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TRANSIENT NATURE OF *TOXOPLASMA GONDII*-INDUCED BEHAVIORAL CHANGES IN MICE

Štěpánka Hrdá, Jan Votýpka, Petr Kodym*, and Jaroslav Flegr†
Department of Parasitology, Charles University, Viničná 7, 12844 Prague 2, Czech Republic

ABSTRACT: Many parasites induce specific changes in host behavior that promote the transmission of their infective stages between hosts. Toxoplasmosis in rodents is known to be accompanied by specific behavioral changes (shift in activity level, learning capacity, and novelty discrimination) that can theoretically increase the chance of infected animals being eaten by the definitive host, the cat. However, toxoplasmosis is also accompanied by many pathological symptoms. It is not known whether the behavioral changes are products of manipulation activity of the parasite or only nonspecific by-products of pathological symptoms of toxoplasmosis. Here, we compared the dynamics of development of behavioral and pathological changes in *Toxoplasma gondii*-infected mice. The results showed that the maximum reduction of mouse activity corresponded with the peak of pathological symptoms, and also that maximum increase of reaction times corresponded with the peak of development of tissue cysts in the brains of infected mice. Behavioral changes were only transient and disappeared before the 12th wk postinoculation. The results suggest that the behavioral changes in infected mice reported by many authors and observed in our experiments could be nonspecific by-products of pathological symptoms of toxoplasmosis rather than specific products of manipulation activity by the parasite.

Toxoplasma gondii is a coccidian protozoan with a dixenic life cycle (Hutchison, 1965). Sexual reproduction of the parasite is accomplished only in a definitive host, e.g., the cat or other felids. Any homeothermic animal can serve as an intermediate host. After the acute phase of infection (promoted by tachyzoites), tissue cysts (containing the population of bradyzoites) are formed mainly in the neural and muscular tissues of the intermediate host. The parasite is transmitted to the definitive host (and also to other intermediate hosts) by carnivory. It is usually supposed that such transmission can be facilitated by manipulation activity of the parasite through induction of behavioral changes in the intermediate hosts that lead to an increased probability of predation (Holmes and Bethel, 1972; Barnard and Behnke, 1990; Poulin, 1995).

Toxoplasma gondii is one of the classical models for the study of manipulation activity by parasites. Specific behavioral effects of latent toxoplasmosis have been demonstrated in numerous studies with laboratory mice and rats. Infected mice have impaired motor performance (Hutchison, Aitken, and Wells, 1980b; Hay, Aitken et al., 1983), deficit in learning capacity and memory (Witting, 1979), higher activity levels both in novel and familiar environment (Hutchison, Bradley et al., 1980; Hay, Hutchison et al., 1983; Hay, Aitken, Hair et al., 1984; Hay et al., 1985), and lower ability to discriminate between familiar and novel surroundings (Hutchison, Aitken, and Wells, 1980a; Hay, Aitken et al., 1983; Hay, Aitken, and Graham, 1984). Infected rats also have higher activity levels (Webster, 1994), lower neophobia (Webster et al., 1994), and reduced learning capacity (Witting, 1979). However, in contrast to other coccidia (Hoogenboom and Dijkstra, 1987; Voříšek et al., 1998), the adaptive nature of these changes in host behavior (influence on the probability of being captured by a predator) has not been tested in any predation experiment.

The fact that *T. gondii* cysts are predominantly localized in the brain tissue suggests that the behavioral changes could be nonspecific by-products of the relatively serious parasitic disease. Inflammatory responses in the host brain and encephalitis are common symptoms associated with acute or latent infection (Hunter et al., 1992; Suzuki and Joh, 1994). It is highly probable that such processes might induce a broad spectrum of behavioral changes. To decide whether the observed behavioral changes are nonspecific products of *T. gondii* pathological activities or products of manipulation activity of the parasite, it would be important to compare the dynamics of development of behavioral changes with development of toxoplasmosis-related pathogenic symptoms. If maximal intensity of behavioral changes coincides with the peak of symptoms of acute toxoplasmosis, induction of changes by nonspecific pathological processes would be the most parsimonious explanation of the phenomenon. On the other hand, if the behavioral changes are typical of the latent phase of *T. gondii* infection, manipulation activity of the parasite can be suspected. However, to date such studies have not been carried out.

