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# Parasite-Induced Behavioral Change: Mechanisms

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## Abstract

Animal behavior and parasitism are more tightly linked than commonly thought. One of the most astonishing phenomena in host–parasite antagonistic interactions is ‘host manipulation,’ that is, the ability of a parasite to alter the behavior of its host in ways that appear to increase parasite fitness at the expense of host fitness. The mechanisms by which a parasite hijacks the behavior of its host have been explored using ethopharmacological and immunocytochemical approaches or carrying out a large-scale proteomic study on manipulated host’s brain. These few mechanistic studies have confirmed both the complexity of host manipulation by parasites and the importance of understanding the molecular cross-talk between a host and its manipulative parasite.

- (1) From phenotypic behavioral changes to altered gene expression.
- (2) Multidimensionality and mechanisms of parasite manipulation.
- (3) Investigating host’s neuromodulatory pathways.
- (4) Screening the host’s proteome and transcriptome.
- (5) The search for the ‘molecules of manipulation’ in excretory-secretory products of manipulative parasites.

## Keywords

Crustaceans; Ethopharmacology; Insects; Neuromodulator; Parasite; Proteome; Rodents; Serotonin; Transcriptome

Parasites can manipulate the behavior of their hosts to their own benefit – this is what evolutionary parasitology studies tell us. But let us go a step further and take up the challenge raised by these manipulative parasites messing with the brains of their hosts and giving our own brains a serious puzzle. How can a so-called ‘simple’ (not ‘regressed’) parasite hijack the behavior of its host, which in some instances might be a so-called ‘higher’ vertebrate? Is there anything like a ‘manipulative molecule’ secreted by the parasite to directly target its host’s CNS and specifically modulate the behaviors affecting transmission success? Or does manipulation come as a fortuitous side-effect of the infection on the host immune system and metabolism?

Despite the growing number of studies reporting on behavioral manipulation by parasites, the proximate mechanisms underlying this phenomenon have been investigated in only a few of them (**Table 1**), and no mechanism has been completely elucidated. This article aims at reviewing these few cases, where the mechanisms of parasite manipulation have been investigated. However, we use here a broader approach, looking for a causal connection between altered host behavior and the modulation of gene expression in both the host and the parasite. The analysis is focused on the mechanisms underlying changes in behavior that increase parasite transmission success (strictly speaking, parasite-induced behavioral manipulation). Mechanisms associated with disease-related behavioral disorders, such as immune-generated alteration of the CNS, will not be addressed here.

The phenomenon of parasite manipulation can be fully understood only if the demonstration of a fitness gain for the parasite (ultimate cause) is coupled to the identification of the mechanisms underlying the observed behavioral changes (proximate causes). Understanding proximate causes of manipulation will contribute to our evolutionary analysis in two ways: (1) it will help evaluate the costs a parasite pays to invest in manipulation and whether these costs are shared with investment in parasite survival (i.e., defense against the immune system) and (2) it will reveal how complex and specific the manipulation process is. These two criteria are currently acknowledged as important in assessing the adaptive significance of manipulation.

The mechanisms involved in parasite manipulation have been explored since the pioneering work on rodents infected with *Toxoplasma gondii* and on the amphipod *Gammarus lacustris* infected with the acanthocephalan bird parasite *Polymorphus paradoxus*. Since then, several studies have attempted to identify the changes in host neurophysiology or gene expression associated with parasite manipulation (**Table 1**; Tain *et al.*, 2006). The expected complexity of the interactive network connecting a host and its manipulative parasite comes from the modulatory connections between the neuronal, hormonal, and immune systems of the host. The investigation of proximate mechanisms therefore relies on an integrative approach combining behavioral ecology, neurophysiology, pharmacology, molecular biology, and biochemistry.

