The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study

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SUMMARY

Toxoplasma gondii, a cosmopolitan protozoan parasite, is known to induce behavioural alterations in rodents and may exert an effect on human personality and behaviour. The mechanism of parasite-induced alterations in host behaviour has not been described, but it was hypothesized that development of *Toxoplasma* tissue cysts in the brain could affect the dopaminergic neuromodulatory system. In this study, we tested the effect of latent *Toxoplasma* infection on mouse behaviour associated with activity of the dopaminergic system, i.e. locomotion in a novel environment and exploration test. Additionally, we examined the behavioural response of *Toxoplasma*-infected mice to a selective dopamine uptake inhibitor, GBR 12909. In both genders, *Toxoplasma* infection decreased locomotion in the open field. Infected females displayed an increased level of exploration in the holeboard test. GBR 12909 induced suppression in holeboard-exploration in the infected males, but had an opposite effect on the controls. These results suggest an association between *Toxoplasma gondii* infection and changes in the dopaminergic neuromodulatory system.

Key words: parasite, dopamine, exploration, holeboard, GBR 12909.

INTRODUCTION

Toxoplasma gondii is an intracellular heteroxenic protozoan parasite of felines. Although rodents are natural intermediate hosts of *T. gondii*, toxoplasmosis has been observed in an unusually wide range of vertebrates, including humans. In the intermediate host, *T. gondii* undergoes a rapid asexual reproduction during the acute phase of infection. In humans, acute infection can be accompanied by moderate fever, cervical lymphadenopathy and asthenia. After the acute phase of infection, resistant tissue cysts develop in different host tissues, most commonly in the brain and muscles. Tissue cysts are infectious for both intermediate and definitive hosts – felines. In the intestinal epithelium of cats *T. gondii* undergoes the sexual part of reproduction, which results in the formation of resistant oocysts (Frenkel, 1988).

According to the manipulation hypothesis, the dixenic life-cycle imposes strong selection pressure on the parasite towards evolving mechanisms which increase the probability of its transmission to the definitive host (Holmes and Bethel, 1972). Behavioural alterations described in *Toxoplasma*-infected rodents compared with non-infected animals are thought to be due to such parasite-induced manipulation. In mice, *T. gondii* infection was shown to be associated with impairment of memory and with learning-deficits (Piekarski *et al.* 1978; Witting, 1979), hyperactivity in the open field (Hay *et al.* 1983, 1984*a*), an increase of voluntary wheel running (Hay *et al.* 1985), decreased ability to discriminate between familiar and novel stimuli (Hay *et al.* 1984*b*; Hutchison *et al.* 1980*a*), and impairment of motor performance and coordination (Hutchison *et al.* 1980*c*). In infected rats, deficits in learning (Piekarski *et al.* 1978; Witting, 1979), decreased neophobia and increased trappability (Webster *et al.* 1994), hyperactivity in a familiar environment (Webster, 1994), increased exploration of a novel object (Berdoy *et al.* 1995), and decreased avoidance of the odour of cat urine (Berdoy *et al.* 2000) have all been described.

The physiological mechanisms of *Toxoplasma*-induced alterations in animal behaviour have not been elucidated. According to one hypothesis, behavioural alterations may simply result from the by-products of inflammation and encephalitis associated with *Toxoplasma* infection (Hay *et al.* 1983; Hrdá *et al.* 2000). However, it is known that *Coxiella*

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burnetti or *Leptospira icterohaemorrhagiae* also cause encephalitis in rodents, but do not cause alterations in animal behaviour (Webster, 1994). A second hypothesis is that development of *Toxoplasma* tissue cysts in the brain may affect levels of certain neurotransmitters and their metabolites. Berdoy *et al*. (2000) have suggested that diminished fear of a predator in infected rats may be similar to the decrease of predator avoidance after administration of N-methyl D-aspartate (NMDA) receptor antagonists or serotonin antagonists. In mice with latent *Toxoplasma* infection, increase of dopamine concentrations up to 114% in the whole brain homogenate over controls was demonstrated (Stibbs, 1985). Results of studies concerning the effects of latent *Toxoplasma* infection on human personality also suggest that behavioural alterations could be due to the influence of the parasite on certain components of the dopaminergic system. Thus it has been reported that infected men scored significantly lower than controls in novelty seeking, one of the four temperament dimensions of the biosocial model of personality developed by Cloninger (Flegr *et al*. 2003). Novelty seeking is thought to be negatively correlated to basal dopaminergic activity (Cloninger *et al*. 1993; Hansenne *et al*. 2002). However, the relationship between dopamine and novelty seeking in humans has not been definitely proved (Corr and Kumari, 2000; Gebhardt *et al*. 2000). An association between dopaminergic activity and intensity of response to novel stimuli was clearly demonstrated in rats (Dellu *et al*. 1996; Kabbaj and Akil, 2001). Several authors have described alterations in response to a novel environment in *Toxoplasma*-infected rodents (Berdoy *et al*. 1995; Hay *et al*. 1984*b*; Hutchison *et al*. 1980*b*).

