Published in final edited form as:
Microbes Infect. 2010 July ; 12(7): 528–537. doi:10.1016/j.micinf.2010.03.009.

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

Maria Gulinello[a,*], **Mariana Acquarone**[b], **John H Kim**[a], **David C. Spray**[c], **Helene S. Barbosa**[d], **Rani Sellers**[e], **Herbert B. Tanowitz**[b], and **Louis M. Weiss**[b]

[a]Behavioral Core Facility, Department of Neuroscience,1410 Pelham PkwyS K925 Albert Einstein College of Medicine, Bronx, NY 10461

[b]Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Forchheimer Building room 504, Bronx, NY, 10461

[c]Dept Neuroscience Department of Neuroscience,1410 Pelham PkwyS K840 Albert Einstein College of Medicine, Bronx, NY 10461

[d]Laboratório de Biologia Estrutural, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Av. Brasil 4361, 21041-361, Rio de Janeiro, RJ, Brasil

[e]Histopathology Core Facility, Department of Pathology,1301 Morris Park Avenue, Price Center/ Block Research Pavilion room 158, Albert Einstein College of Medicine, Bronx, NY 10461

Abstract

Toxoplasma gondii is a ubiquitous intracellular parasite which chronically infects 30 to 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 demonstated 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.

[*]**Corresponding Author** Behavioral Core Facility Albert Einstein College of Medicine of Yeshiva University Dominick P. Purpura Department of Neuroscience Rose F. Kennedy Center RM 925 1410 Pelham Pkwy S., Bronx, NY 10461 phone: 718-430-4042 fax: 718-430-8821 maria.gulinello@einstein.yu.edu .

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 to 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 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.

Toxoplasma 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 show 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 to 9 weeks after initial infection at a time when chronic infection has developed, as demonstrated by the presence of tissue cysts containing bradzyoties, 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 *Toxoplasma 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 at 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 behavioral and functional profile by observational assessment of mice. Assessments were divided into five behavioral domains: general activity/arousal, motor and autonomic, reflexes, sensory and general condition. The details of each observation and list of tests are summarized in table 1. Most observations used an ordinal scale such that 0-1 represented normal or average behavior, negative numbers indicated deficits, hypoactivity or absence of normal behaviors and positive

numbers indicate hyper-reactivity or higher incidence of abnormal behavior. Some tests could produce absolute, rather than ordinal values. Activity, for instance was assessed and number of grid crosses in 1 min. Table 1 (results) details these numerous assays and the units of measurement for each assay not employing an the ordinal scale.

2.2.2 Open field—The open field has been extensively validated, ethologically and pharmacologically in both mice and rats [32]. Behavior was recorded manually for 6 min 16 inch square acrylic white box. Exploration was assessed as the number of rears (defined as raising both forepaws off the ground, except when grooming) and general locomotor activity was scored as the number of grid crosses (defined when the animal crossed a grid past the midline of the body).

2.2.3 Object Recognition and Object Placement Tests—Recognition and spatial memory were assessed in the novel object recognition and placement tasks, respectively essentially as described previously [33]. These tasks utilize the innate tendency of rodents to preferentially explore novel objects and are similar to tests conducted in humans [34,35]. In the object recognition test mice were placed in the open field and allowed to freely explore two identical, nontoxic objects (e.g., plastic, glass, or ceramic items) and the duration of exploration of each object (in seconds) were recorded during a 3 min (trial 1). After a retention interval of 45 min, mice returned to the same open field for 3 min (trial 2) with one of the familiar objects and a novel object. The time spent exploring both the novel and familiar objects (in seconds) was recorded. Exploration of the objects was defined as any physical contact with an object (whisking, sniffing, rearing on or touching the object). These data can be represented an exploratory preference score (time spent exploring the novel object divided by the total time spent exploring both objects × 100). An exploratory preference score of 50% indicates chance performance, while scores higher than chance reflect intact memory. All the objects have been extensively validated previously to ensure that no intrinsic preferences or aversions exist and that the animals explore all the objects for similar durations (given the same trial duration).

Spatial memory was assessed in a similar way as described above. Mice were allowed to explore two identical objects for 5 min in the arena. High contrast visual cues were placed on the walls of the open field. After a retention interval of 15 min, mice were returned to the open field for 3 min with the objects in different placement within the box. Normal animals preferentially explore the displaced object. As in the object recognition task, an exploratory preference score of 50% indicates chance performance, while scores higher than chance reflect intact memory.

2.2.4 Balance beam—Motor coordination deficits were measured by the number of missteps and the latency to cross a round beam [36]. Before the test, all mice were pre-trained on a wide plank to encourage reliable crossing. The start side was brightly lit and the other site had a small, darkened chamber that contained a palatable food (chocolate cereal) to encourage the mice to cross. Mice were allowed to walk across two times so that they rapidly traversed the plank without reversals or stopping. Immediately after pre-training, animals were assessed on the test beam (2.5-cm diameter, 50-cm long).

