Journal of Helminthology (2001) **75,** 125–135 DOI: 10.1079/JOH200169

Toxocara in the mouse: a model for parasite-altered host behaviour?

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Abstract

The objective of this paper is to critically evaluate the significance of parasite-altered host behaviour in the *Toxocara* mouse model particularly in the light of the Manipulation Hypothesis. Murine behaviours were examined in both outbred and inbred strains of mice infected with different doses of *Toxocara canis* ova. Behaviours investigated included activity, exploration, response to novelty, anxiety, learning, memory and social behaviour. Subsequent modifications to the behaviour of infected mice were investigated with respect to dose administered and larval accumulation in the brain. There was substantial variation in the number of larvae recovered from brains of individual mice, which received similar doses of *Toxocara* ova. Furthermore, the numbers of larvae recovered at different doses differed significantly between an outbred and inbred strain of mouse. Alterations in infected host behaviour occurred and were related to the number of larvae recovered from the brain. For social behaviour in outbred mice, a high infection in the brain reduced levels of aggressive behaviour and increased levels of flight and defensive behaviours. In contrast, outbred mice with a low infection in the brain displayed a greater level of risk behaviour in respect of predator odour and the light/dark box compared to control or high infection mice. Post-infection, outbred mice were more immobile whereas inbred mice showed reduced immobility and increased digging and climbing. Impaired learning ability was observed in outbred mice with moderate and high levels of infection in the brain compared to control and low infection mice. *Toxocara* infection has an impact upon a diverse range of murine behaviours with little evidence for a specific and hence an adaptive alteration. Many of the effects on murine host behaviour by *Toxocara* are likely to be pathological side effects of infection rather than as a consequence of adaptive host-manipulation. Observed changes in murine behaviour may be relevant to human toxocariasis.

Introduction

Parasites are now known to have profound and varied effects upon their hosts' behaviour. In the last decade, researchers have sharpened their focus to enquire whether these changes reflect adaptive manipulation by the parasite in order to enhance transmission success or are merely accidental and pathological side-effects of infection. A third explanation maybe host defence – hosts may behave differently in order to rid themselves of parasites, conserve energy or enhance their fitness in some other way. Moore & Gotelli (1990) provided a detailed and critical review of all the relevant studies undertaken and argued for more rigour in the conduct of such studies, particularly with respect to the use of predation experiments to prove increases in transmission success among parasitized intermediate hosts. Subsequently, Poulin (1995) outlined a set of criteria that a host–parasite modification must satisfy before being described as a parasite adaptation. These criteria included complexity of the behavioural alteration, purposiveness of design, convergence and fitness effects. After assessing a number of studies using these criteria,

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https://doi.org/10.1079/JOH200169 Published online by Cambridge University Press

he concluded that the most convincing evidence related to experimental demonstration of fitness benefits where higher host vulnerability to predation was observed but this was a relatively rare occurrence (for e.g. Bethel & Holmes, 1973, 1977; Moore, 1983; Poulin *et al.*, 1992). In a recent elegant illustration of this same point, Webster *et al.* (2000) demonstrated how *Tenebrio* beetles (which act as intermediate hosts of the tapeworm *Hymenolepis diminuta*) showed changes in host behaviour which could be concluded to increase susceptibility to predation by the definitive host, the rat, but in fact, when a predation experiment was performed, there was no evidence that rats consumed more infected than uninfected beetles.

In the present paper the significance of the effect of *Toxocara canis* infection on the behaviour of one of its paratenic hosts, the mouse, will be critically assessed using the data from a series of experiments which examined a broad range of host behaviours. *Toxocara canis* infection in the mouse provides a useful host–parasite system to select for the study of parasite-altered host behaviour. Adult worms attain maturity in the intestine of canids but second stage larvae can infect a wide range of paratenic hosts, including humans and mice. In these abnormal hosts, the immature stages of the parasite undergo a somatic migration through the organs of the body but fail to reach maturity as adult worms in the intestine. If ingested by an appropriate definitive host, larvae in murine tissue can develop to adulthood. In a study of 16 species of small mammals in Slovakia, Dubinsky *et al.* (1995) recorded the highest seroprevalence of 32% in a sample of *Mus musculus* which were predominant in rural and montane regions.

The model system in mice therefore provides several opportunities for investigation (Holland, 1997). Firstly, it can act as a model for human infection and observed changes in murine behaviour, particularly at low dose levels, may be relevant to humans with toxocariasis. Secondly, it can be used to test the hypothesis that parasite-altered host behaviour may enhance the probabilities of predation of infected paratenic hosts and hence, enhanced transmission of the parasite. Considerably less attention has been paid to parasites in paratenic or accidental hosts, like *Toxocara* in mice, compared to intermediate host–parasite systems.

