistic effects (Jékely et al. 2018). More generally, convergent signaling is a common feature of neuropeptides. Particularly in areas of the brain that control major hormonal systems, the same neuron receives input from multiple peptides that modulate the effect of one another: as a result, the signal is not encoded by individual peptides but by their specific combination (Jékely et al. 2018). Although this strategy permits high levels of flexibility and context-dependence, it may

also contribute to protect key signaling pathways from manipulative parasites.

Pulsatile Signaling

A striking feature of many hormones and neuromodulators is that they are not secreted continuously, but in timed pulses. Different molecules have different secretion periods, ranging from minutes (e.g., insulin, oxytocin) to hours (e.g., melatonin, anterior pituitary hormones, gonadal and adrenal steroids; Veldhuis et al. 2008). The feedback loops that give rise to pulsatile secretion can be rather complex, and may be regulated by multiple excitatory and inhibitory mechanisms that integrate inputs from the same cell, other cells, and other organs through hormone receptors (see Veldhuis et al. 2008; Lightman and Conway-Campbell 2010; Leng 2018). Crucially, it is often the case that brief pulses produce stronger physiological and behavioral effects than exposure to constantly elevated levels of the same signaling molecule. For example, estrogen administration to female rats requires about 48 hours to produce the maximal behavioral response, but two brief estrogen pulses a few hours apart are just as effective as 24 hours of hormonal priming (see Pfaff et al. 2004). In many instances, constant elevation does not just fail to elicit a strong response but leads to inhibition or desensitization. For example, steady-state levels of gonadotropin releasing hormone (GnRH) suppress the hypothalamic-pituitary-gonadal axis instead of stimulating it (Pfaff et al. 2004).

The decoding of pulsatile patterns takes place in receiving cells, from the activation of receptors to the regulation of gene expression. These processes may be "tuned" to a specific frequency so that both slower and faster pulses fail to produce the maximal response (Lightman and Conway-Campbell 2010); or may exhibit different responses to different frequencies (for example, slower GnRH pulses favor secretion of follicle-stimulating hormone in the pituitary, whereas faster pulses favor luteinizing hormone; see Leng 2018). Interestingly, frequency and amplitude patterns for the same molecule may vary systematically, both across related species and across an individual's life stages (see Crockford 2003 for a detailed review of variation in thyroid hormones).

What is the functional rationale of pulsatile signaling? Researchers have pointed out that the discrete format of pulses may make them more resistant to noise, as compared with graded concentration changes. Moreover, pulsed signals can encode more information by exploiting both frequency (timing of pulses) and amplitude modulation (amount released per pulse). Some models also suggest that pulsatile secretion can also be more energy efficient than continuous release (Walker et al. 2010; Faghih et al. 2015). In summary, pulsatile signaling has no shortage of potential advantages. An additional possibility that has been overlooked so far is that pulsed signals—whatever their original function—may have become widespread in internal communications because they are an effective countermeasure against hijacking by parasites. To begin, releasing a pulse of a neuroactive substance demands a concentrated metabolic effort over a brief period of time (regardless of the long-term energetic efficiency of the system). Even more importantly, microbes would have to synchronize their activity in time—which would require additional computational and metabolic resources—and do so with the correct frequency: pulses that are too slow or too fast would fail to elicit a strong response, or may even bring about the opposite effect. Although pulsatile codes are not impossible to break, from the standpoint of manipulative parasites they are significantly more challenging than their analog counterparts.

Individualized Signatures

Defensive complexity works by slowing down the evolution of effective manipulative signals in the parasite population. Likewise, the evolution of manipulation would be hampered if each individual host used a somewhat different version of the same basic molecular code—for example, variations in the combination of neuromodulators and/or receptors, or in the optimal frequency

of pulsatile signals. Selection for countermeasures can be expected to favor a certain amount of stochastic variation in the parameters of neural and hormonal signaling, leading to the development of individualized "signatures." In principle, microbes can evolve within a host and converge on its individual code thanks to their short generation time (Spottiswoode and Busch 2019); however, the distinctive features of behavioral manipulation dramatically reduce the effectiveness of this process. In many common scenarios (including trophic transmission and bodyguard manipulations), the manipulation strategy undergoes *one* selective event per individual host (e.g., successful versus unsuccessful predation), irrespective of the parasite's replication rate prior to the event. Other types of transmission (e.g., sexual transmission) allow for multiple selection events within the same individual host (e.g., one per copulation); but as the parasite becomes more adapted to the unique physiology of the current host, it inevitably becomes *less* adapted to that of the next (a partial exception would apply to parasites that are preferentially transmitted between relatives).

This hypothesis is clearly speculative but not implausible; it is consistent with the idea that biological systems employ randomness as a cryptographic device in order to protect information flows from detection and exploitation (Krakauer 2017). The use of stochastic signatures for self/nonself recognition has been documented in various biological systems under selection by parasites, including the major histocompatibility complex (MHC) in the vertebrate immune system, polymorphic egg markings in birds targeted by brood parasites, and olfactory signatures against social parasites in insect colonies (Summers et al. 2003; Spottiswoode and Busch 2019).