**Table 1** Review of studies attempting to identify the changes in host's neurophysiology or proteome associated with parasite manipulation in invertebrates

Host	Parasite	Manipulated behavior	Method of investigation	References
Grasshopper, <i>Meconema thalassinum</i> (Orthopteran insect)	Hairworm, <i>Spinichordodes tellinii</i> (Nematomorph)	Seeking water and jumping into it	Brain proteome (host) and parasite proteome	Biron <i>et al.</i> (2005)
Cricket, <i>Nemobius sylvestris</i> (Orthopteran insect)	Hairworm, <i>Paragordius tricuspidatus</i> (Nematomorph)	Seeking water and jumping into it	Brain proteome (host) and parasite proteome	Biron <i>et al.</i> (2006)
Mosquitoe <i>Anopheles gambiae</i> (Dipteran insect)	<i>Plasmodium berghei</i> (Protozoa: Apicomplexa)	Increased biting rate	Brain proteome (host)	Lefevre <i>et al.</i> (2007a)
Tsetse fly <i>Glossina palpalis gambiense</i> (Dipteran insect)	<i>Trypanosoma brucei</i> (Protozoa: Sarcosomastigophora)	Increased probing, Extended engorging duration	Brain proteome (host)	Lefèvre <i>et al.</i> (2007b)
Amphipod <i>Gammarus insensibilis</i> (Crustacea)	Flatworm <i>Microrhalius papillorobustus</i> (Platyhelminthes: Trematoda)	Negative geotaxis: water surface	Brain proteome (host)	Ponton <i>et al.</i> (2006)
Amphipod <i>Gammarus pulex</i> (Crustacea)	Thorny-headed worm <i>Polymorphus minutus</i> (Acanthocephala: Polymorphidae)	Positive phototactism	Immunocytochemistry on the brain (5-HT)	Helluy and Thomas (2003) Ponton <i>et al.</i> (2006)
Amphipod <i>Gammarus pulex</i> , <i>Gammarus roeselii</i> (Crustacea)	Thorny-headed worm <i>Pomphorhynchus laevis</i> , <i>Pomphorhynchus tereticollis</i> (Acanthocephala: Pomphorhynchidae)	Negative geotaxis: water surface Positive phototactism, Increased drifting behavior and activity	Brain proteome (host) Ethopharmacology (phototactism), Immunocytochemistry on the brain (5-HT)	Tain <i>et al.</i> (2006)
Amphipod <i>Gammarus lacustris</i> (Crustacea)	<i>Polymorphus paradoxus</i> (Acanthocephala: Polymorphidae)	Attraction to chemical cues from fish predator Clinging behavior	Ethopharmacology (clinging), Immunocytochemistry on the nerve cord (5-HT)	Helluy and Holmes (1990) Mavnard <i>et al.</i> (1996)

Source: a. NO is a neuromodulator involved in memory, neuronal development as well as immune defense.

b. ATP can also play a role as neuromodulator.

c. Inducing apoptosis in the brain can alter the chemical signals in the brain (inflammatory immune responses), as reported in vertebrates infected with certain viruses or protozoans.

## From Phenotypic Behavioral Changes to Altered Gene Expression

Changes in host behavior following infection are not necessarily profitable to the parasite. They may actually benefit the host through compensating for the effect of infection or getting rid of the parasite. Alternatively, they can be pathological side-effects, with no benefits for the host or for the parasite. When beneficial to the parasite, changes in host behavior can result from a combination of direct and indirect effects of a parasite on its host's CNS. The most likely indirect effect relies on the connection between the neuronal and immune systems: the host's immunological response to infection can be involved in changing the host's behavior into a behavior that favors parasitic transmission. Therefore, the methods used to investigate mechanisms of parasite-induced behavioral changes must not only identify the biochemical or physiological changes in manipulated hosts, but also demonstrate that these changes are indeed the proximate cause of behavioral manipulation.

More precisely, we have to identify the following

- (1) The functional connection between an altered behavior and the corresponding genes expressed in the host.
- (2) The parasite's biochemical signal (in the excretory/secretory (E/S) parasite products) targeting host's genes, whether it corresponds to 'manipulative molecules,' or molecules with a broader spectrum, including physiological targets.
- (3) The causal link between some of those genes and the direct target of the parasite's E/S products.

To that end, several complementary approaches are possible

- The exploration of specific neurophysiological pathways by means of ethopharmacology and techniques used on candidate proteins (immunohistochemistry or immunocytochemistry, HPLC-ED, etc.). This approach can establish a functional link between a neuromodulatory pathway and the observed altered behavior, without establishing how the parasite directly hijacks the neurophysiology of its host.
- The differential screening of the host proteome or transcriptome between infected-manipulated individuals and non-manipulated ones (uninfected and infected), to reveal proteins or mRNA associated with a manipulated phenotype (as the cause or the consequence of altered behavior and physiology).
- The proteomic analysis of parasite's E/S products followed by the identification of the biological fractions modulating host's behavior. Proteomic tools applied to the analysis of E/S products screen for molecules released by a manipulative parasite that could trigger the observed phenotypic changes.