The primary aim of the present work was to investigate and describe the dynamics of development of different pathological and behavioral effects of toxoplasmosis in laboratory mice to reveal whether the behavioral effects are the by-products of the pathogenic processes of acute toxoplasmosis or specific results of manipulation activity of the parasite. We monitored the health status of infected animals and performed a battery of ethological tests at different times postinfection (3, 6, and 12 wk). Our results suggest, that all behavioral changes observed in the infected mice have only a transient character. Therefore, it is likely that they are the by-products of the pathogenic processes of acute toxoplasmosis rather than specific results of manipulation activity of the parasite.

MATERIALS AND METHODS

Animals and infection

The F1 progeny of crosses between mouse inbred strains BALB/c (females) and C57BL/10J (males) was used. Animals were housed in groups of 4–5 mice in Plexiglas cages (36 × 20 × 16 cm) at room temperature 21 ± 1 C, 50 ± 10% relative humidity, and approximately 12-hr light/dark cycles. Water and standard pellet food were given ad libitum. The *T. gondii* was 01529/38 strain isolated from a pig in 1978 in Czechoslovakia.

Ten-week-old mice were inoculated perorally with a 0.5-ml brain

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* National Diagnostic Laboratory for Toxoplasmosis, National Institutes of Public Health, Šrobárova 48, 10042 Prague 10, Czech Republic.
† Corresponding author. Department of Parasitology, Faculty of Sciences, Viničná 7, 128 44, Prague 2, Czech Republic.

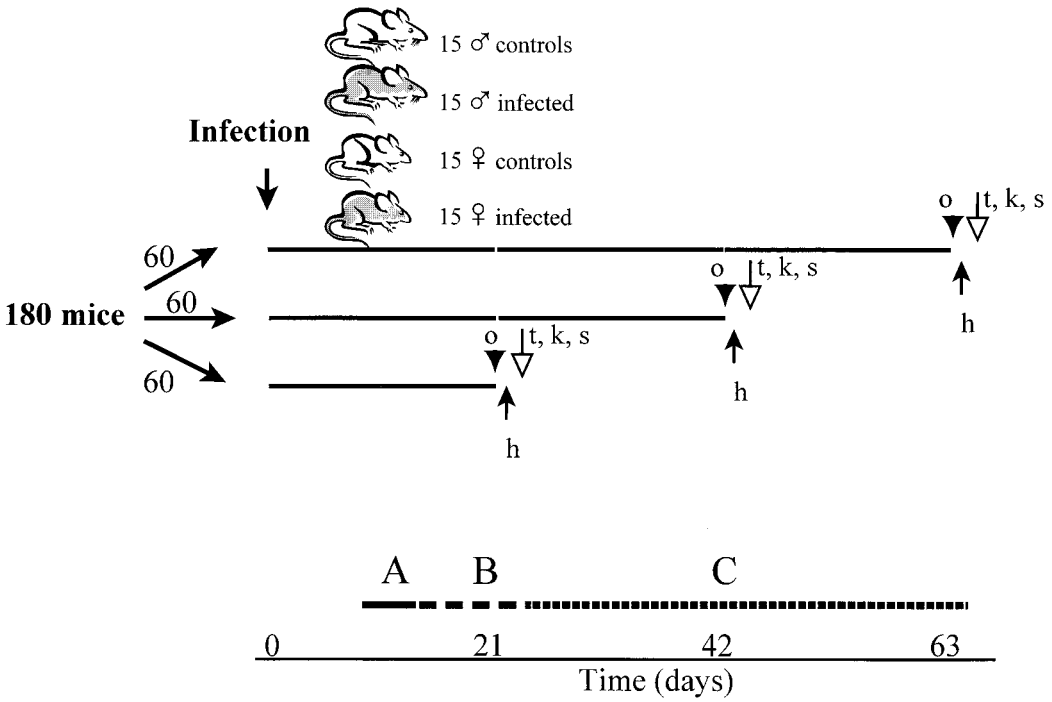

FIGURE 1. Experimental setup. o, open-field test; h, hot-plate test; t, tail-flick test; k, mice killing; s, serological test for anti-toxoplasma immunity. A, B, and C indicate acute, postacute, and latent stages of toxoplasmosis, respectively.

suspension in phosphate-buffered saline, pH 7.2 (PBS) (infected), or 0.5 ml of PBS (controls). The brain suspension was prepared from homogenized and pepsin-digested brains of mice infected 3 mo earlier. The suspension was diluted with PBS so that every experimental animal received the equivalent of 1 bradyzoite cyst. Mice were randomly divided into 3 groups that were tested 3, 6, and 12 wk postinfection (PI). Each group consisted of about 60 animals evenly divided into 4 groups (infected/controls, males/females); see Figure 1.

