ANNUAL REVIEWS

Annual Review of Entomology

Neuroparasitology of Parasite–Insect Associations

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Annu. Rev. Entomol. 2018. 63:471–87

The *Annual Review of Entomology* is online at ento.annualreviews.org

https://doi.org/10.1146/annurev-ento-020117-043234

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Keywords

behavioral manipulation, host, parasites, neurobiology, extended phenotype

Abstract

Insect behavior can be manipulated by parasites, and in many cases, such manipulation involves the central and peripheral nervous system. Neuroparasitology is an emerging branch of biology that deals with parasites that can control the nervous system of their host. The diversity of parasites that can manipulate insect behavior ranges from viruses to macroscopic worms and also includes other insects that have evolved to become parasites (notably, parasitic wasps). It is remarkable that the precise manipulation observed does not require direct entry into the insect brain and can even occur when the parasite is outside the body. We suggest that a spatial view of manipulation provides a holistic approach to examining such interactions. Integration across approaches from natural history to advanced imaging techniques, omics, and experiments will provide new vistas in neuroparasitology. We also suggest that for researchers interested in the proximate mechanisms of insect behaviors, studies of parasites that have evolved to control such behavior is of significant value.

INTRODUCTION

Some parasites have evolved the ability to precisely and adaptively manipulate the behavior of animals they exploit, turning their hosts into vehicles that behave in ways to promote parasite survival and transmission. Since most animals are insects, it is not altogether surprising that many case studies of animals that are manipulated by parasites are insects (51). Furthermore, since many insect taxa have themselves evolved parasitic modes of life (6, 21), we also find that some insects have evolved the ability to manipulate the behavior of other insects. Although alteration of host behavior by parasites is a widespread phenomenon, the underlying mechanisms are only beginning to be deciphered. Effects of parasites on host behavior can be due to the parasites directly manipulating the nervous system and the associated muscular system or indirectly manipulating the immune system and/or the host's metabolism. In this review, we survey the effects of parasites on host behavior, emphasizing those resulting from a direct manipulation of the host's nervous system. Our focus is on parasitic insects that infect other insects (e.g., wasps, flies, and strepsipterans) and parasites of insects (e.g., viruses, fungi, and worms). We use the term parasitic insect (6) rather than parasitoid since not all the examples we discuss involve host death, a hallmark of the definition of a parasitoid (21).

Neuroparasitology is an emerging branch of biology that deals with parasites that can control the nervous system of the host (1, 3, 66). The ability of parasites to alter the behavior of their hosts has recently generated an unusual level of interest from both scientists and nonscientists. One reason is that some parasites can alter the behavior of their host in such a way as to suggest a hijacking of the host's ability to act of its own volition. Many organisms, including viruses, bacteria, fungi, apicomplexans, diverse worms, and even other insects, have evolved to control the insect brain. Here, we explore this diversity to ask two questions. The first is whether there are a limited number of pathways in the brain of insects that can be controlled. The second related question asks whether studying neuroparasitology will allow us to accelerate our understanding of the proximate mechanisms of insect behavior.

Investigating the proximate mechanisms underlying any behavioral manipulation presents an opportunity to achieve new insights in biology and to discover and characterize new genes, metabolites, proteins, and developmental pathways that underlie the expression of behavior. More specifically, such investigation provides insights into the mechanisms of parasite transmission and the mechanisms of insect behavior as parasites have evolved to be neuroengineers tapping into existing neuronal circuits in the host. Each example of a parasite controlling the host nervous system therefore represents an independent experiment across evolutionary time where two organisms (host and parasite) express genes that ultimately affect the behavior in the same individual (the host). Although our understanding of the neural mechanisms of parasitic manipulation is at an early stage, there have been some major advances over the past few years. Part of these advances has been due to recent developments in neuroscience, bioimaging, and molecular technologies, as we discuss in this review. For instance, recent years have seen an increased effort in visualizing parasites and their effects at nanometer-scale resolution, deciphering the composition of the parasite secretome at the molecular level, through the use of metabolomic, proteomic, and transcriptomic approaches. The advent of gene editing tools such as RNA interference (RNAi) and CRISPR Cas-9 (clustered regularly interspaced short palindromic repeats–CRISPR associated protein 9) that work in nonmodel organisms sets the scene for testing the functional role of the parasite's genes in altering behavior in the host.

Our overarching goal in this review is to capture the excitement of these recent developments and argue how many fundamental features of insects can be better understood if we determine the pathways used by parasites to control their hosts. We briefly introduce the reader to prominent examples of insect behavior being controlled by parasites, ranging from viruses to other insects.

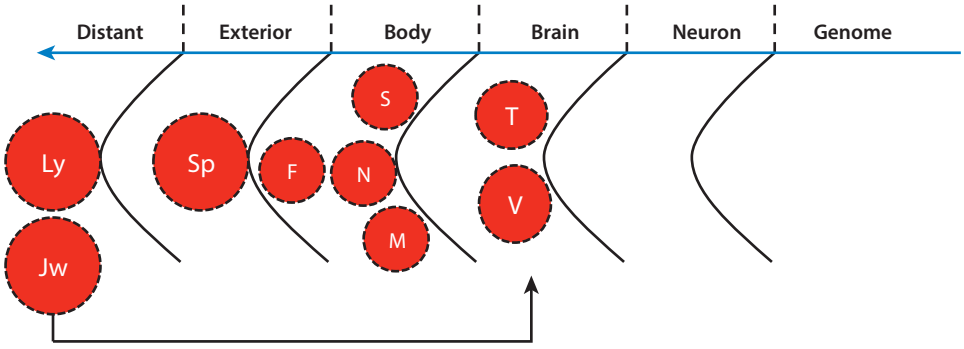

Figure 1

A spatial framework for manipulation of insect behavior by parasites. The top line and arrow represent the pathway to behavioral expression in insects from genes to the observed behavior where it has an effect on some distant component of the environment (e.g., mate, competitor, food, inorganic components). The parasites (*red circles*) work in the opposite direction to affect behavior. They can do this from the environment (distant from the host), host exterior, and host body interior and brain. The parasites are lycaenid caterpillars (Ly), jewel wasps (Jw), spiders (Sp), fungi (F), nematodes (N), mermithids (M), Strepsiptera (S), trematodes (T), and viruses (V). See text for further details. The arrow from jewel wasps shows how brain entry is possible from outside the host's body.

