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REVIEW

Parasites: evolution's neurobiologists

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Summary

For millions of years, parasites have altered the behaviour of their hosts. Parasites can affect host behaviour by: (1) interfering with the host's normal immune–neural communication, (2) secreting substances that directly alter neuronal activity *via* non-genomic mechanisms and (3) inducing genomic- and/or proteomic-based changes in the brain of the host. Changes in host behaviour are often restricted to particular behaviours, with many other behaviours remaining unaffected. Neuroscientists can produce this degree of selectivity by targeting specific brain areas. Parasites, however, do not selectively attack discrete brain areas. Parasites typically induce a variety of effects in several parts of the brain. Parasitic manipulation of host behaviour evolved within the context of the manipulation of other host physiological systems (especially the immune system) that was required for a parasite's survival. This starting point, coupled with the fortuitous nature of evolutionary innovation and evolutionary pressures to minimize the costs of parasitic manipulation, likely contributed to the complex and indirect nature of the mechanisms involved in host behavioural control. Because parasites and neuroscientists use different tactics to control behaviour, studying the methods used by parasites can provide novel insights into how nervous systems generate and regulate behaviour. Studying how parasites influence host behaviour will also help us integrate genomic, proteomic and neurophysiological perspectives on behaviour.

Key words: parasitic manipulation, *Manduca*, wasp, psychoneuroimmunology, brain, nervous system, host behaviour.

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Introduction

Over the last 500 million years, parasites across phyla have evolved mechanisms to elude, inhibit and subvert host defence mechanisms (Schmid-Hempel, 2011). Some parasites also directly or indirectly interact with host nervous systems, leading to a change in host behaviour (Moore, 2002; Thomas et al., 2005). Such interactions occurred early in host/parasite evolution (Hughes et al., 2011). In some cases, the change in host behaviour enhances parasitic transmission (Thomas et al., 2005), suggesting that evolution has selected for parasites capable of manipulating host nervous systems, just as parasites have been selected to manipulate host immune systems. We are beginning to understand how parasites gain control of the brain of their hosts (Thomas et al., 2005; Lefèvre et al., 2009; Adamo, 2012).

Neuroscientists can also manipulate behaviour (e.g. Purves et al., 2012). Decades of research have led to a detailed, although still incomplete, understanding of the mechanisms underpinning the neural control of behaviour at the molecular, cellular and systems levels (Purves et al., 2012). Using this knowledge, neuroscientists have designed targeted interventions, such as drugs and neurosurgery, which alter behaviour in predictable ways. However, there are limits to the behaviours neuroscientists can alter, and to what extent (Purves et al., 2012). Neuroscientists may be able to learn more about the brain, and how to control it, by studying how parasites alter host behaviour. Parasites have millions of years of experience in changing brain function.

In this paper I will compare what is known about the mechanisms parasites use to alter the behaviour of their hosts with how neuroscientists typically manipulate behaviour. Such a comparison will allow us to suggest: (1) as yet unexplored mechanisms that parasites may use to change host behaviour and (2) new methods of neural modification.

How neuroscientists manipulate behaviour

Neuroscientists can control the behaviour of their study subjects using a few common methods: (1) ablation and/or stimulation of specific neurons or brain areas, (2) exposure of the brain to pharmacological agents that interfere with synaptic transmission and neuronal signalling and (3) genomic- and proteomic-based methods, such as inserting or deleting genes important for neuronal function, or altering the production of particular proteins.

How parasites manipulate behaviour

This special volume of *The Journal of Experimental Biology* details what we know about how parasites influence host behaviour in a variety of host–parasite systems. Parasites appear to use at least three broadly defined mechanisms (omitting destruction of sensory structures and/or muscles): (1) psychoneuroimmunological mechanisms, (2) neuropharmacological mechanisms and (3) genomic- and proteomic-based mechanisms. Although methods 2 and 3 are also used by neuroscientists, parasites use different approaches (see below).

Psychoneuroimmunological mechanisms

Both vertebrates and invertebrates contain bidirectional connections between the immune system and the nervous system (Adamo, 2006; Adamo, 2008a; Dantzer et al., 2008). Some of these connections appear to be phylogenetically ancient (Ottaviani and Franceschi, 1996; Adamo, 2008b). The immune system releases factors (e.g. cytokines) that alter neural function, resulting in