Experimental procedures

Mice were observed daily for signs of altered behavior and visual physical signs that might indicate that they were in discomfort or distress (lethargy, ruffled fur, shuddering, diarrhea). Their weight was recorded once a wk. The first test—open-field test—was assessed exactly 3, 6, and 12 wk PI, respectively. The modified hot-plate test was carried out 2 days after the open-field test, and the tail-flick test was done on the fourth and fifth day after the open-field test. All behavioral experiments were done between 1300 and 1800 hr during daylight in the spring of the year. After each observation (described below), the experimental apparatus was cleaned with ethanol. At the end of the experiments, all mice were examined serologically for anti-*Toxoplasma* antibodies by complement fixation test (Warren and Sabin, 1942). Two animals without the specific antibodies were excluded from statistical analysis. In 23 infected animals, randomly selected from all 3 groups, the number of *T. gondii* tissue cysts was counted in the brain suspension immediately after the last experiment. Suspensions were prepared from brains homogenized in PBS (final volumes 2 ml). The number of cysts was counted in 5 10-μl samples of suspension (magnification ×250).

Activity and exploratory behavior in open-field test

Activity and exploratory behavior of mice were observed in open field, i.e., in an empty glass box (60 × 45 × 40 cm) with the floor optically subdivided into 12 squares (15 × 15 cm). For a 5-min period, the following behavioral activities were scored: number of entered squares, rearing (mouse stands on hind paws with fore paws raised or placed on the box walls), jumping, time spent grooming, and time spent sitting without locomotor activity. Freezing (stiff motionless sitting) and jumping were observed only rarely; therefore, these activities have not been analyzed. The number of fecal boluses deposited (indirect indication of fear and anxiety) was recorded at the end of each experiment.

Monitoring of escaping behavior and antinociception in hot-plate test

The hot-plate test (Woolfe and MacDonald, 1944) is often used for measurement of antinociception. In this type of experiment, latency of the first fore-paw or hind-paw licking is recorded. We modified the apparatus so as also to allow measurement of latency of escape behavior—central nervous system response to adverse stimuli. A mouse was placed on the thermostated metal plate (40 × 20 cm) confined on 3 sides (2 shorter and 1 longer) and on the top (20 cm above the plate) with nontransparent walls. The plate was placed 12 cm above the floor of the glass box (60 × 45 × 40 cm). The plate temperature was 60 ± 0.5 C. Latency of fore-paw licking, latency of the first attempt of escape, and latency of leaving the plate (reaction time) were measured with a stopwatch. Every mouse was tested in 3 consecutive trials separated by a 1-min rest time period, and the latency was calculated as an arithmetic mean from all 3 trials. In case the mouse did not escape from the plate, it was removed after 60 sec.

In our modification of the hot-plate test, the animals can terminate the adverse stimulus by escaping from the plate, and the temperature of the plate was 60 C instead of the usual 55 C. With a lower temperature, many mice did not leave the plate. It has been shown by histopathological analysis that a temperature of 60 C causes no damage to the paw pads of mice left on a hot plate for 60 sec (Ankier, 1974). In our experiments there was no change in latency of fore-paw licking when performing 3 repeated measurements. This suggested an absence of any heat sensitization during the experiment. (The latency of leaving the plate decreased, however, and this was probably caused by the effect of learning.) There was no behavioral effect of the test after the experiment.

Monitoring of antinociception in the tail-flick test

Antinociception can also be measured by the tail-flick test (D'Amour and Smith, 1941). In our apparatus, the tail-flick response was elicited by applying radiant heat from a 40-W electric bulb to the tail. The latency of flicking was measured by digital timer connected to the ap-

paratus. The intensity of the heat stimulus (distance from the bulb) was adjusted with nonexperimental mice so that animals flicked their tail within 3–5 sec. Four tail-flick trials were conducted at 30-sec intervals. Mean latency was calculated from the last 3 trials of the 4-trial series.

Statistical methods

The Statistica® program was used for all statistical testing. An analysis of variance (ANOVA) was used to study the effects of toxoplasmosis (factor TOXO), gender (confounding factor SEX), and their interactions on mouse behavior. Because of a low number of animals (23), we used a nonparametric Kruskal–Wallis ANOVA for a study of difference in number of brain cysts in 3 experimental groups of animals. The latencies measured in the hot-plate test were log-transformed before the analysis to obtain a normal distribution of the data. Correlation between the behavioral traits and the actual weight of animals at the time of testing was examined by multiple linear regression. To exclude the gender effect, standard scores (Z scores) of a standard normal distribution were used instead of the original data. The Z score for every mouse was calculated as $Z = (w - W)/SD$, where w is the weight of the mouse, W and SD are average and standard deviation, respectively, of the weights of mice of the same gender and series (both infected and uninfected). Z scores for the behavioral traits (number of squares, time spent by sitting, number of rears, the first trial of latency of escape, and the mean of all 3 trials of latency of escape) were calculated similarly. All differences and correlations were examined by 2-tailed tests.

