The Journal of Experimental Biology 216, 56-66

doi:10.1242/jeb.073668

REVIEW

Comparing mechanisms of host manipulation across host and parasite taxa

Kevin D. Lafferty[1,2,*] and Jenny C. Shaw[2]

[1]Western Ecological Research Center, US Geological Survey, USA and [2]Marine Science Institute, University of California, Santa Barbara, CA 93106, USA

*Author for correspondence (lafferty@lifesci.ucsb.edu)

Summary

Parasites affect host behavior in several ways. They can alter activity, microhabitats or both. For trophically transmitted parasites (the focus of our study), decreased activity might impair the ability of hosts to respond to final-host predators, and increased activity and altered microhabitat choice might increase contact rates between hosts and final-host predators. In an analysis of trophically transmitted parasites, more parasite groups altered activity than altered microhabitat choice. Parasites that infected vertebrates were more likely to impair the host's reaction to predators, whereas parasites that infected invertebrates were more likely to increase the host's contact with predators. The site of infection might affect how parasites manipulate their hosts. For instance, parasites in the central nervous system seem particularly suited to manipulating host behavior. Manipulative parasites commonly occupy the body cavity, muscles and central nervous systems of their hosts. Acanthocephalans in the data set differed from other taxa in that they occurred exclusively in the body cavity of invertebrates. In addition, they were more likely to alter microhabitat choice than activity. Parasites in the body cavity (across parasite types) were more likely to be associated with increased host contact with predators. Parasites can manipulate the host through energetic drain, but most parasites use more sophisticated means. For instance, parasites target four physiological systems that shape behavior in both invertebrates and vertebrates: neural, endocrine, neuromodulatory and immunomodulatory. The interconnections between these systems make it difficult to isolate specific mechanisms of host behavioral manipulation.

Key words: behavior modification, trophic transmission, microhabitat, neuromodulation, immunomodulation, site of infection, activity.

Received 12 April 2012; Accepted 5 July 2012

Introduction

Are parasites sophisticated puppet masters of their host's behaviors? Many studies have demonstrated that differences in behavior between infected and uninfected hosts are beneficial to parasites. In other cases, the logical expectation for parasite adaptation is strong. In either case, proof is elusive and differences in behavior between infected and uninfected hosts do not, on their own, imply manipulation by a parasite. For instance, pathogenic parasites can affect host behavior in ways that do not benefit the parasite. For our Review, we assume that the examples we give are parasite adaptations, but readers should understand that support for this assumption varies.

Parasites target four physiological systems that shape behavior in both invertebrates and vertebrates: neural, endocrine, neuromodulatory and immunomodulatory (Adamo, 2002; Adamo, 2013; Beckage, 1993; Escobedo et al., 2009; Helluy, 2013; Moore, 2002; Thomas et al., 2005; Thompson and Kavaliers, 1994). These systems are connected and communicate *via* neurotransmitters, hormones, and neuromodulatory and immunomodulatory chemicals. As a result, identifying the precise mechanisms that underlie host behavior modification has proven a complex task. Moreover, chemically mediated modifications might be the result of parasite- and host-secreted substances acting in response to one another, further obscuring the mechanistic basis of the alterations (Adamo, 2013; Thomas et al., 2005). Less sophisticated parasites could manipulate host behavior by energetic drain or damaging key systems such as the central nervous system (CNS) (though targeting the CNS is arguably a sophisticated accomplishment).

In this review of manipulative parasites, we discuss how various types of behavioral manipulations are distributed across host and parasite taxa. The bulk of our Review concerns the ways that parasites manipulate their hosts and how factors such as site of infection vary across host and parasite taxa.

Distributions of manipulation across taxa

Does the type of host or parasite affect what kinds of behavioral manipulations evolve? Poulin (Poulin, 1994) conducted a meta-analysis to investigate how host and parasite taxa affected the magnitude of changes in host activity or microhabitat choice. He found that non-trophically transmitted nematodes had the largest effects on host behavior. Acanthocephalans affected only host microhabitat choices, and cestodes had large effects on host activity. In addition, manipulation of microhabitat choices was stronger in vertebrate hosts than in invertebrate hosts. Because there has been considerable research done on manipulative parasites in the last two decades, we wanted to explore the effect of host and parasite taxa with new data and a different approach.

To understand how manipulation is distributed among hosts and parasites, we summarized the available literature on behavioral modification for trophically transmitted parasites, starting with Moore's comprehensive review (Moore, 2002), which we updated using recent literature (Table 1). Several species have been studied for some parasite genera (e.g. *Polymorphus*), often with similar results obtained across species. This suggested that species within a genus were not independent and so we chose not to use species as our unit of replication. We were interested in parasite group (e.g.

Table 1. Summary of parasite manipulations by genus

Genus	Intermediate host	Site	Behavior change	Risk type
Acanthocephala				
Acanthocephalus	Invertebrate	Body cavity	Both	Contact
Corynosoma	Invertebrate	Body cavity	Both	Both
Moniliformis	Invertebrate	Body cavity	Both	Both
Neoechinorhynchus	Invertebrate	Body cavity	Microhabitat	Contact
Octospiniferoides	Invertebrate	Body cavity	Microhabitat	Contact
Plagiorhynchus	Invertebrate	Body cavity	Activity	Contact
Polymorphus	Invertebrate	Body cavity	Microhabitat	Both
Pomphorhynchus	Invertebrate	Body cavity	Microhabitat	Contact
Profilicolis	Invertebrate	Body cavity	Both	Both
Sphaerechinorhynchus	Vertebrate	Body cavity		Reaction
Cestoda				
Anomotaenia	Invertebrate	Body cavity	Activity	Reaction
Confluaria	Invertebrate	Body cavity	Both	Contact
Diphyllobothrium	Invertebrate	Body cavity/muscle	Activity	Reaction
Diplocotyle	Invertebrate	Body cavity	Both	Both
Echeneibothrium	Invertebrate	Muscle	Both	Both
Echinococcus	Vertebrate	Other	Activity	Reaction
Eubothrium	Invertebrate	Body cavity	Activity	Contact
Hymenolepis	Invertebrate	Body cavity	Both	Both
Ligula	Vertebrate	Body cavity	Both	Both
Polypocephalus	Invertebrate	CNS	Activity	Contact
Psilostomum	Vertebrate	CNS	Microhabitat	Reaction
Raillietina	Invertebrate	Body cavity	Both	Both
Schistocephalus	Vertebrate	Body cavity	Both	Both
Spirometra	Vertebrate	Other	Activity	Reaction
Taenia	Vertebrate	Muscle	Activity	Reaction
Nematoda				
Baylisascaris	Vertebrate	CNS/muscle	Activity	Reaction
Dispharynx	Invertebrate	Body cavity	Microhabitat	Contact
Muellerius	Invertebrate	Muscle	Microhabitat	Contact
Myrmeconema	Invertebrate	Body cavity	Microhabitat	Contact
Pseudoterranova	Vertebrate	Muscle	Activity	Reaction
Pterygodermatites	Invertebrate	Body cavity	Activity	Reaction
Skrjabinoclava	Invertebrate	Body cavity	Activity	Contact
Tetrameres	Invertebrate	Muscle	Activity	Reaction
Toxocara	Vertebrate	CNS/muscle	Activity	Reaction
Trichinella	Vertebrate	Muscle	Activity	Reaction
Protozoa				
Frenkelia	Vertebrate	CNS		
Sarcocystis	Vertebrate	CNS	Activity	Contact
Toxoplasma	Vertebrate	CNS/muscle	Activity	Both
Trematoda (Digenea)				
Ascocotyle	Vertebrate	Other	Activity	Reaction
Brachylecithum	Invertebrate	CNS	Activity	Reaction
Crassiphiala	Vertebrate	Other	Activity	Reaction
Curtureria	Invertebrate	Muscle	Both	Both
Dicrocoelium	Invertebrate	CNS	Both	Contact
Diplostomum	Vertebrate	CNS	Microhabitat	Contact
Euhaplorchis	Vertebrate	CNS	Microhabitat	Both
Gymnophallus	Invertebrate	Muscle	Both	Both
Gynaecotyla	Invertebrate	Body cavity	Activity	Contact
Maritrema	Invertebrate	Body cavity	Both	Contact
Microphallus	Invertebrate	CNS/body cavity	Both	Contact
Nanophyetes	Vertebrate	Muscle	Activity	Reaction
Ornithodiplostomum	Vertebrate	CNS	Activity	Reaction
Plagioporus	Invertebrate	Body cavity	Both	Both
Plagiorchis	Invertebrate	Muscle	Activity	Contact
Psilostomum	Vertebrate	CNS	Both	
Ribeiroia	Vertebrate	Muscle	Activity	Reaction
Telogaster	Vertebrate	Muscle	Activity	Reaction

CNS, central nervous system.

cestodes *versus* acanthocephalans) as a factor, so we also did not control for phylogeny. Our solution was to consider parasite genera instead of species as the unit of replication. To focus our question, we only considered examples for which authors have posited a behavioral change that might make intermediate hosts more susceptible to predation by final hosts. We had information on host manipulation from 55 parasite genera, distributed among the following parasite taxa: three protozoans, 10 acanthocephalans, 17 digeneans, 15 cestodes and 10 nematodes. For two genera, we could not ascertain details of the behavioral manipulation. We divided the reported infected host behaviors into three types: altered microhabitat choice and changes to activity (increased or decreased). Because parasites may manipulate more than one behavior at a time (Poulin, 2013), we kept track of parasite genera for which there was evidence of more than one type of manipulation [though we note that increased study is likely to find more types of changed behavior (Perrot-Minnot and Cézilly, 2013)]. Thirty genera had decreased activity and 12 genera had increased activity. The relative frequency of increased or decreased activity was independent of whether microhabitat effects were observed in a genus.