We take a spatial view of manipulation, examining parasites that manipulate the host from different distances, including from outside the body, inside the body, and directly inside the brain (**Figure 1**). We discuss how much of the early work necessarily focused on phenomena and natural history but how, in recent years, a number of emerging models have allowed us to move beyond descriptive studies to fundamental insights into how insect brains and behavior are controlled. Since we are not even close to discovering the full diversity of the ways parasites alter insect behavior, we advocate that phenomenological work through natural history studies is still critical. But now it can be done in lockstep with proximate-level studies (**Figure 2**) for maximum understanding of the many organisms that can only survive and reproduce by controlling the insect brain.

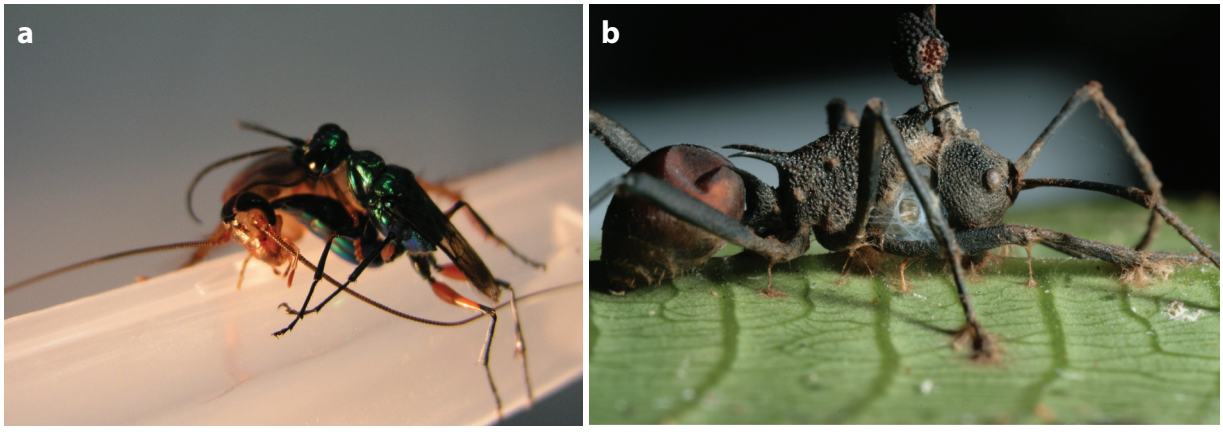

Figure 2

Diverse examples of parasites that control insect behavior that exemplify both the range of tactics and diversity of parasites. (*a*) The emerald jewel wasp has a two-step strategy, where manipulation is by the parent female wasp. (*b*) The zombie ant fungus; in this example, control is held by a collective of fungal cells. See text for details.

EXAMPLES OF BEHAVIORAL MANIPULATION

There are now many examples of parasites affecting the behavior of their host in ways that appear to increase the fitness of the parasite at the cost of host fitness. However, only a few experimental studies have demonstrated that the altered behaviors in the host lead to demonstrable benefits for parasite fitness (44, 68). In the early days of studies of parasites that alter behavior, much effort was invested into discussions of how host behavioral manipulation could be considered adaptive for the parasite when the only data available was the altered behavior (as opposed to data on the proximate mechanisms). This was probably a necessary step in the nascent field but is generally viewed today as having been a limited approach (28, 52, 56). Where studies of the phenomena show consistent and highly stereotyped, behavioral changes in animals infected by parasites in a manner that reasonably appears to benefit parasite transmission at cost to host fitness, then these are likely examples of behavioral manipulation (56). The majority of work to date has been, of necessity, phenomenological, and this has been very useful and should be continued, as we discuss in the conclusion. In the following sections, we survey some of these examples. In certain cases, we understand some details of the underlying mechanisms, whereas in other cases, we are still speculating. We present a schema (**Figure 1**) to organize our approach; the figure shows manipulation from a distance (the parasite is outside the body) to manipulation from inside the brain.

Neurological Control at a Distance

A textbook example of mutualism was the tending of lycaenid caterpillars by ants (55). In exchange for protection, the caterpillars would provide their ant bodyguards with sugar-rich secretions from specialized structures called dorsal nectaries (55). The reciprocal nature of the relationship, in which the ants get food and the caterpillars get protection from predators, strongly suggests an evolved mutualism. Such mutualisms between ants and plant-feeding insects like hemipterans are common (41). One example study by Hojo et al. (25) focused on the caterpillar *Narathura japonica*, which feeds on oak trees (*Quercus glauca*) in Japan and is tended by ants (*Pristomyrmex punctatus*). The caterpillar provides the ants a secreted substance from its dorsal nectaries in return for protective services, where the ants protect the caterpillar from predators and parasitoids. Hojo et al. (25) found that the secretion by the caterpillar made the attendant ants more aggressive and less likely to move away from the caterpillar, thereby reducing the chances that the caterpillar would be targeted by predators. Ants would tend the caterpillars even if sugar rewards were artificially stopped, implying an asymmetry in rewards from a situation of mutualism (where both benefit) to parasitism (where just the caterpillar benefits). Although the caterpillar was external to the ant's body, the researchers found elevated levels of dopamine (but not the other biogenic amines they surveyed). This research was considered important because an apparent example of mutualism turned out to be parasitism instead because ants invested more energy in protecting than was justified by the benefits they received from the caterpillars. But for researchers interested in parasites that have evolved the ability to manipulate behavior, it is of significant interest because it shows how brain chemistry of the manipulated host can occur even if the parasite is physically separated from the host it controls (29).