2.2.5 Gait analysis—The footprint test was used to compare the gait of control mice with that of *T. gondii* infected mice. Before the test, mice received several pre-training trials during which they habituated to the runway (65-cm long, 8-cm wide, 15-cm high) to foster reliable crossing of the runway during the test. The start of the runway was brightly lit and the end contained a darkened enclosed chamber with Cocoa Krispies↗ cereal pieces. Immediately after the pre-training sessions, footprints were recorded on a white paper on the runway floor after applying non-toxic tempera paint to the paws. Front paws were painted red and rear paws blue. Mice were then allowed to walk down the runway, and 4 sets of footprints were collected for

each animal. The footprint patterns were analyzed using the program Image J (NIH). Stride length was measured from the middle of one rear paw to the middle of the next footprint of the same paw (see fig 3D). Foot drags were counted as a total incidence in the set of 4 tracks, and were identified as smearing either before or after foot placement. The number of missteps was also analyzed as a total incidence in 4 sets of tracks and stringently defined as paw misplacement within an identifiable normal stride. The number of misplacements was likely under-estimated due to these strict criteria.

2.3 Histopathology

Five controls and ten infected mice were humanely euthanized using CO_2 gas after the conclusion of behavioral testing. The brains were carefully removed and fixed in 10% neutral buffered formalin (Fisher Scientific). All brains were sectioned coronally and routinely processed to paraffin blocks. A 5 μm section of brain from each sample was taken at or close to 6 Bregma levels: 1.3 mm; 0 mm; −2.1 mm; −6.2 mm; −7 mm; 8.0 mm. Slides were routinely stained by hematoxylin and eosin and evaluated microscopically by a board certified veterinary pathologist (RSS). Brain samples were evaluated for the severity and distribution of inflammation, the numbers of cysts, tissue destruction/loss, edema, and cell death. Gross examination of muscle tissues demonstrated them to be normal. In our previous studies with *T. gondii* infection in C57BL/6 mice no pathology was seen in leg muscle tissue.

2.4 Statistical analysis

Data are displayed as means ± SEM. Statistical analysis was performed using JMP (SAS: Cary, NC) using ANOVA for all parametric data (open field, balance, beam, preference scores, gait etc). Welch's correction was applied to means testing of the gait analysis data, as the variances were not equal. For all ordinal data from the functional observation battery, statistical analysis was performed using an ordinal logistic model.

Two infected animals had severe enough ataxia that it was not possible to accurately or reliably determine strides, and these were excluded from the gait analysis data. These two animals were also unable to rear and explore the objects in the cognitive tests without falling, stumbling and or circling, and were also excluded from analysis in these data.

3 RESULTS

3.1 Pathology

All levels of the brain and brainstem had some minimal to moderate lymphohistiocytic, plasmacytic, and rarely neutrophilic meningoencephalitis with gliosis. The cerebrum was more significantly affected than the brainstem and the lesions, which included glial nodules and rarefaction of brain parenchyma. Lesions in the brain and brainstem were multifocal and random. Intact protozoal cysts (*T. gondii.)* were present multifocally throughout many sections and were generally intact and unassociated with an inflammatory or cellular reaction. The least affected region tended to be the cerebellar folia, which occasionally had areas of gliosis and white matter loss, but which were generally normal in appearance by hematoxylin and eosin staining.

3.2 Functional Observation Battery

When assessed in a primary function screen [31], mice infected with *T. gondii* exhibited the most robust differences compared to control mice in sensory, motor and general condition domains (see table 1) . Measures of activity and arousal were generally normal except for significant differences in positional passivity (the struggling response to a tail hold). Most reflexes were grossly normal. In the sensorimotor domain, *T. gondii*-infected mice exhibited

abnormal pelvic elevation, ataxia and poorer grip strength compared to controls. In addition, infected mice had deficits in visual placing, indicating poor visual acuity. Whisker mobility was abnormally low or absent in almost all T. gondii mice and infected mice exhibited a striking lack of orientation response to whisker stimulation. Body weight was lower in *T. gondii* mice, who also exhibited stereotyped behaviors, including retropulsion, tail dorsoflexion (Straub tail) and circling. When returned to the home cage, social behavior was monitored as normal (immediate exploration of, contact with or return to "huddle" with cage mates). All subjects immediately (under 20 sec) returned to cage mates and thus exhibited normal social behavior as measured by this assay (data not shown) consistent with previous reports of normal social interaction in chronically infected subject [25,27].

3.3 Motor Coordination

Motor coordination deficits were evident in both the balance beam assay and gait analysis. *T. gondii* mice had significantly more slips in the balance beam (fig 2A) and a significantly longer latency to cross the beam (fig 2B). It is notable that despite the motor difficulties, all infected animals continued attempting to cross the beam until they reached the end.

Analysis of gait revealed multiple abnormal measures, including higher incidence of mis-steps (putting a paw down in the middle of a stride, fig 3A), shorter stride length (fig 3B), and higher incidence of foot dragging (fig 3C) in the rear paws. The incidence of foot dragging in the front paws was not significantly higher in infected mice compared to controls (data not shown). In addition to differences in absolute measures of stride length, there was also significantly more variability in stride length in T. gondii mice compared to controls.