The complexity of signaling pathways provides abundant opportunities for stochastic deviations, including genetic and epigenetic mutations as well as developmental noise. In fact, even individual neurons that secrete the same molecule (e.g., oxytocin) within the brain of a single individual are not completely alike—each shows a slightly different pattern of activity, expresses slightly different combinations of receptors, and so on (Anderson et al. 2016; Leng 2018). It is easy to see how selection for countermeasures may lead to release or even amplify existing sources of stochastic variation, instead of buffering or correcting them through canalization processes (see Debat and David 2001; Dworkin 2005; Hiesinger and Hassan 2018). At the same time, genetic and developmental noise are often deleterious, and the potential benefits of decanalization must be weighed against the inevitable costs (including loss of robustness in molecular pathways; see Salathé and Soyer 2008). One should note that adaptive stochastic variation expressed as a countermeasure to parasites should look very similar to nonadaptive variation due to imperfect buffering and canalization; thus, devising empirical tests of this hypothesis is going to be particularly challenging.

INCREASE ROBUSTNESS

Despite multiple layers of preventive countermeasures, a parasite may still be able to gain access to the brain, break the signaling code (or bypass signaling through genomic or immunological means), and carry out a manipulative attack. The host's problem is now one of damage control: the goal is to maintain the functionality of behavior in the face of the parasite's attack (Salathé and Soyer 2008; Foster 2011). In this section I discuss some potential strategies that the host can use to increase the brain's ability to withstand perturbations—that is, its *robustness* (see Kitano 2004; Krakauer 2006; Alderson and Doyle 2010; Flack et al. 2012). For convenience, I distinguish between *passive* strategies that make the brain's architecture intrinsically resistant to manipulation and damage; *reactive* strategies that actively respond to manipulation attempts; and *proactive* strategies that are deployed before manipulation occurs. Some of the mechanisms I review are nonspecific: although they can function as countermeasures to manipulation, they also protect the brain against other sources of damage, malfunction, and noise—for example, nonmanipulative pathogens, strokes, seizures, and chemical imbalances caused

by any number of internal or external factors (including deleterious mutations). Pinpointing the role of parasites in the evolution of those mechanisms is not going to be an easy task. More intriguingly, a manipulation perspective suggests that the brain may contain as yet undiscovered robustness mechanisms that are specifically targeted to parasite intrusions.

Passive Robustness

The architecture of a system is an important factor in its ability to withstand perturbations. Redundancy and modularity are two common properties of biological systems that contribute to increase their robustness (see Kitano 2004; Krakauer 2006; Alderson and Doyle 2010; Flack et al. 2012). When multiple components perform identical or overlapping tasks, the system becomes more resistant to damage and failure. For example, heat avoidance (negative thermotaxis) in *C. elegans* is controlled by three distinct types of thermosensory neurons, which respond in different combinations to different environmental conditions. This functional overlap allows *C. elegans* to successfully avoid heat damage even following the loss of one neuron type (Beverly et al. 2011). In the vertebrate hypothalamic-pituitary-gonadal axis, the release of follicle-stimulating hormone is initiated by GnRH but regulated by several peptides that have both stimulatory and inhibitory effects (Leng 2018). As noted earlier, robustness through partial redundancy is a plausible benefit of complex neuropeptide signaling, in which multiple neuromodulators converge on the same neuron (Jékely et al. 2018). Mathematical models show that when signaling networks evolve under threat of interference by parasites they tend to become more redundant; at the same time, parasites select for robust connection patterns that can withstand the loss of any individual molecule (Salathé and Soyer 2008).

Modularity can also promote robustness by decoupling the functions of different components (functional modularity) and/or separating them in space (anatomical modularity). In modular systems, the effect of perturbations can be contained and isolated, so that the system as a whole maintains a degree of functionality even if one of the components fails. On the other hand, if a specialized module fails the organism may completely lose the ability to perform the corresponding function—another example of robustness-fragility tradeoffs (see above; Kitano 2007). The fact that most parasites show little or no anatomical specificity when they attack the brain (Adamo 2012, 2013) might be viewed as a response to the modularity and redundancy of behavioral processes.

Although redundancy and modularity increase a system's robustness, they also make it harder to coordinate the activity of multiple components with rapidity and efficiency. For example, if different aspects of behavior (e.g., foraging, mating, and predator avoidance) were controlled by modularized biochemical pathways with limited crosstalk, parasites would need to separately hijack each of the pathways in order to manipulate the host's behavior. However, behavioral coordination would also become significantly more difficult for the host. A common design solution to balance robustness and controllability is the "bow-tie" architecture, in which multiple, partially modularized pathways converge on a small hub of shared processes that link their inputs and outputs (Csete and Doyle 2004; Kitano 2004). Individual pathways can evolve and function in relative independence from one another, but the shared core—the "knot" of the bow tie—permits rapid and efficient control of the system as a whole. The concept of bow-tie architectures has been applied to the organization of cell signaling, metabolism, and immunity (Csete and Doyle 2004; Kitano 2004; Kitano and Oda 2006). Bow-tie architectures are not immune from robustness-fragility tradeoffs: while the knot confers robustness on the system, it also becomes a vulnerable target. Thus, parasites can be expected to concentrate their manipulation attempts on core biochemical processes that regulate multiple pathways at once (Kitano and Oda 2006). And, indeed, parasites tend to target neurotransmitters such as dopamine and serotonin, ubiquitous second messengers like cyclic adenosine monophosphate (cAMP), or key transcription factors such as NF-κB and

c-Myc (Adamo 2013; Perrot-Minnot et al. 2014; Cheeseman and Weitzman 2015; Herbison 2017).