We conducted two types of analyses. For the first analysis, we followed Poulin (Poulin, 1994) in contrasting parasite-altered host activity with parasite-altered host microhabitat choice. Types of behavior differed between altered activity (26), altered microhabitat (11), and altered activity and microhabitat (16). In other words, manipulation was more likely to alter activity than microhabitat choice. To determine whether this tendency was the same across host and parasite taxa, we recorded the taxon of the parasite and grouped hosts into vertebrates and invertebrates (finer grouping of hosts into phyla did not alter the results). For statistical comparisons among parasite taxa, we excluded the protozoans because of the small sample size for this category. We sought logistic regression models that allowed independent variables to compete, choosing final models based on Akaike's information criterion (Burnham and Anderson, 2004). In cases where singularities were apparent in the comparisons, we restricted analyses to parasite taxa with representatives in several categories.

Host behavior was significantly associated with a univariate analysis of parasite taxon (χ^2=15.2, d.f.=6, P=0.012; Fig. 1) because more acanthocephalans (all of which were reported from invertebrate hosts) were more frequently associated with altered microhabitat choice than with altered activity in their hosts (consistent with Poulin, 1994), whereas other groups were associated more with host activity. The effect of acanthocephalans indicated a singularity problem with an analysis of host taxon, because all reported acanthocephalans parasitized the body cavity of invertebrate hosts, making it impossible to determine whether the difference between invertebrate and vertebrates hosts was driven by host taxon, parasite taxon or site of infection. A multivariate analysis that excluded the acanthocephalans and protozoans found a significant effect of host taxon (χ^2=7.6, d.f.=2, P=0.02), but interaction between host taxon and site of infection was significant (χ^2=14.6, d.f.=4, P=0.006). Fig. 2 shows that activity was a more common effect of manipulation in vertebrate hosts than microhabitat choice, but the type of behavior in invertebrate hosts did not differ.

Poulin (Poulin, 1994) reached somewhat different conclusions: altered activity and microhabitat choice were equally frequent in his analysis of acathocephalans, nematodes, digeneans and cestodes. In addition, infected vertebrate hosts had more strongly altered microhabitat choices than did invertebrate hosts. However, the approach, response variables and data were different for the two studies. In particular, Poulin's results were driven by non-

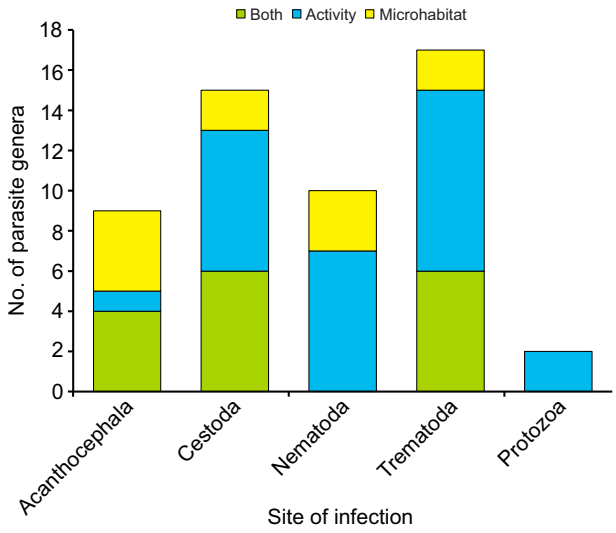

Fig. 1. Type of altered host behavior (activity, microhabitat choice) by parasite taxon. Data represent 55 genera of trophically transmitted parasites. There can be multiple species per genus and multiple studies per species. Altered behaviors were assumed to be adaptive and are categorized according to whether they appeared to impair the host's ability to respond to a predator or cause the host to seek habitats or engage in behavior that put it at increased risked to predation. In many cases, both types of behavioral manipulation were seen for a parasite genus.

trophically transmitted nematodes, which we excluded from our analysis. After excluding nematodes from Poulin's table 1 (Poulin, 1994), we calculated that three of his acanthocephalan species had altered activity whereas six had altered microhabitat choice. For his other parasite taxa, two altered activity and three altered microhabitat choice, a result consistent with our findings that acanthocephalans differ from other parasite taxa in their tendency to manipulate host microhabitat choice.

We also analyzed an alternative grouping of behaviors. The main difference from our previous analysis was that we did not lump increased and decreased activity into a single category. If a parasitized host was described as sluggish, we assumed it would have a decreased reaction to final-host predators, whereas if it was described as more active, we assumed it would have increased contact with predators. Types of behavior were distributed equally between increased contact with predators (19), decreased reaction to predators (20), and both increased contact and decreased reaction (15). Excluding protozoans and acanthocephalans in order to focus on the effects of host taxon (as above), we found that vertebrate hosts were more associated with decreased reaction, whereas invertebrate hosts were more associated with increased contact (χ^2=16.2, d.f.=2, P=0.0003; Fig. 3). However, no residual effect of parasite taxon or site of infection on this categorization of host behavior was evident.

In summary, our two analyses extend Poulin's earlier analysis (Poulin, 1994) with additional data and a different emphasis. Acanthocephalans were more likely to alter host microhabitat choice, while other parasites were more likely to alter host activity. Parasites of vertebrates were more likely to decrease host reaction to predators, while parasites of invertebrates were more likely to increase host contact with predators.

Mechanisms

The mechanisms that underlie behavior modification by parasites are mysterious, and although some components have been

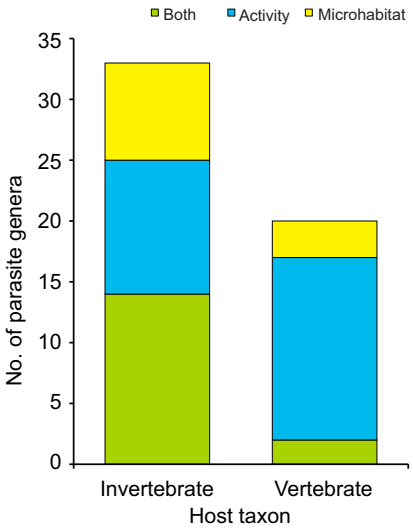

Fig. 2. Type of altered host behavior (activity, microhabitat choice) by host taxon after excluding acanthocephalans and protozoans. Other details as in Fig. 1.

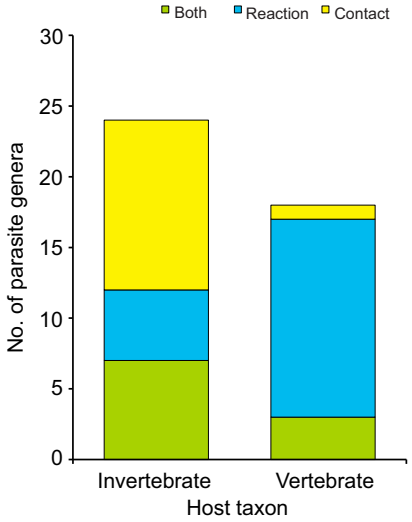

Fig. 3. Type of altered host behavior (host reaction, host contact) by host taxon after excluding acanthocephalans and protozoans. Other details as in Fig. 1.

identified for portions of a behavioral change, delineating all potential mechanisms remains the focus of collaborative research by parasitologists, physiologists and neurobiologists. Here, we discuss energetic drain, site of infection, neuroinflammation and monoamine neurotransmitters as possible common mechanisms of behavior modification by parasites of multiple taxa. However, it appears that mechanisms have been studied in only 19 parasite genera, so it remains difficult to generalize from the current literature.

Energetic drain

Parasites extract energy, in the form of nutrition, from their hosts. If hosts become starved for nutrients, their behavior might change. If energy drain impairs their physiology, they might become more sluggish or display lower physical performance. Alternatively, if a host is drained of energy, it might become more active and increase foraging rates. Either change in behavior could benefit the parasite responsible for the energy drain.