3.4 Open Field

Despite the normal activity/arousal scores in the comparatively short (1 min) test period in the functional observation battery, further open field testing did reveal significant differences in activity (Fig 4A) and exploration (Fig 4B) between *T. gondii* and control mice. Several infected mice exhibited bouts of circling, which also effectively decreases mean number of grid crosses, whereas none of the control mice exhibited this stereotyped behavior. However, analysis of data without the circling mice included still yielded significant differences between *T. gondii* and control mice (data not shown). Although infected mice had fewer grid crosses, this was not because of long periods of immobility that reduced the apparent mean activity, but rather infected mice continued exploration for the total test period. Thus the activity difference was likely to be due to speed and coordination of movement and not to longer durations of inactivity. Even the severely ataxic animals continued to ambulate for the entire test period.

The decreased rearing in infected mice is consistent with the almost total lack of whisking and lack of whisker orientation response demonstrated in the functional observation battery. Thus, though the animals are ambulating, they are doing so without the exploration (rearing, sniffing, whisking) that normally occurs in the open field.

3.5 Cognitive Function

Notably, despite the profound deficits in sensorimotor function, infected mice had comparatively normal cognitive function. Infected mice had normal spatial memory (fig 5A) and normal levels of novel object exploration (fig 5C) in the object placement test after a 15 min retention interval and normal recognition memory (fig 5B) and object exploration (5D) in the object recognition assay after a 45 min retention interval.

4 DISCUSSION

The protozoan parasite *T. gondii* remains as a chronic, latent brain infection throughout the life of the host and may affect host behavior after the acute phase of infection [37]. The decreased activity and exploration during first exposure to the open field reported here (fig 4) is consistent with previous studies in mice with chronic latent infection [29,38], but in contrast to studies reporting higher activity levels in congenitally infected mice or mice in acute phases of infection [39,40]. The lack of rearing and whisking would be consistent with host-parasite interactions that increase the risk of predation, as these (and olfactory) behaviors are a primary means of gathering information for a nocturnal species with poor visual acuity.

The motor coordination deficits reported here are also largely consistent with previous reports [37,41,42] and with the decreased levels of dopamine and other catecholamines reported in the brains of infected mice [43]. Cysts were found throughout the brain consistent with previous studies [24], however, we did not appreciate an increase in cyst density in the amygdalar structures [24]. There were pathologic changes in the cerebellum and in all cortical regions that regulate sensorimotor function. It is possible that skeletal muscle pathology [44] is a contributing factor to the deficits we demonstrate in the balance beam (fig 2) and the gait analysis (fig 3). However, myopathy alone would not explain the lack of whisking and the fact that foot dragging was specific to the hind limbs, nor the high incidence of stereotypies, such as circling, which are more likely to be of central nervous system origin. In previous studies we had not seen significant muscle pathology, nor has this been seen by other investigators [24,37]. In either case, the lack of motor coordination, including decreased stride length, indicative a slower speed of locomotion, would obviously be consistent with the risk of predation and with motor coordination deficits purported to occur in humans [10-12].

Although we found normal cognition (fig 5), some previous studies have demonstrated cognitive deficits in mice after infection with *T gondii* [45-47]. However, in these studies spatial learning was altered in a maze assay while memory was found to be intact [45,46]. Rodents have also been demonstrated to lose their fear of cat urine (cat kariomones), which is a change in an innate hard-wired behavior unrelated to learning or memory [24,48] and to have a decrease in learned fear response [48]. The object recognition and placement tasks essentially only assess memory, so these data are not necessarily inconsistent. In addition, lower locomotor activity and poor motor coordination in infected mice may confound interpretations in maze assays where latency to make a correct choice is one of the primary measures and where lower levels of maze exploration prevents learning. It is thus difficult to distinguish between performance deficits and slower rates of learning. Furthermore, these studies require food deprivation or restriction. Given the significant differences in body weight between infected and control mice, there may confounding effects due to food deprivation effects on stamina or metabolism in infected mice evaluated using a maze assay. There is also a possibility that the cognitive tests used here were simply not sensitive enough or appropriate to detect cognitive deficits should these exist in infected mice. However, we have previously detected cognitive deficits at even shorter retention intervals using these tasks in subjects with no visible pathology [49] or with focal pathology [50] and in various other rodents models . It is also remarkable, given the gross sensorimotor deficits, the lack of normal exploratory activity and the profound and widespread central nervous system pathology evident in infected mice that they should have normal spatial and recognition memory at any retention interval or would in fact perform the task at all. This reinforces our observations of the lack of normal sickness behaviors in these mice, including lethargy, reduced response to novel objects and social withdrawal typical of sick or distressed rodents.

Overall, these studies demonstrate the complex and subtle interactions that have occurred in the evolution of the relationship of *T. gondii* with its intermediate hosts. The parasite 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 facilitating the transmission of genetic information to subsequent generations of parasites. The changes in behavior are specific and do not represent "gross" brain damage or dysfunction, but display specificity. This specificity, however, is not due to targeted destruction of specific areas of the brain, but a subtle dysregulation of brain function. The mechanisms by which *T. gondii* alters behavior deserve further investigation and may involve mimicking of neurotransmitters, or alternations or nitric oxide levels in the brain resulting in the modulation of signaling pathways.

