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The Parasite *Moniliformis moniliformis* Alters the Escape Response of its Cockroach Host *Periplaneta americana*

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*The immature stages of some parasites live in prey animals (intermediate hosts) and only reach reproductive maturity when they are eaten by final host predators. Some of these parasites alter intermediate host behavior in ways that increase the likelihood of predation (parasite transmission). Using the acanthocephalan (*Moniliformis moniliformis*) in the cockroach (*Periplaneta americana*), we show that infected cockroaches experienced a decrease in wind-evoked escape responses, a predator avoidance behavior, that consisted of fewer escape responses, longer latency and higher threshold for escape behavior. We found no correlates of the impairment of the escape behavior in the abdominal portion of the escape neuronal circuitry. This study demonstrates a parasite-induced alteration of a behavior dedicated to predator avoidance.*

KEY WORDS: escape; parasite; cockroach; acanthocephalan; giant interneurons.

INTRODUCTION

Parasitized animals may exhibit alterations in phototaxis, activity levels, substrate preference, thermal preference, foraging behavior, humidity pref-

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erence and a variety of social interactions, to name a few (see Moore and Gotelli, 1990, for review). However, very few altered behaviors can be unambiguously linked to enhanced transmission (but see Molyneux and Jefferies, 1986), especially where intermediate hosts and predation are concerned. The worm-parasite *Moniliformis moniliformis* has a two-host life cycle. It lives as an adult in the intestine of a rodent, and sheds eggs with host feces. When consumed by a cockroach, the egg hatches and the young worm penetrates the cockroach intestinal wall. It matures to an infective stage called a cystacanth in the cockroach hemocoel, and infects a rodent when the cockroach is consumed.

The escape response of the cockroach is a well-studied behavior with an unambiguous function. In this response, the displacement of wind-sensitive hairs on the cerci, two appendages located on the rear-end of the cockroach, results in activation of gaint interneurons that ultimately effect turning away and running from the source of a slight wind puff, the sort that can be caused by the approach of a predator (Ritzmann 1993). This response has been shown to be important in predator avoidance (Camhi 1984). Any disruption of the escape response would likely have only one outcome—reduced escape from predators. With this in mind, we compared the escape responses of uninfected male *P. americana* to those of conspecific males infected with *M. moniliformis.*

Male cockroaches *Periplaneta americana* that were raised in plastic barrels on a cat chow and water diet were exposed to acanthocephalan eggs using the methods of Gotelli and Moore (1992). Cockroaches were randomly allocated to one of two treatments—exposed or unexposed. Within each treatment, cockroaches were placed in one of at least two containers. After a period of food deprivation, the groups within each treatment were merged, thus disrupting any possible feeding hierarchies, and given applesauce; in the case of exposed cockroaches, this contained acanthocephalan eggs. After exposure to the infected applesauce for one month, the escape behavior of infected and uninfected animals was tested. Immediately following both behavioral and physiological experiments, all animals were dissected and cystacanths were counted.

For the behavioral experiments, cockroaches were pinned through the lateral parts of the abdomen dorsal side up onto a small wax platform coated with vegetable oil. In such a position, cockroaches were able to move their legs in bouts of virtually friction-free ''walking'' and ''running.'' A wind simulator produced repeatable wind puffs of defined velocities delivered from a fixed distance and directed at the resting cockroach's cerci. Wind puffs were delivered to the cerci when the cockroach was standing still, roughly 200–500 msec after a short bout of ''walking'' elicited by a light touch on the metathoracic leg. After a short bout of walking, cock-

roaches are known to respond reliably on most trials (Camhi and Nolen, 1981). The threshold is defined as the wind velocity at which 50% of the trials elicited escape responses. A photocell monitored the movement of the left mesothoracic femur (Fig. 1A). Each animal was exposed to 10 wind stimuli at 1.5 m/sec and the average latency (time between stimulus and leg movement) was calculated. To determine threshold, each cockroach was given two wind stimuli at each of several (increasing) wind velocities; threshold was defined as the velocity at which escape was initiated both times. Finally, we delivered a series of 10 wind stimuli (wind velocity: 1.5 m/sec) at 60-sec intervals and counted the number of escape responses.

For physiological experiments, we measured the response of the giant interneurons. Details of the preparation and recording techniques have been described in Libersat (1992). Briefly, the animal was anesthetized, pinned down on a recording platform, and its abdominal nerve cord was exposed and bathed with cockroach saline during the experiment. To increase the resolution of our giant interneuron spike sampling, we separated the left from the right hemiconnectives of the nerve cord between fourth and fifth ganglia and placed each hemi-connective on a pair of hook electrodes at approximately midway between the two ganglia. Ten wind puffs were delivered at the cerci with a peak velocity of 1.5 m/sec and at 20-sec intervals to prevent the sensory input to giant interneuron synapses from habituating. In all the preparations, such wind puffs elicited a robust burst of giant interneurons spikes. The average total number of spikes and the average latency to the first spike was calculated for each preparation. These parameters were measured and averaged in five infected and five uninfected animals.