coordinated changes in behaviour (Dantzer, 2004). These factors induce 'sickness behaviour' (Hart, 1988), a suite of behaviours and changes in motivational state (e.g. a decreased propensity for reproduction) that is thought to help the animal recover from infection (Hart, 1988; Aubert, 1999; Dantzer, 2004). Cytokines can induce these shifts in behaviour because neurons have receptors for them in specific brain areas (Dantzer et al., 2008). By changing the amount, type or relative ratio of cytokines that the immune system releases, a parasite could produce robust and reliable changes in host behaviour (Fig. 1) (Adamo, 2012). This is not just a theoretical possibility: parasites manipulate the release of factors (e.g. cytokines) from the host's immune system as part of their defence against host attack [e.g. invertebrates (Boucias and Pendland, 1998); vertebrates (Friberg et al., 2010; Hakimi and Cannella, 2011)]. It may be a small evolutionary step from manipulating the host's immune system to prevent destruction to manipulating it to secrete modulators that lead to a change in host behaviour (Fig. 1). In addition to altering cytokine secretion, parasite excretory/secretory products induce an array of other changes in host immune function (Friberg et al., 2010). Some of these changes also lead to direct and indirect effects on neural function [e.g. changes in blood–brain barrier performance (Bentivoglio et al., 2011)]. Therefore, immune–neural–behavioural connections may be pre-adapted for parasitic manipulation (Adamo, 1997; Adamo, 2002).

Although there are no definitive examples of parasites hijacking host immune and/or neural communication systems, there are examples that suggest that this does occur (e.g. Helluy and Thomas, 2010; Helluy, 2013). Parasites that alter host behaviour by inducing the host to secrete immune-derived compounds would be expected to produce a form of sickness behaviour in their host, only more extreme or distorted in order to enhance parasitic transmission (Fig. 1). One plausible example of this is the effects of the schistosome parasite *Tricholbilharzia ocellata* on its snail host, *Lymnaea stagnalis*. The parasite secretes/excretes compounds that interfere with host immune function, allowing the survival of the parasite within the host (de Jong-Brink et al., 1997; de Jong-Brink et al., 2001). Infection also leads to a reduction in egg-laying in the snail, and the energy the host would have invested in reproduction is redirected to support parasitic growth (de Jong-Brink et al., 2001). Infection by *T. ocellata* results in the host secreting schistosomin, a molluscan cytokine-like molecule (de Jong-Brink et al., 2001). Egg-laying is suppressed in part by the effects of schistosomin on the physiology of the snail's neuroendocrine cells (caudodorsal cells) that prevents them from triggering egg-laying (de Jong-Brink, 2001). Although the function of schistosomin in the uninfected snail is unclear, it is thought to depress reproduction during unfavourable conditions (de Jong-Brink, 1995). It appears that the parasite secretes a compound that induces the host to activate this immune–neural connection, leading to benefits for the parasite (de Jong-Brink, 1995).

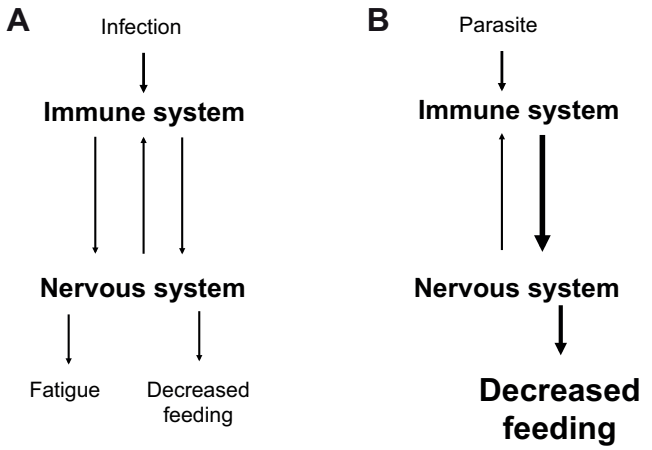

Fig. 1. Changes in the amount and/or ratio of cytokines released by the immune system can produce specific changes in host behaviour. (A) Infection of a normal animal induces the release of cytokines, resulting in adaptive changes in behaviour. (B) Some parasites alter the pattern of normal cytokine release during an infection, inducing abnormal host behaviour. Width of arrows indicates strength of connection.

