Toxoplasma gondii strain-dependent effects on mouse behaviour

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Abstract: *Toxoplasma gondii* reportedly manipulates rodent behaviour to increase transmission to its definitive feline host. We compared the effects of mouse infection by two Type II strains of *T. gondii*, Prugniaud (PRU) and ME49, on attraction to cat odour, locomotor activity, anxiety, sensorimotor gating, and spatial working and recognition memory 2 months post-infection (mpi). Attraction to cat odour was reassessed 7 mpi. At 2 mpi, mice infected with either strain exhibited significantly more attraction to cat odour than uninfected animals did, but only PRU-infected mice exhibited this behaviour 7 mpi. PRU-infected mice had significantly greater body weights and hyperactivity, while ME49-infected mice exhibited impaired spatial working memory. No differences in parasite antibody titres were seen between PRU- and ME49-infected mice. The present data suggest the effect of *T. gondii* infection on mouse behaviour is parasite strain-dependent.

Keywords: *Toxoplasma*, behaviour, strain dependency

Toxoplasma gondii is an obligate intracellular protozoan parasite found worldwide. It infects nucleated cells of all mammals, including humans. However, it requires a cat host for sexual reproduction. Therefore it has been suggested that *T. gondii* manipulates rodent behaviour to increase transmission efficiency (Webster 2001).

Rodents have an innate fear of cat odours (e.g., Dielenberg et al. 2001). Infection with Type II *T. gondii*, strains Prugniaud (PRU) and ME49, has been shown to decrease avoidance of cat odour and increase attraction to it without significantly affecting general anxiety and fear, suggesting specificity in the type of manipulation by the parasite (Berday et al. 2000, Vyas et al. 2007a, Lambert et al. 2008). Many studies on the behavioural effect of *T. gondii* infection have focused on the early latent phase (e.g., 4–10 weeks post-infection), after bradyzoite establishment in the central nervous system (Hrdá et al. 2000, Skallová et al. 2006, Hodkova et al. 2007, Vyas et al. 2007 a, b). Work on behavioural manipulation later in the latent phase of the parasite (e.g., >5 months post-infection) is limited (Hermes et al. 2008). Although the behavioural effects of PRU and ME49 have been studied before, a direct comparison of these strains has not been reported.

The aim of this study was to directly compare the effects of latent infection of two strains of Type II *T. gondii*,

PRU and ME49, on mouse behaviour. We hypothesized that manipulation effects for direct cat transmission (e.g., cat odour attraction) would be similar between strains, while behaviours not directly associated with transmission to cats could be differentially affected. To this end, attraction to cat odour, open field activity, anxiety, pre-pulse inhibition (PPI) of the acoustic startle, and spatial working and recognition memory were tested in mice 2 months post-infection (mpi). In addition, to evaluate duration of manipulation for direct transmission, we reassessed attraction to cat odour 7 mpi.

MATERIALS AND METHODS

Animals. Female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were used in this study. Animal protocols were reviewed and approved by the Animal Care and Use Committee of Johns Hopkins University (JHU). Mice were housed 5 per cage in the JHU animal facility with 14.5/9.5 hours of light/dark cycle and free access to food and water.

***Toxoplasma gondii* culture and infection.** Prugniaud (PRU) and ME49 tachyzoites were maintained by passage in human foreskin fibroblast monolayers. Tachyzoites were released from fibroblasts by using, in succession, 18-, 23-, and 27-gauge needles. Parasites were separated from cell debris by filter sterilization (Polycarbonate Membrane Filter, Whatman) and resuspended in Dulbecco's phosphate buffered saline (DPBS). Mice (9 weeks old) were either mock-infected with sterile DPBS or

infected with 400 tachyzoites (2 parasites/ μ l) intraperitoneally. All behavioural studies were performed on the same cohort 2 mpi, and only aversion to cat odour was reassessed 7 mpi.

Infection determination. Infection was confirmed via serology as described by Xiao et al. (2009). Briefly, sera were collected from mice 9 mpi and IgG antibodies to *T. gondii* measured by microplate enzyme immunoassay.