Energy drain can lead to malaise, which interferes with defense. For example, the most dangerous part of being a mosquito is taking a blood meal from a host that does not want to be bitten. Humans are adapted to hear and feel mosquitoes and are capable of swatting and killing them. This response helps hosts avoid blood loss and reduce exposure to diseases vectored by mosquitoes, such as malaria. Malaria is a taxing disease, inducing periodic fevers and requiring the host to replenish blood cells consumed by the parasite. Individuals in the height of a malarial fever are often incapacitated, and this leaves them exposed to mosquitoes, which land to take an uninterrupted blood meal. For instance, experiments have demonstrated that mice infected with malaria no longer try to avoid mosquitoes in the laboratory (Day and Edman, 1983). Similarly, infected hosts might have fewer energy reserves or the ability to power muscles needed to escape from predators. The larval tapeworm *Echinococcus granulosus* appears to incapacitate intermediate host moose by lodging in the lungs, hypothetically reducing lung capacity and making it easier for wolves to attack moose (Joly and Messier, 2004).

Energy drain might also influence habitat selection. Taking risks to forage might place the host in situations where it is more likely to be eaten by a predator that acts as the final host for the parasite. Fish infected by larval tapeworms have higher oxygen demands, causing them to spend more time at the surface (Giles, 1987). The energy taken by parasites might affect foraging decisions. A hungry host might be more likely to avoid shelter to increase foraging rates (Milinski, 1984).

The advantage to energy drain as a behavioral manipulation is that taking energy from the host is something that parasites are already selected to do. This is an excellent example of how behavioral manipulations do not have to be sophisticated. However, energy drain leads to general changes in behavior that might not target the specific predator or vector of the host, leading to inefficient transmission or putting the host at risk to other sources of danger that do not benefit the parasite.

Site of infection

Manipulative parasites occupy a range of sites in their hosts. They are found in the body cavity, muscles, CNS and other parts of the host. Here, we begin by examining whether there are any patterns in site use across host or parasite taxonomy. Then, we focus on parasites that infect the host's CNS.

To better understand the distribution of parasites among sites within a host, we expanded our analysis of the data (above) to consider the site of infection of manipulative parasites infecting vertebrates and invertebrates. We defined the site of infection as body cavity, muscle, CNS or other (heart and skin). For simplification purposes, parasites that were found in the CNS and other tissues were categorized as CNS.

We found patterns related to site of infection in trophically transmitted parasites. Most (27) genera occurred in the body cavity, 12 were associated, at least in part, with the CNS, 12 were in the muscles and four occurred in other tissues. Parasite taxa significantly differed in their most common site of infection (χ^2=30.3, d.f.=12, P=0.0026; Fig. 4). The two protozoans used in the analysis occurred in the CNS, and acanthocephalans only occurred in the body cavity. Cestodes were often in the body cavity, but occupied other sites as well. Nematodes tended to occur equally in the muscles and body cavity. Trematodes had the most diverse site use, with several examples in the body cavity, muscles, CNS

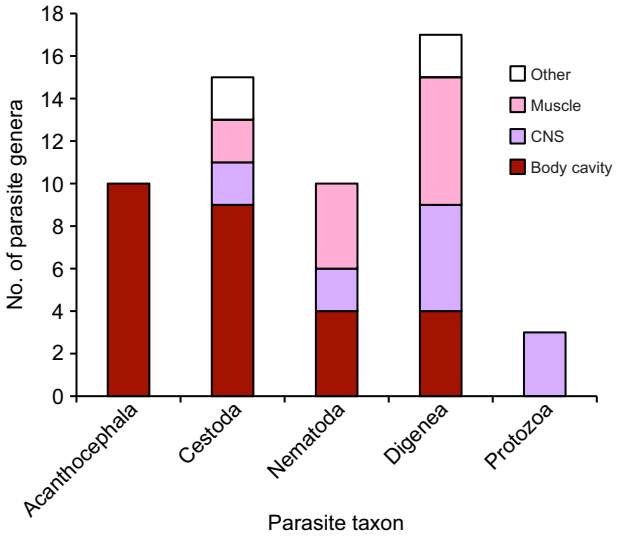

Fig. 4. Site of infection by parasite taxon. Sometimes authors did not define the site of infection, so other literature was used. Note that body cavity is a common default given for parasites of invertebrates. Parasites that had some involvement with the central nervous system (CNS) were all categorized as being in the CNS.

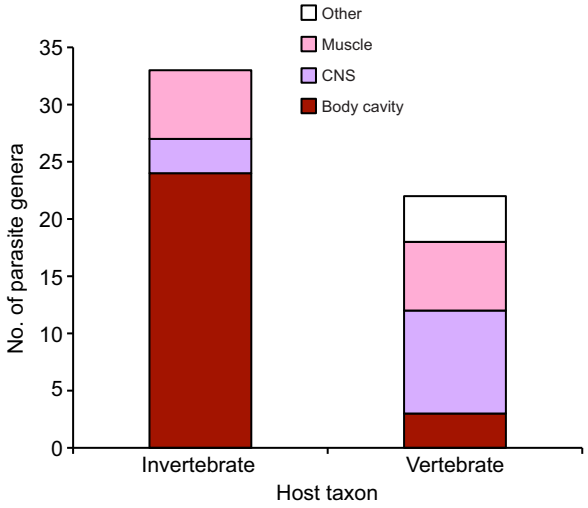

Fig. 5. Site of infection by host taxon after excluding acanthocephalans and protozoans. Other details as in Fig. 4.

and other sites such as the skin. The site of infection varied by whether the host was vertebrate or invertebrate (χ^2=22.0, d.f.=3, P=0.0001), but as before, this was potentially confounded by the uniform host use of acanthocephalans and protozoans. After excluding these groups, there was still a tendency for relatively fewer parasites of vertebrates to be in the body cavity and relatively fewer parasites of invertebrates to be in the nervous system (χ^2=16.9, d.f.=12, P=0.0007; Fig. 5), with no residual effect of parasite taxon. However, bias may be related to host taxon, because of a lack of detail given for the site of infection in invertebrate hosts compared with vertebrate hosts (e.g. the default site of infection for invertebrates is often the 'hemocoel').

Parasites can change host behavior by occupying and damaging key organ systems. For instance, *Diplostomum spatheceum* cercariae penetrate the skin of a freshwater fish and migrate to the eye, where they encyst in the lens and develop into metacercariae. Heavy infections result in a parasite-induced cataract. Infected fish spend more time feeding at the surface (Crowden and Broom, 1980), presumably because the cataracts impair their visual ability to hunt aquatic crustaceans, which can render them more visible to bird predators. Infected hosts have reduced escape responses and are more susceptible to simulated predation in a laboratory setting (Seppälä et al., 2004; Seppälä et al., 2005). In another example, the rhizocephalan barnacle *Sacculina carcini* first destroys the androgenic gland of its host crab, resulting in host feminization of males (Høeg, 1995). Transgendered crabs produce no offspring yet behave like females and care for the eggs of the barnacle parasite as if they were their own. Damaging organs can be an effective way to manipulate a host, and in the case of rhizocephalan-infected crabs, it can be the psychiatric equivalent of a lobotomy.

The key organ system for the coordination of host behavior is the CNS. Parasites that occupy the CNS seem well situated to manipulate behavior either through damage or more subtle manipulation. In fact, a wide variety of parasite taxa invade the CNS of their hosts, including protozoans, digeneans and parasitoid insects (Adamo, 2002; Adamo, 2013; Escobedo et al., 2009; Klein, 2003; Libersat et al., 2009; Moore, 2002). It was once thought that the key advantage of parasitizing the CNS was to escape the immune system (Szidat, 1969). We now know that the brain contains native immunoreactive components, where microglia, astrocytes and even neurons have pathogen-detecting receptors on their cell surfaces (Falsig et al., 2008). In fact, the brain can increase its immune reactivity when invaded by parasites or pathogens, which can be enhanced through transport by specific blood–brain barrier transporter proteins (Erickson et al., 2012). Therefore, the key benefit of parasitizing the CNS appears to be behavioral manipulation.

Toxoplasma gondii is a protozoan parasite that infects warm-blooded vertebrates, such as rodents, as intermediate hosts and cats as definitive hosts (see Flegr, 2013; McConkey et al., 2013; Vyas, 2013; Webster, 2013). In the intermediate host, *T. gondii* reproduces asexually (acute toxoplasmosis) before forming tissue cysts in organs, muscle and, in particular, the CNS (chronic or latent toxoplasmosis). Infected rodents increase their exploratory behavior and are even attracted to the scent of cat urine, which should increase their susceptibility to predation by cats and the transmission of *T. gondii* to its final host (Berdoy et al., 2000; Vyas et al., 2007; Webster and McConkey, 2010). Curiously, *T. gondii* has demonstrated an affinity for infecting the amygdala in rodents, an area central to processing fear (Berenreiterová et al., 2011). Reactivation of a chronic infection, usually in immunocompromised individuals, can cause inflammation of the brain (toxoplasmic encephalitis), whereupon immune reactivity increases and dendritic cells recruit to the infection site (John et al., 2011).