Acknowledgments

This work was supported by NIH Grants AI39454 (LMW) and NIH D43TW007129 (MA) and NCI P30CA013330 to RS

5 REFERENCES

[1]. Dubey JP. History of the discovery of the life cycle of Toxoplasma gondii. International Journal for Parasitology 2009;39:877–882. [PubMed: 19630138]

[2]. Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: from animals to humans. Int J Parasitol 2000;30:1217–1258. [PubMed: 11113252]

[3]. Henriquez SA, Brett R, Alexander J, Pratt J, Roberts CW. Neuropsychiatric disease and Toxoplasma gondii infection. Neuroimmunomodulation 2009;16:122–133. [PubMed: 19212132]

[4]. Yolken RH, Dickerson FB, Torrey E. Fuller. Toxoplasma and schizophrenia. Parasite Immunol 2009;31:706–715. [PubMed: 19825110]

[5]. Carruthers VB, Suzuki Y. Effects of Toxoplasma gondii infection on the brain. Schizophr Bull 2007;33:745–751. [PubMed: 17322557]

[6]. Flegr J. Effects of toxoplasma on human behavior. Schizophr Bull 2007;33:757–760. [PubMed: 17218612]

[7]. Webster JP, Lamberton PH, Donnelly CA, Torrey EF. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on Toxoplasma gondii's ability to alter host behaviour. Proc Biol Sci 2006;273:1023–1030. [PubMed: 16627289]

[8]. Prandota, J. Autism spectrum disorders may be due to cerebral toxoplasmosis associated with chronic neuroinflammation causing persistent hypercytokinemia that resulted in an increased lipid peroxidation, oxidative stress, and depressed metabolism of endogenous and exogenous substances. Research in Autism Spectrum Disorders In Press. 2009. Corrected Proof

[9]. da Silva RC, Langoni H. Toxoplasma gondii: host-parasite interaction and behavior manipulation. Parasitol Res 2009;105:893–898. [PubMed: 19548003]

[10]. Havlicek J, Gasova ZG, Smith AP, Zvara K, Flegr J. Decrease of psychomotor performance in subjects with latent 'asymptomatic' toxoplasmosis. Parasitology 2001;122:515–520. [PubMed: 11393824]

[11]. Flegr J, Klose J, Novotna M, Berenreitterova M, Havlicek J. Increased incidence of traffic accidents in Toxoplasma-infected military drivers and protective effect RhD molecule revealed by a large-scale prospective cohort study. BMC Infect Dis 2009;9:72. [PubMed: 19470165]

[12]. Yereli K, Balcioglu IC, Ozbilgin A. Is Toxoplasma gondii a potential risk for traffic accidents in Turkey? Forensic Sci Int 2006;163:34–37. [PubMed: 16332418]

[13]. Arendt G, Hefter H, Figge C, Neuen-Jakob E, Nelles HW, Elsing C, Freund HJ. Two cases of cerebral toxoplasmosis in AIDS patients mimicking HIV-related dementia. J Neurol 1991;238

[14]. Lyte, M.; Gaykema, RPA.; Goehler, LE.; Moselio, S. Encyclopedia of Microbiology. Academic Press; Oxford: 2009. Behavior Modification of Host by Microbes; p. 121-127.

[15]. Webster JP. Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. Microbes and Infection 2001;3:1037–1045. [PubMed: 11580990]

[16]. Lamberton PH, Donnelly CA, Webster JP. Specificity of the Toxoplasma gondii-altered behaviour to definitive versus non-definitive host predation risk. Parasitology 2008;135:1143–1150. [PubMed: 18620624]

[17]. Howe DK, Honore S, Derouin F, Sibley LD. Determination of genotypes of Toxoplasma gondii strains isolated from patients with toxoplasmosis. J Clin Microbiol 1997;35:1411–1414. [PubMed: 9163454]

[18]. Dubey JP. Strategies to reduce transmission of Toxoplasma gondii to animals and humans. Veterinary Parasitology 1996;64:65–70. [PubMed: 8893464]

[19]. Berdoy M, Webster JP, Macdonald DW. Fatal attraction in rats infected with Toxoplasma gondii. Proc Biol Sci 2000;267:1591–1594. [PubMed: 11007336]

[20]. Webster JP. The effect of Toxoplasma gondii on animal behavior: playing cat and mouse. Schizophr Bull 2007;33:752–756. [PubMed: 17218613]

[21]. Afonso E, Lemoine M, Poulle M-L, Ravat M-C, Romand S, Thulliez P, Villena I, Aubert D, Rabilloud M, Riche B, Gilot-Fromont E. Spatial distribution of soil contamination by Toxoplasma gondii in relation to cat defecation behaviour in an urban area. International Journal for Parasitology 2008;38:1017–1023. [PubMed: 18325523]

[22]. Afonso E, Thulliez P, Gilot-Fromont E. Transmission of Toxoplasma gondii in an urban population of domestic cats (Felis catus). International Journal for Parasitology 2006;36:1373–1382. [PubMed: 16989836]