The first two approaches must compare infected-manipulated individuals with nonmanipulated ones (infected by a nonmanipulative stage of parasite and uninfected), to specifically identify neurophysiological or biochemical changes associated with manipulation. Still, will these pathways or molecules in the host's repertoire be the direct target of parasite? The third approach is thus necessary to identify the initial molecular dialog setting up behavioral manipulation.

Several inferences emerge from the astonishing fact that parasites increase their own transmission success by taking control of their host's behavior. (1) The molecular cross-talk between a host and a parasite that results in fine-tuned phenotypic alterations is probably complex and intimate. (2) A parasite manipulating the behavioral flexibility of its host so that it performs the appropriate behavior likely uses either molecular mimicry (biochemical evolutionary convergence) or highly conserved molecules (phylogenetic inertia). The parasite may thereby control some of its host modulatory pathways by usurping signaling processes. (3) Changes in host behavior are often a mix of direct and indirect effects, and it may prove difficult to differentiate between the two. Investigations of proximate mechanisms involved in parasite manipulation must keep these inferences in mind.

## Multidimensionality and Mechanisms of Parasite Manipulation

The capacity of a parasite to manipulate several behavioral and physiological traits together has been largely ignored in most empirical studies so far, although a review of studies on the same host-parasite systems shows that manipulative parasites generally modify more than a single dimension in the phenotype of their hosts. For instance, the acanthocephalan fish parasite *Pomphorhynchus laevis* reverses the photophobic behavior of its host *Gammarus pulex* and its antipredatory behavior in reaction to olfactory cues, and increases its activity and its drifting behavior. Several physiological changes have been reported as well in *G. pulex* infected with *P. laevis*, such as increased hemolymph protein titers (in particular haemocyanin), reduced O<sub>2</sub> consumption, increased glycogen content, fecundity reduction, and immunosuppression. In wild rats infected with the protozoan *T. gondii*, changes in activity and in motivational level in various contexts have been reported. *T. gondii*-infected rats were found to be significantly less neophobic toward food-related novel stimuli. In outdoor captive environment, they were more likely to be trapped than their uninfected conspecifics, and their propensity to approach a mildly fear-inducing object was higher than that of uninfected rats (reduced neophobia). Alteration of innate behavior (such as neophobia) extends to the reversal of antipredatory behavior from a strong aversion to a preference for cat-treated areas in infected rats. This 'fatal attraction' is expected to increase the chances of transmission of *T. gondii* to its feline definitive host. Such multidimensionality of manipulation makes sense from an ecological and evolutionary point of view: having the 'vehicle' host reaching the right place at the right time (through being predated by, or stinging, or biting the next host species in the cycle) probably involves several behaviors related to environmental sensing and microhabitat choice. In parasites with a direct life cycle, transmission by contact or wounding can be increased by modulating a number of social behaviors, such as aggression and exploration. Several cue-oriented behaviors are generally altered in infected invertebrates (among phototaxis,

chemotaxis, rheotaxis or wind-evoked behavior, geotaxis, etc.) that together contribute to increased transmission success of the manipulative parasite.

Are these multiple dimensions of a manipulated phenotype functionally independent? Or do the proximate mechanisms of manipulation have 'pleiotropic effects'? The best argument supporting the hypothesis of 'pleiotropic effects' lies in the functional connection between host's neuronal, immunological, and endocrinal/metabolic systems, be the host an invertebrate or a vertebrate. Because the very first conditions for a parasite to develop are to successfully establish in a host and exploit its energy reserves, some mechanisms must exist that allow the parasite to interact with its host's physiology, especially the host's immunity. As pointed out by several authors, the evolutionary transition leading to parasite manipulation may simply consist in an extension of the effect of the parasite on the immune system of its host to its neuronal system. Targeting diverse and flexible neuromodulatory pathways to induce adaptive behavioral change in its host would thereby be a small evolutionary step. The understanding of proximate mechanisms of parasite manipulation allows us to test this evolutionary and functional scenario.