Toxocara is known to produce several clinical entities in humans associated with widespread tissue invasion (Beaver *et al.*, 1952; Taylor *et al.*, 1988) and eye involvement (Shields, 1984) but the neurotrophic behaviour of the larvae which results in an accumulation of larvae in the murine brain (Sprent, 1955; Dunsmore *et al.*, 1983; Skerrett & Holland, 1997) as the infection progresses is likely to be of particular significance to parasite-altered behaviour. Exposure to *Toxocara*, as measured serologically, is known to be widespread in humans, for example 31% of Irish schoolchildren were found to exhibit positive serology at a cut-off titre of 1:50 (Holland *et al.*, 1995). A number of cases of infection of the human brain have been recorded in the literature (Hill *et al.*, 1985).

Experimental procedures

Details of mouse maintenance, infection and post-mortem procedures, the apparatus and methodologies used in the behavioural tests are described in detail by Cox & Holland (1998, 2001a,b). Relevant aspects of the methods will be highlighted in the appropriate section of the paper.

Observations on behavioural alterations in *T. canis*-infected mice

This section will summarize the behavioural alterations in *Toxocara*-infected mice for four major categories of behaviour. First, an important aspect of the work was the quantification of the number of larvae found in the brains of individual mice and the assessment of the relationship between larval burden in the brain and observed changes in behaviour. It should be noted that the brain was the only organ examined for the presence of larvae in this study. This was because data from a previous investigation using LACA mice infected with doses of 100, 1000 and 3000 *T. canis* ova revealed that by day 26 of infection larvae had accumulated in the brain and very few or no larvae were recorded from the liver, lungs, kidney and muscle (Skerrett & Holland, 1997). Previous research had demonstrated that *T. canis* larvae stabilize in the brain between days 35 and 45 (Burren, 1971). In the present experiments, the infection was allowed to establish for 30 days before testing began. It was considered that behavioural effects due to the presence of parasites in the brain would be most conspicuous at this time.

The number of larvae recovered from the brains of individual mice: individual variation, influence of dose and strain

In two of the experiments, LACA (inbred) mice received a single dose of 2000 ova per mouse (tables 1 and 2) (Cox & Holland, 1998). The individual variation in larval recovery was large despite the fact that the mice received a single dose, and on the basis of this, mice were divided into two groups, described as low or high *T. canis* larval recovery. In four further experiments, LACA mice (and NIH (outbred) mice for one experiment only) received one of four *T. canis* ova doses – 100, 1000, 3000 and trickle (1000 ova over 28 days, i.e. 250 per week). For the LACA mice this resulted in three larval intensity groupings described as low, moderate and high, and for the NIH mice, which carried lower numbers of larvae in the brain, two larval intensity groupings called low and moderate (table 2). The comparison between LACA and NIH mice revealed significantly lower numbers of larvae recovered from the brains of NIH mice for all dose groups except the 100 dose (table 1). Within strains, fewer larvae were recovered after a trickle dose of 1000 ova compared to a single dose of 1000 ova. It should be noted that in the present review, more attention is paid to the relationship between observed behavioural changes and larval burden in the brain rather than with dose of *Toxocara* ova administered.

Assessment of behavioural alterations in T. canis *infected mice*

The following categories of behaviour were investigated:

1. Baseline activity
2. Isolation-induced social behaviour

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Table 1. The mean number of *Toxocara canis* larvae (±SE) recovered from the brains of outbred LACA mice and inbred NIH mice infected with varying doses of *T. canis* ova. Adapted from Cox & Holland (2001a).

LACA strain			NIH strain				
Dose	Mean ± SE (Range)	% of dose	Dose	Mean ± SE (Range)	% of dose	t statistic	*P* value
100 n = 9	4.4±1.1 (0–9)	4.4	100 n = 5	3.2±1.4 (0–7)	3.2	−0.494	≤0.494
1000 n = 8	59.8±15.2 (14–142)	5.9	1000 n = 6	10.8±2.7 (3–20)	1.1	−3.202	≤0.015
3000 n = 9	183.1±49.7 (66–157)	6.1	3000 n = 4	39.2±5.7 (30–54)	1.3	−2.872	≤0.020
Trickle n = 7	35.5±5.9 (12–55)	3.5	Trickle n = 6	4.8±1.3 (0–9)	0.4	−5.202	≤0.002
2000 Expt A n = 25	54.3±42.8 7–146	2.2					
Expt B n = 22	66.2±33.1 10–129	3.3					

3. Responses to novelty and anxiety using four different paradigms:

   (i) Light/dark box
   (ii) Novel odours
   (iii) The 'T'-maze
   (iv) The elevated plus maze