RESULTS

Body weight and health status

On day 14 PI, weight was significantly reduced in infected mice in comparison with controls (ANOVA, factors: TOXO and SEX, $F_{1,162} = 105.8$, $P < 0.0000$). There was a clear difference, however, in the dynamics of weight recovery between infected males and females (Fig. 2). Any difference between infected and uninfected females disappeared by 4 wk PI. The difference between infected and uninfected males was significant (ANOVA) until 5 wk PI; however, a nonsignificant weight difference of about 3 g was evident until the end of the experiment (12 wk PI).

During days 9–14 PI, symptoms of ill health were observed in all infected mice. Lethargy, ruffled fur, hunched posture, shuddering, and running eyes were observed in most animals. After day 14 PI, all these symptoms of acute illness disappeared. No mice died during the test period.

Serology confirmed the anti-*Toxoplasma* immunity in every mouse except 1 male and 1 female. The number of tissue cysts in the brain was microscopically examined in 6 animals from the 3-wk series (40, 40, 80, 80, 160, and 200; $\bar{x} = 100$), 10 animals from the 6-wk series (80, 80, 80, 120, 120, 120, 120, 120, 200, and 200; $\bar{x} = 124$), and 7 animals from the 12-wk series (40, 80, 80, 80, 120, 120, and 160; $\bar{x} = 97$). The mean number of cysts in all series was 110/brain (SD = 48.6, range 40–200) and did not differ significantly between the series ($H_{[2,N=23]} = 1.94$, $P = 0.38$).

Open-field test

Mice were less active in the acute stage of infection (3 wk PI), whereas no differences in activity between the infected and controls were observed in later stages (6 or 12 wk PI).

Fewer squares were entered by infected mice, 3 wk after the inoculation, than their controls (ANOVA, factors: TOXO and SEX, $F_{1,55} = 13.39$, $P < 0.0006$). No significant difference in the number of entered squares between infected and control mice was observed 6 or 12 wk after the inoculation (Fig. 3A). The infection–sex interaction was not significant in any observed period.

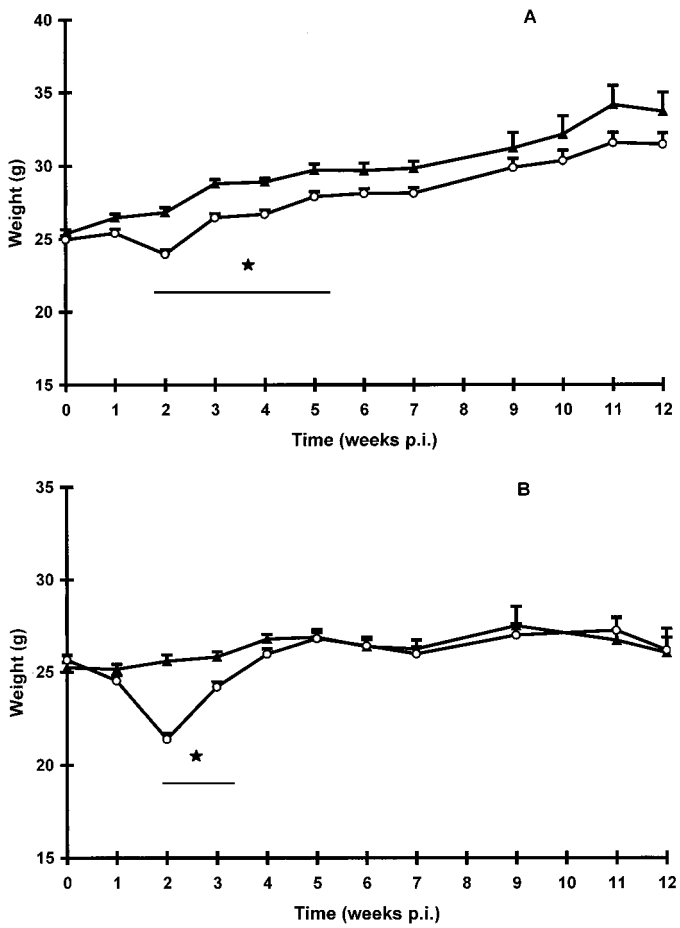

FIGURE 2. Changes in body weight during the time postinfection in males (**A**) and females (**B**). ○, Infected mice; ▲, controls, the figure shows means and standard errors (SE); stars (★) indicate significant (ANOVA) differences ($P < 0.05$).