Brain Entry and Control Via an Ovipositor

Parasites that are external to their host can bring about behavioral change by precisely inserting their ovipositor into the host and injecting compounds that affect behavior. In insects, the

Hymenoptera are numerically the most common insect parasites (6, 21). One key feature that enables this lifestyle is the possession of a sting that allows hosts to be subdued and/or be injected with eggs that subsequently consume the host. In some parasitic insects, the ovipositor is precise enough to inject eggs into nervous tissue, which is beneficial, as the egg, or subsequent larva, is less subject to assault from specialized cells, such as macrophages (6). In many parasitic wasps that lay their eggs into wood inhabiting insects, the ability to locate the host in wood and precisely inject an egg relies upon a precise motor control of the ovipositor (67). The jewel wasp, *Ampulex compressa*, which uses live cockroaches (*Periplaneta americana*) as a food supply for its developing larva, can both identify the nervous tissue and precisely direct its stinger to find a specific location inside the host's brain (17) (**Figure 2**). The adult female wasp stings its host twice: The first sting into the prothoracic ganglion paralyzes the cockroach's prothoracic legs for 3–5 min. Once the cockroach is disarmed of its front legs, the wasp inserts its stinger through the membrane between the neck and the head capsule to precisely locate and inject into the head ganglia (24). This precision is due to the ability of the ovipositor to recognize brain tissue from other tissues inside the head cavity (17).

Control of the Brain While Attached to the Host Body

In other wasps, the egg is laid onto the host and, from this position, the developing parasite (technically an ectoparasite) makes a hole through the exoskeleton and manipulates host behavior from outside the body. The manipulation of spider web spinning by a wasp larva illustrates this type of host/parasite interaction. Adult female *Hymenoepimecis argyraphaga* ichneumonid wasps sting and paralyze a host spider (*Plesiometa argyra*) on its orb web (14). Just before being killed by the wasp larva, the adult spider is induced to spin a modified web that protects the wasp larva during pupation. The adult wasp stings the spider through its mouth, leading to an immediate paralysis, and this suggests a direct targeting of the brain similar to the jewel wasp discussed above (38). Paralysis facilitates the wasp positioning of the egg to prevent it from being destroyed by the spider. Once the adult female wasp lays an egg on the spider's abdomen, it leaves and the spider resumes normal activity. When the larval wasp hatches, it commences feeding on the host's hemolymph through an opening in the cuticle. When ready to pupate, the larva presumably injects a substance into the spider that triggers the weaving of an atypical protective web that functions to protect the developing pupal wasp from predators. The wasp larva apparently manipulates the spider, which normally uses a web-building program, to engage only a subroutine of this program. In a recent investigation of web weaving under controlled conditions in the lab, infection of theridiid spiders (*Neottiura bimaculata* and *Theridion varians*) by the polysphinctine wasp *Zatypota percontatoria* results in the formation of a web type that is normally seen in spiders only during the winter. Such a winter web protects the spiders during a period when they do not hunt. The webs are either dense structures (as in the case with *N. bimaculata*) or a cupola structure (as with *T. varians*), which are normally built in leaf litter or under bark (40). Following manipulation of the spider's behavior by the larval wasp, the formation of these winter webs during the summer serves to protect the wasp pupa too (40). Such modification of web building has been further examined in another association between the ichneumonid spider *Reclinervellus nielseni* and its host spider *Cyclosa argenteoalba* (63). They discovered that both resting and cocoon webs exhibit similar structural features, suggesting that the cocoon web has roots in the innate resting web. This would imply that the effect of the wasp on its host spider is at the neurological level. The mechanism is unknown, but recent work, which we discuss in the section titled Neuronal Correlates of Behavioral Manipulations below, suggests that ecdysteroid-related components may be responsible for the manipulation, further implicating manipulation at the level of the host's brain.

Exiting the Host to Control Behavior

In the previous examples, insect parasites are external to the host they control. In the next example, parasites are inside the host's body cavity, feeding in the host and emerging from the body before manipulation occurs. This is observed in braconid parasitoid wasps infecting various caterpillars. There are three species of wasp in which this has been observed: *Glyptapanteles* sp. infecting *Thyrinteina leucocerae*, *Cotesia glomerata* infecting *Pieris brassicae*, and *C. congregate* infecting *Manduca sexta* (47). First, the wasp stings and injects her eggs into the caterpillar. The caterpillar quickly recovers from the attack and resumes feeding. The braconid larvae mature by feeding on the host, and after two weeks, up to 80 fully grown larvae emerge from the host prior to pupation. One or two larvae remain within the caterpillar while their brothers and sisters begin to pupate. After emergence of the majority of the larval wasps to pupate, the remaining larvae take control of the caterpillar behavior by an unknown mechanism, causing the host to snap its upper body back and forth violently, deterring predators and protecting their pupating siblings (22). Unparasitized caterpillars do not show this behavior. To investigate the impact of such manipulation on the parasite fitness in the field, the guarding caterpillar was removed from half of the batches. The presence of bodyguard hosts resulted in a twofold reduction in mortality of braconid pupae (22). It is still unclear how the parasitoid larva induces this spectacular manipulation of the host, but it is tempting to speculate that the suicidal larva interacts with the caterpillar nervous system to induce this pathological behavior.