[23]. Dubey JP. Comparative infectivity of oocysts and bradyzoites of Toxoplasma gondii for intermediate (mice) and definitive (cats) hosts. Veterinary Parasitology 2006;140:69–75. [PubMed: 16647212]

[24]. Vyas A, Kim SK, Giacomini N, Boothroyd JC, Sapolsky RM. Behavioral changes induced by Toxoplasma infection of rodents are highly specific to aversion of cat odors. Proc Natl Acad Sci U S A 2007;104:6442–6447. [PubMed: 17404235]

[25]. Gonzalez LE, Rojnik B, Urrea F, Urdaneta H, Petrosino P, Colasante C, Pino S, Hernandez L. Toxoplasma gondii infection lower anxiety as measured in the plus-maze and social interaction tests in rats: A behavioral analysis. Behavioural Brain Research 2007;177:70–79. [PubMed: 17169442]

[26]. Dantzer R. Cytokine, sickness behavior, and depression. Immunol Allergy Clin North Am 2009;29:247–264. [PubMed: 19389580]

[27]. Arnott MA, Cassella JP, Aitken PP, Hay J. Social interactions of mice with congenital Toxoplasma infection. Ann Trop Med Parasitol 1990;84:149–156. [PubMed: 2383095]

[28]. Hay J, Hair DM, Graham DI. Localization of brain damage in mice following Toxoplasma infection. Ann Trop Med Parasitol 1984;78:657–659. [PubMed: 6532335]

[29]. Hrda S, Votypka J, Kodym P, Flegr J. Transient nature of Toxoplasma gondii-induced behavioral changes in mice. J Parasitol 2000;86:657–663. [PubMed: 10958436]

[30]. Derouin F, Garin YJF. Toxoplasma gondii: Blood and tissue kinetics during acute and chronic infections in mice. Experimental Parasitology 1991;73:460–468. [PubMed: 1959573]

[31]. Rogers DC, Fisher EM, Brown SD, Peters J, Hunter AJ, Martin JE. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm Genome 1997;8:711–713. [PubMed: 9321461]

[32]. Wilson RC, Vacek T, Lanier DL, Dewsbury DA. Open-field behavior in muroid rodents. Behav Biol 1976;17:495–506. [PubMed: 788698]

[33]. Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. Exp Brain Res 1997;113:509–519. [PubMed: 9108217]

[34]. Kessels RP, Haan E.H. de, Kappelle LJ, Postma A. Selective impairments in spatial memory after ischaemic stroke. J Clin Exp Neuropsychol 2002;24:115–129. [PubMed: 11935430]

[35]. Salame P, Burglen F, Danion JM. Differential disruptions of working memory components in schizophrenia in an object-location binding task using the suppression paradigm. J Int Neuropsychol Soc 2006;12:510–518. [PubMed: 16981603]

[36]. Stanley JL, Lincoln RJ, Brown TA, McDonald LM, Dawson GR, Reynolds DS. The mouse beam walking assay offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by benzodiazepines. J Psychopharmacol 2005;19:221–227. [PubMed: 15888506]

[37]. Hermes G, Ajioka JW, Kelly KA, Mui E, Roberts F, Kasza K, Mayr T, Kirisits MJ, Wollmann R, Ferguson DJ, Roberts CW, Hwang JH, Trendler T, Kennan RP, Suzuki Y, Reardon C, Hickey WF, Chen L, McLeod R. Neurological and behavioral abnormalities,ventricular dilatation, altered cellular functions, inflammation, and neuronal injury in brains of mice due to common, persistent, parasitic infection. J Neuroinflammation 2008;5:48. [PubMed: 18947414]

[38]. Skallova A, Kodym P, Frynta D, Flegr J. The role of dopamine in Toxoplasma-induced behavioural alterations in mice: an ethological and ethopharmacological study. Parasitology 2006;133:525–535. [PubMed: 16882355]

[39]. Hay J, Hutchison WM, Aitken PP. Congenital Toxoplasma infection and response to novelty in mice. Ann Trop Med Parasitol 1983;77:437–439. [PubMed: 6639188]

[40]. Hay J, Hutchison WM, Aitken PP, Graham DI. The effect of congenital and adult-acquired Toxoplasma infections on activity and responsiveness to novel stimulation in mice. Ann Trop Med Parasitol 1983;77:483–495. [PubMed: 6660954]

[41]. Hay J, Aitken PP, Hutchison WM, Graham DI. The effect of congenital and adult-acquired Toxoplasma infections on the motor performance of mice. Ann Trop Med Parasitol 1983;77:261–277. [PubMed: 6625726]

[42]. Hutchison WM, Aitken PP, Wells BW. Chronic Toxoplasma infections and motor performance in the mouse. Ann Trop Med Parasitol 1980;74:507–510. [PubMed: 7469564]

[43]. Stibbs HH. Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. Ann Trop Med Parasitol 1985;79:153–157. [PubMed: 2420295]

[44]. Tonino P, Finol HJ, Marquez A. Skeletal muscle pathology in mice experimentally infected with Toxoplasma gondii. J Submicrosc Cytol Pathol 1996;28:521–526. [PubMed: 8933735]