Total time spent stationary was increased in the group of mice infected 3 wk compared to their control (ANOVA, factors: TOXO and SEX, $F_{1,55} = 12.07$, $P < 0.001$). The duration of sitting was not significantly changed by infection 6 or 12 wk PI (Fig. 3B). The infection–sex interaction was not significant in any observed period.

The infection did not change the number of rears and time spent grooming in any observed period. The number of fecal boluses deposited during the test did not differ between infected and control mice. No differences among 3 control groups (3, 6, and 12 wk PI) in any behavioral traits were observed.

Linear regression analyses showed that correlation between activity and body weight existed only in the group of mice infected by *T. gondii* for 3 wk. Positive correlation was found between Z scores calculated for number of squares and Z scores calculated for weight of mice ($F_{1,28} = 9.56$, $P < 0.005$, $R^2 = 0.25$, β [slope of a regression line] = 0.504) (Fig. 4A); negative correlation was found between Z scores calculated for time spent by sitting without activity and Z scores calculated for weight of mice ($F_{1,28} = 7.26$, $P = 0.012$, $R^2 = 0.21$, $\beta = -0.45$)

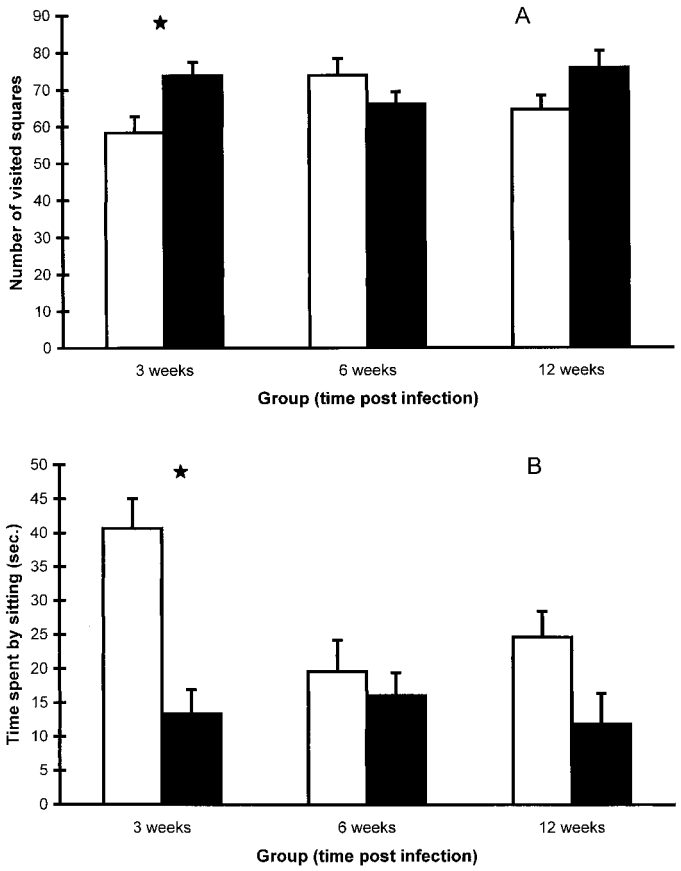

FIGURE 3. Number of visited squares (**A**) and time spent without any activity (**B**) in a 5-min test period of mice infected with *T. gondii* (light columns) and controls (dark columns). The figure shows means and standard errors (SE); stars (★) indicate significant differences ($P < 0.01$).