## Investigating Host's Neuromodulatory Pathways

Biogenic amines (serotonin, dopamine, octopamine among others) and other chemical signals such as neuropeptides or the gas nitric oxide (NO) play a neuromodulatory role in numerous sensory, motor, and endocrine functions, in both invertebrates and vertebrates. They modulate the behavioral or physiological responses of an organism to external information, according to its internal status. By 'manipulating' these neuromodulatory pathways in its host's CNS, a parasite could adjust its host's behavioral response to reflect the parasite's own interest. The E/S products of the parasite would thus be akin to the venom of several predators or parasitoid wasps manipulating the monoaminergic system of their hosts to improve prey handling and use.

Several studies have shown a major role of biogenic amines and neuropeptides in the physiological and behavioral alterations induced by parasites (Table 2). The 'candidate neuromodulatory pathway' approach to parasite manipulation targets simple tropisms or cue-oriented behaviors such as phototaxis, geotaxis, chemotaxis, thermal gradient sensitivity (in biting or sucking vectors of warm blood animals), and reflectance (Table 2). In vertebrates, several viruses and protists increase their hosts' exploratory behavior or aggression, two behaviors suspected to enhance parasite transmission either by predation or by conspecific wounding/contact respectively. These behavioral effects have been related to changes in concentrations or receptor binding of amines (dopamine, serotonin) or opioids.

However, few studies have combined ethopharmacological analysis to biochemical techniques (immunohisto- or cytochemistry, western blot, and ELISA) to establish or invalidate the involvement of a neuromodulator in parasite manipulation of behavior. One pioneer ethopharmacological study investigated the role of several neuromodulators in the behavioral alterations induced by *P. paradoxus* in its intermediate host *G. lacustris*. Uninfected individuals injected with serotonin responded to mechanical stimulation by skimming to the water surface until clinging to floating material and exhibited positive phototaxis, two behavioral mimics of amphipods infected with this parasite of dabbling ducks. Immunocytochemistry on the nerve cord of amphipods infected with *P. paradoxus* revealed an increase in the number of varicosities exhibiting serotonin-like immunoreactivity in the third thoracic ganglion. Serotonin was altered either in the amount or in the number of local storage and release sites along neural fibers in *P. paradoxus*-infected amphipods, but not in *G. lacustris* infected with *Polymorphus marilis*, a parasite of diving ducks inducing positive phototactism but no escape response.

Exogenously supplied serotonin can mimic the effect of parasitism in other amphipod-acanthocephalan systems: *G. pulex*–*P. laevis* and *Pomphorhynchus tereticollis*. Injection of serotonin in uninfected *G. pulex* reversed their reaction to light, mimicking the positive phototactism of gammarids infected with these two fish parasites. The serotonergic activity in the brains of infected-manipulated gammarids was significantly increased, compared to that of four controls: uninfected *G. pulex*, *G. pulex* infected with *P. tereticollis* but not manipulated, *G. pulex* infected with the bird acanthocephalan *Polymorphus minutus* (not altering phototaxis), and a nonmanipulated sympatric amphipod species *Gammarus roeseli*, infected with *P. laevis* (Fig. 1).

In *Gammarus insensibilis* infected by the cerebral trematode *Microphallus papillorobustus*, immunocytochemistry on brain has revealed the degeneration of discrete sets of serotonergic neurons: immunoreactivity to serotonin (5-hydroxytryptamine or 5-HT) was decreased in the optic neuropils but increased in the olfactory lobes. This imbalance in brain serotonergic activity is suspected to contribute to the behavioral alterations reported in this brackishwater amphipod species, in particular, positive geotactism and attraction to light. In vertebrates, several viral and protozoan parasites infecting the CNS of their rodent hosts alter neurochemical pathways in the brain. In the brains of infected mice and rats for instance, rabies virus decreases 5-HT and GABA neurotransmission, and *T. gondii* increases the concentration of dopamine and decreases the concentration of norepinephrine. These changes in neuromodulatory pathways may be linked to elevated aggression exhibited by infected rodents (and exploratory and fearless behavior in the case of *T. gondii* infected rats). These behavioral alterations presumably enhance the transmission of rabies virus by increased conspecific biting and of *T. gondii* by increased predation rate.

Although the exploration of these neurophysiological changes can provide evidence that a neuromodulator plays a key role in one or few behavioral dimensions of parasite manipulation, it also has several limitations. First, the neuromodulatory and signaling network is complex: several neuropeptides or amines may act together to modulate a given behavior, while a single neuromodulator may regulate several behaviors. If this may fit well with the multiple dimensions of parasite-induced alteration on host's phenotype, it makes the full understanding of the underlying neurophysiological process difficult. Second, showing a change in brain CNS does not establish a causal connection with the manipulative process.