In another example, the parasitic wasp *Cotesia congregata* appears to use the immune–neural connections of its host to suppress host feeding (Adamo, 2005). Decreasing host feeding increases parasite success (Adamo, 1998). During wasp larval development, the host caterpillar (*Manduca sexta*) feeds normally (Adamo et al., 1997; Miles and Booker, 2000). However, approximately 8 h prior to wasp emergence from the host, both feeding and host locomotion decline (Adamo et al., 1997). Caterpillars are eating machines; this decline in feeding is a profound change in their behaviour. In part, the decline in feeding is produced by a dramatic increase in the caterpillar's neurohormonal levels of octopamine that lasts for days (Adamo et al., 1997). By a still unknown mechanism, the wasp larvae are able to severely retard the breakdown of octopamine (Adamo, 2005). This increase in octopamine depresses feeding by desynchronizing the patterned neural output of the frontal ganglion, a part of the host's central nervous system (CNS), reducing the caterpillar's ability to swallow (Miles and Booker, 2000). Octopamine levels within the CNS are also altered (Adamo and Shoemaker, 2000). Octopamine is released during both stress and immune responses in other insects [e.g. orthopterans (Adamo, 2010)], and appears to be one aspect of immune–neural communication that is manipulated by the wasps. The wasps probably also initiate a cytokine storm on exiting the host. Host hemocyte numbers plummet as the wasps exit the host, presumably because many are used to form clots covering the holes created by the exiting wasps (Adamo, 2005). However, this response will also result in the release of cytokines, such as paralytic peptide (Skinner et al., 1991). Injections of paralytic peptides into *M. sexta* results in reduced locomotion [i.e. paralysis (Skinner et al., 1991)]. Under normal conditions, paralytic peptides are thought to help heal wounds by increasing hemocyte 'stickiness' (Yu et al., 1999) and the behavioural changes are also thought to enhance recovery (Skinner et al., 1991). An exaggerated cytokine response could lead to changes in host behaviour that benefit the wasp (Fig. 2).

Psychoneuroimmunological mechanisms have the added benefit that parasites do not need to reside within the brain to have a neurobiological effect. Vertebrates (Purves et al., 2012) and many invertebrates [e.g. insects (Nation, 2008)] have an effective blood–brain barrier that prevents most molecules from entering the brain from the blood. Substances secreted by a parasite would have to confront this barrier. However, immune-derived molecules typically have privileged routes of entry into the brain (e.g. Dantzer et al., 2008), circumventing the blood–brain barrier problem.

Neuropharmacological mechanisms

Not all changes in host behaviour resemble sickness behaviour. Some behaviours appear to be novel behaviours for the host (Moore, 2002). In these cases, the evidence suggests that at least some parasites secrete substances that act directly on the host's nervous system. Secreting substances that affect the nervous system

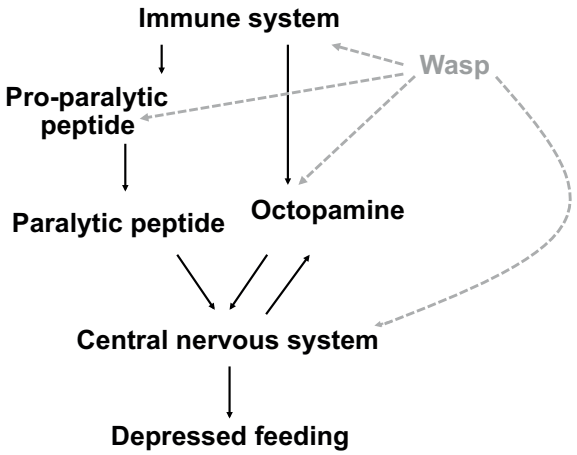

Fig. 2. A schematic of the immune–neural connections of the host, the caterpillar *Manduca sexta*, which are thought to be exploited by the parasitic wasp *Cotesia congregata*. During emergence from its host, the wasp provokes host immune responses. These include the release of the biogenic amine octopamine (released from both neural and immune sources) and probably the activation of the insect cytokine, paralytic peptide. Both octopamine and paralytic peptide act on the central nervous system of the host to depress feeding. By still unknown mechanisms, the parasitoid suppresses the normal pathways that curtail these responses, resulting in an exaggeration and prolongation of this immune-generated host behavioural response. The reduction in feeding benefits the wasp. The wasp also has other direct and/or indirect effects on the host's central nervous system. See 'Psychoneuroimmunological mechanisms' and 'Multiple mechanisms to alter single behaviours' for references.

is not unusual; many, if not all, organisms make substances capable of altering neuronal activity. For example, animals have hormones and neuromodulators that can resculpt the connections between neurons, enabling an animal to change its behaviour depending on the environment (Huber, 2005). These chemical connections within the CNS give animals behavioural plasticity, a trait necessary for survival in unpredictable environments. However, this plasticity comes at a price. It opens up the animal to manipulation by any organism (e.g. a parasite or neuroscientist) that can co-opt these chemical connections.

Parasites appear to rely heavily on neuropharmacological methods to alter host behaviour (Beckage, 1997; Pryor and Elizee, 2000; Klein, 2003; Adamo, 2012). Biogenic amines such as dopamine, octopamine and serotonin are key neuromodulators that are commonly affected by parasitism (Adamo, 2012; Helluy, 2013; Libersat and Gal, 2013; Webster et al., 2013). Altering the functioning of these critical neural systems in non-parasitized animals causes predictable changes in behaviour [e.g. serotonin in Crustacea (Weiger, 1997)]. However, there are two issues that need further study. First, how parasites manipulate host neuromodulatory systems (i.e. the identity of the parasite-produced neuropharmacological agent) remains unknown for almost all parasitic manipulators. Second, the change in the behaviour of a manipulated host is often not the same as the change in behaviour induced by selectively manipulating a single neuromodulator.