The aim of this study was to clarify the effects of latent toxoplasmosis on mouse behaviour associated with the activity of the dopaminergic system. Therefore, ethological tests were focused on motor activity in a novel environment and exploration. Additionally, we examined the effect of a selective dopamine re-uptake inhibitor, GBR 12909, on mice behaviour in the holeboard test. We hypothesized that if the development of *Toxoplasma* tissue cysts in the brains of mice induced changes in the dopaminergic system, the effects of GBR 12909 on the behaviour of infected and control mice might be quantitatively or even qualitatively different.

MATERIALS AND METHODS

Animals and infection

Sixty female and 60 male F1 crosses between mouse inbred strains BALB/c (females) and B10A (males), obtained from Velaz (Czech Republic), were used. The F1 crosses between 2 inbred mouse strains have identical genotype but, in contrast to the parental strains, they are heterozygous in many genes. It was shown that behavioural patterns of F1 crosses between various mouse strains are comparable (Lipp and Wolfer, 2003). Mouse strain crosses are known to be healthier and more resistant to *T. gondii* than inbred mouse strains (Darcy and Santoro, 1994). The mice were housed in unisex groups of 7–8 in plastic cages with wood shaving bedding and nesting material and maintained under a 12-h light : dark cycle (lights on at 04:00 h). Food and water were available *ad libitum*. All experiments were performed in accordance with present Czech legislation and were approved by the IRB Faculty of Science, Charles University, Prague.

At 10 weeks of age, mice were inoculated perorally with 0·5 ml of brain suspension in saline, which contained the equivalent of 10 tissue cysts of the avirulent cyst-forming HIF strain of *Toxoplasma gondii*, isolated in 1993 in the Czech Republic from the cerebrospinal fluid of a male HIV-positive patient (Kodym *et al*. 2002). The brain suspension was prepared from brains of CBA/J mice infected 3 months earlier. Controls received perorally 0·5 ml of saline. Six of the male mice inoculated with *T. gondii* and 4 of the male controls died during the inoculation.

Mice were regularly observed for symptoms of illness and their weights were recorded. At the end of each experiment, all animals were serologically examined for anti-*Toxoplasma* antibodies by complement-fixation test. In 16 randomly selected infected mice (8 females and 8 males), the number of tissue cysts was quantified in the brain suspension 13 weeks p.i. Brains of selected mice were homogenized in 1 ml of saline. The number of cysts was counted in ten 15 μl samples of each suspension under $\times 250$ magnification.

Drugs and solutions

GBR 12909 1-[2-[bis(4-fluorofenyl)metoxy]-etyl]-4-[3-fenylpropyl]piperazin (Tocris) was dissolved in sterile 0·9% saline solution and administered i.p. in a volume of 120 μl. Each mouse received the drug at a concentration of 10 mg/kg. Controls received 120 μl of sterile saline.

Apparatus and procedures

Behavioural testing started 10 weeks after infection. With regard to the great number of animals used in the experiment, males and females were tested in separate trials. The tests on females were performed immediately after the tests on males were completed. All experiments were carried out during the dark phase between 16:00 and 23:00 h. The behaviour of the mice was videotaped by a video camera positioned directly above the apparatus. All of the videotapes were analysed using The Observer

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software (Noldus). The analyses of behaviour were done blind.

Open field test of activity and exploratory behaviour

The open field consisted of a black plastic arena, 60 × 60 × 40 cm, divided into 9 squares (20 × 20 cm). The arena was lit by 2 red bulbs (15 W). The mouse was placed in a corner square, facing the walls, and videotaped for 10 min. The following parameters were recorded: the total number of squares crossed, the total number of rears, time spent grooming, time spent sitting and the relative number of central square entries (number of central square entries/total number of squares crossed). Between each mouse, faeces and urine were removed, and the arena was cleaned using a detergent and dried. The test was repeated 48 h later to assess habituation.

Holeboard test of exploratory behaviour

Exploratory behaviour was assessed 48 h after the second open field test. The holeboard consisted of a black plastic arena, 60 × 60 × 40 cm, with a false floor, 2·5 cm high, divided into 9 squares, each 20 × 20 cm. There were 16 holes (2 cm diameter) in the floor in a symmetrical pattern. The arena was lit by 2 red bulbs (240 W). Each mouse was placed in a corner square, facing the walls, and videotaped for 10 min. The number and duration of head dips into the holes, time spent sniffing at the holes and the number of squares crossed, were recorded. Head dipping was defined as a hole entry up to the ears. After each test session, faeces and urine were removed, and the arena was cleaned using a detergent and dried prior to the next mouse.