Another species of braconid wasp, *Dinocampus coccinellae*, manipulates its host even after leaving the host's body and in the absence of residual siblings, who remain inside the host. This wasp also induces guarding behavior in its ladybug host (*Coccinella septempunctata*). Initially, the single wasp larva develops inside the body of its host (65), but after about 20 days, a single prepupal larval-stage wasp emerges from the ladybug's abdomen and spins a cocoon between its legs. Once the wasp larva has emerged, the ladybug remains alive but paralyzed, twitching its body to keep the single wasp pupa safe from potential predators. Thus, the ladybug occupies a similar role to the *Glyptapanteles* caterpillar discussed above. Given that the wasp pupa is outside of the ladybug body, and no siblings remain inside the ladybug body, how is manipulation occurring? A possible explanation comes from an elegant study of *D. coccinellae* and its ladybug host, *Coleomegilla maculata*. It appears the ovipositing adult female wasp injects a virus, *Dinocampus coccinellae paralysis virus* (DcPV), together with an egg. The virus is apparently responsible for the behavioral change because of its invasion of the ladybug's brain (13). We discuss the mechanisms in detail the Neuronal Correlates of Behavioral Manipulations section.

Controlling the Brain from the Body Cavity

It is possible to control the host insect brain from different areas of the body that are distant from the brain (**Figure 1**). Classic examples of this are parasitic nematomorph and mermithid worms that have convergently evolved abilities to provoke suicidal behavior in their insect hosts. Crickets and ants infected by nematomorph and mermithid both enter water to allow for emergence of the worm from the body (46, 64). Since both crickets and ants are terrestrial, they either die or are eaten by aquatic predators (60). The worms do not physically touch the brain, and, at least for hairworms, we know that the brains of behaviorally manipulated insects have differential expression profiles of certain proteins (discussed in the next section). Another example where manipulation of host behavior apparently does not require entry into the brain is the zombie ant fungi that manipulate ants into biting and grasping vegetation before dying (**Figure 2**) (31). The adaptive significance of such manipulation for the fungus is that by biting the underside of leaves or twigs, the dying

ant provides a safe platform for the development and reproduction of the fungus. Such a high position of the dead ant also increases the parasite's spore dispersal from the ant cadaver (4, 31, 44). Histological work showed a large number of fungal cells in the body cavity and between the muscle fibers of the host but none in the brain (30). Another example of controlling the brain from the body cavity occurs in strepsipteran parasites, which manipulate female social wasps, solitary bees, and ants (9, 34, 35) to leave the nest and occupy distinct areas of the environment that promote parasite reproduction. These parasites are unusual in that they have an extreme form of sexual dimorphism, with females remaining neotenic and endoparasitic (with just her head emerging from the host) and males being free living and flying as adults. Mating occurs as the free-flying male inserts his aedeagus into the head of the female, whose body protrudes from another insect. In the case of male strepsipterans infecting ants, the challenge is to mate with a female of the same species who occupies the body of a cricket. Mating is not possible inside the nest either because wasps and bees are aggressive toward the male strepsipteran or because the ant nest does not contain crickets infected with female strepsipterans. Hence, because mating is not possible inside the confines of the nest, the infected social insects leave the nest to allow the males to emerge and mate (34). Because, in the example, the parasite resides in the abdomen, manipulation occurs without physical contact with the brain. Studies of paper wasps have shown distinct changes in the volume of the corpora allata (58, 62), and the possible mechanisms are discussed in the next section.

Migration to the Brain to Enable Manipulation

In the jewel wasp example discussed above, the parasite envenomates the brain, using its ovipositor with surgical precision. The virus injected with the egg of a braconid wasp (DcPV) is also found inside the host brain. As the virus first resides in the body cavity floating in the hemolymph, the circulation caused by the heart pumping presumably carries it to the brain (and it obviously has some ability to travel across the blood–brain barrier). In some systems, however, active migration occurs. One of the most famous examples of behavioral manipulation is that exerted by the liver fluke on its second intermediate host, an ant. The adult form of the liver fluke (*Dicrocoelium dendriticum*) occupies the liver of its mammalian host. After mating, the eggs are excreted in the feces. One of the relevant intermediate hosts is an ant that gets infected when it feeds on snail slime (the preceding host). Most of the liver fluke cercariae make their way to encyst in the ant hemolymph until one or two move to the ant's cerebral ganglia (57), inducing the ants to ascend and bite grass (as done by ants infected by *D. dendriticum*) or simply ascend and remain motionless on the grass (as done by ants infected by *D. hospes)* (10, 11, 45, 57). In both cases, the result is the same: consumption of ants (and thus the parasite) by grazing herbivores. Different species of trematode occupy either the ventral subesophageal ganglion, which is in close contact to the origin of the mandibular nerves (*D. dendriticum*), or the antennal lobes (*D. hospes*) (57). Worth noting is that the species of trematode that induces ant biting behavior (*D. dendriticum*) is found next to the subesophageal cerebral ganglion known to control the mouthparts.

NEURONAL CORRELATES OF BEHAVIORAL MANIPULATIONS

Manipulation of host behavior is an impressive phenomenon in nature. Richard Dawkins, who coined the term extended phenotype to better understand such behaviors, considered parasite manipulation of host behavior as his "personal epitome of Darwinian adaptation, the *ne plus ultra* of natural selection in all its merciless glory" (12, p. xi). The general fascination with so-called zombie behaviors further drives home the effectiveness of one organism controlling, in such a precise manner, the behavior of another. Although remarkable, it is important to point out that

there has to be a causal link between the genes of the parasite and the resultant behavior. Our goal in presenting a spatial view of parasite extended phenotypes is to emphasize this take-home message (**Figure 1**). In the next section, we explore what we already know about these chains linking parasite genes to host behavior.