[45]. Witting PA. Learning capacity and memory of normal andToxoplasma-infected laboratory rats and mice. Parasitology Research 1979;61:29–51. [PubMed: 543216]

[46]. Piekarski G. Behavioral alterations caused by parasitic infection in case of latent toxoplasma infection. Zentralbl Bakteriol Mikrobiol Hyg A 1981;250:403–406. [PubMed: 7197863]

[47]. Hodkova H, Kodym P, Flegr J. Poorer results of mice with latent toxoplasmosis in learning tests: impaired learning processes or the novelty discrimination mechanism? Parasitology 2007;134:1329–1337. [PubMed: 17445326]

[48]. Vyas A, Kim SK, Sapolsky RM. The effects of Toxoplasma infeciton on rodent behavior are dependent on dose of the stimulus. Neuroscience 2007;148:342–348. [PubMed: 17683872]

[49]. Li Y, Vijayanathan V, Gulinello ME, Cole PD. Systemic methotrexate induces spatial memory deficits and depletes cerebrospinal fluid folate in rats. Pharmacol Biochem Behav 2009;94:454–463. [PubMed: 19887080]

[50]. Gulinello M, Lebesgue D, Jover-Mengual T, Zukin RS, Etgen AM. Acute and chronic estradiol treatments reduce memory deficits induced by transient global ischemia in female rats. Horm Behav 2006;49:246–260. [PubMed: 16125703]

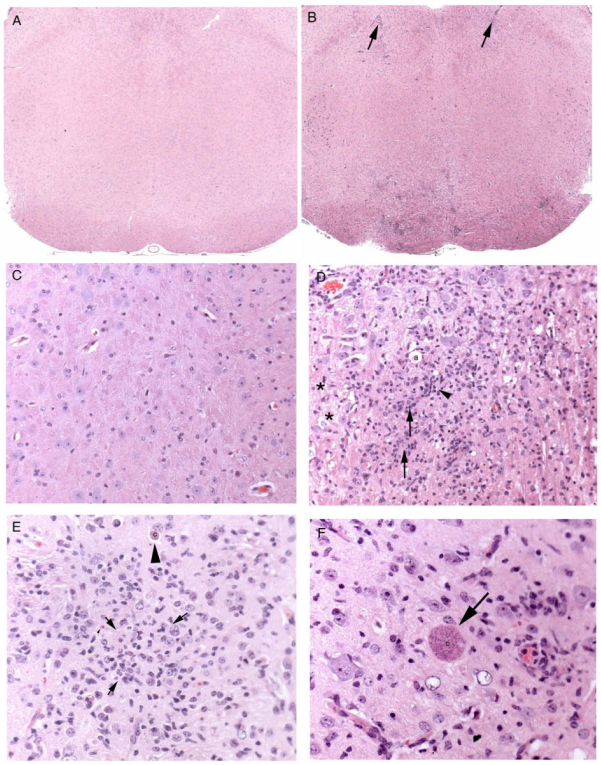

Figure 1.

Histological findings in brain stained with hematoxylin and eosin. **(A)** Normal brainstem (25x magnification). **(B)** Toxoplasma treated mouse brainstem. There is extensive lymphocytic perivascular inflammation (arrows) and focally extensive inflammation at the base of the brainstem (boxed area) (25x magnification). **(C)** Normal brainstem (200x magnification). **(D)** *T. gondii* infected mouse brainstem. There are nodules of glial cells (arrows), rarefaction of white matter (*), infiltrates of lymphocytes (arrow heads), and axonal swelling (a). (200x magnification). **(E)** *T. gondii* infected mouse cortex. There are glial cell aggregates (surrounded by arrows) and a protozoal cyst (large arrowhead) (200x magnification). **(F)** *T. gondii* infected mouse brainstem. There is a single large protozoal cyst (arrow) with no apparent inflammatory reaction (200x magnification).