Recordings of electrical signals were stored on video tape (Data Neurocorder), digitized with a NB.MIO.16 analog to digital board (National Instruments) and analyzed with a data acquisition system featuring a window discriminator function for spike counting (Spike Studio; Eli Meir). All data were analysed with Mann-Whitney *U*-tests: for latency and threshold measurements, averages of score for each of the two wind puff conditions were submitted to Mann-Whitney *U*-tests. If a treatment effect occurred, we expected an impaired escape response; therefore, we used one-tailed tests.

The behavioral data showed clear effects of infection on a variety of escape response attributes. The wind puff stimulus delivered to the cerci of an uninfected animal typically elicited an escape response (Fig. 1A) lasting a few seconds (range 1.6–7.3 sec) with a very short latency. In an infected animal, the same stimulus resulted in an escape response that often lasted for less than a second (range 0.6–2.3 sec) with increased latency. In addition, the high stepping rate at the onset of the escape response was significantly faster in uninfected animals (range 12–20 steps/sec) than in

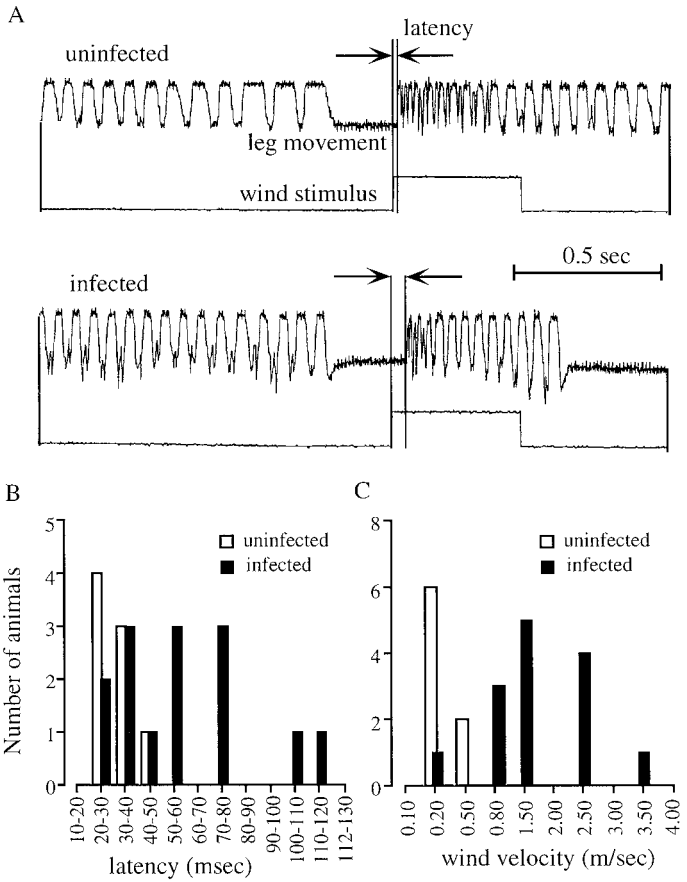

Fig. 1. Comparison of the wind-evoked escape response in tethered infected and uninfected cockroaches. A. Escape response of uninfected cockroach (top) and infected cockroach (bottom). For each panel, top trace is the recording of leg movement and bottom is the wind stimulus. Each downward deflection in the top trace corresponds to one step cycle of a middle leg. The wind stimulus is always delivered after a brief bout of stationary walking (see methods). Note the greater latency and shorter duration of the escape response in infected cockroaches, as well as the lower initial stepping rate. B. The latency of escape response for infected animals (black bars; $n = 14$) is significantly longer than that of uninfected animals (open bars; $n = 8$). C. Threshold of escape response is significantly higher in infected animals (black bars; $n = 14$) than in uninfected animals (open bars; $n = 8$).

infected counterparts (range 2–10 steps/sec; $P < 0.05$). With supra-threshold stimuli (1.5 m/sec) uninfected cockroaches exhibited a significantly lower ($P < 0.05$) latency (mean ± SD: 38.1 ± 1.8 msec; range 25–35, $n = 8$) than infected animals did (mean ± SD: 50.9 ± 30 msec, range 27–115, $n = 14$; Fig. 1B). The behavioral threshold for the escape behavior is defined as the velocity of the wind that elicits this behavior (Fig. 1C). The wind velocity that elicited escape responses from uninfected animals (mean ± STD: 0.35 ± 0.2 m/sec, range 0.2–0.5, $n = 8$) was significantly ($P < 0.05$) lower than that eliciting this behavior in infected ones (mean ± SD: 1.35 ± 1.6 m/sec; range: 0.2–3.5 m/sec, $n = 14$, Fig. 1C). Thus, acanthocephalan infected animals had significantly higher escape thresholds. In addition, uninfected animals exhibited a significantly higher percentage of escape responses ($P < 0.05$) to wind puffs (90 ± 14; range 80–100, $n = 8$) than did infected cockroaches (55 ± 7; range 10–70; $n = 14$).