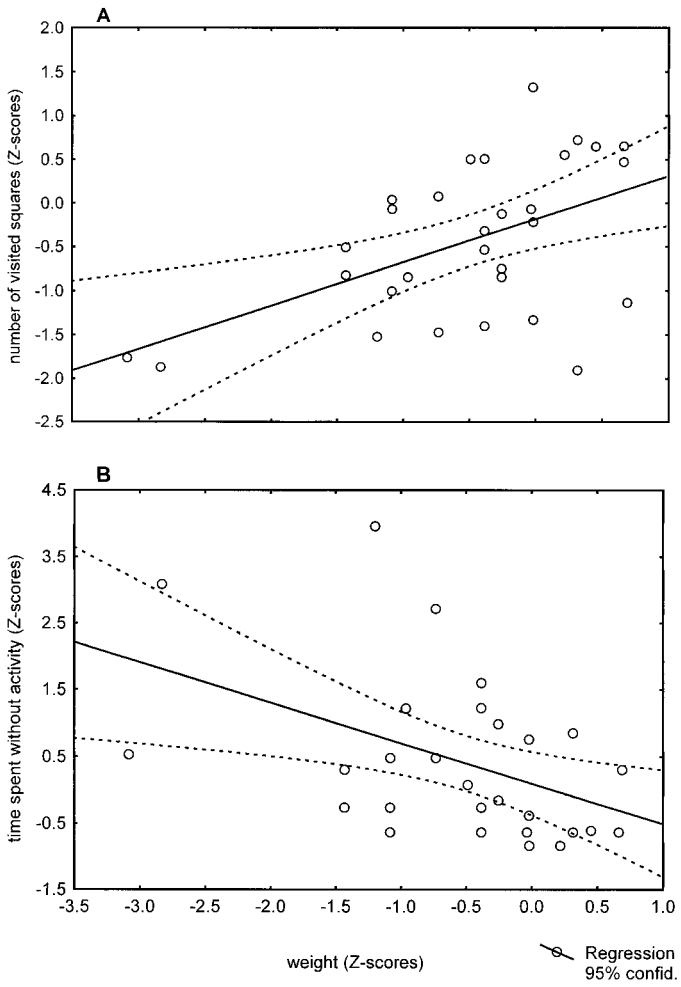

FIGURE 4. Correlation between weight of mice infected 3 wk and number of visited squares (**A**) ($y = -0.183 + 0.493x + \epsilon$) or time spent without activity (**B**) ($y = 0.097 - 0.604x + \epsilon$). Confidence bands around the fitted (regression) line indicate the area where the true fitted line (in the population) falls with 95% probability.

(Fig. 4B). No correlation was found in the other 5 experimental groups of mice, neither infected nor control.

Hot-plate test and tail-flick test

Toxoplasma infection did not affect the threshold of pain response measured as licking latency in hot-plate test or flicking latency in tail-flick test. There were no differences between infected and control mice at any observation period.

Toxoplasma gondii-infected mice had shorter latency of the first attempt to escape than controls 3 wk PI (ANOVA, factors: TOXO and SEX, $F_{1,55} = 7{,}49$, $P = 0.008$). No changes in this latency were observed either 6 or 12 wk PI. Whereas differences between the 3 series (3, 6, and 12 wk) were minimal, the effect of sex was evident (ANOVA, factors TOXO and SEX, $F_{1,165} = 83.9$, $P < 0.0001$). Females attempted to escape later than males. The infection–sex interaction was not significant.

The latency of successful escape from the hot plate (reaction time) was prolonged 6 wk PI (ANOVA, factors: TOXO and SEX, $F_{1,52} = 8.4$, $P = 0.0056$). No changes in this latency were observed either 3 or 12 wk PI. Latency of successful escape in the first trial was longer in the group infected 6 wk compared to their controls (ANOVA, factors: TOXO and SEX, $F_{1,46} = 8.61$, $P = 0.005$) (Fig. 5). The second and the third trials were less affected. The increase in the reaction time was nonsignif-

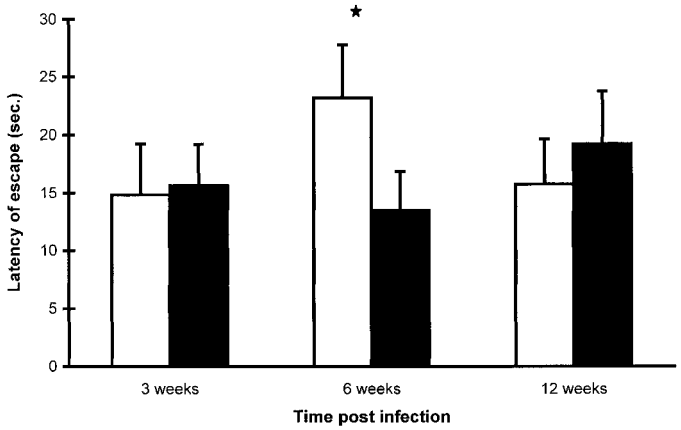

FIGURE 5. Latency of escape from hot plate of mice infected with *T. gondii* (light columns) and controls (dark columns). The figure shows means and standard errors (SE) for the first trials; stars (★) indicate significant differences ($P < 0.01$).

icant for the second (ANOVA, factors: TOXO and SEX, $F_{1,52} = 3.46$, $P = 0.069$) as well as for the third (ANOVA, factors: TOXO and SEX, $F_{1,52} = 2.38$, $P = 0.16$) trial. No differences among 3 control groups (sham-infected for 3, 6, and 12 wk) were observed. Comparison of males and females detected a highly significant effect of sex (ANOVA, factors: TOXO and SEX, $F_{165} = 78{,}6$, $P < 0.0001$); females needed more time for escape. The interaction between infection and sex was not significant.