Reactive Robustness

Negative feedback is arguably the most basic form of reactive robustness (Kitano 2004; Krakauer 2006; Alderson and Doyle 2010; Khammash 2016). Feedback-regulated systems are homeostatic: perturbations that move the system away from the set point are detected and corrected with adjustments in the opposite direction, potentially very rapidly if the feedback loop has high "gain" (that is, if deviations from the set point elicit a strong compensatory response; see Bechhoefer 2005; Frank 2018). Negative feedback is a general strategy for robustness, and a pervasive feature of neural and endocrine systems at all levels of analysis (e.g., Pfaff et al. 2004; Davis 2006; Del Giudice et al. 2018; Leng 2018). Even the BBB may contribute to the feedback regulation of brain neurochemistry by modulating the efflux of neurotransmitters such as dopamine, serotonin, and GABA (see above). From the host's perspective, signaling pathways can be made more resistant to neuropharmacological manipulation through stiffer feedback regulation. The price to pay is loss of flexibility: as the system becomes more effective in blocking external interferences, it also becomes slower and less adaptable (Bechhoefer 2005). In addition, higher feedback gain makes the system more robust against slow perturbations but increasingly unstable against rapid fluctuations—a point of fragility that can be exploited by parasites to affect the host's behavior (Csete and Doyle 2002; Kitano 2007). More generally, feedback-regulated systems are particularly vulnerable to noise coming from sensors (Bechhoefer 2005); thus, parasites may attempt to disrupt homeostasis by targeting the receptors and cellular pathways that receive and relay feedback signals.

Negative feedback is a basic building block of robust systems; it is also a general purpose strategy with little functional specificity. More sophisticated forms of reactive robustness can be implemented by specialized mechanisms designed to detect manipulation attempts and respond in flexible, strategic ways (see Weinersmith and Earley 2016; Massey and Mishra 2018). The first question to ask is: How can the brain detect a manipulation attempt? When parasites hijack a signaling pathway and significantly alter its activity, they should typically induce a compensatory response in the feedback mechanisms that stabilize the pathway (unless they also manage to disrupt the feedback channels; see above). If this is the case, unusual activation patterns of neural and endocrine feedback mechanisms (e.g., atypically large or sudden responses) may be interpreted as potential cues of ongoing manipulation. Other detection mechanisms may be subtler and more attuned to indirect forms of manipulation—for example, intracellular probes may monitor key second messengers and transcription factors for anomalous changes in their activity, or sense the presence of specific molecules produced by the parasite.

Once a potential manipulation attempt has been detected, various adaptive responses are possible. A straightforward option is to directly counteract the biochemical mechanisms employed by parasites. For example, the expression of noncoding RNAs in host cells responds to cues of infection and inflammation, and contributes to regulate multiple immune-related genes. Intriguingly, noncoding RNA expression partly depends on the specific pathogen infecting the host (Duval et al. 2017; zur Bruegge et al. 2017). Unsurprisingly, host RNAs are themselves targeted by parasites because of their immunoregulatory activity (Duval et al. 2017; zur Bruegge et al. 2017). Some of these RNA molecules may interfere with the messenger and noncoding RNAs injected by the parasite, initiate epigenetic changes that counteract their activity, or even regulate the expression of the parasite's own genes. The fact that some noncoding RNAs in the host are strongly induced by parasite-derived molecules is usually interpreted as evidence of parasite manipulation (for a recent example involving toxoplasma see Menard et al. 2018). However, it is also possible that certain host RNAs function as components of molecular mechanisms that detect and adaptively respond to manipulative attacks. In

mammals, a large fraction of noncoding RNA is specifically expressed in brain cells, and our understanding of its many functions is still very patchy (Briggs et al. 2015). Some brain-expressed RNAs may contribute to reactive robustness against behavior-altering parasites, as either "sensors" or interference mechanisms.

On a more macroscopic level, the host may adjust the parameters of feedback mechanisms (e.g., increase feedback gain, lower the set point) to constrain the response of signaling pathways that might have been hijacked, thus dampening the effects of the parasite's manipulative effort. If signals are carried by partially redundant pathways, the brain may respond by silencing or attenuating the suspicious pathway—effectively switching its internal communications to safer and plausibly intact channels. These countermeasures do not need to be centrally coordinated and may be implemented via self-organizing processes (e.g., signaling pathways may automatically readjust or temporarily "shut off" if their activity patterns become consistent with a hijacking attempt). Such active countermeasures would have costs and undesirable side effects; in particular, any defensive shift toward tighter regulation and lower redundancy of signals can be expected to compromise the flexibility of the organism's behavior. Moreover, manipulation detection mechanisms are costly and can themselves become targets of parasite attacks, thus introducing new points of fragility into the system.

The brain's reactive robustness may contribute to explain why, at least in some instances, it has proven difficult to reproduce the behaviors induced by parasites by simply altering the level of key signaling molecules. Some apparently "paradoxical" effects—e.g., increasing dopamine makes rats more fearful rather than less—are consistent with the existence of compensatory mechanisms that get triggered by sudden, anomalous changes in brain biochemistry. What I am suggesting is that those mechanisms may be more than general purpose homeostatic devices (e.g., Adamo 2013); instead, some of their features may be *specifically* designed to detect and respond to parasite intrusions (for a similar argument see Weinersmith and Earley 2016).