For example, the protozoan intracellular parasite *Toxoplasma gondii* forms cysts inside the neurons and glia of its intermediate host, the rodent (Gonzalez et al., 2007). Infected rodents become more attracted to cat urine, among other behavioural changes, and these changes probably lead to increased parasite transmission (Webster et al., 2006). The cysts inside the brain secrete tyrosine hydroxylase (Prandovszky et al., 2011). This enzyme is a rate-limiting step in the synthesis of at least two neuromodulators: dopamine and norepinephrine (Cooper et al., 2003). *In vitro*, neurons containing cysts release more dopamine than controls (Prandovszky et al., 2011). Therefore, in this system, we have a plausible mechanism explaining how the parasite (*T. gondii*) can manipulate a specific neurotransmitter system.

However, increasing dopamine release in non-parasitized rodents does not lead to the same changes in behaviour observed in the infected host. For example, infected rodents show a decline in some measures of anxiety (Gonzalez et al., 2007), and exhibit an increase in exploratory behaviour (Webster et al., 1994). Dopamine levels in the brains of non-parasitized rodents can be increased using the drug L-DOPA, which increases the amount of dopamine precursor available to neurons (Cooper et al., 2003), similar to the effects of increasing the amount of tyrosine hydroxylase. However, rodents treated with L-DOPA show the opposite behaviour of that exhibited by infected animals. Rodents treated with L-DOPA show an increase in anxiety (Eskow Jaunarajs et al., 2011). For example, rats given L-DOPA show less exploratory behaviour than controls (Eskow Jaunarajs et al., 2011). The effect of L-DOPA on attraction to cat urine has not been tested.

Recent research (Vyas, 2013) has shown that *T. gondii* also influences testosterone and arginine vasopressin (AVP) levels. These results suggest that multiple mechanisms are probably involved in producing the changes in host behaviour (see Webster et al., 2013).

Both intra- and extra-CNS parasites of gammarids alter the host's serotonergic neurotransmitter system (Adamo, 2012; Helluy, 2013; Perrot-Minnot and Cézilly, 2013). This effect is thought to be caused by a neuropharmacological agent secreted by the parasites (Helluy, 2013; Perrot-Minnot and Cézilly, 2013). Gammarids are crustaceans, and serotonin is a key neuromodulator mediating their escape behaviours (Weiger, 1997). Escape behaviours are altered in infected gammarids, making them more prone to predation by the definitive host (Helluy, 2013). Pharmacological manipulations of the serotonergic system of infected and uninfected gammarids suggest that the changes in serotonin are causally related to the change in behaviour (Helluy, 2013; Perrot-Minnot and Cézilly, 2013). However, non-serotonergic mechanisms appear to be involved as well, at least for trematode infections (Helluy and Thomas, 2010; Helluy, 2013).

The parasitic wasp *Ampulex compressa* uses multiple neuropharmacological agents to zombify its cockroach host (Libersat and Gal, 2013). The wasp venom, used by the wasp to change host behaviour, is a cocktail of substances (Libersat et al., 2009). Low-weight components of the venom abolish neural activity by blocking both acetylcholine and gamma-aminobutyric acid (GABA) mediated synaptic transmission. This blockade leads to transient paralysis. The venom of a second sting contains dopamine and/or a dopamine agonist. The increased dopamine levels produce excessive grooming (Libersat et al., 2009). The most dramatic change in behaviour occurs after the excessive grooming when the cockroach becomes hypokinetic (zombie-like). This change in behaviour involves alterations to the cockroach's octopaminergic system (Rosenberg et al., 2007). Therefore, manipulation of host behaviour involves multiple compounds influencing a variety of neurotransmitter systems (Gal and Libersat, 2008).