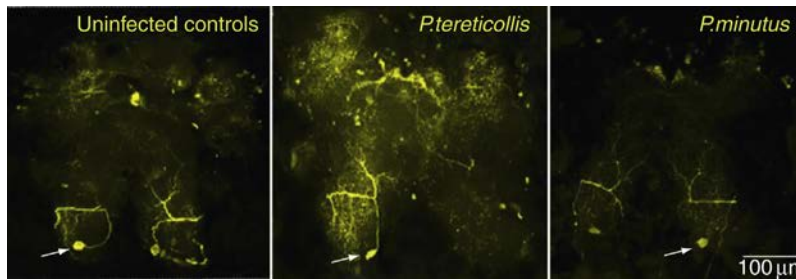
**Table 2** Review of studies suggesting the involvement of certain neuropeptides or biogenic amines in parasite-induced alteration of invertebrate hosts' behavior (used as intermediate hosts by trophically transmitted parasites or as food store and shelter by parasitoid larvae)

Host species	Parasite species	Technics	Results	References
Amphipod <i>Gammarus lacustris</i>	<i>Polymorphus paradoxus</i> (Acanthocephalan)	<ul style="list-style-type: none"> <li>– Injection: serotonin, dopamine, octopamine, norepinephrine</li> <li>– Immunocytochemistry (anti-5-HT) on nerve cord</li> </ul>	<p>Only 5-HT injection mimics clinging behavior</p> <ul style="list-style-type: none"> <li>– Increased 5-HT immunoreactivity in the third thoracic ganglion (increase in varicosities)</li> </ul>	<p>Helluy and Holmes (1990)</p> <p>Maynard <i>et al.</i> (1996)</p>
Amphipod <i>Gammarus insensibilis</i>	<i>Microphallus papillorobustus</i> (Trematode)	Immunocytochemistry (anti-5-HT) on the brain	Serotonergic activity depressed in specific areas of the brain	Helluy and Thomas (2003)
Amphipod <i>Gammarus pulex</i>	<i>P. laevis</i> , <i>P. tereticoelis</i> (Acanthocephalan)	<ul style="list-style-type: none"> <li>– Immunocytochemistry (anti-5-HT)</li> <li>– Injection of serotonin</li> </ul>	<ul style="list-style-type: none"> <li>– Increase in brain 5-HT immunoreactivity in infected amphipods (correlates to their degree of manipulation of phototaxis)</li> <li>– Injection of 5-HT to uninfected animals mimics positive phototaxis of infected ones</li> </ul>	Tain <i>et al.</i> (2006)
Crab <i>Macrophthalmus hirtipes</i>	<i>Maritrema</i> (Trematode)	HPLC-ED on brain extracts	Increase in 5-HT content in the brain of crabs coinfecting with both parasite	Poulin <i>et al.</i> (2003)
Crab <i>Hemigrapsus crenulatus</i>	<i>Proflicollis</i> (Acanthocephalan)	HPLC-ED on hemolymph extracts	Increase in hemolymph dopamine content, but not serotonin, in infected crabs	Rojas and Ojeda (2005)
Moth <i>Manduca sexta</i>	<i>Cotesia congregata</i> (Hymenoptera Braconidae)	<ul style="list-style-type: none"> <li>– HPLC-ED on hemolymph extracts</li> <li>– Injection of octopamine or blood from postemergence parasitized larvae</li> </ul>	<ul style="list-style-type: none"> <li>– Increased octopamine content of the brain, thoracic ganglia, and abdominal ganglia</li> <li>– Injection mimics the decreased peristaltic activity in the foregut (related to decreased feeding)</li> </ul>	Adamo and Shoemaker (2000)
<i>Periplaneta americana</i> (and other cockroaches) <i>Ampulex compressa</i> (Hymenoptera, Sphecidae)	<ul style="list-style-type: none"> <li>– Injection (dopamine)</li> </ul>	<ul style="list-style-type: none"> <li>– Injection of dopamine mimics venom-induced grooming</li> <li>– GC-MS and HPLC-ED on venom</li> <li>– Immunocytochemistry (anti-dopamine)</li> <li>– Electrophysiology</li> </ul>	<ul style="list-style-type: none"> <li>– Pharmacological depletion of monoamines mimics venom-induced nonparalytic hypokinesia and reduced-escape response</li> <li>– Dopamine present in the venom</li> <li>– Decrease in octopamine neurons activity in the thorax, modulated by input from descending neurons from the brain, themselves modulated by venom injection</li> </ul>	Miles and Booker (2000)
				Anon