One of the classic examples of host behavior modification involves the liver fluke *Dicrocoelium dendriticum* in its second intermediate ant host. Adult worms reproduce in the digestive tract of ruminant livestock (sheep and cattle), releasing their eggs with the host's feces. The eggs are ingested by first intermediate host pulmonate snails, which cough up slime balls containing infective cercariae. Ants become infected after ingesting the slime ball, which are a preferred meal for an ant. Once inside the ant's digestive tract, the cercariae migrate through the body and encyst in the ant's hemocoel, except for one that encysts in the subesophageal ganglion, which control the mandibles. Infected ants display a curious behavior at sundown, when temperatures drop,

where they ascend to the tip of a blade of grass, clamp down with their mandibles and remain motionless for the duration of the night and next dawn. Once temperatures increase, they climb back down and resume normal activity until the next evening. This modified behavior places ants in prime position to be eaten by grazing livestock – which tend to graze in the evenings and early mornings – thus solving parasite's dilemma of getting from the ant to an herbivore and increases its chances of completing its life cycle.

Digeneans of the family Diplostomidae often parasitize the brain of their fish second intermediate hosts and develop into adults in the digestive tract of bird final hosts. Diplostomid metacercariae are large relative to the fish's brain and encyst just beneath the surface of the optic lobes, an area engaged in visual processing (Muzzall and Kilroy, 2007; Shirakashi and Goater, 2005). *Ornithodiplostomum ptychocheilus* metacercariae have been shown to disrupt visual processing and locomotion in their hosts (Shirakashi and Goater, 2005), an impairment that could interfere with the fish's ability to detect and escape from a bird predator. However, this occurs before the metacercaria becomes infective to the final host, so the disruption in optomotor activity might be a pathological side effect of infection (Shirakashi and Goater, 2005).

Euhaplorchis californiensis (Heterophyidae), another brain-encysting digenean, alters the behavior of its second intermediate fish host, the California killifish (*Fundulus parvipinnis*). Infected killifish display conspicuous swimming behaviors, which render them 30 times more likely to be eaten by bird final-host predators (Lafferty and Morris, 1996). This increased susceptibility to predation by birds might be due to a parasite-induced suppression of the fish's stress response. Interestingly, *E. californiensis* metacercariae aggregate on the brainstem in low-intensity infections (Shaw et al., 2009). Brain-encysting cercariae navigate to the brain along spinal nerves and would therefore enter the brainstem first, facilitating encystment on the brainstem (Hendrickson, 1979). Perhaps *E. californiensis* found a way to capitalize on its initial default location. Interestingly, site preference is also exhibited by brain-encysting metacercariae in gammarids (see Helluy, 2013). *Microphallus papillorobustus* encysts in the protocerebrum of its gammarid host (*Gammarus lacustris*) in single infections (Helluy and Thomas, 2003). Although the sample size was too low to indicate a significant trend, this suggests that parasites can evolve a preference for specific brain regions.

To conclude, parasites occur in a wide variety of sites in their hosts. The main site of infection in invertebrate hosts is the body cavity, whereas a wider diversity of sites is used in vertebrate hosts. Larval acanthocephalans in our sample data only infected the body cavity, which was also a common site of infection for cestodes. Digenean trematodes used the greatest variety of sites. Many genera use the host CNS, at least in part. Counter to expectations, immune responses do occur in the CNS. Although this means the CNS is not a safe haven for parasites, it does place them in an ideal position to manipulate behaviour, and, as discussed below, they can use the host's immune defenses to achieve this.

Immune system

It is feasible that parasites, having to negotiate the host's immune system, would evolve ways to capitalize on the host's innate defense mechanisms (Adamo, 1997; Adamo, 2013; Helluy and Thomas, 2003; Thomas et al., 2005). Further, heritable manipulative strategies that increase parasite fitness should be selected for [see Poulin (Poulin, 2010) and references therein]. Many studies reveal the possible links between host immune responses and the resultant physiological and behavioral changes that parasites might exploit as strategies to enhance transmission (see Adamo, 2013; McCusker and Kelley, 2013). Studies involving neuroinflammation are discussed below.

Neuroinflammation is a common immune response of the brain to injury or invading pathogens. Several studies indicate that parasites may incorporate host neuroinflammatory responses into their behavior modification strategy, such as nitric oxide (NO), rodlet cells and alteration of neuromodulators. Helluy and Thomas (Helluy and Thomas, 2010) found elevated NO activity around the cyst wall of cerebral metacercariae in gammarid hosts. One of the main effector molecules of the immune system, NO targets tumors and pathogens in both invertebrates and vertebrates (Bogdan, 2001; Helluy and Thomas, 2010), so it should come as no surprise to see elevated NO activity near invading parasites. However, NO also functions as a neurotransmitter (although it is not stored in synaptic vesicles and is synthesized as needed) that can influence brain monoaminergic activity. NO has been shown to influence serotonin (5-HT) and dopamine (DA) release in rodents (Dunn, 2006; Frisch et al., 2000; Helluy and Thomas, 2010), and therefore elevated NO activity likely plays a role in the decreased serotonergic activity observed in the brains of infected gammarids (Helluy and Thomas, 2003; Helluy and Thomas, 2010).

Teleost fish possess rodlet cells, so named for the rod-shaped structures present within each cell, whose primary function appears to be to respond to tissue injury, especially in the cases of parasitic infection (Dezfuli et al., 2007; Matisz et al., 2010; Schmachtenberg, 2007). Brain-encysting metacercariae evoke a strong response by rodlet cells in freshwater minnows, where rodlet cells surround the offending worm and increase in density as more damage is incurred by developing metacercariae (Matisz et al., 2010). Rodlet cells are not found in the brains of uninfected conspecifics (Dezfuli et al., 2007). Rodlet cells are thought to be a part of a larger inflammatory response that includes increased vascularization and a proliferation of fibroblasts to the site of injury (Matisz et al., 2010). The latter is intriguing when considering that brain-encysting *E. californiensis* metacercariae express three types of fibroblast growth factors on the surface of their cysts (Shaw et al., 2009). Fibroblast growth factors stimulate proliferation of fibroblasts, cells that are ubiquitous in all types of connective tissues and produce collagen proteins and other components of the extracellular matrix. They also activate in response to tissue damage to promote healing and scar formation. In the CNS, fibroblasts have a neuroprotective role and can promote neurogenesis (Erickson et al., 2012; Guillemot and Zimmer, 2011). It remains unclear why metacercariae would promote fibroblast activity near their cysts walls, but inhibition of the fibroblast growth factors appeared to disrupt the ability of the metacercariae to aggregate *in vitro* (J. LaClair and K.D.L., unpublished data).

Parasitic infection elicits host immune responses that are designed to overcome the invading parasite. Not only do parasites have to negotiate the host's immune defenses in order to establish an infection, the durable nature of the infection means that they must continually evade or deceive the constant vigilance of the immune system. In addition, many parasites demonstrate the ability to exploit host immune defense mechanisms for their own benefit. However, a growing body of research delineates the extensive cross-communication between the immune system and other neuromodulatory systems (Demas et al., 2011; Lowry et al., 2007; Webster Marketon and Glaser, 2008; Thompson and Kavaliers, 1994), so that a parasite-exerted effect on the immune system may, in turn, influence a neuromodulator pathway. These systems

interact through redundant signaling molecules (e.g. cytokines, monoamine neurotransmitters and stress hormones), which facilitate reciprocal feedback interactions. Moreover, recent studies demonstrate that parasites can alter host neuromodulator activity, as discussed below.

Neuromodulation

Neuromodulators are molecules that affect long-term physiological changes as opposed to fast-acting chemical messaging at the synapse. Hormones are a good example of neuromodulators, because they effect changes in the body that can last weeks, months and even years. However, the traditional distinction between neurotransmitters and neuromodulators has become blurred, as many neurotransmitters have been found to enact long-lasting physiological changes in addition to their transient roles in synaptic transmission. Also, a chemical can act as a neurotransmitter or neuromodulator depending on its site of action, e.g. in vertebrates, noradrenaline (norepinephrine, NE) and DA are released from adrenal glands as hormones, but both also are neurotransmitters throughout the body and CNS (Nelson, 1995). Some of the best-known examples of host behavior modification involve parasite-induced changes in neuromodulator activity. Many host–parasite systems and the mechanisms involved have been reviewed in depth (Adamo, 2013; Adamo, 2002; Kavaliers et al., 1999; Klein, 2003; Lefèvre et al., 2009; Libersat et al., 2009; Moore, 2002; Poulin, 2010; Thomas et al., 2005; Thompson and Kavaliers, 1994) and will be summarized here.

Monoamine neurotransmitters are potent neuromodulators. These include the catecholamines DA, adrenaline (epinephrine, EP) and NE, the indoleamine 5-HT, and octopamine (OA), found in invertebrates. Monoamines influence many types of behaviors in vertebrates and invertebrates, including those related to activity, movement, stress, social activity and reproduction (Fabre-Nys, 1998; Libersat and Pflueger, 2004; Nelson, 1995; Nelson and Trainor, 2007; Øverli et al., 2007; Weiger, 1997; Winberg and Nilsson, 1993). Parasite-induced changes in OA, DA and 5-HT feature prominently in host–parasite systems of various taxa, which indicate a significant level of evolutionary conservation in the structure and function of these chemicals (Libersat and Pflueger, 2004; Pflüger and Stevenson, 2005; Weiger, 1997; Winberg and Nilsson, 1993; see also Helluy, 2013; Libersat and Gal, 2013; McConkey et al., 2013; Vyas, 2013; Webster et al., 2013). In comparison, EP and NE have not yet been shown to be significant in altered host behaviors (Helluy and Holmes, 1990; Øverli et al., 2001; Shaw et al., 2009; Stibbs, 1985). For this reason, we focus on OA, 5-HT and DA.