Holeboard test of exploratory behaviour after i.p. administration of GBR 12909

The effects of selective dopamine re-uptake inhibitor GBR 12909 on behaviour in infected mice were assessed 6–7 days after the first holeboard test. The male mice were tested in the 16-hole holeboard described above. A strong decrease in the exploratory activity in males due to habituation in the 16-hole holeboard was observed and, because of this, the female mice were tested in a novel 4-hole holeboard, formed by inserting a new false floor that was divided into 16 squares, 15 × 15 cm. Four central squares contained 2 cm diameter holes. It is supposed that this modified version can better provide independent measures of exploration and motor activity (File, 2001). Each mouse was placed into the arena 40 min after the intraperitoneal administration of GBR 12909 (10 mg/kg) or saline, when the effect of the drug on mice activity was approximately at a peak (Irifune *et al*. 1995). The number and duration of head dips into the holes, time spent sniffing at the holes, time spent grooming, number of squares crossed and number of rears were recorded over a 10 min period. Inbetween each mouse, faeces and urine were removed, and the arena was cleaned using a detergent and dried.

Data analysis

Statistica® v.6.0 software was used to analyse the data. The parametric assumptions of the data were verified using Kolmogorov-Smirnov and Lilliefors tests. Homogeneity of variance was tested using the Levene test. Data without normal distribution were transformed using transformation formula $X' = \log(X)$. General linear models (GLM) were used to study the effects of toxoplasmosis, gender (confounding factor), and their interactions on each of the behavioural variables recorded. Actual weight of the animals was included as a covariate in all analyses. To assess habituation in the open field, the value of each behavioural trait, recorded in the first test, was included as a covariate, and the value of the same trait in the second test was included as a dependent variable. To determine whether infected and control animals differentially responded to GBR 12909, GLM, followed by Tuckey HSD *post hoc* test, were carried out. The results were considered statistically significant when $P < 0{\cdot}05$.

RESULTS

Clinical appearance

A significant negative effect of *Toxoplasma* infection on mouse weight was observed on day 11 p.i. in males ($F(1,44) = 12{\cdot}57$; $P < 0{\cdot}001$) and day 12 p.i. in females ($F(1,57) = 33{\cdot}96$; $P < 0{\cdot}001$), (Fig. 1). No typical symptoms of acute toxoplasmosis, i.e. lethargy, ruffled fur, hunched posture, were observed in infected mice.

Complement-fixation test confirmed anti-*Toxoplasma* antibodies in every inoculated mouse. All control mice were *Toxoplasma*-negative. The mean number of tissue cysts, microscopically examined in 16 randomly selected infected mice, was 46·25/brain (S.E.M. = 5·07).

Open field test

In the first test session, infected mice crossed significantly fewer squares ($F(1,101) = 9{\cdot}15$; $P = 0{\cdot}003$) and reared significantly less ($F(1,101) = 9{\cdot}38$; $P = 0{\cdot}003$) than controls (Table 1A). GLM showed no significant effect of toxoplasmosis on the relative number of central square entries, but there was a significant interaction between gender and infection ($F(1,101) = 8{\cdot}79$; $P = 0{\cdot}004$). Infected males entered the central square more often (Table 1B), infected females less often (Table 1C) than controls.

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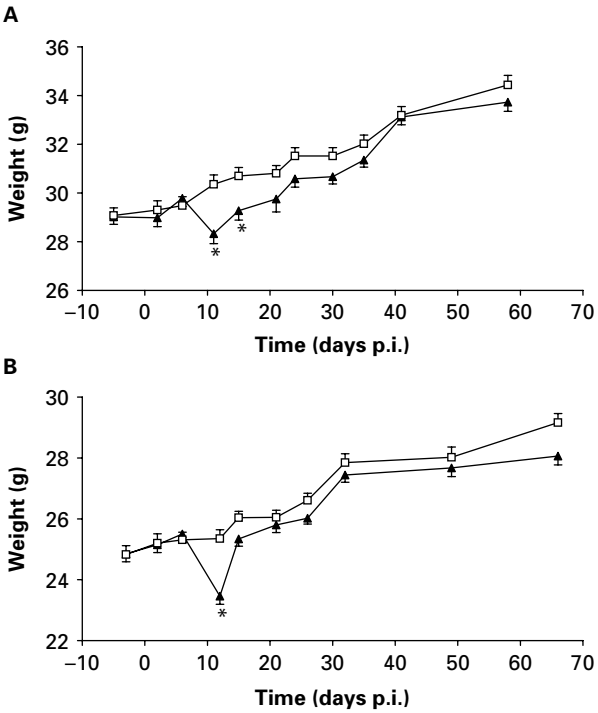

Fig. 1. Changes in body weight during *Toxoplasma* infection in males (A) and females (B). (▲) Mice infected with *T. gondii*; (□) Control mice. (*) Significant difference between infected mice (males $n=23$; females $n=30$) and controls (males $n=24$; females $n=30$) (GLM, $P<0{\cdot}05$). Each point represents mean and S.E.M.