Aversion to domestic cat urine. Mice were habituated to an open-field box (48 $\times$ 45 $\times$ 21 cm) for 5 min daily for three consecutive days prior to testing. On testing day, 50-ml vial caps containing either 1-ml of undiluted domestic cat (gift from Dr. David Ryugo, Johns Hopkins University School of Medicine) or domestic dog (a pet) urine were placed in diagonal corners. Mice were placed in the centre and allowed to freely explore for 10 min while being videotaped. The position of the odours was altered between each mouse to avoid positional bias. Time spent near each odour was recorded using Stopwatch+ (Centre for Behavioural Neuroscience) by individuals blind to the type of odour. Occupancy was calculated as the percent time spent near each odour for the first 5 min of the 10-min trial to evaluate early responses to cat odour. Other behavioural tests were run as described by Pletnikov et al. (2008) and explained briefly below.

Novelty-induced activity. Novelty-induced activity in the open field was assessed over a 30-min period using activity chambers with infrared beams (San Diego Instruments Inc., San Diego, CA, USA). Horizontal and vertical activities, stereotypic activities, and time spent in the centre or along the walls (thigmotaxis) of the chamber were automatically recorded.

Spontaneous alternation and spatial recognition memory. Y-maze consisting of three arms of equal length, interconnected at 120°, was used as described by Melnikova et al. (2006). The test included two sessions, which evaluate spatial working and recognition memory, respectively. In the first session we scored the number of alternations done by the mouse when all three arms are visited, without entering the same arm twice in a row. After 5–7 days, the second session was performed and consisted of two trials. During trial 1, one maze arm was blocked and the mouse allowed to freely explore the other two arms for 5 min. Trial 2 began after a 30-min delay. During trial 2, the mouse was allowed to freely explore all three arms for another 5 min, taking advantage of the innate tendency of mice to explore novel unexplored areas (e.g., the previously blocked arm). Mice with intact recognition memory prefer to explore novel over familiar arms, whereas mice with impaired spatial memory enter all arms equally. Motor activity was recorded as number of arms visited for the whole duration of the test. Time spent in the blocked arm during the first 2 min of the 5-min test period was used to assess spatial memory in mice as the first 2 min of testing have been shown to be most sensitive to manipulations (Melnikova et al. 2006).

Anxiety. The elevated plus maze is a cross of two arms that are open to the environment and two arms that are enclosed by side and end walls. The arms are connected by a central area. The maze is elevated from the floor (75 cm). Mice were placed in the central area of the maze facing a closed arm. Exploratory behaviour was recorded via a video camera. The number of open- and closed-arm entries and the time spent in both types of arms were scored. An entry was defined as all four paws in one arm. Anxiety was scored as the percentage of time spent in the open arm in relation to the total 5-min test.

Sensorimotor gating. Pre-pulse inhibition of the acoustic startle (PPI) was used to assess sensorimotor gating. Mice were acclimatized in startle chambers (San Diego Instruments, Inc., San Diego, CA) to a 70-dB background noise (continuous throughout the session) for 5 min, followed by the presentation of ten 40 ms 120 dB white noise stimuli at 20 s inter-stimulus intervals (habituation). After habituation, mice were left in the enclosure for 5 min without presentations of any startle stimuli. Immediately after, the pre-pulse inhibition (PPI) session was begun, consisting of six presentations of each trial type in a pseudorandom order. The types of trials include: pulse-alone (120-dB, 100-msec, broadband burst), omission of stimuli, and four pre-pulse–pulse combinations consisting of a 20-msec pre-pulse broadband burst at either: 74, 78, 82, 86 or 90 dB, presented 80-msec before the pulse. Startle reactivity and PPI were evaluated by separately comparing absolute values of the startle amplitudes for different types of trials between three groups. Compared to using percentage values of PPI, the absolute values approach provides a better evaluation of startle reactivity both at pulse-alone and pre-pulse trials (Swerdlow et al. 2000).

Statistical analysis. Data were tested for normality and if failed were subjected to rank transformation. Afterwards, data were analysed using ANOVA with strain of the parasite and time (when applicable) as independent variables. In addition, planned Student's *t*-test was used to evaluate cat attraction between the two strains of parasite, and $p < 0.05$ was considered significant.