4. Learning and memory

Baseline activity

Alterations in activity are one of the most widespread changes observed in infected hosts under laboratory and field conditions (Moore & Gotelli, 1990). Poulin (1995) has described changes in activity as simple traits thereby not fulfilling the criterion of complexity. It has been suggested that without evidence from predation experiments both decreases and increases in intermediate host activity can be argued to lead to greater susceptibility to predation and thus enhanced parasite transmission (Moore & Gotelli, 1990; Poulin, 1995). Webster (1994) compared the effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid rats. She demonstrated that out of six parasite species detected in wild rats, *T. gondii* was the only parasite which required predation by a definitive host to complete its life cycle and was the only species associated with higher activity levels in infected compared to uninfected rats.

In *Toxocara* infected mice, activity was assessed by monitoring six different independent categories of murine host behaviour – ambulation (general movement), grooming, rearing, digging, climbing and immobility as originally described by Hutchinson *et al.* (1980a). Within each behavioural category, the duration of time spent at each behaviour and the number of short and long bouts performed were recorded over a 20 min period.

Table 2. The cut-offs employed for *Toxocara canis* larval intensity in the murine brain of outbred LACA and inbred NIH strains of mice and behavioural tests.

		LACA strain			NIH strain[1]		
Behavioural test	Dose received	Cut-offs	Mean ± SE (n)	Range	Cut-offs	Mean ± SE (n)	Range
Baseline activity Exploratory behaviour Response to novelty Anxiety (elevated plus maze) Learning and memory	100, 1000, 3000 trickle	Low Moderate High	5.8±4.9 (11) 38.7±11.5 (10) 169.3±131.9 (12)	0–15 27–55 66–555	Low Moderate	4.3±2.8 (14) 29.4±13.7 (7)	0–9 13–54
Social behaviour	2000	Low (≤50) High (>50)	26.2±12.2 (10) 96.5±36.9 (15)	7–45 54–146			
Anxiety (predator odour and light/dark box)	2000	Low (≤70) High (>70)	40.2±20.1 (11) 92.2±20.1 (11)	10–66 68–129			

[1] Baseline activity was the only behavioural test performed on NIH mice.

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The activity of LACA and NIH mice differed prior to infection. LACA mice spent more time immobile compared to NIH mice, which ambulated and climbed more. Variations in the amount of time spent at a given activity were also observed between pre-assigned dose groups of mice of similar strain prior to infection. Post-infection, LACA mice spent more time immobile and this was particularly pronounced for mice which had received the 3000 and trickle doses or were defined as the high larval intensity group. Ambulation also decreased in the mice who had received the 3000 and trickle doses whereas the opposite was the case for the control and the 100 and 1000 dose group. In contrast, for NIH mice more categories of behaviour changed post-infection with immobility and ambulation decreasing post-infection and digging and climbing increasing.

In conclusion, the effect of infection on LACA mice particularly at high infection levels, is debilitation manifested as immobility. In contrast, the effect on NIH mice, which carry lower burdens of larvae in their brains, is to increase certain activities. What emerges from this experiment is that the strain of mouse selected in studies of parasitism on host behaviour is important – pre-infection behaviour differs between strains as does the effect of infection. Yan *et al.* (1994) demonstrated variation in susceptibility and behavioural change among genetic strains of beetle infected with *H. diminuta*.

Other studies, which examined the effect of *T. canis* infection upon murine activity, have provided contrasting results (Dolinsky *et al.*, 1981; Hay *et al.*, 1985, 1986). This is not surprising given that activity level is very sensitive to environmental and endogenous stimuli and may also be attributable to strain. Festing & Greenwood (1976) reported that strain and age differences irrespective of infection can influence behaviours such as wheel running. Our studies bear this observation out, i.e. that endogenous strain differences in activity patterns may actually overide the effects of infection.

Isolation-induced social behaviour

Social interactions of male mice infected with *T. canis* and their uninfected counterparts were assessed using methods employed by Arnott *et al.* (1990). The behaviour of mice was subdivided into a number of broad categories – non-social, social and antagonistic behaviours, which were further divided into aggressive, ambivalent and flight behaviours. A description of the social postures and individual elements of these broad categories of behaviour has been described by Grant & Mackintosh (1963)and Mackintosh (1981). Infected mice received a single dose of 2000 *T. canis* ova, which resulted in the creation of two larval intensity groupings (see tables 1 and 2). Precise details of the infections and mouse grouping procedures are provided in Cox & Holland (1998).

Changes in social behaviour were most pronounced in mice with high numbers of larvae in the brain compared to control mice and mice with low infections. Heavily infected mice displayed significant reductions in aggressive behaviours combined with high levels of flight and defensive behaviours compared with control and lightly infected mice (table 3).