Proactive Robustness

By definition, reactive mechanisms wait for evidence that a manipulation attempt may be taking place. The logic of proactive robustness is to *anticipate* the parasite and deploy countermeasures even before manipulation occurs. Proactive processes may operate on different time scales. In the short term, the brain may use nonspecific cues of immune activity (e.g., inflammatory cytokines) as a warning sign that a parasite is invading the body, and respond with preventive measures to make manipulation more difficult. Information about the activity of the immune system reaches the brain through multiple routes: both cytokines and leukocytes can cross the BBB, and inflammatory signals are relayed by the vagus nerve and other afferent neural pathways (Quan and Banks 2007; Capuron and Miller 2011). It is well established that inflammation modulates brain neurochemistry; specifically, inflammatory signals induce substantial changes in the activity of major signaling pathways, including the synthesis and metabolism of serotonin and dopamine (e.g., Capuron and Miller 2011; Baganz and Blakely 2013). On the standard view, these neurobiological responses bring about sickness behavior—lethargy, loss of appetite, sleepiness, and so on (McCusker and Kelley 2013). It is also possible that one of their functions is to prevent manipulation, for instance, by tightening the feedback regulation of key pathways or reducing the activity of vulnerable mechanisms. Intriguingly, even some aspects of sickness behavior might be interpreted as proactive countermeasures: to give just one example, lethargy may thwart behavioral manipulations designed to transport the parasite to a different habitat. The activation of decoy receptors and the expression of noncoding RNAs can also be used as warning cues of impending manipulation attempts. In some cases, these mechanisms can be so specific that they permit accurate recognition of the invading parasite, allowing the host to deploy a tailored response.

On a longer time scale, recurrent infections by the same parasite over evolutionary time may lead organisms to preemptively

compensate for the manipulative effects of that parasite. To illustrate, consider a hypothetical animal targeted by a serotonin-increasing parasite. If infection is so common and predictable that it can be treated as an expected feature of the environment, the host may evolve lower levels of serotonin as a proactive countermeasure (in turn, this would select for stronger manipulation by the parasite, setting the stage for reciprocal escalation). A downside of preemptive strategies is *evolved dependence* (de Mazancourt et al. 2005): if brain physiology and behavior are designed to function optimally when the parasite is present, the *absence* of the parasite will lead to inappropriate or fitness-reducing behaviors (Weinersmith and Earley 2016; see also Johnson and Foster 2018). When a parasite is common but the frequency and intensity of infection varies across generations, the host species should not evolve fixed physiological adjustments, but plastic responses triggered by cues of infection. Weinersmith and Earley (2016) provide a detailed discussion of these and other scenarios.

Constraints on the Evolution of Countermeasures

At various points in the preceding sections, I have noted that the evolution of manipulation strategies is constrained by their metabolic and computational costs (see Adamo 2013; Herbison 2017). In fact, I have argued that hosts may increase the costs of neural and hormonal signaling precisely as a strategy against parasite manipulation. I now look at the other side of the coin, and consider how the same factors constrain the evolution of effective countermeasures by the hosts. I then discuss the lack of documented examples of adaptive behavior manipulation in our own species.

metabolic and computational constraints

Most of the countermeasures examined in this paper require the host to invest in higher metabolic expenditures, additional neural machinery (which is costly to build and maintain), and/or more complex computations (which are also energetically expensive; see Sterling and Laughlin 2015; Faisal and Neishabouri 2017). It follows that animals with smaller metabolic budgets should be more limited in their ability to evolve effective defenses against parasites. But energy availability is not the only factor in play. A small body size means that the maximum size of the brain is also severely limited; the problem is exacerbated in animals that fly or jump and thus need to minimize total body weight. In turn, miniaturized nervous systems face tremendous computational constraints (Niven and Ferris 2012). Smaller neurons generate more spontaneous noise, and cannot host enough mitochondria to sustain high firing frequencies. Smaller axons transmit information more slowly and less accurately; at the same time, error correction strategies are hard to implement because they require higher energetic expenditures and/or more complex neural circuits (Niven and Ferris 2012; Zylberberg et al. 2016; Faisal and Neishabouri 2017). Body temperature is another relevant factor. Somewhat counterintuitively, the noise introduced by random action potentials increases at lower temperatures; as a consequence, cold-blooded animals cannot afford to reduce the size of axons as much as warm-blooded animals can (Faisal and Neishabouri 2017).

It follows that the brains of small, cold-blooded animals must rely on relatively simple computations, and may not be able to afford sophisticated countermeasures to manipulation. For example, insect brains employ many computation-saving shortcuts for perception and decision-making, and use a small number of "command" neurons to control complex behavioral patterns (Sterling and Laughlin 2015). In contrast, animals with larger (and warmer) brains can evolve multiple layers of protection and considerable amounts of redundancy. This plausibly contributes to explain why the most striking instances of complex behavioral manipulation involve insects (e.g., ants), small crustaceans (e.g., gammarids), and mollusks (e.g., snails). The same considerations may explain why direct manipulation of neurotransmitter levels can have such divergent outcomes in different species. Injecting serotonin in

gammarids successfully mimics the effects of helminth infection (for this and other examples see Perrot-Minnot and Cézilly 2013); but raising dopamine in rats fails to reproduce the symptoms of toxoplasma, and in fact seems to favor the opposite behaviors (see Adamo 2013).