Genomic- and proteomic-based mechanisms

Over the last three decades, neuroscientists have used genomic- and proteomic-based methods to selectively manipulate the expression of genes and the production of proteins important for neural

function (e.g. Venken et al., 2011). Molecular neuroscientists use targeted techniques to: eliminate specific neuronal cell populations, inhibit neural activity (e.g. block synaptic transmission by interfering with vesicle recycling) and increase neural activity (e.g. by blocking a membrane repolarization pump) (Venken et al., 2011). Molecular neuroscientists cannot control all genes important for neural function and behaviour, and typically manipulate only a few at a time (Venken et al., 2011). For example, a strain of fruit flies (*Drosophila melanogaster*) has been engineered to lack a working tyramine β-hydroxylase gene. This prevents mutant flies from synthesising octopamine from tyramine, leaving them without one of the fly's major neuromodulators (Monastirioti et al., 1996). As would be predicted by Sombati and Hoyle's (Sombati and Hoyle, 1984) hypothesis that octopamine sets the threshold for the production of most motor behaviours, these octopamine-less flies can still fly, but they show a greatly reduced ability to initiate and maintain flight (Brembs et al., 2007).

Like molecular neuroscientists, parasites influence the gene expression of their subjects (i.e. hosts) [e.g. *T. gondii* (Hakimi and Cannella, 2011)]. However, many of the methods neuroscientists use to alter gene expression are not used by parasites (e.g. X-ray mutagenesis). Parasites have their own mechanisms. Intracellular pathogens (e.g. bacteria) secrete compounds into host cells that alter important second messenger systems (e.g. cAMP) leading to changes in both gene expression and protein production (McDonough and Rodriguez, 2012). These changes in host cellular physiology allow the parasite to manipulate host immune responses (e.g. cytokine production) to ensure its survival in the host (Hakimi and Cannella, 2011; McDonough and Rodriguez, 2012). For example, *T. gondii* secretes molecules into the host cell, including the protein kinase IKK (Hakimi and Cannella, 2011). *Toxoplasma gondii* uses kinase IKK to increase the level of phosphorylated IκB-α in the infected host cell, which leads to the activation of NF-κB (Rahman and McFadden, 2011). NF-κB is a transcription factor and can alter the expression of genes involved in immune function (Gilmore, 2006) and neural signalling (Meffert et al., 2003). Therefore, although parasites may use different mechanisms than molecular neuroscientists, they could, theoretically, alter neuronal function by manipulating the host's genome and/or proteome.

Altering host genomic and/or proteomic function may be a common method of exerting control over host behaviour (see Biron et al., 2005; Biron et al., 2006; Biron and Loxdale, 2013). For example, a virus (baculovirus, *Lymantria dispar* nucleopolyhedrovirus) causes its caterpillar host to climb to an elevated position prior to death, allowing the escaping infectious viral particles to rain down on new hosts. The virus produces this behaviour by inducing its caterpillar host to secrete a virally encoded enzyme into its bloodstream (O'Reilly et al., 1992). This enzyme inactivates one of the host's major hormones, 20-hydroxyecdysone (O'Reilly et al., 1992). Normally, ecdysteroid hormone concentration exhibits a circadian rhythm (Steel and Vafopoulou, 2006). As day approaches, changes in 20-hydroxyecdysone levels are thought to induce caterpillars such as *Lymnatria dispar* to leave their feeding positions in the tree canopy and climb down to hide in bark or soil until dark (Hoover et al., 2011). By inactivating the hormone, the infected insect lacks the hormonal signal to stop feeding, climb down from the leaves and hide (Hoover et al., 2011). Therefore, by making a protein encoded in the virus's genetic code, the host inactivates one of its own hormones, altering its behaviour in ways that benefits the parasite (Fig. 3) (Hoover et al., 2011).

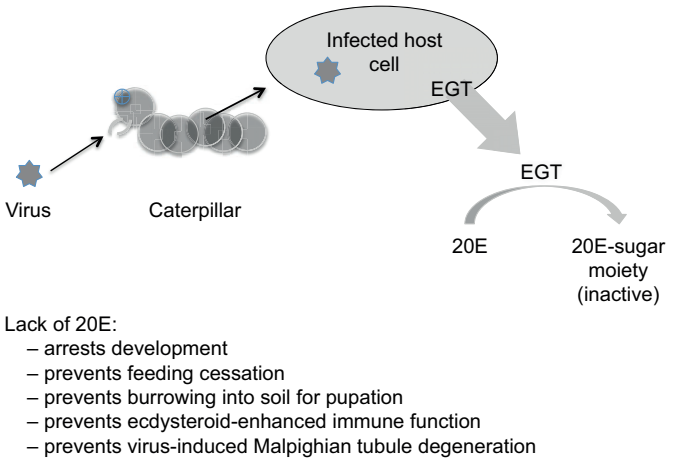

Fig. 3. Schematic of parasitic control *via* a genomic mechanism. A virus (e.g. *Lymantria dispar* nucleopolyhedrovirus) enters the caterpillar *per os*. The viral DNA encodes a protein, that is made by the host, secreted and then inactivates a major host hormone (20E). EGT, ecdysteroid uridine 5′ diphosphate (UDP)-glucosyltransferase (viral gene product); 20E, 20-hydroxyecdysone. For references, see O'Reilly (O'Reilly, 1995) and Spindler et al. (Spindler et al., 2009).