Habituation in the open field

The number of crossed squares significantly decreased from trial 1 to trial 2 in both infected and control mice ($F(1,98)=65{\cdot}98$; $P<0{\cdot}001$). Conversely, the number of rears increased between trials ($F(1,98)=21{\cdot}95$; $P<0{\cdot}001$). There was no significant effect of infection or significant gender-infection interaction in the case of a decrease in crossed squares or increase of rears in the second trial.

Holeboard test

Infected mice spent significantly more time sniffing at holes than controls ($F(1,98)=9{\cdot}04$; $P=0{\cdot}003$), (Fig. 2A, Table 1). There was no significant interaction between gender and infection. In separate analyses, the infected females displayed more exploratory activity, i.e. spent more time head dipping, than the controls ($F(1,57)=4{\cdot}30$; $P=0{\cdot}043$). There was no significant effect of infection on exploratory activity in male mice (Fig. 2B).

Effects of GBR 12909 on activity in the holeboard

Male and female mice were analysed separately, because different modifications of the holeboard test were used in this trial (16-hole holeboard in males and 4-hole holeboard in females). The response to i.p. administration of GBR 12909 was significantly different in the infected males and the controls (Table 2). GBR 12909 significantly decreased time spent grooming in the controls (Tukey HSD; $P<0{\cdot}001$), but had no significant effect on the infected males. There was a significant interaction between infection and GBR 12909 in the case of the frequency of head dips (GLM; $F(1,41)=5{\cdot}03$; $P=0{\cdot}030$) and the time spent head dipping (GLM; $F(1,41)=4{\cdot}66$; $P=0{\cdot}037$). In response to GBR 12909, frequency of head dips was significantly lower in the infected males than in the controls (Tukey HSD; $P=0{\cdot}036$). The effect of GBR 12909 on the infected males was opposite to the effect detected on the controls (Fig. 3). Despite the significant interaction between the infection and GBR 12909, Tukey *post hoc* comparison showed no differences between groups in time spent head dipping.

There were no significant differences between infected and control females in response to administration of GBR 12909 in the 4-hole holeboard (Table 3, Fig. 4).

DISCUSSION

Toxoplasma-infected mice (males, as well as females) showed hypoactivity in a novel environment. Infected female mice showed more exploration than controls in the holeboard test. In infected males, a different behavioural response to the selective dopamine reuptake inhibitor GBR 12909 was observed.

Clinical appearance

The outcome of a *T. gondii* infection depends on the virulence of the parasite strain, on the size of the challenge dose, on the initial port of entry of the parasite and on the host species, and on the immunological and genetic status of the host (Darcy and Santoro, 1994). Most of the studies concerning *T. gondii*-induced behavioural alterations in mice were performed with intraperitoneally infected animals using a relatively high challenge dose, mostly 20 tissue cysts per mouse (Hutchison *et al.* 1980*a*, *b*, *c*). Intraperitoneal inoculation is undoubtedly unnatural and can result in different pathogenicity than peroral inoculation (McLeod *et al.* 1989). By using peroral inoculation and a low challenge dose, we tried to achieve a similar-to-natural and therefore more biologically relevant outcome of *T. gondii* infection.

In our study, the typical symptoms of acute toxoplasmosis were not apparent, except for body weight reduction during the second week p.i. The weight reduction probably coincided with the onset of cellular and antibody immune response (Denkers and Gazzelini, 1998; Lee *et al.* 1999). During the

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Table 1.
(A) Open-field and holeboard tests (both genders together)

			Infected ($n=53$)		Controls ($n=54$)	
Test	Measure	Trial	Mean	S.E.M.	Mean	S.E.M.
Open field	Squares crossed	Trial 1	179·3	3·9	196·9	3·9**
		Trial 2	168·7	4·9	181·9	5·2
	Rears	Trial 1	94·8	3·1	105·3	2·4**
		Trial 2	105·5	4·2	106·9	3·1
	Time spent grooming (sec)	Trial 1	9·3	0·8	8·1	0·6
		Trial 2	10·1	0·8	10·4	0·8
	Time spent sitting (sec)	Trial 1	1·5	0·3	1·3	0·3
		Trial 2	5·0	1·2	5·1	1·2
	Entries to central square (%)	Trial 1	15·3	0·7	15·0	0·5
		Trial 2	12·4	0·5	12·7	0·5
Holeboard	Head dips		46·3	2·4	42·6	2·4
	Time spent head dipping (sec)		86·1	5·5	73·2	5·5
	Time spent sniffing at holes (sec)		92·4	2·5	81·4	2·5**
	Squares crossed		143·2	4·0	145·9	4·0

** Significant differences between infected and control mice (GLM, $P<0·01$).

(B) Open-field and holeboard tests (males)