Source: a: NO is a neuromodulator involved in memory, neuronal development as well as immune defense.

b: ATP can also play a role as neuromodulator.

c: Inducing apoptosis in the brain can alter the chemical signals in the brain (inflammatory immune responses), as reported in vertebrates infected with certain viruses or protozoans.



**Fig. 1** 5-HT immunoreactivity (yellow) within the brains of uninfected *Gammarus pulex*, *P. tereticollis*-infected *G. pulex*, and *P. minutus*-infected *G. pulex*. Arrows show position of tritocerebrum giant neuron (TGN) cell body. No differences in brain anatomy from infected and uninfected individuals were observed. Bar shows 100 μm.

## Screening the Host's Proteome and Transcriptome

In the few host–parasite systems to which it has been applied, the proteomic approach appeared sensitive enough to detect proteome differences between infected and noninfected hosts that can be attributed to the manipulative syndrome. Indeed, the use of comparative screening of whole proteome or transcriptome between infected hosts and uninfected hosts appears a powerful means to cope with the predicted complexity of proximate mechanisms involved in parasite manipulation, if several conditions are met (e.g., the quality of controls run, the access to database allowing protein identification, and other limitations listed here). Proteins or transcripts differentially produced and specifically associated with the manipulative process can be identified, if one compares manipulated hosts with uninfected and infected nonmanipulated hosts. The analysis of infected nonmanipulated hosts (i.e., usually containing a developmental stage of the parasite not infective to the next host) is an important control to run, to distinguish the proteins or transcripts specifically associated with the manipulative process from the ones produced in response to infection. Similarly, noninfected hosts exposed to the same environmental conditions as infected manipulated ones should be analyzed (in addition to noninfected ones in their natural environment) to distinguish the proteins or transcripts specifically associated with the manipulative process from the ones produced in response to the environmental changes associated with manipulation (for instance, living at the surface instead of the bottom of a body of water). The differences in brain proteome between infected-manipulated hosts and controls are either in the presence/absence, the quantity, or the posttranslational processing of certain proteins.

From the studies reviewed (Table 3) it seems that the alteration of the CNS is a common feature in the proteome of infected manipulated animals. In addition, key metabolic pathways are often perturbed, as well as proteins involved in cellular stress (HSP, other chaperones), immunomodulation, or oxidative damage. Alteration in energy metabolism in the brains of infected blood-feeding insects can be interpreted as a parasite strategy to manipulate vector-feeding behavior by inducing a nutritional stress. Interestingly, several proteins putatively involved in similar behavioral modifications in different host–parasite systems belong to the same family. For instance, differential expression of proteins from the family (CRAL–TRIO) implicated in the vision process has been found in the brain of the wood cricket *N. sylvestris* infected by the manipulative nematomorph *P. tricuspis* and in the brain of *G. insensibilis* infected with the bird trematode *M. papillorobustus* (Table 3). Such pattern is suggestive of a limited ‘jeu des possibles,’ with molecular convergence or conserved proximate mechanisms being the only way to alter the behavior of phylogenetically distant hosts such as an amphipod and an insect.

However, despite their power in investigating the molecular basis of parasite manipulation, proteomic studies have several limitations

- Some peptides might be undetected because of their low concentration, specific pI, or small size (for instance, neuropeptides).
- Most proteomic studies have focused on differential expression of proteins; however posttranslational modifications of proteins might be involved as well in the modulation of the host's phenotype induced by parasites, and their importance in interpreting transcriptional data has been emphasized by some authors.
- Protein identification becomes problematical in nonmodel organisms: it relies on cross-species protein identification, and therefore, on either highly conserved proteins or proteins known from organisms closely related to the host. For instance, the power of proteomic studies on *Gammarus* amphipods in revealing proteins specifically linked to the manipulative process was limited by the impossibility of identifying 27 out of 72 proteins spots differentially present or absent from the brain of *G. insensibilis* manipulated by *M. papillorobustus* and 60 out of 68 proteins spots differentially present or absent from the brain of *G. pulex* manipulated by *P. minutus*.