As mentioned earlier, we still do not know the details of how gammarid parasites alter serotonergic transmission in their host. However, a proteomic study showed that the presence of the trematode *Microphallus papillorobustus* in the brain of its host, the gammarid *Gammarus insensibilis*, led to an increase in the amounts of the enzyme aromatic-L-amino acid decarboxylase in its host (Ponton et al., 2006). This enzyme is part of the synthetic pathway for biogenic amine neurotransmitters (Cooper et al., 2003). Such an increase could raise the levels of all biogenic amines.

Parasites show little neuroanatomical specificity in their attack

Neuroscientists rely heavily on their knowledge of neuroanatomy to change specific behaviours in their subjects. Neuroscientists have shown that precisely defined neural circuits subserve specific behaviours and/or behavioural states in animals (Purves et al., 2012). Decades of ablation and stimulation studies of targeted brain regions in both vertebrates and invertebrates demonstrate that specific behaviours can be manipulated by controlling neural activity in prescribed areas of the brain (Carew, 2000). For example, prey-catching behaviour in toads can be controlled by stimulating or inhibiting specific groups of cells in the toad brain (Carew, 2000). By targeting specific neuroanatomical regions, parasites could exert control over particular host behaviours without disrupting other behaviours. This would allow the host to retain behaviours necessary for both host and parasite survival.

Although parasites have impressive abilities for infesting specific organs and subsections of organs within a host (Roberts and Janovy, 1996), their ability to selectively attack specific brain regions is modest (Adamo, 2012). Parasites do not limit their attack to only those brain areas controlling specific behaviours (see Adamo, 2012). For example, *T. gondi* intracellular cysts are found in most areas of the brain, not just in those areas thought to be involved in the response to cat odour (Berenreiterová et al., 2011). Berenreiterová et al. (Berenreiterová et al., 2011) hypothesise that if parasites infest wide regions of the brain, the odds are good that at least one parasite will hit neural areas crucial for the targeted host behaviours.

Like *T. gondii*, the rabies virus is widely disseminated in the host's brain (Laothamatas et al., 2008). Nevertheless, the occurrence of the pathogen is not entirely random. Midline CNS structures such as the thalamus, brain stem and basal ganglia are preferentially infected in both dogs and humans (Thanomsridetchai et al., 2011).

The trematode *M. papillorobustus* tends to attack the protocerebrum, a particular part of its gammarid (*G. insensibilis*) host's CNS (Helluy and Thomas, 2003). However, it can occur in different regions of this brain area (Helluy and Thomas, 2003). Similarly, the entire serotonergic system is altered in infected *G. insensibilis* (Helluy and Thomas, 2003).

Therefore, parasites do not migrate exclusively to specific brain areas, but they are not random in their attacks either. They show some specificity for particular brain regions, but they also infest those areas not currently considered to be involved in the behaviours changed by the parasite. This lack of specificity may be due, in part, to the evolution of parasitic manipulators from parasites that merely reside in the brain. In that case, unless a lack of specificity resulted in the expression of behaviours that decreased parasite transmission, there would be little selection pressure for precise neuroanatomical specificity. And, when closely examined, hosts typically do show some effects of parasitism on non-target behaviours (Cézilly and Perrot-Minnot, 2005), as might be expected when multiple brain areas are affected. However, these changes in host behaviour may have few fitness consequences for the parasite.

Lessons from neuroscience for researchers studying parasite-induced changes in behaviour

Some methods that neuroscientists use to alter gene expression may also be used by parasitic manipulators. For example, neuroscientists use RNAi to control gene expression (Venken et al., 2011). Some parasites are also thought to use small, pathogen-encoded pieces of RNA (e.g. microRNAs) to alter host gene transcription (Hakimi and Cannella, 2011). This possibility, however, has not been examined within the context of parasite behavioural manipulation.

Neuroscientists have shown that epigenetic mechanisms, such as altering methylation patterns on DNA, can have predictable effects on behaviour (Weaver, 2011). Parasitologists have shown that parasites are capable of inducing epigenetic changes in their host (Cossart, 2011). Therefore, it seems plausible that parasitic manipulators could use epigenetic mechanisms to alter host gene transcription, and hence influence host behaviour (Poulin, 2010). However, there are no studies as yet that address this possibility.

Borrowing novel techniques from neuroscience will also help advance the field. For example, *in vivo* optogenetic techniques can target specific neurons (e.g. Adamantidis et al., 2011), allowing researchers to alter the activity of infected neurons to test how these changes contribute to the change in host behaviour.