Although parasites can evolve subtler and more indirect means of manipulation, their computational capabilities are ultimately limited by their size. As the size and complexity of the host's brain increase relative to the parasite, the disparity may become so extreme that the host is able to "outcompute" its adversary, making complex manipulations effectively impossible. The parasite may still be able to alter the host's behavior in nonspecific ways (e.g., sickness, brain damage), but is unable to induce the kind of coordinated pattern required for trophic transmission or bodyguard manipulation. Although this argument is admittedly speculative, it is consistent with the fact that complex behavioral manipulations have not been documented in larger, warm-blooded animals (see Lafferty and Kuris 2002).

other constraints

Besides energetic and computational resources, many other factors can constrain the evolution of host countermeasures. Generally speaking, selection for enhanced protection will not occur if infection is sufficiently rare (e.g., the parasite is uncommon), the cost of manipulation is sufficiently low (e.g., the host dies only after reproduction), and/or the cost of effective countermeasures is sufficiently high. In turn, the balance between costs and benefits depends on the specific outcomes of manipulation and the host's ecology and life history.

An interesting example in this regard is provided by eusocial species (including many species of ants, bees, and wasps), which are characterized by group living in colonies and reproductive division of labor. In eusocial species, nonreproductive individuals are relatively expendable, and there are strong selective pressures to protect other members of the colony from parasite infections (*social immunity*; see Meunier 2015). Mechanisms of individual immunity only increase the survival of a single organism, and may be selected against if their cost detracts from investment in group-beneficial adaptations (Cotter and Kilner 2010). A similar tradeoff may exist in the evolution of countermeasures to manipulation. Consider a trophic parasite that is relatively rare, kills the infected individual through predation, but is not directly transmitted to other colony members. In this case, the benefits of expensive countermeasures would mainly accrue to the individual, with relatively little impact on the colony. All else being equal, the scope for the evolution of countermeasures against the parasite should be more constrained if the host is eusocial (Hughes 2012). In principle, this hypothesis can be tested empirically by comparing patterns of manipulation and countermeasures among related species with different social systems. Even in noneusocial animals, the costs and benefits of individual defense may vary depending on the life history of a species and the sex and life stage of a particular individual (see Cotter and Kilner 2010).

behavioral manipulation in humans

With their large, complex, and energetically expensive brains, humans seem unlikely targets for adaptive behavioral manipulations. Moreover, our extended life history and prolonged investment in brain development (Kaplan et al. 2007) suggests that we should invest in mechanisms that protect our hard-won "neural capital" from deterioration and external attacks. Modern humans have few predators and very low predation rates, making trophic transmission an unworkable option. On the other hand, predation—for example, by felines such as leopards and tigers, raptors such as hawks and eagles, or reptiles such as snakes and crocodiles—has been a significant pressure throughout primate evolution (Hart 2007). Indeed, there is evidence that our hominid ancestors were prey as much as predators; even present-day hunter-gatherers are not immune from the risk of being killed and eaten by snakes (e.g., pythons) and other large animals (see Hart and Sussman 2009; Headland and

Greene 2011). Although our long life span may diminish the potential benefits of trophic strategies (Lafferty and Kuris 2002), it is possible that our brains (and some of our present-day parasites) carry the traces of a past in which transmission through predation was a reality (Libersat et al. 2018). An intriguing study by Poirotte et al. (2016) suggested that toxoplasma-infected chimpanzees develop attraction to leopard urine; however, this finding is based on a very small sample and must be regarded as anecdotal until replicated.

Alternatively, we could be susceptible to manipulation as an incidental byproduct, if parasites adapted to trophic transmission in other animals happened to have similar effects on our brain mechanisms. In line with this scenario, some researchers have found evidence that toxoplasma infection in humans leads to permanent alterations in personality and behavior—for example, increased risk-taking, impulsivity, and extraversion; reduced cognitive concentration and novelty seeking; and higher suspiciousness in males, but lower suspiciousness in females (reviewed in Flegr 2013). Unfortunately, research on this topic tends to suffer from methodological limitations, including unconstrained multiple testing (with the possibility of cherry-picking) and inconsistent measures across studies (see Martinez et al. 2018). More recently, some large-sample studies have yielded interesting if somewhat scattered findings, namely increased risk of self-harm in women (Pedersen et al. 2012); higher scores on some aggression and impulsivity measures, with possible differences by sex (Cook et al. 2015); no effects on personality, but lower performance on some cognitive measures (Sugden et al. 2016); and higher levels of entrepreneurial behaviors (an indirect manifestation of risk-taking; Johnson et al. 2018). Taken at face value, these data are consistent with the idea that toxoplasma alters human neurobiology as a byproduct of manipulation in other species, causing subtle behavioral changes but no dramatic modifications of personality.