DISCUSSION

In our study, the symptoms of acute toxoplasmosis in mice culminated during the week 2 PI. The locomotor activity was reduced significantly week 3 PI, but not in the 6 and 12 wk PI. Prolonged latency of escaping from the hot plate was observed in mice 6 wk PI, but it returned to normal later.

The weight reduction and other symptoms of health problems probably coincided with the onset of cellular and antibody immune response (Shirahata and Shimizu, 1975; Hunter et al., 1992; Roberts et al., 1995). Oral infection with *T. gondii* is known to stimulate a strong interferon (INF)-γ- and tumor necrosis factor (TNF)-α-mediated cellular immune response that polarize immunity reactions toward an early Th1 response (Denkers and Gazzinelli, 1998; Lee et al., 1999). The weight loss that is probably mediated by increase of TNF level (Bluthe et al., 1994) was described by Johnson (1988). He also measured body temperature of infected animals and showed that fever started 8 day PI and culminated 12–14 days PI. This time period is considered the acute stage of the infection. In observation of sex differences, Roberts et al. (1995) found that female mice are more sensitive to pathogenic symptoms of toxoplasmosis than male mice (as determined by higher mortality levels within 4 wk PI). We also observed that the weight of infected females was reduced more than the weight of infected males in acute stage. In contrast, in the latent stage the weight of infected females returned back to the level of controls, whereas the weight of infected males was lower for the entire course of the experiment. We suppose that the lowered weight of infected males in the latent stage of infection was caused by the fact that the females had already reached their final weight, whereas the males were still growing at the time of inoculation rather than by differences in resistance between the sexes.

Changes in locomotor activity of infected animals have been observed by several authors. During the first 2 wk PI, the activity of mice was investigated by Chao et al. (1992), who observed reduction of voluntary running to less than 30% of normal levels by day 12 PI. From day 12 PI, locomotor activity was slowly recovering to normal levels; however, the experiment was terminated before complete recovery was reached (at day 14 PI). Chao et al. (1992) argued that the observed fatigue resulted from stimulation of the immune system as it coincided with high serum levels of transforming growth factor-beta in these mice. It has also been observed that maximum sickness behavior corresponds to the peak of plasma interleukin (IL)-1 in mice (Bluthe et al., 1994; Segreti et al., 1997).

We extended these results by finding that locomotor activity remains lowered 3 wk PI, but returns to normal 6 wk PI. Moreover, our observation of a highly significant positive correlation between locomotor activity of infected mice and their weight strongly supports the view that these changes are due to acute illness. As no correlation between the weight and activity was observed in the group of uninfected mice, we can suppose that mice with the most severe symptoms of acute disease were less active because of their illness rather than for their (illness-associated) lower weight.

We observed that the activity of infected mice was not significantly different from controls in the latent stage of infection. This is not in agreement with some other published findings. Witting (1979), using a different ethological method (exploration in a deep maze), found a significant decrease of locomotor activity of infected mice and rats at 2 and 3 mo PI. In contrast, Webster (1994) observed an increase in locomotor activity in experimentally and also naturally infected rats. Hutchison, Bradley et al. (1980), Hay, Hutchison et al. (1983), and Hay, Aitken et al. (1984) described the hyperactivity in mice with congenital and also adult-acquired toxoplasmosis infected for 14 and 28 wk. They supposed that hyperactivity could be a consequence of histopathological damage to the central nervous system associated with the toxoplasmic encephalitis and with development of cysts in the brain. These considerations were supported by the findings of Anderson (1970) that hyperactivity of rats can be provoked also by nontoxoplasmic, experimentally inflicted limbic lesions. The hyperactivity was observed also in mice with experimental cerebral toxocariasis (Hay et al., 1986). On the other hand, from 6 parasites studied by Webster (1994), only *T. gondii* induced hyperactivity in rats, despite 2 other parasites also causing the encephalitis.