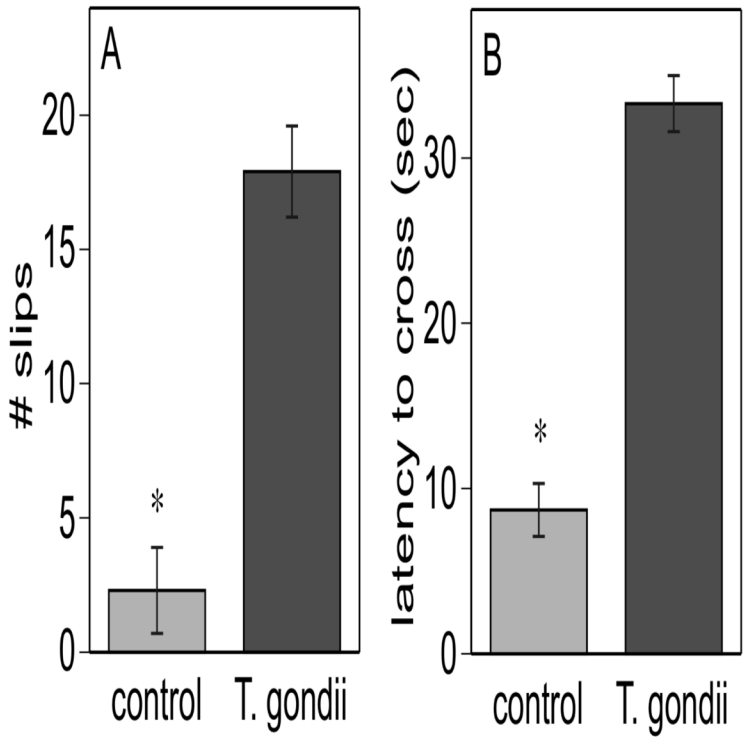

Figure 2.
Motor coordination deficits assessed as the number of slips **(A)** and the time to cross **(B)** a 120 cm long and 2.5 cm diameter beam. * indicates significant differences between *T. gondii* infected and control mice, $p < 0.001$.

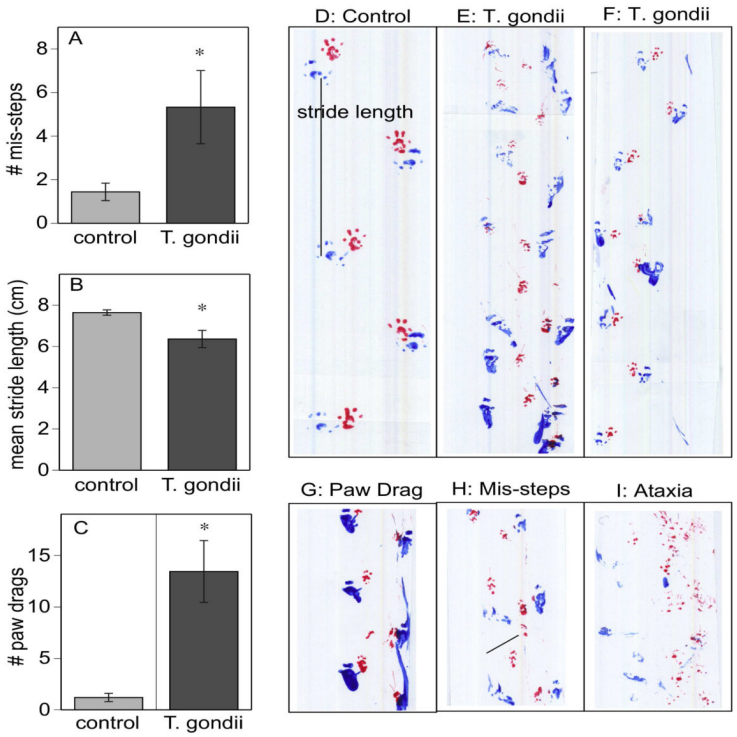

Figure 3.

Gait deficits assessed as the number of mis-steps **(A)** and the mean stride length **(B)** and the number of rear paw drags **(C)**. * indicates significant differences between *T. gondii* infected and control mice, $p < 0.05$. Representative footprints of a control mouse **(D)** compared to *T. gondii* infected mice **(E and F)** clearly show examples of the shorter and more variable stride pattern, paw misplacements, weaving gait and other abnormalities. Examples of foot drag **(G),** paw misplacement **(H)**, and gross ataxia **(I)** from *T. gondii* infected mice are also illustrated.

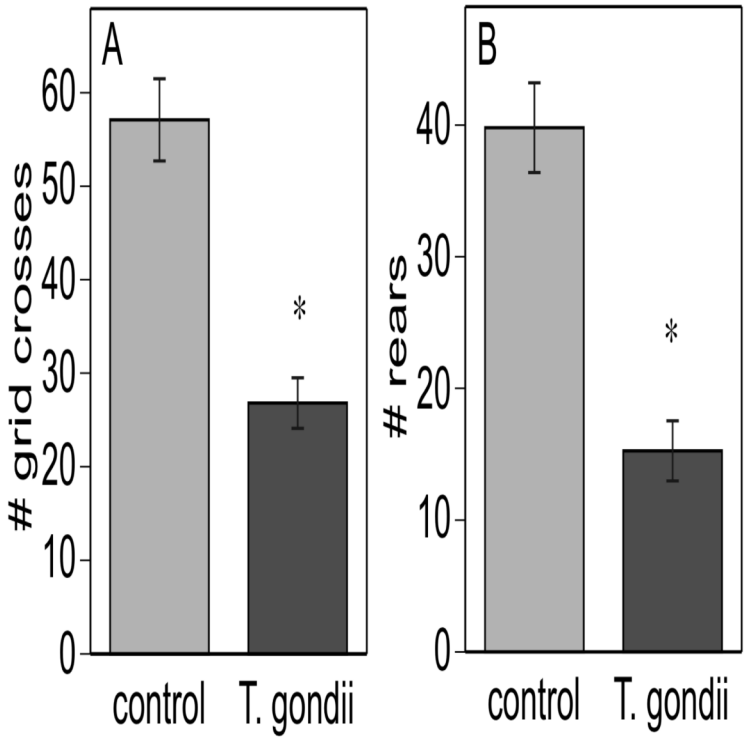

Figure 4.
Open field behavior in a 6 min test period indicates that *T. gondii* infected mice have lower levels of general locomotor activity **(A: grid crosses)** and exploratory activity **(B: number of rears)** and the number of rear paw drags. * indicates significant differences between *T. gondii* infected and control mice, $p < 0.005$.