RESULTS

We found that both strains of parasite caused comparable cat odour attraction in infected mice 2 mpi, but had a differential effect 7 mpi (Fig. 1). At 2 mpi, compared to control mice, infected mice spent significantly more time near the cat odour. In addition, infected mice spent more time near the cat odour than dog odour (Fig. 1A). At 7 mpi, PRU-infected mice continued to spend more time near cat odour than dog odour, as compared to controls (Fig. 1B). In contrast, ME49-infected mice now spent comparable time near dog and cat odours, similar to control mice.

Although the effects of the two parasite strains were similar on cat attraction behaviour 2 mpi, behaviours not directly associated with manipulation were differentially affected by the two strains of the parasite (Fig. 2). PRU-infected mice had significantly heavier body weights than controls and ME49-infected mice [a group effect, $F(2, 25) = 4.77$, $p = 0.018$], which was abated at 7 mpi [a group effect, $F(2, 25) = 1.32$, $p = 0.287$] (Table 1). PRU-infected mice also showed increased locomotor activity in the open field, as evidenced by the greater number of broken beams compared to control (Fig. 2A). In contrast, only ME49-infected mice exhibited decreased spontaneous alternations in the Y-maze, indicative of impaired spatial working memory (Fig. 2B).

No effect of either parasite strain was found on spatial recognition memory, anxiety, or sensorimotor gating (Fig. 3). There was no difference in spatial recognition memory between groups as evidenced by similar exploration time of the blocked arm during the first 2 min of testing [a group effect, $F(2, 25) = 1.0$, $p = 0.380$] (Fig. 3A).

Table 1. Average body weight and *Toxoplasma gondii* antibody titres of infected and control mice.

		Control	PRU	ME49
Body weight (g)	2 mpi	25.8 ± 1.3	*30.6 ± 1.4	25.1 ± 1.2
	7 mpi	27.3 ± 1.1	29.1 ± 1.8	26.3 ± 0.7
Antibody titres (O.D.)	9 mpi	0.15 ± 0.06	*3.77 ± 0.04	*3.76 ± 0.03

PRU-infected mice weighed significantly more than control and ME49-infected mice 2 mpi. Control, n = 10; PRU-infected, n = 7; ME49-infected, n = 9, * denotes $p = 0.009$ relative to control and $p = 0.016$ relative to ME49-infected mice. No differences in weight were seen 7 mpi.

High antibody titres were present in infected mice as compared to control. Control, n = 3; PRU-infected, n = 6; ME49-infected, n = 7, * denotes $p < 0.001$.

All data are mean values ± SEM.

No differences in anxiety were noted between control and either infected group [a group effect, $F(2, 25) = 0.68$, $p = 0.517$] (Fig. 3B). Finally, control and mice infected with either parasite strain had similar levels of the acoustic startle of the PPI (Fig. 3C).

The serology results confirmed infection with PRU and ME49 [a group effect, $F(2, 15) = 1883$, $p < 0.001$] and found no differences in serum antibody titres between the two groups of infected mice [t-test, $p = 0.847$] (Table 1).

DISCUSSION

The data support our hypothesis that both strains of the parasite comparably manipulate behaviour associated with direct cat transmission and differentially manipulate behaviours not directly associated with feline transmission. However, while mice infected with either parasite demonstrated comparable attraction to cat odour at 2 mpi, only PRU-infected mice exhibited this behaviour 7 mpi.

Our present results appear consistent with previous studies that have reported *Toxoplasma gondii* infection in wild and laboratory rodents causes attraction to cat predator odour (Berdoy et al. 2000, Vyas et al. 2007a, Lamberton et al. 2008). Somewhat unexpectedly, our findings seem to suggest that duration of manipulation, or at least some behavioural manifestations of manipulation, might vary between strains of the parasite. Future work is clearly needed to further our understanding of possible permanent and transient aspects of the manipulation effects of *T. gondii*.