A number of studies of social interactions among mice (Freeland, 1981; Rau, 1983, 1984; Edwards, 1988; Arnott *et al.*, 1990) and rats (Berdoy *et al.*, 1995) infected with different parasites have been undertaken. Rau (1983) described how laboratory mice infected with *Trichinella spiralis* were less likely to be dominant in pairwise interactions and that *T. spiralis* could reverse existing dominance (Rau, 1984). Similarly Freeland (1981) showed that mice infected with *Heligmosomoides polygyrus* were prevented from becoming behaviourally dominant over their uninfected counterparts. Arnott *et al.* (1990) reported that infected laboratory mice were more likely to be territorially aggressive when paired with an uninfected and previously unencountered mouse whereas Berdoy *et al.* (1995) demonstrated that the parasite had no significant effect on the establishment or maintenance of social status or mating success in laboratory/wild hybrid rats during competitive mating situations. These authors discussed an interesting explanation for the lack of a relationship between *Toxoplasma gondii* and social status or mating success (Berdoy *et al.*, 1995). The authors suggest that it is beneficial to the parasite to infect all social categories of rats in a colony – subordinates are more likely to be susceptible to predation and dominants are more likely to reproduce and hence transmit the parasite transplacentally. In contrast, *Toxocara canis* in mice can only be transmitted via predation.

Response to novelty and anxiety

Four different paradigms were used to explore the relationship between response to novelty and anxiety in *T. canis*-infected mice versus controls. All the mice used in these experiments were of the LACA outbred strain but the doses administered differed.

The four approaches were: (i) exploration and response to novelty in mice infected with four doses of *T. canis* using the 'T' maze; (ii) anxiety in mice infected with four doses of *T. canis* using the elevated plus maze; (iii) fear-induced exploration in mice infected with a single dose of *T. canis* using the light/dark apparatus; (iv) fear-induced exploration in mice infected with a single dose of *T. canis* using novel odours.

The 'T' maze. The 'T' maze was used to investigate general exploratory behaviour and response to novelty by the introduction of a running wheel into the apparatus. The response of the mice to an unfamiliar environment was assessed over a two-day period and after habituation. Mice received one of four doses of *T. canis* ova, which resulted in the creation of three larval intensity groupings (Cox & Holland, 2001b) (table 2).

Initially, exploration after habituation was observed to decrease significantly in high intensity mice and then on day 2 exploration declined in all the infected groups. The response to novelty was similar for the control, low and moderately infected group with a divergence in the high intensity group. The high intensity group spent less time in the novel arm and made fewer investigations of the novel object.

The elevated plus maze. The elevated plus maze (Pellow *et al.*, 1985) is a novel conflict paradigm in which the drive to explore conflicts with the negative drive to avoid

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Table 3. The mean numbers of categories of behaviour and individual elements of those categories for the control, low and high *Toxocara canis* larval intensity groups. Adapted from Cox & Holland (1998).

Behaviour	Control Mean ± SE	Low Mean ± SE	High Mean ± SE	*P* value
Category				
Aggressive	40.4±28.1	31.6±22.1	18.7±21.5	0.017*
				0.423†
Individual elements				
Threat	2.5±3.2	2.7±3.3	1.2±1.3	0.313*
				0.212†
Aggressive groom	3.8±5.3	2.0±4.9	0.7±2.4	0.002*
				0.043†
Attack	8.1±7.1	6.4±5.3	3.5±4.9	0.098*
				0.576†
Bite	7.8±7.2	6.6±4.9	3.1±4.9	0.051*
				0.84†
Chase	4.4±5.6	2.6±3.2	1.6±3.3	0.122*
				0.603†
Rattle	9.6±8.6	8.5±11.2	5.0±4.8	0.087*
				0.293†
Circle	0.04±0.2	0±0	0±0	0.525*
				0.505†
Zigzag	0.04±0.2	0±0	0±0	0.525*
				0.505†
Walk around	105±2.6	0.6±1.3	0.7±1.2	0.628*
				0.299†
Over	2.5±3.9	3.4±3.2	1.5±3.4	0.345*
				0.335†
Category				
Flight	11.7±14.1	14.8±13.9	23.8±16.7	0.018*
				0.293†
Individual elements				
Evade	1.0±1.7	2.2±1.6	1.7±4.5	0.878*
				0.018†
Retreat	0.8±1.6	1.3±2.1	1.1±2.8	0.848*
				0.655†
Flee	1.7±2.7	2.0±3.3	6.6±7.7	0.051*
				0.611†
On back	2.7±3.9	2.9±4.3	4.2±5.1	0.138*
				0.732†
Kick	2.6±4.2	4.8±6.4	3.8±5.0	0.396*
				0.076†
On bars	1.1±3.4	0.4±0.7	2.4±4.7	0.177*
				0.649†
Freeze	0.4±1.1	0.4±0.9	1.1±1.7	0.041*
				1.000†
Off bars	1.1±3.9	0.6±1.1	2.2±4.8	0.365*
				0.591†