Lessons from parasitic manipulators

Identification of novel mechanisms of behavioural regulation

Many genes are involved in regulating brain function, as neuroscientists have long known. Genes, such as those involved in controlling the expression of ion channels, are crucial for neuronal function (Venken et al., 2011). However, recent work in proteomics and genomics suggests that behaviour can be selectively altered by targeting genes not traditionally thought of as playing a direct role in neuronal signalling. For example, a gene for a desaturase enzyme in *Drosophila* affects its ability to perceive pheromone (Bousquet et al., 2012). The gene is expressed in both neural and non-neural tissue and is thought to be involved in lipid metabolism (Bousquet et al., 2012). It is unclear how changes in the biochemical pathway regulated by this enzyme alter the ability of flies to perceive pheromone (Bousquet et al., 2012). It is not a biochemical pathway that is typically thought of as being important for neurotransmission. Therefore, a change in the expression of a gene not directly related to neural function can cause a specific change in behaviour. Work on parasitic manipulators suggests that such indirect mechanisms may be more central to behavioural control than has been recognised. Proteomic and molecular studies on parasitic manipulators suggest that parasites typically target molecules not traditionally considered crucial for neural transmission (e.g. Hayakawa et al., 2000; Biron et al., 2005; Biron et al., 2006; Ponton et al., 2006; Hoover et al., 2011). For example, proteins involved in neural development appear to play a role in altering host behaviour in some systems (Biron et al., 2006; Ponton et al., 2006), even though neural development is no longer occurring in the adult hosts. Molecules normally associated with neural development may continue to influence neural functioning in the adult. Although this has been known for some developmental factors [e.g. brain-derived neurotrophic factor (Purves et al., 2012)], work on parasites suggests that it may also be the case for many more.

Interestingly, several major pharmaceutical companies (e.g. Novartis, GlaxoSmithKline and AstroZeneca) have recently announced that they will close their neuroscience research divisions (Abbott, 2011). This decision was made because designing new neuropharmacological agents based on neurotransmitters and other molecules known to be crucial for neural communication has not produced drugs capable of targeting specific behaviours and/or mental disorders (Abbott, 2011). Instead, these companies are investing in genomic studies to find new targets (Abbott, 2011). These companies may also profit from studying how parasites alter host behaviour. Some of the molecules altered by parasitic manipulators may also supply new non-traditional drug targets.

Multiple mechanisms to alter single behaviours

Neuroscientists typically manipulate a single neural component to alter a subject's behaviour (e.g. blocking a single channel). However, parasites appear to use multiple mechanisms to change the behaviour of their host (e.g. Eberhard, 2010). For example, as discussed previously, schistosomes shut down host egg-laying by inducing the release of schistosomin, exploiting an immune–neural connection. However, schistosomes also downregulate the expression of the gene for caudodorsal cell hormone, a hormone crucial for egg-laying (de Jong Brink et al., 2001). The parasitic wasp *C. congregata* not only alters immune–neural connections in its host, but also induces the accumulation of neuropeptides in the host's cerebral neurosecretory system (Zitnan et al., 1995) (Fig. 2).

Why do parasites use multiple methods to alter host behaviour when neuroscientists can evoke similar effects using just one? For example, the rabies virus is thought to induce increased aggressive behaviour in its host by directly invading a variety of brain areas and by changing the levels of a number of cytokines (Laothamatas et al., 2008). Neurobiologists can increase aggression in many mammals by raising the amount of a single cytokine (IL-1) in a single brain area [i.e. the periaqueductal grey area (Zalcman and Siegel, 2006)].

The lack of neuroanatomical specificity may necessitate multiple mechanisms. Invading multiple brain areas is likely to lead to frequent parasitic effects on non-target behaviours. Multiple mechanisms may help focus the effects on particular neural circuits,

just as drugs are sometimes given to patients to counter-act the side effects of neuroleptics (e.g. see Weinberger et al., 2011). Moreover, neural systems have homeostatic mechanisms that help the brain retain normal function even if there is increased synthesis of a particular neurotransmitter (e.g. see Cooper et al., 2003). For example, many neural functions are controlled by transcription factors (Benito et al., 2011). Because of the overlapping effects between different transcription factors, even if one is inhibited, other transcription factors can compensate to maintain normal neuronal function (Benito et al., 2011). Such redundancy makes it difficult to induce specific behavioural changes, and may explain why parasites need multiple mechanisms. Parasites may also require multiple mechanisms because of the long-term behavioural changes that they need to induce in their hosts. Host behavioural changes are often permanent (see Moore, 2002), whereas neuropharmacological effects induced by neuroscientists tend to be temporary (Cooper et al., 2003).