Strain differences in behavioural manipulation may be due to differences in prolonged cyst burden. For instance, although at 1 mpi high PRU cyst density was found in many brain regions (Vyas et al. 2007a) only few ME49 cysts were observed in more chronic infections (Hermes et al. 2008). Future studies will directly compare cyst number and localisation of PRU and ME49 in different brain regions and at different time points to determine whether this is in fact a factor in the manipulation by ME49.

Our data are also in line with studies that have shown infected mice display hyperactivity and learning and memory impairment (Witting 1979). For example, studies on mice and rats with adult and congenital infection found hyperactivity in infected mice compared to con-

Fig. 1. PRU- and ME49-infection increases mouse attraction to cat odour during the first 5 min of the 10-min trial. **A** – Infection increased occupancy near the cat odour 2 mpi. Control, n = 10; PRU-infected, n = 6, * denotes $p = 0.032$ relative to control; ME49-infected, n = 8, # denotes $p = 0.05$ relative to control. **B** – Increased occupancy near cat odour 7 mpi. Control, n = 10; PRU-infected, n = 6, * denotes $p = 0.066$ relative to control; ME49-infected, n = 8. Error bars denote SEM.

trols between 2 and 7 mpi (Webster 1994, Hay et al. 1983, 1985, Hutchinson et al. 1980), similar to what we found with PRU-infected mice. In contrast, a study performed in infected mice and rats found decreased locomotor activity 2–3 mpi (Witting 1979). Yet another work did not find any significant difference in activity between infected and control mice 3 mpi (Hrdá et al. 2000), consistent with our finding with ME49-infected mice. These discrepancies suggest strain-dependence in behavioural effects of *T. gondii*. Although these behavioural changes may not directly increase transmission of *T. gondii* to cats, both locomotor hyperactivity and impaired spatial working memory could potentially increase chances of being captured by a predator.

Similar to putative strain-dependent effects in cat attraction, behavioural effects not related to direct manipulation may be due to a number of factors. For instance, it is possible that cyst localization differs between strains. It has been shown that the amygdala of PRU-infected mice contain high cyst density (Vyas et al. 2007a). It is

Fig. 2. Differential effects of PRU- and ME49-infection on mouse behaviour. **A** – Increased locomotor activity in open field of PRU- but not ME49-infected mice. Control, n = 10; PRU-infected, n = 6; ME49-infected, n = 8. **B** – Impaired spatial working memory in ME49- but not PRU-infected mice. Control, n = 10; PRU-infected, n = 6; ME49-infected, n = 8, * denotes $p = 0.056$ relative to control (Student's *t*-test). Error bars denote SEM.

Fig. 3. No effect of infection on spatial recognition memory, anxiety, or sensorimotor gating. **A** – No difference in spatial recognition memory between PRU- and ME49-infected mice and controls. Control, n = 10; PRU-infected, n = 7; ME49-infected, n = 9. **B** – No changes in anxiety between infected and controls. Control, n = 10; PRU-infected, n = 7; ME49-infected, n = 9. **C** – No difference in pre-pulse inhibition of acoustic startle between infected and controls. Control, n = 10; PRU-infected, n = 7; ME49-infected, n = 9. X-axis: p4, p8, p12, p16 and p20 are intensities of different pre-pulse trials above the background noise level (70 dB). Error bars denote SEM.

conceivable that individual strains may have variable tropism to other brain regions, differentially affecting other behaviours than just innate fear. Behavioural differences may also be due to infection of different cell types. It is also possible that strains elicit varying immunological responses, resulting in dissimilar alterations in neurotransmission as the three clonal lineages of *T. gondii* differ in their modulation of cytokine secretion (Saeij et al. 2005). However, direct comparison of the effects of different strains within and between lineages on behavioural manipulation, neurobiology, and neuroimmunology need to be done to better understand mechanisms of behavioural effects caused by this parasite. Differential susceptibility of behaviours to parasite strains might partly explain the broad spectrum of behavioural abnormalities in ani-

mals and clinical manifestations in humans infected with *T. gondii* (Flegr 2007, Torrey and Yolken 2007).

In conclusion, our data indicate that infection with *T. gondii*, PRU and ME49, produced comparable attraction to cat odour in mice 2 mpi. We also found that the degree of attraction and the effects on other mouse behaviours may be influenced in a parasite strain-dependent manner.

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