* Control and low burden comparison.
† Control and high burden comparison Adapted from Cox & Holland (1998).

open or exposed areas. The apparatus consists of two open arms painted white, two enclosed arms painted black with an open roof elevated 50 cms off the ground. The arms extend from a central platform. Infected mice received one of four doses of *T. canis* ova, which resulted in the creation of three larval intensity groupings (see table 2).

Moderate and high intensity mice spent more time in the open arms compared to the controls. When the number of times the mice entered the open arm was expressed in terms of the total number of arm entries (open and enclosed arms) these were significantly higher for the infected mice and particularly so for the moderate intensity mice. When the time spent in the open arm was expressed in terms of the total time spent by each mouse in both open and enclosed arms, the trend was similar in that infected mice spent more time than the controls, and this was particularly pronounced for both the moderate and high intensity mice.

These results imply that more intensely infected mice were less anxious and less inhibited to open areas which could increase the risk of being located by a predator. This study incorporated two elements which are infrequently used in the elevated maze test – time spent on

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the central platform, which may be more aversive than the areas protected by walls and the number of approaches/avoidances towards the open arm which is a good measure of the cautiousness of the animal. No differences were observed among the four groups for time spent on the central platform but the controls clearly showed more approaches/avoidances compared to the infected groups.

The light/dark box. Investigation of exploration in a novel environment and reaction to open and exposed areas was investigated in the standard light/dark box. Mice received a single dose of 2000 *T. canis* ova, which resulted in the creation of two larval intensity groupings (Cox & Holland, 1998) (table 2). Mice with low infection in the brain (analagous to the moderate group in the elevated maze and 'T' maze experiments; see table 2) displayed differences in behaviour compared to the control and high intensity group which displayed similar behaviour in the light/dark box. This difference was statistically significant for the time spent in the light area of the box which was significantly longer for the low burden group compared to the control and the high burden group.

Novel odours. A number of authors have recommended that predation experiments represent the best way of evaluating the adaptive significance of parasite-altered host behaviour and its relevance to enhanced transmission (Moore & Gotelli, 1990; Poulin, 1995; Webster *et al.*, 2000). Although some studies have been performed on the predation of invertebrate intermediate hosts by vertebrate definitive hosts (for e.g. Bethel & Holmes (1977) using amphipods; Urdal *et al.* (1995) using copepods; Webster *et al.* (2000) using *Tenebrio* beetles), predation experiments using vertebrate prey are likely to be logistically difficult and may be ethically unacceptable. A notable exception was a study by Vorisek *et al.* (1998) which utilized mice experimentally infected with *Sarcocystis dispersa* and a feral long-eared owl in a predation experiment. We attempted an experiment which would simulate the response of *Toxocara canis*-infected mice to a potential predator by means of the presentation of predator and non-predator odours (Cox & Holland, 1998).

The response to predator and non-predator odours by *T. canis*-infected and uninfected mice was investigated by exposing mice to odours in a restricted environment, the Y maze (Kavaliers & Colwell, 1995a). The predator odour was from the litter tray of a feral cat, the non-predator odour was from the litter tray of laboratory bred rabbits and the control odour was unused cat litter alone. The experiment was run over a three-day period using the following combinations – predator odour versus control odour, predator odour versus non-predator odour and non-predator odour versus control odour. Mice received a single dose of 2000 *T. canis* ova, which resulted in the creation of two larval intensity groupings (Cox & Holland, 1998) (table 2).

When the predator odour versus control odour was compared on day 1, mice with low infection showed a significant preference for the predator odour arm whereas the control group showed no preference for either arm. The high infection group demonstrated a significant preference for the control arm. When the predator odour versus the non-predator odour was compared on day 2, there was no significant difference between the three groups for the time spent in the predator odour arm. The time spent in the non-predator odour arm was significantly less in the low infection group compared to the control. Intra-group comparisons revealed that the control group showed no preference for either arm, while the low and high groups showed a significantly greater preference for the predator odour arm. When the non-predator versus the control odour arm was compared on day 3, there was no significant difference in the time spent in the non-predator and control odour arm of the maze between the three groups of mice. Intra-group comparisons showed that the control and low infection groups showed a significantly greater preference for the non-predator odour arm compared to the high infection group.