The extent of brain tissue damage could possibly play a major role in the character of changes in the activity of infected mice (hypoactivity/hyperactivity). In the *Toxocara*–mouse model, the degree of behavioral change correlated with the number of larvae recovered from the brain of each individual mouse (Cox and Holland, 1998). No data concerning the *Toxoplasma*-induced brain histopathology were available in the behavioral studies (including the present study). However, the extent of encephalitis can be indirectly estimated from the number of tissue cysts in the brain tissue (Suzuki and Joh, 1994; Brown et al., 1995). In our experiments, we tried to avoid strong pathological symptoms of toxoplasmosis by using relatively resistant F1 hybrid mice. The mice used have the major histocompatibility complex gene L^d that is associated with lower cyst burden and weaker symptoms of encephalitis (Brown et al., 1995). By using a low infection dose, we achieved an intensity of infection with only about 110 tissue cysts per brain, which is approximately the value we usually observed in wild mice. In contrast, Hutchison's and Hay's groups reported the presence of several hundreds to thousands of cysts in the brains of their experimentally infected mice (Hutchison, Bradley et al., 1980; Hay, Hutchison et al., 1983; Hay, Aitken et al., 1984). The difference of 1 or 2 orders of magnitude in the cyst numbers between their studies and our work (and corresponding difference in brain damage) could explain the absence of the hyperactivity in our experiments.

The probability of transmission of *T. gondii* from intermediate to definitive host by predation could be positively influenced by the ability of *T. gondii* to slow down the reaction of infected host to dangerous or novel stimuli, e.g., presence of a cat. In our experiments the stimulus was represented by an elevated hot plate. The reaction time (latency of escape) of a

mouse in this experimental set-up could be influenced by pain perception (hyperalgesia or analgesia) of infected animals, their locomotor activity, and their ability to recognize and react to novel stimuli.

Changes in pain perception are relatively common in early stages of various infections. It has been reported for *Trypanosoma brucei brucei* (Kristensson et al., 1994) and *Eimeria vermiformis* (Colwell and Kavaliers, 1993) infections. Within several days after *T. gondii* infection, we found hyperalgesia in male mice (measured in classical hot-plate test) that was significant by 9–14 days PI but disappeared before 3 wk PI (data not shown). In the present work, hyperalgesia could be responsible for a decrease in latency of the first attempt to escape 3 wk PI. However, a strong influence of hyperalgesia or analgesia was ruled out by the absence of differences in licking latency in the hot-plate test as well as in flicking latency in an independent tail-flick test.

The results of the open-field test suggest that changes in locomotor activity probably did not affect reaction time. Animals infected for 3 wk with reduced activity did not differ in time of escape from the hot plate from uninfected animals. On the contrary, 6-wk-infected animals without reduced activity in the open-field test had a prolonged reaction time compared to their controls.

The infected animals differed from the controls mainly during the first trial, but not in the second and third trials when they became familiar with the experimental setup. Therefore, we suppose that the transient changes in latency of leaving the hot plate observed 6 wk PI could be caused by impaired perception of novel stimuli (Hay, Aitken et al., 1983). Our data, as well as the results of other authors (Werner and Pichl, 1969; Burke et al., 1994), suggest that the peak of development of *T. gondii* cysts in the brain tissue of infected mice is during the second month PI. This finding corresponds with the time of maximal impairment of latency of escape from the hot plate.

In contrast to previous studies, our work was primarily focused on the dynamics of the developmental changes in mouse behavior during the first 3 mo of *T. gondii* infection. We confirmed that mice are less active in the acute stage of the infection. The hyperactivity observed by some authors in the latent stage could probably be observed only in animals with massive experimental infections that can hardly occur under natural conditions. We also found temporarily prolonged reaction time during the progression to latent phase. However, the correspondence of maximal reduction of activity with maximal symptoms of acute disease, as well as the correspondence of the maximum increase in reaction time with the assumed term of maximal development of tissue cysts in the brain of mice, suggest that both observed behavioral effects of toxoplasmosis are probably only the by-products of pathological processes associated with acute and postacute parasitosis.

Generally, our results suggest that the usual model for testing a manipulation hypothesis, the laboratory mouse with its relatively long acute toxoplasmosis (in relation to short life-span), is not the best choice for studying manipulation activity of *T. gondii.* In our opinion, the experiments with more resistant animals (Dubey, 1996), e.g., rats (Webster, 1994; Webster et al., 1994) or observations with human latent toxoplasmosis (Flegr and Hrdý, 1994; Flegr et al., 1996; Flegr and Havlíček, 1999), provide more convincing evidence for manipulation activity of *T. gondii.*

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