			Infected ($n=23$)		Controls ($n=24$)	
Test	Measure	Trial	Mean	S.E.M.	Mean	S.E.M.
Open field	Squares crossed	Trial 1	185·3	7·1	191·8	5·9
		Trial 2	178·2	9·7	174·8	7·5
	Rears	Trial 1	106·5	5·2	117·9	2·7*
		Trial 2	125·9	6·6	118·8	4·7
	Time spent grooming (sec)	Trial 1	12·8	1·2	10·1	0·9
		Trial 2	12·7	1·3	13·8	1·3
	Time spent sitting (sec)	Trial 1	2·0	0·5	2·5	0·5
		Trial 2	10·0	2·6	9·1	2·2
	Entries to central square (%)	Trial 1	20·0	0·8	17·4	0·8*
		Trial 2	13·1	0·7	10·9	0·5*
Holeboard	Head dips		43·2	4·1	45·2	3·4
	Time spent head dipping (sec)		66·3	8·1	67·8	6·6
	Time spent sniffing at holes (sec)		78·8	3·2	72·6	3·3
	Squares crossed		162·6	5·1	163·5	5·6

* Significant differences between infected and control mice (GLM, $P<0·05$).

(C) Open-field and holeboard tests (females)

			Infected ($n=30$)		Controls ($n=30$)	
Test	Measure	Trial	Mean	S.E.M.	Mean	S.E.M.
Open field	Squares crossed	Trial 1	174·7	4·2	200·9	5·2**
		Trial 2	161·4	4·4	187·6	7·1*
	Rears	Trial 1	85·1	2·6	95·4	2·7*
		Trial 2	89·4	3·3	96·6	3·1
	Time spent grooming (sec)	Trial 1	6·4	0·8	6·6	0·6
		Trial 2	8·2	0·9	7·7	0·6
	Time spent sitting (sec)	Trial 1	1·1	0·4	0·3	0·2
		Trial 2	2·0	0·6	2·0	0·8
	Entries to central square (%)	Trial 1	174·7	4·2	200·9	5·2
		Trial 2	11·7	0·7	14·3	0·7*
Holeboard	Head dips		48·8	2·1	40·5	3·4
	Time spent head dipping (sec)		102·4	6·6	77·5	8·4*
	Time spent sniffing at holes (sec)		103·7	2·7	88·3	3·1**
	Squares crossed		128·5	3·5	131·9	4·2

** Significant differences between infected and control mice (GLM, $P<0·01$).
* Significant differences between infected and control mice (GLM, $P<0·05$).

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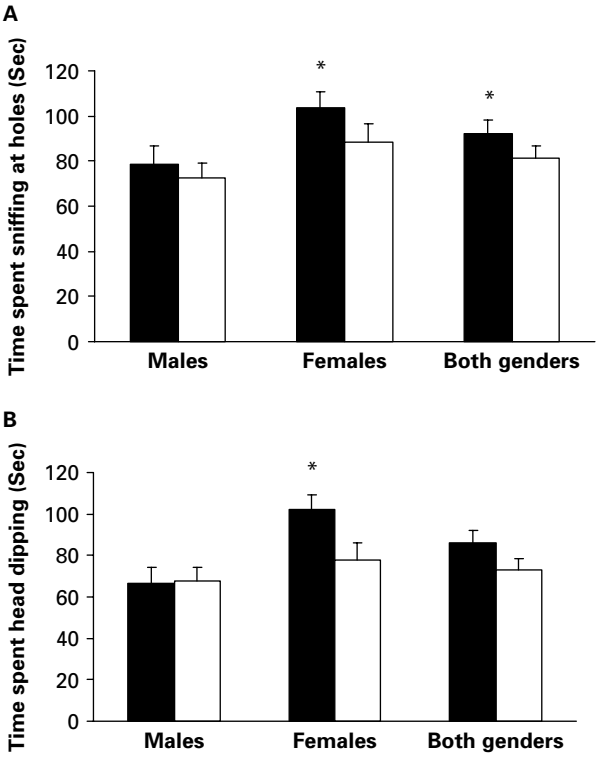

Fig. 2. Time spent sniffing at holes (A) and head dipping (B) in the holeboard test. (■) Mice infected with *T. gondii*; (□) Control mice. (*) Significant difference between infected mice (males $n=23$; females $n=30$) and controls (males $n=24$; females $n=30$) (GLM, $P<0{\cdot}05$). Height of columns shows mean and S.E.M.

third week after inoculation, the body weight of infected mice rose again and, at the start of the ethological experiments, no differences in weight between infected and control mice were observed. The intensity of infection was, on average only 46 tissue cysts per brain which is approximately the value we have usually observed when examining brains of wild mice (unpublished data).