Subsequent to observing behavioral differences between infected and uninfected cockroaches, we measured the response of the giant interneurons in the same individuals. We found no statistically significant difference ($P = 0.673$) in the mean latency of giant interneurons' action potentials between the infected (6.3 ± 2 msec) and the uninfected (5.8 ± 0.8 msec). Likewise, we found no significant difference ($P = 0.805$) in the mean number of giant interneuron action potentials between the infected (85.2 ± 23.1) and the uninfected (82.6 ± 12.8).

Although the alteration of host behavior by parasites is a widespread phenomenon, underlying mechanisms are often poorly understood. However, it is well-known that parasites may have profound effects on host reproduction and development (Beckage, 1985, 1991; Hurd, 1990; Thompson, 1990; Thompson and Kavaliers, 1994; De Jong-Brink, 1995) by affecting the endocrine or neuroendocrine system.

Clearly, all behavior is intimately associated with neuronal function, and some parasites are known to influence the nervous system directly through compounds that affect that function. Kavaliers and Colwell (1992, 1994) have investigated the role of opioids in parasitized mammals. They have discovered that internal parasites such as coccidia and schistosomes may actively produce opioids and related neuropeptides and may influence the endogenous opioid activity of the host. Opioid levels affect social interactions, reproductive behavior, and general locomotion. Parasitized rodents display decreased wariness of predators and increased opioid-related analgesia that may vary over the course of the infection. Thompson and Kavaliers (1994) argue that such effects may be widespread and may explain many behavioral alterations in parasitized animals.

In our experiments, the escape behavior, which is certainly one of the best understood systems in terms of neural circuitry, is altered by an infec-

tive acanthocephalan parasite. This is especially notable because the parasite is trophically transmitted, and the escape response is unambiguously dedicated to predator avoidance. Moreover, these changes took place in cockroaches with relatively light (<10 cystacanths) infections. The escape response is modified in ways that probably increase predation risk for infected animals. Infected cockroaches took longer to respond to the stimulus, required greater stimulation, and exhibited fewer escape responses than infected ones. Whether these alterations in the escape behavior of cockroaches can be directly linked to an increase in parasite transmission to final host is currently under investigation.

Although we can not rule out the possibility that the parasites decrease the cockroach's stamina, infected cockroaches appear to perform like normal cockroaches during other behavioral tasks. For instance, infected and non-infected animals respond equally quickly to synthetic cockroach pheromone components (Carmichael *et al.,* 1993). Also the infection does not affect velocity of the cockroach's movements (Gotteli and Moore, 1992). In addition, the parasite does not damage any internal tissues and infected cockroaches live as long as noninfected ones.

We found no correlates of the impairment of the escape behavior in the abdominal portion of the escape circuit, i.e., the wind evoked response of the giant interneurons. Impairment in the escape circuitry could take place at several nodes in the escape circuitry such as, for example, at the giant interneuron to thoracic interneurons connections in the thorax. Cockroach escape behavior is modulated under specific behavioral contexts. For example, during grooming and quiescence, cockroaches are less likely to escape a wind stimulus and the escape latency increases (Camhi and Nolen, 1981; Watson and Ritzman, 1996). Unsuccessful escape responses to wind stimuli in these behavioral contexts have been associated with a decrease in the response of thoracic neurons of the escape circuitry with no change in response of the abdominal giant interneurons. These changes in the neuronal responses could be mediated by monoamines such as octopamine and dopamine which are known to enhance and serotonin to decrease synaptic efficacy between the giant interneurons and the thoracic interneurons (Casagrand and Ritzmann, 1992). The mechanism by which the thoracic portion of the escape circuit might be impaired in animals infected with *Moniliformis moniliformis* remains to be explored.

Although parasite-induced behavioral alterations are ubiquitous (Moore, 1993), we know little about mechanisms influencing them (Helluy and Holmes, 1990; Thompson and Kavaliers, 1994). An understanding of mechanisms would greatly assist us in placing such alterations in an evolutionary context that could then be used as a predictive framework for unstudied host-parasite associations. Cockroaches have proven to be

among the most fruitful subjects for neuroethological study, and the acanthocephalan-cockroach model may prove equally rewarding for the neuroethological study of host-parasite associations.

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