These four paradigms explored various aspects of exploration, response to novelty, fear and anxiety. In general, infected mice were less exploratory and less responsive to novelty and less aversive with respect to open and lighted spaces and predator odours. Exploration is an essential but risky behaviour carried out by small rodents. One of the adaptive values of successful exploration lies in an enhanced ability to escape from predators (Montgomery & Gurnell, 1985). The exploratory behaviour of the mice in the 'T' maze differed between the groups with low and moderate intensity mice being more exploratory compared to the controls and high intensity mice showing less exploration. Over the two days of habituation, exploration was reduced in all groups as the mice became more familiar with the environment but this reduction was more pronounced for the low and moderate intensity groups compared to the high intensity group. This implies that normal exploration is impaired in the high intensity group of mice. In addition, the high intensity group spent less time in the novel arm and made fewer investigations of the novel object compared to the control and the two other infected groups. It is likely that these reductions in exploration and response to novelty result from the non-specific debilitatory effect of heavy infection rather than enhanced neophobia.

Burright *et al.* (1982) infected mice with graded doses of *T. canis* and showed that they differed in their response to a familiar and novel environment. Mice infected with a dose of 1000 larvae never entered the novel environment whereas mice which received a lower dose of 500 and 250 ova did; however, control mice entered the novel environment on more occasions and spent significantly more time than any of the infected groups. Dolinsky *et al.* (1981) also showed that infected outbred mice, which received a single dose of 1000 ova, showed a longer latency to enter a novel cage compared to control mice. In contrast, Hay & Aitken (1984) found outbred mice, infected with 1000 ova, to be less cautious and to display greater preference for a blocked novel arm in a Y maze compared to control mice.

Changes in exploration have also been observed for the protozoan parasite *Toxoplasma gondii* that, like *Toxocara*

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canis, has a predilection for the brain in its intermediate or abnormal hosts. Laboratory mice have been shown to be less responsive to a novel arm in a Y maze although their activity was greater during habituation and test trials (Hutchinson *et al.*, 1980b; Hay *et al.*, 1983, 1984). In contrast, *Toxoplasma gondii*-infected wild and hybrid rats also displayed increases in activity but were significantly more responsive to novel stimuli than control rats, indicating marked reductions in normal neophobic behaviour associated with rats (Webster, 1994; Webster *et al.*, 1994; Berdoy *et al.*, 1995).

In the predator odour test, we hypothesized that if the cat odour evoked a particularly threatening stimulus, the control mice would avoid the arm containing the source of the stimulus, however, this was not observed. The subjects used in the experiment had never been exposed to these odours before so it was assumed that possible risk assessment evoked by the odour might result from an innate recognition of the cat odour as a danger signal. It has been shown that predator odour aversion is usually innate, including the response of laboratory and wild rats to cat odour (see Berdoy *et al.*, 2000). In our case the possibility exists that the chosen predator odour was not strong enough to elicit a fear response at least in the control mice.

However, in the present study, behavioural differences were observed between the groups of mice which indicates that infection did reduce anxiety or cautiousness in the mice. Initial analysis by infected versus controls produced no discernible difference between the groups and this may be explained by the divergence of the response of the high infection group from the low infection group. *Toxocara canis*-infected mice have been shown previously to exhibit less cautious behaviour on exposure to a new environment by displaying a greater preference for a previously blocked novel arm in a Y maze (Hay & Aitken, 1984). This is similar to the response of infected mice in the present study, particularly the low infection group. Mice with low infection demonstrated a preference for the predator odour arm on day 1, which was followed by a significant predator odour preference by high and low infection groups on day 2. The fact that control mice explored the two arms of the maze equally on days 1 and 2 and showed no odour preference until day 3 when the predator odour was absent and the apparatus had become familiar would indicate that these mice were more cautious. The tendency to explore the aversive area of the novel environment was greatest in mice with low infection on initial exposure. The results from this experiment indicate that mice infected with *T. canis* can display lower levels of anxiety in a threatening situation which in turn is dependent upon the larval number in the brain. These results are mirrored in the observations from the light/dark box where mice with low infection in the brain spent more time in the light side of the box.

