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Original article

Acquired infection with *Toxoplasma gondii* in adult mice results in sensorimotor deficits but normal cognitive behavior despite widespread brain pathology

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Abstract

Toxoplasma gondii is a ubiquitous intracellular parasite which chronically infects 30–50% of the human population. While acquired infection is primarily asymptomatic several studies have suggested that such infections may contribute to neurological and psychiatric symptoms. Previous studies in rodents have demonstrated that *T. gondii* infection does not just kill its host, but alters the behavioral repertoire of an infected animal, making it more likely that predation with occur completing the parasite life cycle. The aim of the present study was to evaluate the behavioral changes in C57BL/6 mice chronically infected with the avirulent *T. gondii* (ME49, a Type II strain), in a comprehensive test battery. Infected mice demonstrated profound and widespread brain pathology, motor coordination and sensory deficits. In contrast, cognitive function, anxiety levels, social behavior and the motivation to explore novel objects were normal. The observed changes in behavior did not represent "gross" brain damage or dysfunction and were not due to targeted destruction of specific areas of the brain. Such changes point out the subtle interaction of this parasite with its intermediate hosts and are consistent with ideas about increased predation being an outcome of infection.

Keywords: *Toxoplasma gondii*; Latent infection; Motor coordination; Cognitive tests; Social behavior; Parasitology

1. Introduction

The protozoan *Toxoplasma gondii* is a definitive parasite of cats which has as intermediate hosts all warm blooded animals, including humans [1]. Worldwide, approximately 2 billion people are chronically infected with *T. gondii* and it has been estimated that *T. gondii* chronically infects 30–50% of the human population [2]. Infection is found throughout the world in the tissues of food animals [2]. While infection with *T. gondii* has generally thought to be primarily asymptomatic in immune competent humans, recent studies have suggested that infection may contribute to the development of various neurological and psychiatric symptoms [3–10] and that negative outcomes resulting from initial or chronic infection may be under-diagnosed [10–13]. These studies in humans are, however, associative and cannot prove if infection causes the behavior or the behavior increases the risk of infection. There are, however, several lines of evidence in experimental

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animals that suggest that specific host–parasite interactions influence the behavior of intermediate hosts in important ways and that these behavioral changes increase the likelihood of horizontal transmission [14–16]. Systematic analysis of the behavioral outcomes of infection in intermediate hosts may have implications for understanding and controlling the transmission of the parasite and also for elucidating the host–parasite interactions in regulating the specific outcomes of infection.

T. gondii has several life cycle stages. The tachyzoite invades cells, replicates rapidly and results in dissemination of infection. The bradyzoite is found in latent infections as tissue cysts. The sporozoite is found in oocysts shed by cats and is the product of sexual reproduction. Infection can occur in intermediate hosts (including humans) due to the ingestion of tissue cysts, the ingestion of oocysts, or by congenital infection during acute infection in a pregnant host. There has been a clonal expansion of *T. gondii* lineages with three main lineages in the United States and Europe, Type I, Type II and Type III, which are defined by their ability to form cysts in mice (Type II and III) and by their acute virulence (i.e. Type I are lethal to mice during acute infection) [17].

Latent infection persists during the lifespan of the intermediate hosts by the formation of cysts in muscle, neurons and glia. In congenitally infected children, *T gondii* infection causes mental retardation, seizures and loss of vision. Reactivation of latent infection with the transformation of bradyzoites back into tachyzoites can occur in immune suppressed patients, such as individuals with AIDS, and can be fatal [18]. The central nervous system (CNS) is the most common site of such reactivation infections, resulting in Toxoplasmic encephalitis.

Any behavior in an intermediate hosts (i.e. rodents) that influences the likelihood of predation will increase the frequency of transmission to the definitive host (i.e. cats) facilitating completion of the sexual stage of the parasitic life cycle and the potential for recombination and reassortment of genetic loci [9,14,15,19,20]. Carnivorism of infected intermediate hosts, such as rodents, with subsequent oral-fecal transmission is thought to be the most prevalent route of transmission due to cats [21–23]. Several lines of evidence suggest that specific host–parasite interactions may alter the behavior of infected rodents and increase the risk of predation [14,15]. Rodents infected with *T. gondii* [10–12] show reduced fear specifically toward feline predators that does not generalize to non-feline predators [16,19,24], which may lead to an augmented rate of predation and multiplication of the parasite through an increased number of life cycles. Chronically infected adult rodents also show reduced anxiety and risk assessment [25]. Notably, the social withdrawal associated with sickness behavior in most rodents [26] is also not evident in rodents chronically infected with *T. gondii* [25,27].

Though several studies demonstrate behavioral changes in rodents infected with *T. gondii* the precise effects of adult-acquired, chronic latent infection are still unclear. Many studies have primarily examined the behavioral and pathological effects of congenital infection [27,28], and few studies have included behavioral assays in multiple behavioral domains. Furthermore, the parasite load, anatomical location of parasite stages and behavioral outcomes change during the course of the infection [29,30] and interpretations of behavioral outcomes in the acute phase of infection are complicated by the high levels of tachyzoites in critical peripheral tissues, such as muscle, heart and lungs. The aim of the present study was to evaluate the behavioral changes in C57BL/6 mice chronically infected with the avirulent *T. gondii* strain ME49 (a Type II strain), in a comprehensive test battery. The mice were tested 7–9 weeks after initial infection at a time when chronic infection has developed, as demonstrated by the presence of tissue cysts containing bradyzoites, and acute infection has resolved, as demonstrated by the absence of tachyzoites.

2. Materials and methods

2.1. Subjects and infection protocol

Experiments were performed in accordance with the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine. Eight-week-old C57BL/6 male mice (Jackson Laboratories) were infected with 10^3 *T. gondii* bradyzoites of the Type II ME49 strain diluted in 200 μL of PBS (20 infected mice) and injected intraperitoneally or were injected only with PBS (10 control mice). A total of 10 of the infected mice died within 3 weeks after initial infection during the development of chronic infection. Mice were then housed 5 animals per cage and behavioral testing began 7 weeks after infection. This time point was chosen for a variety of reasons. First, active parasite levels in critical peripheral organs (i.e. lungs) are absent by this time, making further deaths unlikely in surviving subjects. Second, parasite load in the brain is reasonably stable from this point in chronically infected mice [30]. The colony was documented to be free of a number of standard viral, bacterial, and parasitic agents including Mouse Hepatitis Virus (MHV), Epidemic Diarrhea of Infant Mice (rotavirus, EDIM), Theiler's Murine Encephalomyelitis Virus (TMEV, GDVII), Mouse Parvovirus (MPV1) (MPV2) (VP2) general (NS1), Minute Virus of Mice (MMV), Mouse Adenovirus I and II (MAD), Ectromelia (Ectro), Lymphocytic Choriomeningitis Virus (LCM), *Mycoplasma pulmonis* (*M. pulmonis*), Pneumonia Virus of Mice (PVM), Reovirus (Reo), Sendai Virus (Sendai), Mouse Cytomegalovirus (MCMV), Mouse Norovirus (MNV), Ectoparasites (mites) by microscopic evaluation of fur plucks and Endoparasites (pinworms) by cecal and colon float and anal tape test.

2.2. Behavioral assays

Mice were tested behaviorally starting at 7 weeks after infection in the open field, object placement, object recognition, balance beam, grip strength, gait analysis and functional observation battery, in that order.

2.2.1. Functional observation battery

This primary screen was based on the SHIRPA protocol [31]. This is a standard method that provides a quantitative