OA appears to be analogous to NE in invertebrates, where EP and NE have a much lower physiological significance than in vertebrates (Libersat and Pflueger, 2004; Roeder, 1999; Roeder et al., 2003). OA is an important neurotransmitter and hormone that regulates other neuromodulators and influences many behaviors, including fight-or-flight reactions, stress, aggression, locomotion and feeding (Adamo, 2010; Beckage, 1997; Libersat and Pflueger, 2004; Sneddon et al., 2003). Two well-known host–parasite systems demonstrate significant host behavior modification in association with altered OA activity. Tobacco hornworm caterpillars (*Manduca sexta*) infected with parasitoid wasp larvae (*Cotesia congregata*) stop feeding and moving 1 day before the larvae emerge (Beckage, 1997). After emergence, the wasps settle on their host's cuticle and pupate, which takes several days. The altered host behaviors appear to be critical for survival of the wasps, because otherwise the caterpillar may turn around and consume the attached larvae (Adamo, 2002). These behaviors coincide with a rise in OA levels in the host's hemolymph, a physiological change that appears to be due to the presence of the wasp larvae and not the wasp venom (Adamo, 2002). The decrease in feeding appears to be due in part to the increased OA, which reduces foregut contractions – the caterpillar's swallowing mechanism – through neuronal disruption of the frontal ganglion (Adamo, 2002). Although the larvae do not appear to be the source of the excess OA, the exact mechanism of how the larvae induce the surge in OA remains unknown (Adamo, 2002; Adamo, 2010).

OA is a neurotransmitter for motor pathways in invertebrates, and it appears to be disrupted by an acanthocephalan parasite that alters the locomotion of its host, which ends up benefiting the parasite. Gammarids (*G. lacustris*) infected with larval acanthocephalans *Polymorphus paradoxus* display a positive phototaxis and an unusual clinging behavior, where they swim up to the surface and respond to disturbance by clinging to vegetation or floating material (Helluy and Holmes, 1990). These altered behaviors make the infected gammarids more susceptible to being eaten by grazing waterfowl, which are the final hosts for *P. parodoxus*. OA injected into infected gammarids suppresses their clinging response (Helluy and Holmes, 1990).

Parasite-induced changes in the indoleamine 5-HT have been documented in several host–parasite systems with striking behavior modification. Gammarids that are infected with either digenean metacercariae (*M. papillorobustus*) or acanthocephalan cystacanths (*Polymorphus* spp. and *Pomphorhynchus* spp.) are photophilic, which is a behavior associated with elevated 5-HT activity (Adamo, 2013; Helluy and Holmes, 1990; Lefèvre et al., 2009; Moore, 2002; Tain et al., 2006). Attraction to light can be induced in uninfected gammarids by increasing 5-HT content *via* direct injection or pharmaceutical drug (Guler and Ford, 2010; Tain et al., 2006; Tain et al., 2007). 5-HT injected into uninfected *G. lacustris* also elicits the aberrant clinging behavior displayed by individuals infected with the acanthocephalan *P. parodoxus* (Helluy and Holmes, 1990). Examinations of neuronal architecture in the CNS reveal increased serotonergic immunoreactivity (staining) in gammarids infected by different acanthocephalan species, which are not in direct contact with the brain [table 3.1 in Lefèvre et al. (Lefèvre et al., 2009) (see also Maynard et al., 1996; Tain et al., 2006)]. In contrast, gammarids infected with *M. papillorobustus*, the only parasite infecting its brain, exhibit lower serotonergic immunoreactivity in a specific optic area (Helluy and Thomas, 2003). The decreased immunostaining in *M. papillorobustus*-infected gammarids could be due, in part, to parasite-associated disruptions in the morphology of serotonergic neurons, although degenerate neurons were also found at the opposite end of the brain from the metacercaria, suggesting that the degeneration could have resulted from decreased serotonergic innervation (Helluy and Thomas, 2003). Immunostaining can reveal discrepancies in steady-state serotonergic content; however, the information does not indicate the direction of change. An increase in 5-HT staining can indicate either a decrease in neurotransmitter release or an increase in synthesis to compensate for an increase in release (Adamo, 2013; Helluy and Thomas, 2003; Tain et al., 2006). An additional type of complementary analysis would be to measure both 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the gammarid brains using HPLC; altered relative concentrations of 5-HT and 5-HIAA would indicate the direction of turnover (Øverli et al., 2001; Shaw et al., 2009). For example, a decrease in 5-HT and an increase in 5-HIAA would indicate an increase in 5-HT metabolism and therefore turnover.

Altered 5-HT metabolism also underlies changes in host behavior in fish infected by larval helminths. Three-spined sticklebacks (*Gasterosteus aculeatus*) infected with the larval tapeworm *Schistocephalus solidus* exhibit a variety of altered behaviors, including increased surfacing and impaired escape responses, which should make infected fish more susceptible to predation by birds, the final hosts for *S. solidus* (Barber et al., 2000; Øverli et al., 2001). Infected fish display elevated 5-HT activity in their brainstems, as indicated by an increase in the ratio of 5-HT to its metabolite, although this change is characteristic of fish under chronic stress, and *S. solidus* does harm its host (Øverli et al., 2001). In contrast, killifish infected with *E. californiensis* display a parasite-induced suppression of brainstem serotonergic activity, an initial component of the fish's innate stress response. The normal stress response in fish consists of an initial rise in brainstem serotonergic activity, which launches a cascade of other physiological processes, including a surge of circulating stress hormones (Winberg and Nilsson, 1993). Disruption of this initial physiological stress response could ultimately impair the killifish's escape response.

The striking changes in 5-HT-altered behaviors in vertebrates and invertebrates demonstrate the ubiquity of 5-HT and its widespread functionality in animals of varying complexity. 5-HT is a major neuromuscular neurotransmitter used in the most primitive animals (e.g. cnidarians), and its roles expand into neuromodulator and neurohormone in more complex animals, where it controls additional physiological functions and behaviors, including stress responses and immune challenges (Weiger, 1997). A stress response consists of activation of the hypothalamic-pituitary-adrenal axis, where the brain signals the release of stress hormones and monoamines, which in turn activate and regulate lymphocytes and other immune effector proteins such as cytokines (Webster Marketon and Glaser, 2008). It is plausible that any neurochemical that is instrumental in the physiological stress response will influence the immune response (Adamo, 2010; Wendelaar Bonga, 1997; de Jong-Brink et al., 2001; Demas et al., 2011; Escobedo et al., 2009; Webster Marketon and Glaser, 2008), and parasite-induced changes in host neuroactive chemicals might have arisen through reciprocal communication by both systems (Adamo, 2013; Adamo and Baker, 2011; Escobedo et al., 2009; Thomas et al., 2005). For example, 5-HT can activate and regulate the hypothalamic-pituitary-adrenal axis activity in mammals (hypothalamic-pituitary-interrenal in fish), a system of neuroendocrine pathways that coordinate physiological response to stress and disease through common effector molecules such as neuromodulators (Chaouloff, 2000; Clements et al., 2003; Webster Marketon and Glaser, 2008; Winberg et al., 1997) and is in turn regulated by immune proteins (Dunn, 2006).

Altered DA activity has been demonstrated to drive behavioral changes observed in striking examples of behavior modification by parasites in crustaceans. DA stimulates activity in crustaceans and is associated with escalated fight posture and behavior (Sneddon et al., 2003). Shore crabs (*Hemigrapsus crenulatus*) infected with cystacanths of the acathocephalan *Profilicollis antarticus* are more active than uninfected ones (Haye and Ojeda, 1998). Hemolymph DA is increased in infected crabs, which could contribute to the increased activity (Rojas and Ojeda, 2005).

Parasite-dependent changes in brain DA activity are also evident in killifish infected with *E. californiensis* metacercariae. Infected killifish have increased DA activity in their brainstem (Shaw et al., 2009). DA is associated with heightened locomotion, aggression, dominance and reproductive behavior in fish and mammals (Winberg and Nilsson, 1993; Fabre-Nys, 1998), and the elevated dopaminergic activity in killifish might contribute to the increased displays of conspicuous swimming activity (Shaw et al., 2009). However, dopaminergic neurons in the brainstem also inhibit serotonergic neurons that project from the brainstem to the midbrain, and thus might contribute to the parasite-induced suppression of brainstem 5-HT activity seen in infected killifish (Shaw et al., 2009).