It is difficult to assess why mice with lower brain infection in two of the experiments which received a 2000 ova dose of *T. canis* ova showed a greater deviation than the high infection group. The difference may be attributable to several causes. Firstly, the explanation may lie in brain function and the site which larvae occupy although we have no evidence to confirm or refute this suggestion. Several studies, based upon a single dose of *T. canis* ova, reported larvae in a non-random distribution within the brain (Burren, 1971; Dolinsky *et al.*, 1981; Summers *et al.*, 1983). Larvae were recorded from the heavily myelinated tracts of the corpus callosum, internal and external fornix capsules, cerebellar peduncles and the cerebellar medulla (Summers *et al.*, 1983). No studies, however, have simultaneously examined the relationship between dose and larval position in the brain and observed changes in behaviour. Secondly, the baseline behaviour of mice with high larval burdens may differ from that of the mice with low burdens and control mice, in that they had naturally higher levels of neophobic or cautionary behaviour. The issue of pre-infection behavioural variability has already been demonstrated for a simpler type of behaviour like activity. Finally, mice with heavy burdens may, depending upon the behavioural test employed, show little specific alteration in behaviour but more the general manifestations of debilitation which by coincidence mirror those of the controls for these particular behavioural tests.

Learning and memory

In this experiment, mice were exposed to a water finding apparatus which is designed to examine the ability of mice to gather information from a novel environment and to remember the location of a specific resource within that environment (Ettenberg *et al.*, 1983). The latency to relocate the resource, in this case a water tube, after a period of deprivation was considered to be an indication of memory capacity of the mice (Cox & Holland, 2001b).

Moderate and high intensity mice showed a greater latency to enter the alcove, find the water tube and to drink from it compared to controls, although these differences did not reach statistical significance. This was particularly pronounced for the time it took the mice to find the drinking tube – with virtually double the time being taken by the moderate and high intensity mice compared to the low intensity group and the controls. In addition, the control group contained the highest percentage of mice which actually drank once they found the water tube compared to the infected groups in which the proportion of mice that actually drank was much lower (Cox & Holland, 2001b).

These results suggest possible memory impairment among moderate and high intensity mice. It should be noted that during the training period, all the infected mice showed reduced exploration compared to the controls and this could affect the ability of the animal to remember the location of the tube or result in the failure of the mouse to identify this resource on the training day. Furthermore, the drive to enter the alcove may be influenced more by a desire to locate the alcove itself, as a consequence of the murine preference for narrow, dark places, than a need to locate the water source.

Toxocara canis infection in laboratory rats (Olson & Rose, 1966) and laboratory mice (Dolinsky *et al.*, 1981) has been shown to affect learning. Poor spatial learning has been demonstrated in mice with light and moderate infections of the intestinal nematode *Heligmosomoides*

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Table 4. Summary of the behavioural alterations observed in LACA and NIH mice in relation to the intensity of *Toxocara canis* larvae in the brain.

	LACA strain		NIH strain	
Type of behaviour	Specific element of behaviour	Effect/group	Specific element of behaviour	Effect/group
Activity	Immobility	Increase high burden	Immobility Digging Climbing	Decrease high burden Increase high burden Increase low and high
Social behaviour	Aggression	Decrease high burden	ND	ND
	Flight	Increase high burden	ND	ND
Response to novelty and anxiety				
T-maze	Exploration	Decrease high burden	ND	ND
	Investigation of novel object	Decrease high burden	ND	ND
Light/dark	Time spent in light area	Increase low burden	ND	ND
Predator odour	Time spent in predator odour arm	Increase low burden	ND	ND
Elevated maze	Time spent in open arm	Increase moderate and high	ND	ND
Learning and memory	Time to find water tube	Increase moderate and high	ND	ND
	% mice which drank	Decrease moderate and high	ND	ND

ND, not done.

polygyrus (Kavaliers & Colwell, 1995b) and it has been suggested that the poorer performance shown by infected mice could result in an inability to gain the same information from environmental cues as control mice.

Conclusions

Mice infected with the parasite *T. canis* show changes in a wide range of behaviours compared to control mice (table 4). Alterations in behaviour are generally related to the burden of larvae in the brain. Mice who harboured particularly heavy burdens (produced as a consequence of receiving doses of 3000 eggs) show changes in behaviour, which are most likely to be explained by debilitation. For example, immobility was significantly increased in heavily infected LACA mice and exploration in the 'T' maze was most significantly reduced in this same group. In humans, Taylor *et al.* (1988) described abdominal pain, nausea, limb pains and lethargy among a large group of other non-specific symptoms associated with raised titres to *Toxocara* (>0.3 optical density).

Therefore do these changes result from a specific manipulation or a side effect from the presence of the parasite in the brain? We conclude that for most of the experiments the evidence points to a non-specific side effect of infection, which shows a relationship with intensity of infection. Mice with higher numbers of larvae in their brains are less active, less exploratory, less aggressive, more likely to spend time in the open areas of the elevated maze and show some impairment in learning and memory. The only two paradigms that do not conform clearly to these trends are the light/dark box and the predator odour whereby so-called low intensity mice (regarded as moderate depending upon which cut-off you use) diverged from high infection mice and showed decreased aversive behaviour. It should be noted that mice in these two experiments, along with the investigation of social behaviour, received a single dose of *T. canis* ova compared to the four-dose regime in all the other experiments.