Direct Action on the Brain

It is reasonable to conjecture that an effective way to control host behavior is to directly affect the insect brain, either by using an appendage that enters the brain (such as the ovipositor of the jewel wasp) or by invading the brain as in the case of the so-called brain-worm trematodes in ants. In the former example, we have some understanding of the mechanisms of behavioral manipulation. In this system, the jewel wasp uses live cockroaches (*P. Americana*) as a food supply for its developing larva. Instead of using its venom to paralyze the cockroach and then facing the challenge of carrying the larger cockroach to the preselected burrow, the wasp manipulates the cockroach into behaving as a docile and compliant host for the upcoming handling by the wasp and feeding by the larva. Transient paralysis of the front legs occurs after the first thoracic sting. While the short-term effect of the venom, and the intense grooming that follows the head sting, seems to be mediated by neuropharmacological mechanisms [gamma-aminobutyric acid (GABA) and dopamine] (42, 50, 69), we hypothesize that the long-term effect must be mediated by up/down regulation of protein expression. Regarding the short-term effect of the venom (front-leg paralysis) and the midterm effect (grooming), the wasp is taking advantage of the existing neurotransmitters and neuromodulatory systems in the cockroach central nervous system (GABA and dopamine). It first suppresses motor movements of the front legs (GABA) to neutralize their possible interference with the second head sting. It then induces grooming motor behavior (dopamine), the purpose of which is unclear.

The long-term effect is to keep the cockroach immobile in the wasp's burrow to allow the feeding behavior of the larval wasp. To accomplish this long-term behavioral manipulation, the wasp injects its venom cocktail inside the head capsule directly into the cerebral ganglia, the subesophageal ganglion (SEG), and the supraesophageal ganglion (brain). The sting to the brain is accurate and aimed to a neuropil termed the central complex (CX), a multisensory higher center known to regulate locomotion. As a result, the cockroach enters a long-lasting lethargic state, which is characterized by a drastic decrease in its ability to initiate and maintain locomotion. In addition, the cockroach displays a reduced responsiveness to mechanical and nociceptive stimuli. Specifically, stimuli that reliably evoke an escape response in an alert cockroach will, at best, induce a brief startle response in a stung cockroach. Moreover, during envenomation by the jewel wasp, cockroaches are exposed to strong nociceptive stimuli. After stinging and injecting venom, the wasp inflicts two strong noxious stimuli that jeopardize the cockroach tegument (cuticle) integrity. Using its strong mandibles, it breaks off the cockroach's antennae to drink hemolymph from the cut end. Surprisingly, the cockroach does not show any defensive behavior in response to these noxious stimuli. Such defensive behaviors to pain are termed nocifensive. The unresponsiveness of the cockroach to stimuli allows the wasp to further handle the cockroach, lead it to a burrow, and lay an egg on the cockroach's cuticle.

Behavioral analysis of the nocifensive behavior of cockroaches stung by the jewel wasp was conducted using a modified Shuttle Box in which escapable foot shocks were administered to the cockroach's legs. Venom-induced analgesia lasts for 72 h, during which time the nocifensive behavioral threshold increases tenfold in stung cockroaches (18). A likely target candidate for such a change in nocifensive behavior is the opioid system, which is known to affect responsiveness to stimuli in insects. To explore this possibility, different opioid receptor agonists or antagonists were

injected in the hemolymph of cockroaches prior to being stung by a wasp. Antagonists significantly decreased the nocifensive threshold in stung individuals, whereas agonists led to an increase in nocifensive threshold in controls (19). Using the antenna-heart preparation as a bioassay, Gavra & Libersat (19) showed the presence of an opiate receptor binding molecule in the venom. The opiate mediation of the stung cockroaches' nocifensive behavior is currently being studied in Libersat's laboratory.

Regarding the long-term effect of the venom (hypokinesia or long-term lethargic state), the venom induces reduced activity in neurons (18). Targeted injection of procaine, a reversible-action potential blocker that reduces or suppresses neuronal activity to mimic venom injection to the CX or the SEG, is sufficient to induce a decrease in spontaneous walking and changes in responsiveness comparable to the natural sting (36). Interestingly, cockroaches display an innate quiescent state that is correlated to daytime, and that behaviorally resembles the venom-induced lethargic state. In this state, a cockroach exhibits diminished mobility and assumes a flaccid posture. Quiescent- and venom-induced lethargic states bear some similarities in that the sensory information carried by abdominal giant interneurons is unaffected in both states. Hence, the absence of motor responses characteristic of escape behavior and generated by the thoracic ganglia in both states must be controlled via descending input from the head ganglia. Emanuel & Libersat (15) show that postural-leg motoneuron ongoing activity is reduced in quiescent and stung animals compared to alert animals. Injection of procaine to prevent neuronal activity into the central complex to mimic the wasp venom injection produces a similar effect on the activity of this postural motoneuron. These results suggest that the venom-induced lethargic state in cockroaches might represent an extreme version of the quiescent state and that both states might be controlled by the head ganglia. If true, this implies that, in the course of the coevolved arms race between a parasite and its host, the parasite has found ways to tap into an existing neuronal circuit that is part of the quiescence-regulating network, to its own benefit. Interestingly, it was shown recently that clock pacemaker neurons involved in regulating the rest cycle in *Drosophila* project to the CX, thereby regulating locomotor activity (5). Hence, the CX could be the last station that controls multiple downstream circuits to orchestrate behavioral rhythms. Because the jewel wasp injects its venom cocktail into and around the CX, we hypothesize that it may have evolved the chemical arsenal to manipulate rest–wake homeostasis in its cockroach prey. There may be other examples where the parasite chemical cocktail is directly injected into the host brain. The manipulation of spider behavior discussed above could be one such case. There, the adult ichneumonid wasp places her ovipositor into the mouth of the spider and possibly, as suggested by Kloss et al. (38), into the brain.