Open field test

Infected mice displayed a lower level of ambulatory activity and reared less than control mice. Rearing is considered to reflect exploration (Deacon *et al.* 2002) while ambulatory activity mainly reflects general activity, but also a level of anxiety or exploration (Rogers *et al.* 1999; Tang and Sanford, 2005). In our experiments we used a black arena and red illumination instead of a white arena lit by extra bright lights. Therefore, we suggest that the observed changes in ambulatory activity may be mainly due to alterations in general activity or exploration and only in a minor part to changes in anxiety.

A low level of horizontal activity, measured by the number of squares crossed in the open field, is reported to be associated with low dopaminergic activity in the nucleus accumbens in rats (Dellu *et al.* 1996; Kabbaj and Akil, 2001). Low rearing may be associated with a low level of dopamine in the ventral striatum and high level of serotonin in the medial frontal cortex (Thiel *et al.* 1999). Hypoactivity in a novel environment is thought to be associated with low dopaminergic activity in the mesolimbic and nigrostriatal systems in mice, too (Jones *et al.* 1996; Puglisi-Allegra and Cabib, 1997).

Hypoactivity of infected mice in the open field, which was observed in our study, is not in agreement with the hyperactivity described by Hay *et al.* (1983). According to Hay *et al.* (1984*b*), infected mice also showed high levels of activity in the Y–maze. In contrast, Hutchison *et al.* (1980*b*) observed hypoactivity in infected mice in the same test. However, the number of crossed squares in the open field and the number of visited arms in the Y–maze probably do not reflect the same behavioural trait (Dellu *et al.* 1996).

There was a significant gender – infection interaction in the case of the relative number of central square entries. Infected males entered the most forbidding central area of the arena more often, infected females less often than controls. Frequent entries to the central square may reflect exploration, whereas avoiding the centre is supposed to be linked to greater anxiety based on the natural aversion of rodents to open spaces (Prut and Belzung, 2003; Tang and Sanford, 2005). However, the open-field test was originally validated only for males. Therefore, a single parameter may not necessarily be associated with the same behavioural trait in both genders. Preference for the forbidding central area of the arena was shown to be associated with low anxiety in male rats and high locomotor activity in female rats (Fernandes *et al.* 1999). Since there was no correlation between locomotor activity (number of squares crossed) and the relative number of central square entries in female mice in our study (data not shown), we suggest that the gender differences observed in this test may be due to the opposite effect of *T. gondii* on male and female mice in this parameter.

In contrast to our study, Hay *et al.* (1983) described an opposite effect of *Toxoplasma* infection on the central area preference. Infected mice (both males and females) entered the centre of the arena less often than controls.

Behaviour of mice in the open field is strongly influenced by the size and colour of the arena, level of illumination and test conditions (Crawley *et al.* 1997; Rogers *et al.* 1999). Hay *et al.* (1983) used a white arena lit by a white light. In such test conditions, changes in activity of infected mice may be due to changes in anxiety, rather than general activity or exploration (Deacon *et al.* 2002). Because our aim was to study activity and exploration above all, not anxiety, a black arena and red illumination were used in our study and the differences in experimental setup might explain the differences between our results and those of Hay's group.

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Table 2. Holeboard test after i.p. administration of 10 mg/kg GBR 12909 (males – original 16-hole version with floor divided into 9 squares, 20 × 20 cm)

(Parameters providing significant differences between infected and control mice ($P<0{\cdot}05$) are highlighted.)

	Infected				Controls			
	Saline ($n=11$)		GBR 12909 ($n=12$)		Saline ($n=12$)		GBR 12909 ($n=12$)	
Measure	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Head dips	**12·27**	**1·55**	**8·58**	**0·96**	**10·25**	**1·06**	**13·58**	**1·56**
Time spent head dipping (sec)	**15·77**	**2·39**	**9·13**	**1·36**	**12·57**	**1·72**	**15·99**	**2·29**
Time spent sniffing at holes (sec)	61·92	4·60	60·14	4·70	50·19	4·51	73·23	7·53
Time spent grooming (sec)	**17·43**	**4·29**	**21·03**	**5·98**	**34·03**	**5·69**	**7·77**	**1·30**
Squares crossed	124·64	8·09	179·50	15·23	120·92	9·75	190·25	8·75
Rears	69·27	4·95	68·67	9·03	57·33	8·46	71·92	4·95

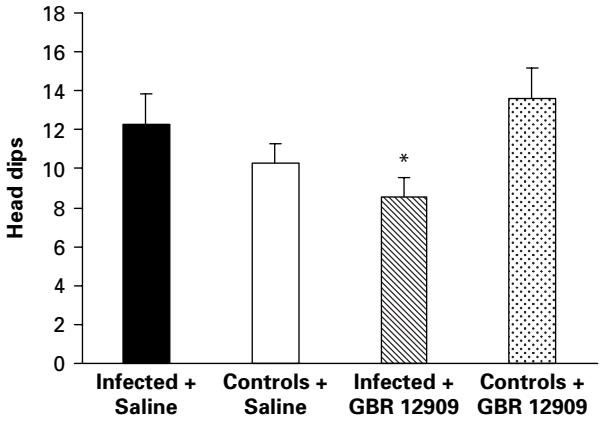

Fig. 3. The number of head dips in the holeboard test after i.p. administration of saline or GBR 12909 in male mice. (*) Significant difference between *Toxoplasma*-infected and control mice in response to GBR 12909 (infected + saline $n=11$; controls + saline $n=12$; infected + GBR 12909 $n=12$; controls + GBR 12909 $n=12$) (Tukey HSD, $P<0{\cdot}05$). Height of columns shows mean and S.E.M.