Several studies implicate alterations of DA activity as a possible mechanism underlying altered behaviors observed in rodents infected with *T. gondii* [see Webster and McConkey (Webster and McConkey, 2010) and Adamo (Adamo, 2013) for thorough reviews]. Brain DA activity is increased in *T. gondii*-infected mice (Stibbs, 1985), and administration of a DA receptor agonist induces novelty-seeking behavior in uninfected mice (Skallová et al., 2006). These results align with the general notion that DA stimulates locomotion, aggression, dominance and reproductive behavior in fish and mammals (Winberg and Nilsson, 1993; Fabre-Nys, 1998).

Anti-psychotic drugs are as effective as anti-parasitic drugs in diminishing *Toxoplasma*-induced alterations of behavior, and the most effective drug is a DA antagonist (Webster et al., 2006). More recently, it was discovered that *T. gondii* expresses genes for tyrosine hydroxylase, the enzyme that synthesizes DA, as well as increases DA metabolism in host neurons (Gaskell et al., 2009; Prandovszky et al., 2011). This heightened signaling activity of infected neurons could drive DA-associated behavior changes, especially in the heavily dopaminergic amygdala and basal ganglia, which are important regions for shaping fear and locomotory behaviors, respectively (Prandovszky et al., 2011).

These parasite-associated changes in monoamine activity in the various host–parasite systems above indicate a high level of conserved function throughout the evolution of invertebrates and vertebrates (Libersat and Pflueger, 2004; Pflüger and Stevenson, 2005; Weiger, 1997; Winberg and Nilsson, 1993). However, the details of the specific mechanisms of action for OA, 5-HT and DA in shaping behavior are dependent on specific receptor types and their locations in different brain and CNS regions (Adamo, 2013; Demas et al., 2011; Fabre-Nys, 1998; Takahashi et al., 2011; Winberg and Nilsson, 1993), and should be accounted for when interpreting mechanistic studies. For example, 5-HT receptors can reduce or increase aggression depending on their location in the vertebrate brain (Summers and Winberg, 2006; Takahashi et al., 2011).

Despite the extensive research described herein, it remains unknown whether parasites alter host neuromodulators by secreting neurochemicals to produce changes in host neurochemical activity, or by secreting chemicals that trigger a host response, leading to altered neurochemical activity (Adamo, 2013; de Jong-Brink et al., 2001; Thomas et al., 2005). The parsimonious explanation would be the latter, given that parasites are often much smaller than their hosts, and it might be too costly for them to produce the exact chemicals needed to achieve the levels of systemic alteration observed in hosts (Adamo, 2013; de Jong-Brink et al., 2001; Thomas et al., 2005).

The complexity and connectedness of neuromodulator systems make it a difficult task for scientists to tease apart the specific mechanisms responsible for discrete changes in behavior. However, the complex nature of these systems might also enable parasites to induce widespread changes in their hosts' behavior with minimal effort. For example, monoamines regulate one another in regulatory feedback loops, e.g. OA and DA stimulate escape

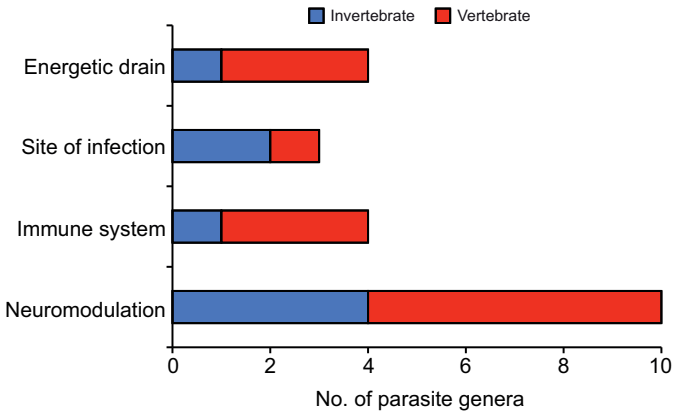

Fig. 6. Types of mechanisms reported for manipulative parasites by host type. Parasites that had some involvement with the central nervous system (CNS) were all categorized as being in the CNS.

behavior in insects, which is counteracted by 5-HT (Gavra and Libersat, 2011). Similarly, DA promotes locomotion, aggression and other dominance behaviors in vertebrates, whereas 5-HT inhibits the same behaviors (Winberg and Nilsson, 1993). In theory, a parasite need only alter the activity of one of the neuromodulators involved in a feedback loop to effect regulatory changes in the other components.

Parasite-induced changes in host neuromodulators might have arisen through reciprocal communication by neuroendocrine and immune systems (Adamo, 2013; Adamo and Baker, 2011; Escobedo et al., 2009; Thomas et al., 2005). Parasites must negotiate and overcome their hosts' immune system, and in doing so, might have capitalized on the behavioral changes that resulted from immune response alterations in physiology. Adamo (Adamo, 2013) notes that it would be but a small evolutionary step for parasites to enact changes in host behavior from attempts to immunosuppress the host. Indeed, parasites use a variety of neurochemical methods to enact changes in host physiology, rather than target a singular neurotransmitter system or pathway (Adamo, 2013; de Jong-Brink et al., 2001; Lefèvre et al., 2009; Thomas et al., 2005). The high degree of interconnection and cross-communication of what is being called the neuroimmunoendocrine system (Escobedo et al., 2009) perhaps enables parasites to affect change across multiple systems with minimal energetic effort. A sustained activation of immune system effector proteins (cytokines) is necessary to maintain resistance to acute and chronic *T. gondii* infections, and this alone could alter neuromodulator activity (Webster and McConkey, 2010). One additional consideration for this hypothesis is the pattern of parasites that are unable to modify the behavior of non-native hosts, indicating a level of local adaptation to the native host immune system and perhaps a clue to mechanisms of behavior modification (Ballabeni and Ward, 1993; Cornet et al., 2010; Tain et al., 2006).

To summarize the section on mechanisms, parasites have many ways that they alter host behavior. Parasite group had a near significant effect (χ^2=35.3, d.f.=24, P=0.064), with acanthocephalans associated with monoamines, cestodes associated with energetic drain and hormones, and digeneans not associated with hormones. Mechanisms were found more or less equally distributed across vertebrate and invertebrate hosts (Fig. 6). These results are too premature to conclude general patterns. However, the mechanisms used by parasites are distributed across groups, so there appear to be multiple, independent evolutions of various mechanisms. The one mechanism available to all parasites is energy drain. This is not a sophisticated mechanism, but it might favor parasite transmission under some situations. The effects of a parasite might be limited to the host tissues on which it lives. For this reason, the site of infection can provide clues to mechanisms of behavioral manipulation. Of all the sites, the CNS is the most linked to the ability to manipulate host behavior and is over-represented compared with other mechanisms (Fig. 5). We agree that the CNS is not free from immune defenses. In fact, parasites are able to use immune defenses to their advantage because inflammation is linked to various neuromodulators. The ability to produce, suppress or activate neuromodulators is the apex of sophistication for manipulative parasites.

Conclusions

In this Review, we have assumed that parasite-associated altered behavior is an adaptation that benefits the parasite. There are credible examples of manipulative parasites from many different taxa. Acanthocephalans differed from other parasites because they were reported only in the body cavity of invertebrates and were more likely to alter host microhabitat than activity. Infected vertebrate hosts were more likely to exhibit impaired reactions to predators, and infected invertebrate hosts were more likely to experience increased contact with predators. Parasites in muscles, the CNS and other tissues were more frequently associated with decreased reaction to predators (impaired response), whereas parasites in the body cavity were more likely to be associated with increased contact with predators. Few studies have shown the three key pieces of evidence needed to understand how parasites manipulate their hosts: a change in behavior associated with parasitism, a positive link between altered behavior and parasite fitness, and mechanisms by which parasites alter behavior. Of these, understanding the mechanisms is the most challenging. Despite these challenges, we have a wealth of information on how parasites can affect the immune system and neuromodulatory chemicals. It seems reasonable to assume that these sophisticated mechanisms have evolved under natural selection to alter host behavior to the parasite's advantage.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

Adamo, S. A. (1997). How parasites alter the behavior of their insect hosts. In *Parasites and Pathogens: Effects on Host Hormones and Behavior* (ed. N. E. Beckage), pp. 231-245. New York: Chapman & Hall.
Adamo, S. A. (2002). Modulating the modulators: parasites, neuromodulators and host behavioral change. *Brain Behav. Evol.* **60**, 370-377.
Adamo, S. A. (2010). Why should an immune response activate the stress response? Insights from the insects (the cricket *Gryllus texensis*). *Brain Behav. Immun.* **24**, 194-200.
Adamo, S. A. (2013). Parasites: evolution's neurobiologists. *J. Exp. Biol.* **216**, 3-10.
Adamo, S. A. and Baker, J. L. (2011). Conserved features of chronic stress across phyla: the effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, *Gryllus texensis*. *Horm. Behav.* **60**, 478-483.
Ballabeni, P. and Ward, P. I. (1993). Local adaptation of the tremadote *Diplostomum phoxini* to the European minnow *Phoxinus phoxinus*, its second intermediate host. *Funct. Ecol.* **7**, 84-90.
Barber, I., Hoare, D. and Krause, J. (2000). Effects of parasites on fish behaviour: a review and evolutionary perspective. *Rev. Fish Biol. Fish.* **10**, 131-165.
Beckage, N. E. (1993). Endocrine and neuroendocrine host-parasite relationships. *Receptor* **3**, 233-245.
Beckage, N. E. (1997). *Parasites and Pathogens: Effects on Host Hormones and Behavior*. New York: Chapman and Hall.