Microarrays provide an alternative to proteomics but have been used so far in a limited number of host–parasite systems (rodents, salmon, mosquitoes, and bees infected with Apicomplexa protists, helminths, or mites). Most studies have performed transcriptional profiling of infected and uninfected individuals or resistant and susceptible strains, to identify the immune and metabolic response to infection. In parasites, microarray profiles coupled to other techniques in functional genomics have been used to discover new drug targets, or to understand the genetic basis of drug resistance. To our knowledge, the method has not yet been

**Table 3** A review of studies screening the proteomes of infected manipulated and noninfected individuals in insects and amphipods. The role of proteins differentially produced has been putatively ascribed to the functioning of the CNS, to metabolic pathways, or to other functions, including immunity and stress response

<i>Differential changes in the brain proteome of infected animals</i>				
	<i>Host-parasite system</i>	<i>Functioning of the CNS</i>	<i>Metabolism</i>	<i>Others</i>
Tsetse flies with mature infection of <i>Trypanosoma gonciii</i>	Dopa decarboxylase (-) (synthesis of dopamine and serotonin)	Glycolytic enzymes	Pheromone and odorant-binding protein (+) Molecular chaperone (stress-activated proteins) Signaling,....	
<i>Anopheles gambiae</i> (mosquitoe) with mature infection of <i>Plasmodium berghei</i> (sporozoite stage in salivary glands)	Tropomyosin (+up)	Metabolic enzymes involved in the production of ATP <sup>b</sup> or in glucose oxidation pathway (+up) Calmodulin (+up) (activation of NO synthase) <sup>a</sup>	Molecular chaperone (HSP20, stress protein)	Several other proteins involved in cell cycle, signaling, etc....
<i>Mecanema thalassinum</i> (grasshopper) during manipulation by <i>Spiniochoradodes tellinii</i> (nematomorph)	Six protein families involved in the development of the CNS (+) (such as Actins, ATPase, Wnt); two in the release of neurotransmitters, one in apoptosis <sup>c</sup>	?		
<i>Nemobius sylvestris</i> (cricket) during manipulation by <i>Paragordius tricuspidatus</i> (nematomorph)	Five protein families involved directly and/or indirectly in the development of the CNS (such as Actins, ATPase, Wnt)	Glucose metabolism	Glutathion S-transferase (oxidative stress)	
<i>Gammarus insensibilis</i> infected with <i>Microphallus papillorobustus</i> (Trematode)	Two proteins implied in the vision process (including CRAL-TRIO) Arginine-kinase (-) (regulating factor in NO synthesis) <sup>a</sup>	?	Nonspecific stress Signal transduction (serine/threonine protein phosphatase) Proteins implied in immunity defenses (PBP-GOBP (pheromone binding protein) (+) and ATP-gua_Ptrans: (-))	
<i>Gammarus pulex</i> infected with <i>Polymorphus minutus</i> (Acanthocephalan)	Aromatic-L-amino acid decarboxylase (-) (synthesis of serotonin) Two proteins implied in the vision process (including CRAL-TRIO) Arginine-kinase (+) (regulating factor in NO synthesis) <sup>a</sup> Tropomyosin (+) (interacts with the development and plasticity of the CNS)	?	Proteins implied in immunity defenses (prophenoloxidase: (+); MAM, sushi and ATP-gua_Ptrans: (+))	

<sup>a</sup>NO is a neuromodulator involved in memory, neuronal development as well as immune defense.

<sup>b</sup>ATP can also play a role as neuromodulator.

<sup>c</sup>Inducing apoptosis in the brain can alter the chemical signals in the brain (inflammatory immune responses), as reported in vertebrates infected with certain viruses or protozoans. Source (-): Not detected in the brain proteome of infected individuals compared to uninfected ones.

(+): Detected in the brain proteome of infected individuals but absent from the one of uninfected individuals.

(-cd) Downregulation.

(+up) Upregulation.



used to screen for ‘manipulative molecules,’ possibly because of the lack of genomic data on the historically best model systems of behavioral manipulation (acanthocephalans or trematodes and their arthropod intermediate hosts). This could, however, be done on mosquitoes/*Plasmodium* (with altered behavior driven by olfactory cues), tsetse flies and *Trypanosoma*, and bee/*Varroa* systems. Indeed, in the later model, a recent microarray-based analysis reveals that resistant and susceptible strains of bee to *Varroa* differ more on olfaction-related genes than on immune genes.