Costs of behavioural manipulation may influence the mechanisms parasites use

When a change in host behaviour enhances parasite fitness, selection should favour parasites that can induce such a change. However, altering host behaviour is likely to have physiological costs for a parasite (Poulin, 2010), and this could limit its evolution if the costs are high. There is evidence that some parasites adopt strategies that allow them to lower the cost of behavioural manipulation (Poulin, 2010), suggesting that such costs can reduce fitness (Maure et al., 2011).

Parasitic manipulation may be most common in host–parasite systems that allow for relatively inexpensive control of host behaviour. For example, if the compounds that parasites use to alter host behaviour are also required for survival in the host, then host behavioural manipulation could be relatively cheap (e.g. manipulating immune–neural connections). However, parasites that alter host behaviour by secreting molecules identical to host neuromodulators are likely to be rare because of the energetic costs of this strategy. Host neuromodulators are usually rapidly metabolized by dedicated biochemical pathways (see Cooper et al., 2003); therefore, parasites would need to constantly synthesise host neuromodulators to maintain them at elevated levels [for a discussion, see Adamo (Adamo, 1997; Adamo, 2002)]. Parasites typically use less energetically expensive methods to alter host neurochemistry. For example, in infected gammarids, a variety of phylogenetically diverse parasites are thought to induce the host to increase serotonin production within its CNS, reducing the direct cost to the parasite (Lefèvre et al., 2009; Adamo, 2012; Helluy, 2013; Perrot-Minnot and Cézilly, 2013). The parasitic wasp *C. congregata* reduces the breakdown of the neuromodulator octopamine, increasing the time levels are elevated in the host, without the parasite having to continuously secrete large amounts of this biogenic amine (Adamo, 2005). Another way around the cost problem is to secrete a substance that is not easily degraded and/or has long-lasting effects [e.g. venoms (Weisel-Eichler and Libersat, 2004; Libersat and Gal, 2013)]. Given that parasitism is often long lasting, this could be a crucial factor in whether parasitic manipulation can evolve. For example, the existence of venom in the hymenopterans may partly explain why parasitic manipulation of behaviour is more common in this group of parasitic insects (e.g. wasps) than in the dipteran (e.g. flies) parasitoids. Dipteran parasitoids do not use venom (Feener and Brown, 1997). Altering host gene expression is another method that could require an initial modest cost (e.g. a secretory product), but could have long-lasting effects on host behaviour. Such 'cheaper' mechanisms are likely to be favoured by evolution.

The life history of a parasitic species may also limit the evolution of 'costly' parasitic manipulation. Parasites that infect hosts with multiple, non-genetically identical offspring (e.g. many parasitic wasps) may have minimal selection pressure towards evolving an energetically expensive method of host manipulation. If manipulation is costly, selection is likely to favour individuals that under-invest (i.e. cheat) in the mechanisms needed for behavioural manipulation. Cheating individuals would then have more energy to invest in their own reproduction, shifting the cost of manipulation to their siblings and half-siblings. Interestingly, in the parasitic wasps, much of the cost of manipulating the host's physiology is borne by the mother wasp. She produces polydnavirus particles and venom, and these are crucial for successful parasitism (Godfray, 1994). This maternal investment probably reduces the cost of manipulation for the offspring, and this probably reduces the energetic advantage that would be accrued by cheating. Such cheating could result in declines in maternal reproductive success.

Systems in which there is a solitary parasite (e.g. some parasitic wasps), or in which the infesting parasites are all clones of one another, may have fewer obstacles to the evolution of more costly mechanisms of host control. Nevertheless, even in these systems, parasites will be under selection to minimize costs to maximize reproduction. This evolutionary pressure is not faced by neuroscientists when they are devising methods of neural control. This pressure may help explain some of the indirect methods many parasites use to control host behaviour. Selection will not necessarily favour the most direct methods of behavioural manipulation, but rather the ones that can achieve the desired result at the lowest cost.

Conclusions

Recent papers (e.g. Adamo, 2012), including those in this special issue, review what is known about how parasites manipulate host behaviour. This information shows that parasites tend to use complex, multipronged methods to alter the behaviour of their hosts. Parasitic manipulation evolved within the context of the manipulation of other host physiological systems necessary for a parasite's survival. This starting point, coupled with the fortuitous nature of evolutionary innovation and evolutionary pressures to minimize the costs of parasitic manipulation, likely contributed to the complex and indirect nature of the mechanisms involved in host behavioural control. This is in contrast with the direct methods used by neuroscientists. Neuroscientists develop methods of behavioural control based on an understanding of neural circuits and their regulation. Given the different approaches of parasites and neuroscientists, continued study of how parasites alter host behaviour is likely to uncover novel mechanisms of behavioural control.

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