Berdoy, M., Webster, J. P. and Macdonald, D. W. (2000). Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc. R. Soc. Lond. B* **267**, 1591-1594.
Berenreiterová, M., Flegr, J., Kuběna, A. A. and Němec, P. (2011). The distribution of *Toxoplasma gondii* cysts in the brain of a mouse with latent toxoplasmosis: implications for the behavioral manipulation hypothesis. *PLoS ONE* **6**, e28925.
Bogdan, C. (2001). Nitric oxide and the immune response. *Nat. Immunol.* **2**, 907-916.
Burnham, K. P. and Anderson, D. R. (2004). Multimodel inference – understanding AIC and BIC in model selection. *Sociol. Methods Res.* **33**, 261-304.
Chaouloff, F. (2000). Serotonin, stress and corticoids. *J. Psychopharmacol.* **14**, 139-151.
Clements, S., Moore, F. L. and Schreck, C. B. (2003). Evidence that acute serotonergic activation potentiates the locomotor-stimulating effects of corticotropin-releasing hormone in juvenile chinook salmon (*Oncorhynchus tshawytscha*). *Horm. Behav.* **43**, 214-221.
Cornet, S., Sorci, G. and Moret, Y. (2010). Biological invasion and parasitism: invaders do not suffer from physiological alterations of the acanthocephalan *Pomphorhynchus laevis*. *Parasitology* **137**, 137-147.
Crowden, A. E. and Broom, D. M. (1980). Effects of the eyefluke, *Diplostomum spathaceum*, on the behaviour of dace (*Leuciscus leuciscus*). *Anim. Behav.* **28**, 287-294.
Day, J. F. and Edman, J. D. (1983). Malaria renders mice susceptible to mosquito feeding when gametocytes are most infective. *J. Parasitol.* **69**, 163-170.
de Jong-Brink, M., Bergamin-Sassen, M. and Solis Soto, M. (2001). Multiple strategies of schistosomes to meet their requirements in the intermediate snail host. *Parasitology* **123**, S129-S141.
Demas, G. E., Adamo, S. A. and French, S. S. (2011). Neuroendocrine-immune crosstalk in vertebrates and invertebrates: implications for host defence. *Funct. Ecol.* **25**, 29.
Dezfuli, B. S., Giari, L. and Shinn, A. P. (2007). The role of rodlet cells in the inflammatory response in *Phoxinus phoxinus* brains infected with *Diplostomum*. *Fish Shellfish Immunol.* **23**, 300-304.
Dunn, A. J. (2006). Effects of cytokines and infections on brain neurochemistry. *Clin. Neurosci. Res.* **6**, 52-68.
Erickson, M. A., Dohi, K. and Banks, W. A. (2012). Neuroinflammation: a common pathway in CNS diseases as mediated at the blood-brain barrier. *Neuroimmunomodulation* **19**, 121-130.
Escobedo, G., López-Griego, L. and Morales-Montor, J. (2009). Neuroimmunoendocrine modulation in the host by helminth parasites: a novel form of host-parasite coevolution? *Neuroimmunomodulation* **16**, 78-87.
Fabre-Nys, C. (1998). Steroid control of monoamines in relation to sexual behaviour. *Rev. Reprod.* **3**, 31-41.
Falsig, J., van Beek, J., Hermann, C. and Leist, M. (2008). Molecular basis for detection of invading pathogens in the brain. *J. Neurosci. Res.* **86**, 1434-1447.
Flegr, J. (2013). Influence of latent *Toxoplasma* infection on human personality, physiology and morphology: pros and cons of the *Toxoplasma*–human model in studying the manipulation hypothesis. *J. Exp. Biol.* **216**, 127-133.
Frisch, C., Dere, E., Silva, M. A. D., Godecke, A., Schrader, J. and Huston, J. P. (2000). Superior water maze performance and increase in fear-related behavior in the endothelial nitric oxide synthase-deficient mouse together with monoamine changes in cerebellum and ventral striatum. *J. Neurosci.* **20**, 6694-6700.
Gaskell, E. A., Smith, J. E., Pinney, J. W., Westhead, D. R. and McConkey, G. A. (2009). A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. *PLoS ONE* **4**, e4801.
Gavra, T. and Libersat, F. (2011). Involvement of the opioid system in the hypokinetic state induced in cockroaches by a parasitoid wasp. *J. Comp. Physiol. A* **197**, 279-291.
Giles, N. (1987). Predation risk and reduced foraging activity in fish: experiments with parasitized and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus* L. *J. Fish Biol.* **31**, 37-44.
Guillemot, F. and Zimmer, C. (2011). From cradle to grave: the multiple roles of fibroblast growth factors in neural development. *Neuron* **71**, 574-588.
Guler, Y. and Ford, A. T. (2010). Anti-depressants make amphipods see the light. *Aquat. Toxicol.* **99**, 397-404.
Haye, P. A. and Ojeda, F. P. (1998). Metabolic and behavioral alterations in the crab *Hemigrapsus crenulatus* (Milne-Edwards 1837) induced by its acanthocephalan parasite *Profilicollis antarcticus* (Zdzitowiecki 1985). *J. Exp. Mar. Biol. Ecol.* **228**, 73.
Helluy, S. (2013). Parasite-induced alterations of sensorimotor pathways in gammarids: collateral damage of neuroinflammation? *J. Exp. Biol.* **216**, 67-77.
Helluy, S. and Holmes, J. C. (1990). Serotonin, octopamine, and the clinging behavior induced by the parasite *Polymorphus paradoxus* (Acanthocephala) in *Gammarus lacustris* (Crustacea). *Can. J. Zool.* **68**, 1214-1220.
Helluy, S. and Thomas, F. (2003). Effects of *Microphallus papillorobustus* (Platyhelminthes: Trematoda) on serotonergic immunoreactivity and neuronal architecture in the brain of *Gammarus insensibilis* (Crustacea: Amphipoda). *Proc. R. Soc. B* **270**, 563-568.
Helluy, S. and Thomas, F. (2010). Parasitic manipulation and neuroinflammation: evidence from the system *Microphallus papillorobustus* (Trematoda) – *Gammarus* (Crustacea). *Parasit. Vectors* **3**, 38.
Hendrickson, G. L. (1979). *Ornithodiplostomum ptychocheilus*: migration to the brain of the fish intermediate host, *Pimephales promelas*. *Exp. Parasitol.* **48**, 245-258.
Høeg, J. T. (1995). The biology and life cycle of the Rhizocephala (Cirripedia). *J. Mar. Biol. Assoc. U. K.* **75**, 517-550.
John, B., Ricart, B., Tait Wojno, E. D., Harris, T. H., Randall, L. M., Christian, D. A., Gregg, B., De Almeida, D. M., Weninger, W., Hammer, D. A. et al. (2011). Analysis of behavior and trafficking of dendritic cells within the brain during toxoplasmic encephalitis. *PLoS Pathog.* **7**, e1002246.
Joly, D. O. and Messier, F. (2004). The distribution of *Echinococcus granulosus* in moose: evidence for parasite-induced vulnerability to predation by wolves? *Oecologia* **140**, 586-590.
Kavaliers, M., Colwell, D. D. and Choleris, E. (1999). Parasites and behavior: an ethopharmacological analysis and biomedical implications. *Neurosci. Biobehav. Rev.* **23**, 1037-1045.
Klein, S. L. (2003). Parasite manipulation of the proximate mechanisms that mediate social behavior in vertebrates. *Physiol. Behav.* **79**, 441-449.
Lafferty, K. D. and Morris, A. K. (1996). Altered behavior of parasitized killifish increases susceptibility to predation by bird final hosts. *Ecology* **77**, 1390-1397.
Lefèvre, T., Adamo, S. A., Biron, D. G., Missé, D., Hughes, D. and Thomas, F. (2009). Invasion of the body snatchers: the diversity and evolution of manipulative strategies in host-parasite interactions. *Adv. Parasitol.* **68**, 45-83.
Libersat, F. and Gal, R. (2013). What can parasitoid wasps teach us about decision-making in insects? *J. Exp. Biol.* **216**, 47-55.
Libersat, F. and Pflueger, H. J. (2004). Monoamines and the orchestration of behavior. *Bioscience* **54**, 17-25.
Libersat, F., Delago, A. and Gal, R. (2009). Manipulation of host behavior by parasitic insects and insect parasites. *Annu. Rev. Entomol.* **54**, 189-207.
Lowry, C. A., Hollis, J. H., de Vries, A., Pan, B., Brunet, L. R., Hunt, J. R. F., Paton, J. F. R., van Kampen, E., Knight, D. M., Evans, A. K. et al. (2007). Identification of an immune-responsive mesolimbocortical serotonergic system: potential role in regulation of emotional behavior. *Neuroscience* **146**, 756-772.
Matisz, C. E., Goater, C. P. and Bray, D. (2010). Density and maturation of rodlet cells in brain tissue of fathead minnows (*Pimephales promelas*) exposed to trematode cercariae. *Int. J. Parasitol.* **40**, 307-312.
Maynard, B. J., DeMartini, L. and Wright, W. G. (1996). *Gammarus lacustris* harboring *Polymorphus paradoxus* show altered patterns of serotonin-like immunoreactivity. *J. Parasitol.* **82**, 663-666.
McConkey, G. A., Martin, H. L., Bristow, G. C. and Webster, J. P. (2013). *Toxoplasma gondii* infection and behaviour – location, location, location? *J. Exp. Biol.* **216**, 113-119.
McCusker, R. H. and Kelley, K. W. (2013). Immune–neural connections: how the immune system's response to infectious agents influences behavior. *J. Exp. Biol.* **216**, 84-98.
Milinski, M. (1984). Parasites determine a predator's optimal feeding strategy. *Behav. Ecol. Sociobiol.* **15**, 35-37.
Moore, J. (2002). *Parasites and the Behavior of Animals*. New York: Oxford University Press.
Muzzall, P. M. and Kilroy, A. L. (2007). *Tylodelphys scheuringi* (Diplostomidae) infecting the brain of the central mudminnow, *Umbra limi*, in Silver Creek, Michigan, USA. *Comp. Parasitol.* **74**, 164.
Nelson, R. J. (1995). *An Introduction to Behavioral Endocrinology*. Sunderland, MA: Sinauer Associates.
Nelson, R. J. and Trainor, B. C. (2007). Neural mechanisms of aggression. *Nat. Rev. Neurosci.* **8**, 536-546.
Øverli, Ø., Pall, M., Borg, B., Jobling, M. and Winberg, S. (2001). Effects of *Schistocephalus solidus* infection on brain monoaminergic activity in female three-spined sticklebacks *Gasterosteus aculeatus*. *Proc. R. Soc. Lond. B* **268**, 1411-1415.
Øverli, Ø., Sørensen, C., Pulman, K. G. T., Pottinger, T. G., Korzan, W. J., Summers, C. H. and Nilsson, G. E. (2007). Evolutionary background for stress-coping styles: relationships between physiological, behavioral, and cognitive traits in non-mammalian vertebrates. *Neurosci. Biobehav. Rev.* **31**, 396-412.
Perrot-Minnot, M.-J. and Cézilly, F. (2013). Investigating candidate neuromodulatory systems underlying parasitic manipulation: concepts, limitations and prospects. *J. Exp. Biol.* **216**, 134-141.
Pflüger, H. J. and Stevenson, P. A. (2005). Evolutionary aspects of octopaminergic systems with emphasis on arthropods. *Arthropod Struct. Dev.* **34**, 379.
Poulin, R. (1994). Meta-analysis of parasite-induced behavioural changes. *Anim. Behav.* **48**, 137-146.
Poulin, R. (2010). Parasite manipulation of host behavior: an update and frequently asked questions. *Adv. Stud. Behav.* **41**, 151-186.
Poulin, R. (2013). Parasite manipulation of host personality and behavioural syndromes. *J. Exp. Biol.* **216**, 18-26.
Prandovszky, E., Gaskell, E., Martin, H., Dubey, J. P., Webster, J. P. and McConkey, G. A. (2011). The neurotropic parasite *Toxoplasma gondii* increases dopamine metabolism. *PLoS ONE* **6**, e23866.
Roeder, T. (1999). Octopamine in invertebrates. *Prog. Neurobiol.* **59**, 533-561.
Roeder, T., Seifert, M., Kähler, C. and Gewecke, M. (2003). Tyramine and octopamine: antagonistic modulators of behavior and metabolism. *Arch. Insect Biochem. Physiol.* **54**, 1-13.
Rojas, J. M. and Ojeda, F. P. (2005). Altered dopamine levels induced by the parasite *Profilicollis antarcticus* on its intermediate host, the crab *Hemigrapsus crenulatus*. *Biol. Res.* **38**, 259-266.
Schmachtenberg, O. (2007). Epithelial sentinels or protozoan parasites? Studies on isolated rodlet cells on the 100th anniversary of an enigma. *Rev. Chil. Hist. Nat.* **80**, 55.
Seppälä, O., Karvonen, A. and Valtonen, E. T. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Anim. Behav.* **68**, 257-263.
Seppälä, O., Karvonen, A. and Valtonen, E. T. (2005). Impaired crypsis of fish infected with a trophically transmitted parasite. *Anim. Behav.* **70**, 895-900.
Shaw, J. C., Korzan, W. J., Carpenter, R. E., Kuris, A. M., Lafferty, K. D., Summers, C. H. and Øverli, Ø. (2009). Parasite manipulation of brain monoamines in California killifish (*Fundulus parvipinnis*) by the trematode *Euhaplorchis californiensis*. *Proc. Biol. Sci.* **276**, 1137-1146.
Shirakashi, S. and Goater, C. P. (2005). Chronology of parasite-induced alteration of fish behaviour: effects of parasite maturation and host experience. *Parasitology* **130**, 177-183.
Skallová, A., Kodym, P., Frynta, D. and Flegr, J. (2006). The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology* **133**, 525-535.