## The Search for the ‘Molecules of Manipulation’ in E/S Products of Manipulative Parasites

Differential screening of infected-manipulated hosts and noninfected or infected and nonmanipulated hosts will not reveal which molecules are actually released by the parasite to induce changes in the proteome or transcriptome. Even if proteomic studies can reveal a protein specifically produced by a parasite during the manipulation process, such as the mimetic protein Wnt by hairworms infecting crickets, the demonstration of its role as a ‘manipulative molecule’ requires that its excretion in the host’s hemolymph is established. Therefore, proteomic analysis of E/S products from manipulative parasites is necessary to identify the parasitic molecules initiating the manipulation process. The separation of biological fractions in the ‘secretome’ allows their biochemical identification and must be followed by the biological testing in vivo of their functional role (in the manipulative process).

Such analysis is currently limited to parasites that can be maintained or cultivated in vitro. The proteomic identification of E/S parasite products also relies on ongoing sequencing (genomic and EST) projects. So far, it has been possible in several nematodes and in the trematode *Schistosoma mansoni*, in the prospect of designing vaccines or drugs. Given these limitations, no such analysis has yet been done to identify the E/S products involved in behavioral manipulation. Studies analyzing E/S products from worms have revealed the release of proteins involved in a diversity of functions, such as stress response proteins/chaperones, antioxidant enzymes, energy metabolism and structural/cytoskeletal proteins, immune evasion, protease inhibitors, and lipid binding. No doubt the proteomic analysis of ‘secretome’ is a promising route to the discovery of the ‘manipulative molecules’ (either directly or indirectly targeting the host’s CNS).

## Conclusion

The most tricky aspect in our quest for the mechanistic basis of parasite-induced behavioral alterations is a causality problem akin, at first sight, to ‘the chicken or the egg’ dilemma. Whenever one spots a change associated with the manipulative process/the manipulated phenotype (be a neurophysiological change, a gene or a protein differentially expressed or produced), is it the cause or a side-effect of parasite manipulation? The reviewed studies addressing the mechanisms underlying parasite manipulation reveal that the alteration of host’s CNS is a common feature of manipulated hosts. Given the diversity of host–parasite systems in which behavioral alterations have been reported, further studies should help in answering several key questions: (1) Have the same constraints on parasitic transmission led to a similar solution (evolutionary convergence of behavioral manipulation)? (2) Are similar behavioral alterations induced by the same biochemical tools (molecular convergence)? For instance, geotaxis is altered in several host–parasite systems. Ants parasitized with parasitic fungus of the genus *Cordyceps* and ants infected with the liver fluke *Dicrocoelium*, as well as the caterpillar *Mamestra brassicae* infected with *M. brassicae* nuclear polyhedrosis virus, climb to the top of a plant. Several amphipods infected with various acanthocephalan species (*G. pulex*/*P. minutes*, *G. lacustris*/*P. paradoxus*, and *G. insensibilis*/*M. papillorobustus*) swim at the water surface. Chemosensing and possibly learning are also altered in several host–parasite systems. Changes in olfactory perception and/or learning have been reported in various host–parasite systems as different as rats infected with *T. gondii*, amphipods infected with acanthocephalans, or dipteran vectors carrying protozoans. Is molecular convergence at the heart of these similar changes? The understanding of the mechanisms underlying parasite manipulation will likely reveal how parasites play tricks on their hosts, guiding them round more or less directly, by using molecular mimics or conserved molecules that affect the host’s flexible neuromodulatory network.

**See also: Cognition:** Non-Elemental Learning in Invertebrates. **Hormones and Behavior:** Immune Systems and Sickness Behavior. **Host-Parasite Interactions:** Disease: Parasite-Modified Vector Behavior; Evolution of Parasite-Induced Behavioral Alterations; Intermediate Host Behavior; Propagule Behavior and Parasite Transmission. **Methodology:** Neuroethology: Methods. **Neurons and Senses:** Nematode Learning and Memory; Neuroethology; Parasitoid wasps: Neuroethology. **Predator-Prey Interactions:** Parasitoids.

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