Even if humans are not a vehicle for trophic transmission, parasites may attempt to control behavior to facilitate direct transmission from an individual to another. In light of our intense sociality and flexible mating patterns, social and sexual behaviors are the most likely candidates for adaptive manipulation. Several authors have speculated that sexually transmitted pathogens may influence a person's sexual attractiveness and/or behavior so as to maximize their transmission, for example, by increasing sexual desire or inducing behaviors that favor promiscuous mating (Cochran et al. 2000; Nesse and Foxman 2011; Heil 2016; Miller and Fleischman 2016; Sarafin et al. 2018). This hypothesis is fascinating, but has yet to be tested empirically. As noted earlier, arguments for adaptive manipulation by gut microbes in our species (e.g., Alcock et al. 2014) are still largely speculative; if supported, they will indicate another plausible source of manipulative pressures. At present, the best-documented behavioral syndrome in humans is the one induced by the rabies virus. The symptoms of "furious rabies"—hydrophobia, extreme salivation, exaggerated reactivity to stimuli, agitation—are similar to those observed in other infected animals (Susilawathi et al. 2012). However, human-to-human transmission is exceedingly rare (Hanlon and Childs 2013), suggesting that the symptoms of rabies in our species are more likely to be nonadaptive byproducts. There are many other parasites that affect human behavior, including poliovirus (the agent of poliomyelitis) and the sleeping sickness protozoan *T. brucei*. However, so far there is no evidence that the behavioral effects of these pathogens represent adaptive manipulations (for a tentative argument about the possible parasite benefits of hypersomnia see Lundkvist et al. 2004).

In total, it is fair to conclude that—if one excludes simple symptoms such as coughing and scratching—there are no established examples of adaptive behavioral manipulation in humans. This can be partly explained by our (current) place in the food chain and long life span. Another possibility is that our brain is so large, complex, and secured by multiple layers of countermeasures that it exceeds the capabilities of parasites. The main piece of evidence against this hypothesis is the ability of the rabies virus to induce a co-

ordinated (if nonadaptive) behavioral syndrome in infected people. Assuming that humans are not victims of behavior-altering parasites, does it mean that defenses against manipulation have become useless in our species? This is a complex question that will require a complex answer and much additional evidence. It is certainly possible that our brain and endocrine systems contain "frozen" countermeasures that evolved at a time when parasite manipulation was a strong selection pressure, became embedded in neural processes, and persist today even if they no longer serve their original function. In fact, some of those ancient mechanisms may have been recycled and modified to perform novel functions, as is the rule in brain evolution (Anderson 2010).

Implications for Neuroscience and Psychopharmacology

implications for basic neuroscience

In the quest to reverse engineer the brain, neuroscientists confront some hard questions about the evolved design of neural systems. To what extent are brain mechanisms optimized for their tasks? Are they efficient and streamlined or wasteful and redundant? On one hand, there is mounting evidence that neural systems, from synapses and neurons to whole brain networks, are relentlessly optimized to process information with maximum energetic efficiency (e.g., Sterling and Laughlin 2015). On the other hand, there are important biological forces that work against selection for efficiency. Evolution is a wasteful process that "tinkers" with what is available at any given time, and can never escape the constraints of previous history; for all of its ability to produce effective and finely tuned adaptations, natural selection is also bound to leave behind a legacy of suboptimal solutions, design compromises, and inefficiencies (e.g., Marcus 2009). The fact that brains are exposed to accidents and malfunctions inevitably gives rise to tradeoffs between efficiency and robustness (see Del Giudice and Crespi 2018); less intuitively, sexual selection for behavioral traits—from courtship displays such as bird songs to manifestations of cognitive ability—often favors the evolution of wasteful, costly mechanisms rather than maximally efficient ones (Miller 2000; Kuijper et al. 2012). Sexual selection may also *decrease* the robustness of some traits, precisely to turn them into reliable indicators of health and genetic quality (see Geary 2015).

On this background, evolutionary conflicts between parasites and hosts are another potential source of inefficiency. In general, competitive interactions push the evolution of biological mechanisms away from simple optimization goals (Foster 2011), and may escalate into wasteful "arms races" in which organisms spend large amounts of resources just to keep ahead of the adversary (Summers et al. 2003). Other scholars have noted that host-parasite conflicts may select for enhanced robustness (Salathé and Soyer 2008; Foster 2011). In this paper, I have argued that robustness mechanisms are only one type of countermeasure; other possible responses include defensive increases in complexity, escalating costs of signaling, toxic signaling molecules, and more. Each of these countermeasures has unique implications for understanding the evolution of brain function. For example, selection for defensive complexity tends to produce complex signals that are intricate and hard to decode—for parasites, but also for the neuroscientists who study them. The fact that the chemical codes employed by neurons and endocrine cells are exceedingly difficult to decipher may turn out to be a specific design feature, rather than just a reflection of their complex functions (Chastain et al. 2012).

Another intriguing possibility is that mechanisms that initially evolved as countermeasures may have been co-opted and exapted to serve other functions, unrelated to the original conflict (Foster 2011). For example, a defensive increase in signaling complexity (e.g., receptor duplication and divergence) may enhance the flexibility of neural signaling, and serve as a springboard for the evolution of novel behavioral patterns. A useful analogy is that of military research, which—under the pressure of ongoing or potential war—generates a multitude of new technologies that are later adopted for everyday

use. The key point of the analogy is that the research and design costs of high-risk innovations are often prohibitive; the logic of conflict justifies large-scale investments that would be unsustainable in a peacetime economy. Likewise, conflict with parasites may fuel the evolution of complex or expensive adaptations that would not evolve otherwise, because their large "R&D costs" would exceed the initial fitness benefits for the host.