Hutchison's and Hay's groups reported the presence of hundreds to thousands of cysts in the brains of their experimentally infected mice, which is significantly higher than in our study. The variation in the cyst numbers among these studies and the corresponding difference in brain damage could partly explain the discrepancy in mice behaviour (Hay *et al*. 1983; Hutchison *et al*. 1980*a*).

It is known that mice behaviour significantly differs among different strains. Puglisi-Allegra and Cabib (1997) have shown that behaviour of particular strains of mice in response to administration of dopamine receptor agonists could vary because of distinct basal dopaminergic activity, density of dopamine receptors and density of dopamine transporters in the brain. Hutchison's and Hay's groups used A strain albino mice in all of their studies. We suggest that if *Toxoplasma* tissue cysts in the brains of mice induced any changes in dopaminergic system, the impact on the behaviour of mice could differ among strains.

Habituation in the open field

Habituation to a novel environment can be used as a non-associative test of learning. Changes in ambulatory activity and rearing in the re-test may provide a measure of contextual memory (Contet *et al*. 2001). It is supposed that the level of habituation of horizontal and vertical activity reflects the ability of adequate evaluation of environmental stimuli in mice (Nieoullon, 1989).

While the number of visited squares was significantly lower in the second trial, numbers of rears increased in both infected and control mice. There were no differences between infected and control animals in the extent of habituation. Therefore, we cannot support the notion that *Toxoplasma*-infected mice display any deficits in contextual memory.

Holeboard test

The holeboard test can provide independent measures of exploration and locomotor activity (File, 2001). In our study, differences in direct exploration between infected and control females were observed. The infected female mice spent significantly more time head-dipping and sniffing at holes than the controls, which reflects a higher level of exploratory activity.

Exploration may be associated with dopaminergic activity in the prefrontal cortex in mice, especially because of the high density of D4 dopamine receptors in this area (Powell *et al*. 2003), although the mesolimbic dopaminergic system is supposed to be associated with exploration as well (Nieoullon, 1989; Viggiano *et al*. 2003).

Our results are not in agreement with Hutchison *et al*. (1980*a*), who described reduced novel object exploration in *Toxoplasma*-infected mice. Infected male mice were reported to prefer the familiar arm to

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Table 3. Holeboard test after i.p. administration of 10 mg/kg GBR 12909 (females – modified 4-hole version with floor divided into 16 squares, 15 × 15 cm)

	Infected				Controls			
	Saline ($n=14$)		GBR 12909 ($n=14$)		Saline ($n=15$)		GBR 12909 ($n=15$)	
Measure	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
Head dips	25·00	4·10	24·93	4·15	17·13	2·16	13·13	2·38
Time spent head dipping (sec)	49·95	11·35	47·00	11·42	27·29	5·59	17·98	4·18
Time spent sniffing at holes (sec)	43·61	2·43	52·92	1·95	40·08	2·92	45·13	3·47
Time spent grooming (sec)	5·49	0·99	3·51	0·96	5·82	1·47	5·94	1·43
Squares crossed	250·80	13·41	344·67	14·72	225·43	11·82	353·64	17·70
Rears	34·14	4·38	31·43	3·75	45·53	5·45	37·27	4·90

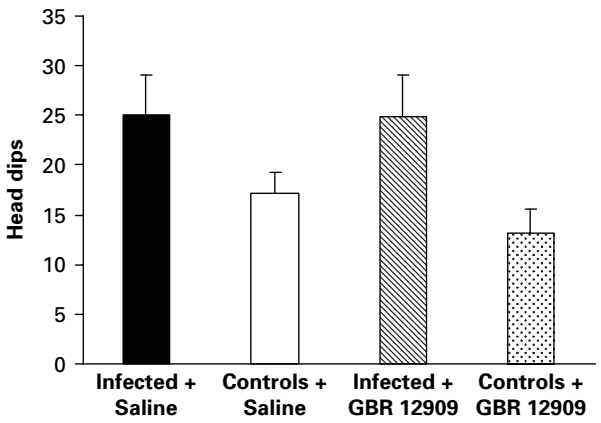

Fig. 4. The number of head dips in the modified 4-hole holeboard test after i.p. administration of saline or GBR 12909 in female mice (infected + saline $n=14$; controls + saline $n=15$; infected + GBR 12909 $n=14$; controls + GBR 12909 $n=15$). Height of columns shows mean and S.E.M.

the novel one in the Y–maze, too (Hay *et al.* 1983*b*; Hutchison *et al.* 1980*b*). In contrast, infected rats (males, as well as females) were reported to spend more time exploring a novel object than controls (Berdoy *et al.* 1995).