This raises an important issue with regard to infective doses used in these experiments. We conclude from our data that alterations in behaviour in relation to larval burden in the brain are more clearly definable when intensity groupings are created in mice that received one infective dose. When mice from several dosage regimes are combined within an intensity grouping we may be missing the influence of visceral effects on the observed behaviours. In this respect some behavioural data may be masked or lost. Therefore by controlling the aspect of dose in relation to investigating larval intensity and behavioural alteration a single dosing regime should be utilized.

The fact that such a wide range of behaviours is affected argues against a specific alteration, which is clearly linked to susceptibility to predation. Furthermore, *T. canis* can be maintained in a wide range of paratenic hosts all of which behave quite differently therefore it is unlikely that the parasite could select for a behavioural trait which if altered would have the same consequences for all hosts. This in turn would suggest that the increased susceptibility is not specific and therefore not adaptable to either host or parasite, as it is unlikely that the correct predator will take the infected host in every confrontational situation. Parasite manipulation of intermediate host behaviour may be considered a true adaptation as, in many cases, the alteration will not occur until the onset of parasite infectivity to the next host (Poulin *et al.*, 1992; Tierney *et al.*, 1993) and is

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therefore more specific. Berdoy *et al.* (1995) concluded from their studies on free-ranging rats infected with *Toxoplasma gondii* that there was a lack of selective benefit to the parasite from altering all host behavioural patterns and that the effect of *T. gondii* was specific (i.e. increased exploratory behaviour and no effect on social status or mating success) rather than general.

Behaviour alterations observed in *Toxocara*-infected mice may still result in animals which are more susceptible to predation. Animals which are debilitated, less active and less aggressive on the one hand but also less able to assess risk will be more vulnerable. The numbers of larvae which reach the brain of infected mice in the wild are likely to be considerably less than those in many of the mice described in these experiments. The provision of a low dose (100 ova) in some of the experiments produced mice with lower burdens in the brain. Dubinsky *et al.* (1995) examined the brains of 476 small mammals from Slovakia and found the numbers of larvae to range from 1 to 13 per brain with the peak average being 4.2 ± 4.1 in animals collected from a suburban location. The numbers of larvae harboured by the low intensity group in this paper are very similar to those described by Dubinsky and colleagues. In a natural situation, wild rodents are likely to be prone to repeated infections which have been shown to result in less accumulation of larvae in the brain (Abo-Shehada *et al.*, 1991). Mice with low burdens in the brain (in the four dose regimes) showed few alterations in behaviour and were often similar to uninfected mice.

The other question that arises from this work is whether these observations are relevant to human hosts infected with *Toxocara*. Human brains are much larger than those of mice and, as in the case of naturally infected rodents, numbers are likely to be low. Despite the fact that *T. canis* larvae have been identified in the brains of humans (see Hill *et al.*, 1985), no data exists on intensity of infection or the relationship between neural involvement and pathogenesis in humans. A small number of investigations concerning the effect of *T. canis* (Nelson *et al.*, 1996; Magnaval *et al.*, 1997) and *Toxoplasma gondii* (Flegr *et al.*, 1996) on human behaviour have been conducted. Nelson *et al.* (1996) did not find a consistently strong relationship between *Toxocara canis* exposure and reduced intelligence, although there was a trend towards a decrease in cognition. The authors could not fully determine whether the observed trend was due to infection or pre-exposure lower intelligence, which is often the case in such investigations. Flegr *et al.* (1996) found a correlation between *Toxoplasma gondii* immunity and certain personality disorders by which males revealed a high disregard for societal rules, whereas women displayed outgoingness and easygoingness. The authors associated these findings with those found in animal models by which rodents infected with *T. gondii* are less anxious and less neophobic (Hutchinson *et al.*, 1980a; Hay *et al.*, 1984; Webster *et al.*, 1994).

To conclude, *Toxocara canis* infection induces behavioural alterations in its murine paratenic host but these changes are likely to be as a consequence of parasite-induced pathology rather than an adaptive mechanism of parasite-altered host behaviour. Clearly, the mechanism(s) by which the parasite alters host behaviour is of importance. The observation of Dolinsky and co-authors (1981) that the administration of lead in conjunction with *T. canis* ameliorated the behavioural alterations observed in mice infected with *T. canis* alone remains unexplained but would suggest that the mechanism may be chemically orientated as opposed to site related. Certainly, this model system may prove to be useful for the investigation of a debilitatory parasitic infection on behaviour.

Acknowledgements

The authors are grateful to Janice Moore for comments on an earlier draft of this paper.

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(Accepted 7 December 2000)
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