IMPLICATIONS FOR PSYCHOPHARMACOLOGY

Using psychoactive drugs to treat psychiatric symptoms is an attempt to alter behavior by pharmacological means. This is also what manipulative parasites do—even though, in the case of psychiatric treatment, the goal is to benefit the patient (Massey and Mishra 2018). If the human brain contains evolved countermeasures to manipulation, the implications for psychopharmacology could be profound. I now briefly discuss some of these implications, using depression as a running example.

A persistent obstacle in the development of psychoactive drugs is that stable behavioral changes are difficult to bring about in a reliable fashion. The acute effects of drugs of abuse such as heroin and cocaine are intense but short-lived, and wash out within hours; in contrast, psychiatric drugs like antidepressants must alleviate symptoms for months or years on end in order to be useful. Not only do antidepressants take weeks to start working, they also tend to induce tolerance in patients. The buildup of tolerance to antidepressants can be gradual, but about 10–20% of patients experience a sudden, rapid loss of effectiveness (tachyphylaxis) during which symptoms reemerge quickly after the initial remission (Fornaro et al. 2019; Kinrys et al. 2019).

The mechanism responsible for tolerance to antidepressants (and most other drugs) are still poorly understood. Researchers have argued that continued administration of the drug may trigger compensatory mechanisms at the level of receptors and/or cellular transduction pathways; similar feedback processes could explain the delayed onset of the drug effects and their decline over time (see Fava and Offidani 2011; Fornaro et al. 2019). It is worth considering the possibility that at least some of these reactive mechanisms may be specifically designed to detect and respond to parasite intrusions. If so, standard pharmacological treatments may unwittingly mimic a parasite attack and trigger specialized defensive responses. Intriguingly, some undesirable "side effects" of the drugs (e.g., behavioral rigidity, loss of motivation) might be best understood as costly yet adaptive features—that is, adaptive in the original context of parasite manipulation, but potentially detrimental in the evolutionarily novel context of psychiatric treatment.

A manipulation perspective may contribute to explain when and why drugs fail, but also help researchers devise more effective treatments. On point, Adamo (2013) contrasted the ways in which parasites and neuroscientists use biochemical manipulations to alter behavior in animals. She argued that parasites can teach two useful lessons to neuroscientists. The first is that parasites tend to attack multiple mechanisms at once instead of focusing on one specific pathway. This pattern is to be expected in light of countermeasures such as robustness and defensive complexity, and may explain why the behavioral changes induced by parasites are often remarkably stable, or even permanent. In the domain of psychopharmacology, one implication is that using multiple drugs to treat a single disorder—for example, depression—may enhance the reliability and long-term efficacy of the treatment. This approach is known as *combination therapy* when the drugs have similar functions but different mechanisms of action (e.g., two or more antidepressants) or *augmentation therapy* when the main drug (for example, an antidepressant) is supplemented with molecules that target different systems (e.g., antipsychotics, anxiolytics, or hormones such as testosterone). Combination/augmentation therapies for depression and other mood disorders have yielded promising results against treatment resistance and tachyphylaxis, but are still underresearched (Ionescu et al. 2015; Kinrys et al. 2019). There are also concerns

about the cumulative effects of multiple drugs on the risk for other conditions (e.g., kidney disorders; Nestsiarovich et al. 2019). A manipulation perspective may help to understand more clearly why some combinations of molecules work better or worse than others, and offer insights into the best attack strategy. If core signaling pathways turn out to be strongly protected against hijacking, it might pay off to take indirect routes, sidestepping the obvious candidate mechanisms and focusing on the vulnerable nodes of the system (e.g., the knots of biochemical bow-tie structures).

The second lesson discussed by Adamo (2013) is that parasites often eschew signaling molecules and their receptors, and instead target genes and proteins that are not directly involved in signaling. One of the most common indirect strategies employed by parasites is to target the immune system. This is also a relatively novel, active research topic in the treatment of depression. The current therapeutic approach is straightforward: in light of the finding that at least some depression subtypes are associated with elevated inflammation biomarkers, anti-inflammatory drugs may be used to treat depressive symptoms. The results of clinical trials have been promising, but heterogeneous and still tentative in many respects (Köhler et al. 2014; Köhler-Forsberg and Benros 2018; Pfau et al. 2018). A closer look at the mechanisms employed by parasites to alter immune functioning is likely to suggest other, more sophisticated avenues for intervention.

Finally, the novel idea that pulsatile signaling may have evolved as a countermeasure is particularly intriguing in relation to pharmacological treatments. At present, most psychiatric drugs are used to bring about a sustained increase (or decrease) in the brain concentration of certain signaling molecules, for example, serotonin or dopamine. Unfortunately, this might be exactly the kind of simple manipulation that pulsatile signals are designed to thwart. Modern delivery technologies can be used to release drugs in pulses rather than continuously (Davoodi et al. 2018). Framing pulsatile signaling as a code to break may indicate new ways to enhance the efficacy of drugs, by delivering them in strategically timed patterns.