Effects of GBR 12909 on activity in the holeboard

GBR 12909 is a selective dopamine re-uptake inhibitor, which specifically binds to the dopamine transporters (DAT). DAT is a critical protein for dopamine regulation and a blockage of it can result in elevation of synaptic levels of endogenous dopamine because of re-uptake inhibition of released dopamine into presynaptic terminals (Irifune *et al.* 1995; Mazei *et al.* 2002).

GBR 12909 was shown to induce hyperlocomotion and stereotyped rearing (Irifune *et al.* 1995). It has been hypothesized that inhibitors of dopamine uptake reduce dopamine metabolism and turnover. Irifune *et al.* (1995) described decreased dopamine turnover (DOPAC/dopamine ratio) in the striatum in mice. On the contrary, Westerink *et al.* (1987) observed no effect of GBR 12909 on dopamine turnover in the striatum of rats. After administration of GBR 12909, Mazei *et al.* (2002) observed an increase of extracellular dopamine concentration in the caudate-putamen, but not in the medial prefrontal cortex of rats. These results indicate, that GBR 12909 may influence the nigrostriatal and mesolimbic systems of dopamine pathways.

The effect of GBR 12909 was significantly different in the infected males and the controls. GBR 12909 significantly decreased the time that the controls spent on grooming, but had no effect on the infected males. After administration of GBR 12909, the number of head dips was significantly lower in the infected males than in the controls. The frequency of head dips was decreased in infected males in response to GBR 12909; conversely it was increased in controls.

Repeated use of the 16-hole holeboard resulted in a strong decrease in the number of head dips in males due to habituation. Therefore, a modified novel 4-hole holeboard was used in females to reduce habituation. In contrast to male mice, there were no significant differences between infected and control females in response to administration of GBR 12909 in the 4-hole holeboard. The alteration in experimental design may not explain the observed gender differences. Both of the tests (16-hole holeboard and 4-hole holeboard) provide independent measures of exploration and locomotor activity and are very similar. Literature suggests that exploratory activity is reduced in holeboard re-tests even after modification of the holeboard in the second trial (Deacon *et al.* 2002).

In the females, the effect of GBR 12909 could be influenced by the stage of the oestrus cycle. Since the stage of the oestrus cycle was not monitored in this study, the risk of false negative results in female mice can be higher than in male mice.

Our results indicate, that GBR 12909 may induce suppression of exploration in *Toxoplasma*-infected mice. Decreased head dipping is reported in rats after administration of low doses of D2 receptor agonists such as apomorphine (Stahle and Ungerstedt, 1986,

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1987). It was hypothesized that the suppressive effect of low doses of apomorphine is attributed to activation of dopamine autoreceptors (Stahle and Ungerstedt, 1987; Irifune *et al*. 1995). According to the second hypothesis, suppression of exploration may be induced by stimulation of a special group of postsynaptic D2 receptors in the nucleus accumbens (Stahle, 1992). It is known that in response to administration of low doses of apomorphine, suppression of exploration is always associated with decrease of locomotion – contrary to the hyperactivity observed in our study. The opposite effect of GBR 12909 on the infected and the control mice could be caused by an impairment of the dopamine pathway integration of the infected animals. Increase in locomotor activity, caused by GBR 12909-mediated elevation of synaptic levels of dopamine in the limbic system, was followed by decreased exploration in the infected mice. Dopaminergic activity in the limbic system is shown to be associated with locomotion (Dow-Edwards and Busidan, 2001; Irifune *et al*. 1995). The activity of dopamine D4 receptors, which are concentrated in the prefrontal cortex, is supposed to be associated with directed exploratory activity (Powell *et al*. 2003). Therefore, we suppose that in the infected mice, an increase of dopaminergic activity in the limbic system was followed by decreased dopamine activity in the prefrontal cortex.

Conclusion

The nature of the previously described and presently observed behavioural alterations in *Toxoplasma*-infected mice, as well as the observed difference in effects of GBR 12909 on the infected and the control male mice suggests that the proximal causes of alterations in mice behaviour induced by *T. gondii* are probably changes in the dopaminergic system. However, because of the high plasticity and complexity of behavioural and neurophysiological mechanisms, it is not possible to satisfactorily explain the mechanism of *Toxoplasma*-induced behavioural alterations without direct examination of levels of dopamine and its metabolites in various brain regions. We suggest, that a combination of neurochemical and ethological studies may be essential to explain the physiological mechanism of *Toxoplasma*-induced alterations in animal behaviour.

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