Until recently, however, only a few studies have dealt with the chemical components in solitary wasp venoms. It may be related to the difficulty in collecting enough venom from a large number of individuals that is necessary for chemical analysis. But technological advances have made possible sequencing and identification of wasp venom components. Chromatography coupled with mass spectrometry is a high-throughput proteomic method that can obtain protein sequences from little venom quantity. Similar to venom found in most venomous animals, wasp venoms consist of proteins, peptides, and a mixture of low–molecular mass compounds (39, 53, 54). In general, venom from wasps that feed externally on the host is aimed at paralyzing the host to secure feeding of the larval offspring on the host cuticle. In contrast, wasps that feed internally use their venom to facilitate parasitization of the offspring inside the host by interfering with the host's immune system and/or host development without paralysis. Given our knowledge of the behavioral phenotypes and associated changes in neuronal network activity, the challenge will be to define causal relationships between individual wasp venom components, their impacts on the expression of protein targets in the cockroach brain, and consequences of such interactions on host behavior.

In comparison to what we know about the jewel wasp, we know essentially nothing about the mechanisms by which the trematode infection of ants leads to the distinctive biting behavior into grasses (*D. dendriticum*) or the resting behavior on grass (*D. hospes*). Both modes of manipulations likely increase the chances of ingestion by an herbivore in which the parasite completes its life cycle (52). What we do know is the position of the parasite in the brain. As mentioned above, *D. dendriticum* occupies the ventral side of the subesophageal ganglion, in close contact to the origin of the mandible nerves, whereas *D. hospes* is found near the antennal lobes. Although we do not know how the manipulation occurs, the presence of two similar yet distinct modes of manipulation apparently requires the parasite to take up different locations. In this system, the parasite presumably releases some chemical that affects the action of these brain regions.

These different organisms (wasp and trematode) show how parasites can directly affect the brain of their insect host. But what is perhaps surprising is that a direct action on the brain is not the default mode of action in cases where parasites have evolved to control host behavior. Although tallying up examples is premature, it is likely true that direct manipulation of the insect brain happens in only a minority of examples where parasites adaptively manipulate insect behavior.

Changes in Biogenic Amines

As the case studies above highlight, parasites can affect behavioral change in their hosts without entering the brain or even entering the body (such as the inducement of ants to become bodyguards to the lycaenid caterpillars) (**Figure 1**). This is emerging as one of the most interesting observations in the field of parasite control of host behavior and clearly an area where we need considerably more detail at the proximate level. The picture arising from different studies is that altered behaviors can be due to changes in biogenic amine profiles. One of the extensively studied manipulations of host central nervous system is that of the behavioral change in *M. sexta* larvae parasitized by the braconid wasp *C. congregata*. Briefly, the female wasp injects a cocktail of venom, a virus, and its eggs into the caterpillar (7). The wasp larva hatches in situ to continue developing by ingestion of nutrients from the host's hemolymph. When the wasps are ready to pupate, they perforate and exit through the host's cuticle and spin cocoons on the body of the host. They will later emerge as adults prior to the host death.

The most conspicuous behavioral change in the host occurs prior to the emergence of the wasp larvae. Spontaneous locomotion and feeding are reduced, leading up to host death. The decline in host feeding and locomotion appears to benefit the parasitoid by preventing the host from feeding on, or dislodging, the wasp cocoons. Ablation of the subesophageal head ganglion in parasitized hosts restores locomotion, suggesting that the wasp larva interferes with this ganglion known to be involved in the initiation of locomotion. Correlated with a decline in feeding, octopamine concentration increases in both cerebral ganglia (the brain and SEG) and hemolymph of parasitized *M. sexta*. Octopamine appears to be a potent neuromodulator of feeding, as injections of this neuromodulator decrease feeding in nonparasitized *M. sexta*, and this can be associated with abnormal activity in the frontal ganglion (48). What causes this increase of octopamine is unknown. A recent study suggests that the exiting wasps induce a stress response involving cytokine and, as a result, an increase in octopamine levels (2). Such increase in otopamine levels has been reported in insects experiencing stress (49). The increase in host octopamine concentrations alone is unlikely to account for the complete behavioral phenotype observed in the postemergent host. And, in fact, other neuromodulators are affected during parasitism. For example, dopamine concentration increases in the hemolymph just prior to wasp emergence (2). Interestingly, in cockroaches stung by the jewel wasp, the activity of thoracic octopaminergic neurons is decreased (59). The spontaneous firing rate of these neurons in stung and brainless animals is approximately 20% that in control

animals. The alteration in octopaminergic neuron activity could be part of the mechanism by which the wasp induces a change in the behavioral state of its prey but also affects its metabolism by reducing the potent glycolytic activator fructose 2,6-bisphosphate in leg muscles.

Dopamine is the biogenic amine that is altered during the manipulation of ant behavior by lycaenid caterpillars (25, 29). In this example of behavioral manipulation, ants that consumed the secretions of the caterpillar are less likely to move away from the caterpillar and more likely to be aggressive. This benefits the caterpillar, as without its retinue of bodyguards, it is more likely to be predated upon or attacked by parasitic insects that would lay eggs inside its body. Since these caterpillars (and many other insects) provide ant bodyguards with sugar secretions, why is it necessary to lace the sugar with some substance to make ants more likely to stay close and act more aggressively? The reason is probably because during certain times of the season, the abundance of sugar rises in the environment and ants could obtain it without the effort of tending a caterpillar. An asymmetry arises in the mutualism between the caterpillar and the ant such that the former requires the latter, but not the reverse. The upshot of this is that caterpillars can affect dopamine levels in ants' brains in a specific manner, as the other surveyed biogenic amines (serotonin, tyramine, and octopamine) are unchanged (25).

It could be that, in some cases, the increase in biogenic amines is probably due to the production of the compound directly by the parasite. As mentioned for the jewel wasp, its venom contains dopamine, which is responsible for inducing grooming in the cockroach host before egg laying (69). It is tempting to speculate that the hidden goal of such a manipulation is to coerce the cockroach host into getting rid of cuticular pathogens to provide a clean and "sterile" surface for the wasp egg.