Suggestions for Future Research

Rethinking behavior manipulation from the standpoint of the host's nervous system raises many fascinating questions and hypotheses, which at this point are necessarily speculative. Because the hypotheses I advance in this paper bear on a variety of disciplines and research areas—each with its own specialized tools and techniques—it is hard to offer general guidelines for testing them. Here I propose some heuristics and examples of how this perspective can be used to extend current thinking in the relevant disciplines. For example, major efforts are underway in molecular neurobiology and immunology to elucidate the roles of glycans, noncoding RNAs, cell adhesion molecules, and other mechanisms involved in parasite-host conflicts. The set of possible interpretations of findings in these areas could be expanded to include host countermeasures against manipulation. To illustrate, one might entertain the idea that noncoding RNAs whose expression is strongly induced by specific pathogens may be part of specialized detection and response mechanisms (see above). Serendipity will obviously play a major role in future discoveries, but this can only happen if researchers are aware that the question exists in the first place.

In other cases, some modeling and simulation work will be necessary before empirical tests can be designed and carried out. This is especially true of hypotheses about the origin of ancient, ubiquitous mechanisms—such as pulsatile signaling, the use of antimicrobial compounds as neurotransmitters/neuromodulators, or co-transmission and convergent signaling in the nervous system. These mechanisms are highly conserved across species, cannot be significantly altered by short-term selection, and may not be amenable to experimental manipulations. Evolutionary models would help determine if they can benefit the host in the presence of parasites, delimit the conditions at which they do, and evaluate the strength of the resulting selec-

tion pressures in the context of other potential advantages (e.g., metabolic efficiency, flexibility). Crucially, formal models may suggest unique predictions that are hard or impossible to derive from the verbal statement of a hypothesis. In the future, it should also become feasible to combine evolutionary models of fitness costs and benefits with detailed mechanistic models of parasite biochemistry (e.g., metabolic network models; see Imam et al. 2015; Zhang and Hua 2016). Present-day metabolic models—which are only available for a few model species, such as *E. coli*—incorporate information about hundreds or thousands of genes and intracellular reactions. Among other things, they can be used to predict the effects of antimicrobial substances on the metabolism and growth rate of bacteria (e.g., Li et al. 2016).

Naturally, comparative research is a crucial source of evidence to investigate the evolution of host countermeasures. Related species or populations may be targeted by different behavior-altering parasites with different strategies, experience infection at different rates, or face different sets of constraints (e.g., energy, temperature, size, life history). At least in some instances, it may be possible to map these ecological variables onto differences in the expression of hypothetical countermeasures. Examples include the biochemical parameters that affect the costs of neural or hormonal signaling (e.g., effective concentrations, receptor expression, clearance rates); CNS-expressed glycans and surface molecules when the parasite manipulates the host from within the brain; or the regulation of influx/efflux through the BBB, particularly when the parasite resides in the host's body and releases neuroactive substances in the circulation. Other useful comparisons could be made between host populations that coexist with the same parasite, but show different levels of susceptibility to manipulation—or, symmetrically, different strains of a parasite that vary in their ability to manipulate the same host species. If comparative molecular data are available for both the host and the parasite, it may be possible to reconstruct the coevolutionary process, and explore the temporal links between the evolution of putative host countermeasures and that of parasite strategies and responses (Table 1). Also, patterns of molecular diversity can provide indirect cues to the existence of host-parasite conflicts and arms races (Summers et al. 2003; see also Massey and Mishra 2018).

"Natural experiments" in which a parasite is introduced or removed from the host's environment can be quite informative, although they are limited to the more rapidly evolvable aspects of host countermeasures. For example, I speculated that hosts may amplify genetic and/or developmental stochasticity to generate individualized signatures in their signaling pathways. This strategy should entail nontrivial costs for the host, and may be quickly reversed (via tighter canalization) as soon as it is no longer beneficial. As an analogy, there is evidence that birds rapidly lose variability in egg markings when they escape brood parasitism (Lahti 2005; see Spottiswoode and Busch 2019).

Finally, countermeasure hypotheses can be explored with a variety of experimental designs. Experimental evolution is an especially promising method in this regard. In a recent study, Hafer-Hahmann (2019) subjected the parasitic flatworm *Schistocephalus solidus* to selection for enhanced versus reduced manipulation strength, finding high heritability and a rapid response to selection. A similar approach could be applied to hosts with short generation times, either by directly selecting for/against resistance to manipulation or by introducing/removing the parasite from the host's environment. Other relevant designs include neurobiological studies in which the action of a parasite is mimicked to study the host's response, and brain lesion or gene knockout studies in which putative countermeasures are inactivated or impaired.

Conclusion

Throughout their long history, brains have been battlegrounds between hosts and parasites for the control of the host's behavior. The unrelenting pressure exerted by parasites must have shaped the evolution of nervous and endocrine systems at all levels, with important consequences even for animals

that are not (or no longer) manipulation targets. If this is true, many aspects of neurobiology are destined to remain mysterious or poorly understood until parasites—the brain's invisible designers—are finally included in the picture. This is not a simple task, and one can anticipate that researchers will face plenty of false starts and dead ends. At the same time, there are good reasons for curiosity and excitement. The road ahead is barely visible, but one can already tell that it leads to strange and interesting places.

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