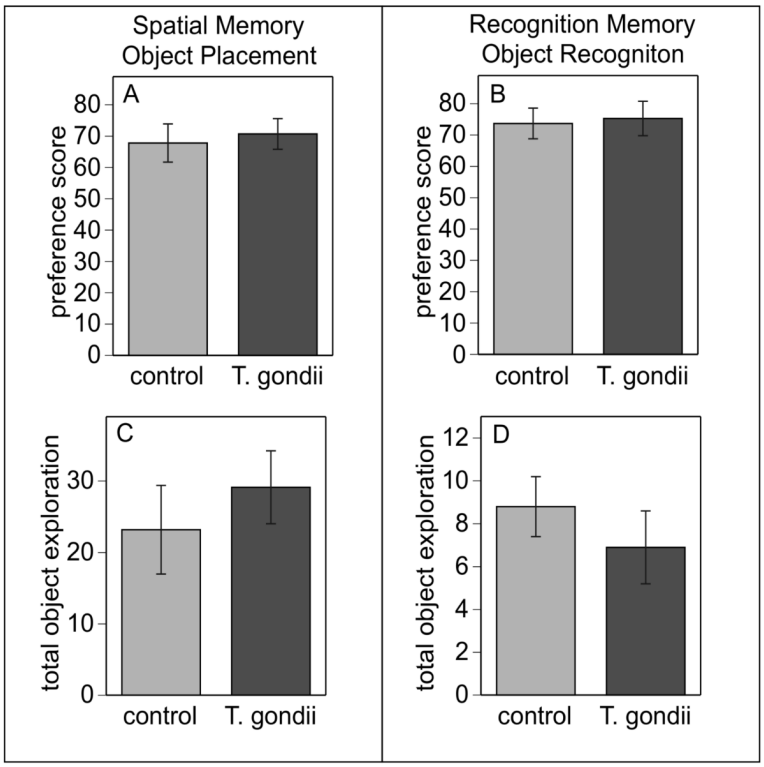

Figure 5.

Cognitive deficits were not evident in *T. gondii* infected mice (Toxo) in either spatial memory **(A)** or in recognition memory **(B)** in the novel object recognition and object placement tasks. Extent of object exploration (sec) was also normal during the trial 1 of each task **(D and E)** in both *T. gondii* infected and control mice

Table 1

Functional Observation Battery

Representative results from a primary screen indicate deficits in several behavioral domains in mice infected with *T. gondii*. An ordinal scale is used to categorize behaviors with normal defined as 0, hyopo-reactive behaviors or absence of normal behaviors as progressively negative numbers and hyper-reactivity or incidence of abnormal behaviors as positive numbers. Where absolute values could be measured (precise number of rears etc), those values and units are indicated in the table and are used in lieu of the ordinal scale. Probability (p) is based on statistical testing as described in the materials and methods section. NS indicates not significant.

	control		SE	T. gondii		SE	p
Activity / Arousal							
Transfer Arousal (latency to move after transfer, sec)	4.1	±	0.7	3.1	±	1.0	NS
Rears (# in 1 min)	6.2	±	1.4	2.7	±	1.4	NS
Activity (grid crosses in 1 min)	33.2	±	5.5	19.1	±	4.0	NS
Touch escape	1.2	±	0.2	0.7	±	0.2	NS
Positional Passivity	2.0	±	0.0	1.0	±	0.4	**0.02**
Sensorimotor							
Pelvic Elevation	0.0	±	0.0	−0.7	±	0.3	**0.02**
Gait	−0.6	±	0.2	−0.8	±	0.3	NS
Tail elevation	0.0	±	0.0	−2.0	±	1.6	**0.04**
Eye	0.0	±	0.0	−0.7	±	0.7	**0.009**
Ataxia	0.0	±	0.0	−1.5	±	0.6	**0.02**
Hind limb use	0.0	±	0.0	−0.9	±	0.4	**0.05**
Whisker mobility	0.0	±	0.0	−0.4	±	0.2	**0.05**
Grip (latency to fall from a wire in sec)	24.4	±	4.6	2.9	±	3.9	**0.005**
Reflexes							
Toe pinch	1.0	±	0.0	1.0	±	0.0	NS
Negative Geotaxis (latency to turn upright after inversion on a grid in sec)	1.55	±	2.3	7.5	±	2.1	NS
Startle	1.5	±	0.3	0.7	±	0.3	NS

	control		SE	T. gondii		SE	p
Visual Placing	1.4	±	0.4	0.0	±	0.5	NS
Whisker stimulation	0.0	±	0.0	−1.0	±	0.3	**0.005**
General condition							
Fur	0.0	±	0.0	−1.1	±	0.1	**0.001**
Stereotyped Behavior	−0.2	±	0.2	−1.4	±	0.3	**0.008**
Weight (g)	26.6	±	1.0	17.5	±	0.9	**0.001**