Sneddon, L. U., Braithwaite, V. A. and Gentle, M. J. (2003). Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc. R. Soc. Lond. B* **270**, 1115-1121.
Stibbs, H. H. (1985). Changes in brain concentrations of catecholamines and indoleamines in *Toxoplasma gondii* infected mice. *Ann. Trop. Med. Parasitol.* **79**, 153-157.
Summers, C. H. and Winberg, S. (2006). Interactions between the neural regulation of stress and aggression. *J. Exp. Biol.* **209**, 4581-4589.
Szidat, L. (1969). Structure development and behaviour of new strigeatoid metacercariae from subtropical fishes of South America. *J. Fish. Res. Board Can.* **26**, 753.
Tain, L., Perrot-Minnot, M.-J. and Cézilly, F. (2006). Altered host behaviour and brain serotonergic activity caused by acanthocephalans: evidence for specificity. *Proc. Biol. Sci.* **273**, 3039-3045.
Tain, L., Perrot-Minnot, M.-J. and Cézilly, F. (2007). Differential influence of *Pomphorhynchus laevis* (Acanthocephala) on brain serotonergic activity in two congeneric host species. *Biol. Lett.* **3**, 69-72.
Takahashi, A., Quadros, I. M., de Almeida, R. M. M. and Miczek, K. A. (2011). Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. *Psychopharmacology* **213**, 183-212.
Thomas, F., Adamo, S. and Moore, J. (2005). Parasitic manipulation: where are we and where should we go? *Behav. Processes* **68**, 185-199.
Thompson, S. N. and Kavaliers, M. (1994). Physiological bases for parasite-induced alterations of host behaviour. *Parasitology* **109**, S119-S138.
Vyas, A. (2013). Parasite-augmented mate choice and reduction in innate fear in rats infected by *Toxoplasma gondii*. *J. Exp. Biol.* **216**, 120-126.
Vyas, A., Kim, S.-K., Giacomini, N., Boothroyd, J. C. and Sapolsky, R. M. (2007). Behavioral changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odors. *Proc. Natl. Acad. Sci. USA* **104**, 6442-6447.
Webster, J. P. and McConkey, G. A. (2010). *Toxoplasma gondii*-altered host behaviour: clues as to mechanism of action. *Folia Parasitol.* **57**, 95-104.
Webster, J. P., Lamberton, P. H. L., Donnelly, C. A. and Torrey, E. F. (2006). Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour. *Proc. R. Soc. Lond. B* **273**, 1023-1030.
Webster, J. P., Kaushik, M., Bristow, G. C. and McConkey, G. A. (2013). *Toxoplasma gondii* infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour? *J. Exp. Biol.* **216**, 99-112.
Webster Marketon, J. I. and Glaser, R. (2008). Stress hormones and immune function. *Cell. Immunol.* **252**, 16-26.
Weiger, W. A. (1997). Serotonergic modulation of behaviour: a phylogenetic overview. *Biol. Rev. Camb. Philos. Soc.* **72**, 61-95.
Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiol. Rev.* **77**, 591-625.
Winberg, S. and Nilsson, G. E. (1993). Roles of brain monoamine neurotransmitters in agonistic behavior and stress reactions, with particular reference to fish. *Comp. Biochem. Physiol.* **106**, 597-614.
Winberg, S., Nilsson, A., Hylland, P., Söderstöm, V. and Nilsson, G. E. (1997). Serotonin as a regulator of hypothalamic-pituitary-interrenal activity in teleost fish. *Neurosci. Lett.* **230**, 113-116.