Protein and Small Molecule Changes in the Host Brain Without Apparent Physical Changes to the Brain

Similar to studies on changes in biogenic amines, a number of studies demonstrate changes in proteins and metabolites of the host brain without any apparent physical changes. If such physical changes exist, they have not yet been investigated and/or such changes are subtle enough to require a thorough investigation before they can be determined. For *M. sexta* larvae parasitized by the braconid wasp *C. congregate*, distinct changes in biogenic amines are accompanied with neuroendocrine changes exemplified by accumulation of neuropeptides in the cerebral neurosecretory system (70). These neuroendocrine changes are evidenced by an increase in immunohistochemical staining for 9 of the 10 different peptides examined in parasitized *M. sexta*. One peptide, proctolin, shows an increase in staining at about the same time as the host's behavior changes. This issue has been further explored in a similar host–parasite association, the moth *Plutella xylostella* and the braconid parasitoid wasp *C. vestalis*. Parasitism alters expression of the host genes encoding neuropeptides (61). Using transcriptomics, 19 genes encoding 17 neuropeptides involved in fundamental aspects of the moth physiology were identified. Parasitism affected the expression of these genes, and the concentration of the molting hormone, 20E, is reduced in parasitized larvae (61).

The molting hormone is also important in the modification of spider web construction by ichneumonid wasps. Recall the parasitized spider webs resemble those normally built immediately before ecdysis, implying that a chemical substance that might be a molting hormone or a precursor chemical of this hormone was injected. Parasitized *Cyclosa* spider species exhibiting modified behavior present higher 20-OH-ecdysone levels than unparasitized individuals (37). Hence, such rises in ecdysone titer could manipulate the spider into manufacturing a natural yet modified web in preparation for ecdysis. In other web building modifications, the host is induced to build web

structures normally observed only in winter, and such building could be artificially induced by declining temperatures under experimental conditions (40). Chemicals produced by the wasp to induce such winter webs are unknown.

In the hairworm model, crickets are induced to commit suicide in water to promote emergence of the worm in a suitable aquatic environment (64). Biron and colleagues (8) used proteomics tools to identify the biochemical alterations that occur in the head of the infected cricket when it is driven to water by the worm parasite. They found differential expression of proteins in the head of the manipulated cricket specifically linked to neurogenesis, circadian rhythms, and neurotransmitter activity.

Physical Changes in Secretory Glands Associated with the Brain

In the strepsipteran system discussed previously, worker wasps that should remain on the nest to engage in colony tasks (building, foraging, brood care, and defense) leave the nest during the peak work time to take up positions in extranidal (out of the nest) aggregations (34). Early work clearly showed that the corpora allata of the wasp brain were highly reduced in parasitized individuals (62). The corpora allata secrete juvenile hormone affecting development of ovaries; this in turn determines whether a female wasp is functionally sterile or a reproductive individual that does not work on the nest (58). Recently, transcriptomic profiles revealed the expression of genes in the wasp brain, resulting in gene expression profiles of worker wasps that were similar to those of reproductives (20). As the reproductive wasps do not work in the nest as workers do, it is possible that the reduced volume of the corpora allata results in a sequence of changes. We still do not know what chemicals the larval strepsipteran produces to induce such changes. It is interesting that, in this system, the parasite enters the larval host (wasp) and thus goes through the complete development of the host from larva to adult (33). This of course offers the potential for affecting adult brain development at multiple stages of wasp development. It would be interesting to determine whether the parasite affects the maturing brain as the host goes from larva to adult, through complete metamorphosis, or the parasite waits until the brain is formed before affecting the corpora allata.

Viral Genomes to Manipulate Insect Behavior

There are a number of interesting examples of insect behavior being manipulated by viral genomes. Clear examples are baculoviruses that control lepidopteran larvae behavior, causing them to remain on exposed foliage of trees where they die, which increases the spread of virions from the insect cadaver (23). Such changes in behavior are attributable to viral genes that prevent the insect larvae from molting or induce hyperactivity. A series of studies have recently uncovered the viral genes involved (23). For instance, a single gene that controls molting in the insect was found for the altered behavior (26). The gene encodes an enzyme known as ecdysosteroid UDP-glycosyl-transferase (EGT); this inactivates insect host ecdysteroid hormones, preventing larval molting. Consequently, larvae continue feeding on leaves on which they die and liquefy, spreading virions onto other leaves and promoting transmission. The viral gene is a result of horizontal gene transfer approximately 70 million years ago and now prevents molting and pupation and permits a buildup of the viral population within the host (27). In other insect–virus parasitic associations, manipulation of host behavior is related to entry of the virus into the brain. For example, honey bees (*Apis mellifera*) infected with *Deformed wing virus* and its variant called *Kakugo* induced changes in learning and aggressiveness (16). (*Kakugo* is a Japanese word for "ready to fight.") Viruses are also striking, as they can apparently serve critical functions for parasites infecting insects.

The previously mentioned parasitic insect *D. coccinellae*, which causes a guarding behavior in its ladybug host (*C. septempunctata*), apparently achieves its behavioral control in collaboration with a viral mutualist, DcPV (13). The larval-stage parasite contains the virus, and just before the larva leaves the host to pupate (and benefits from the bodyguard behavior), it experiences a massive increase in viral replication. This virus enters the brain and specifically glial cells, where it induces atrophy and aberrant behavior in the ladybug host (13).

INTEGRATED APPROACHES TO NEUROPARASITOLOGY

So far, we have examined some case studies and emphasized spatial interactions between host insect and parasite that control host behavior. Case studies are important, but it is critical that they be presented in a synthetic framework if we are to uncover common rules of manipulation. Seeing many studies beginning to decipher the mechanisms responsible for such coordinated behavioral changes in hosts has been encouraging. However, it is beginning to look like a series of case studies where the method of behavioral manipulation depends on the type of host–parasite interaction being considered. This perhaps helps us determine how likely it is that we can answer our first question posed in the introduction: Are there are a limited number of pathways in the brain of insects that can be controlled? In this respect, neuromodulatory systems, and in particular biogenic systems, seem to occur frequently when investigating the interaction between a parasite and its host. The reason is that the behavior of all animals is the outcome of the orchestrated activity of large assemblies of neurons in the central nervous system. Such assemblies can be recruited by chemical compounds known as neuromodulators and neurohormones (43) produced by a single neuron or small group of neurons and released into the central nervous system or periphery, where they affect larger assemblies of neurons to control behavior. To affect such large assemblies, neuromodulators and neurohormones are not necessarily released at synaptic sites, but, importantly, they often act through secondary messengers (neuromodulators) or directly on genes (neurohormones), often modifying gene expression to produce long-lasting effects. As such, they are highly suitable targets for the parasite that often aims at modifying host behavior on long time scales. The idea of chemical coding of specific behaviors raises the possibility that these parasite-induced alterations in host behavior result from changes in the activity of such neuromodulatory systems, as exemplified in several parasite–host associations described in this review. What we can see from these emerging studies into the proximate mechanisms is that behavioral manipulation does not even require a direct control of the brain and the secretion of chemicals into the body cavity can affect key processes, such as molting, which in turn affect host behavior and ultimately transmission (e.g., of baculoviruses). Where the brain is involved, we can see how physical changes in regions, changes in biogenic amines, and changes in protein levels all occur, depending on the system. Speculating on the answer to our first question is still perhaps premature, but so far it does not seem as if there are a limited number of pathways in the brain of insects that can be controlled. Where control of host behavior involves alteration of the muscular system or the immune system, we might find that the number of options is reduced. This could reflect the greater complexity of the nervous systems, which after all encode a very broad diversity of behaviors in insects. Moreover, if not directly injected in the host central nervous system, for any manipulating compounds secreted by the parasite to reach the central nervous system (in particular the brain of the host), it must face the challenge of crossing the blood–brain barrier. Whether this is achieved by diffusion through the ganglionic sheath, active transport, or direct injection, we propose two pathways by which such compounds may affect central neuronal circuits. The first is to use existing neurochemical pathways (hormones or neurotransmitters) and increase their concentrations directly (by injection) or by stimulating/inhibiting secretions

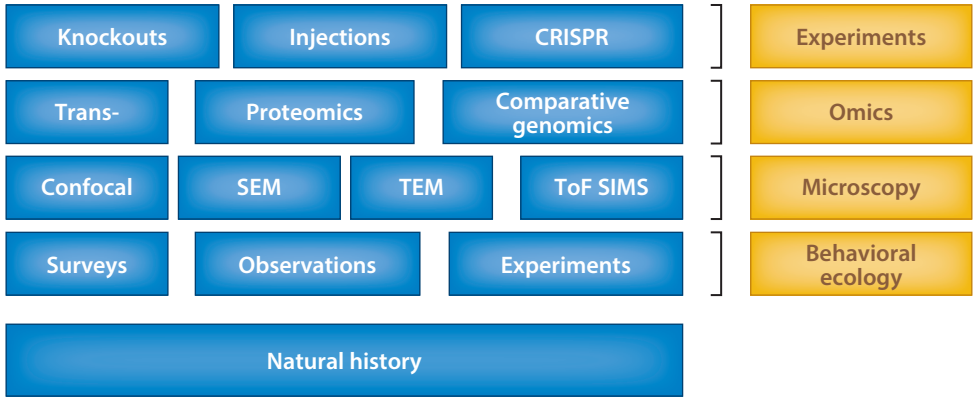

Figure 3

The integrated approach to neuroparasitology. At the base is natural history, where the phenomena of parasites affecting behavior are studied. Resting on this foundation is behavioral ecology, microscopy, omics, and experiments. In blue is a nonexhaustive list of approaches within each of these approaches. Abbreviations: CRISPR, clustered regularly interspaced short palindromic repeats; SEM, scanning electron microscopy; TEM, transmission electron microscopy; ToF SIMS, time-of-flight secondary-ion mass spectrometry.

or directly blocking receptors. The first neuronal manipulation occurs in the extracellular space. The second pathway is to manipulate the neuronal biochemistry via signal transduction pathways and/or gene expression. The latter may require access to the intracellular space.

We suggest that future studies should ideally integrate across different approaches from behavioral studies in ecologically realistic settings to histology, omics, and manipulative experiments where candidate compounds, genes, or modes of action are tested experimentally (**Figure 3**). For example, knockout experiments with baculoviruses and subsequent rescue work were critical for highlighting a gene (*egt*) responsible for the altered behavior (26).

CONCLUSIONS

We opened this article with two questions. First, are there a limited number of ways a parasite can control the behavior of insects that infect? And second, do we gain fundamental insights into neurobiology by studying the parasites that have evolved to control behavior? We have suggested that there are likely diverse ways parasites can control the behavior of insects and that understanding these processes provides fundamental insights into the neural basis of behavior. It is clear that providing a comprehensive picture of how parasites influence host behavior will require the integration of genomic, proteomic, and neurophysiological perspectives. It is also crucial to keep the natural history and organismal biology front and center in these investigations. The phenomena are, after all, what attract us to parasites that control insect behavior. Taken together, clear questions, integrative approaches, and natural history will allow us to discover these new vistas in the neuroparasitology of parasite–insect associations.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

This work was supported by an NSF IOS-1558062 award to D.P.H. F.L. is grateful to his long-term collaborator, Michael Adams of the University of California, Riverside, for his and colleagues' involvement in the omics of the jewel wasp project. The most recent work performed on the jewel wasp and described in this review is currently supported by the United States-Israel Binational Sciences Foundation (2015161). The experiments performed comply with Principles of Animal Care, NIH publication no. 86-23, revised in 1985, and with the current laws of the State of Israel.

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