The relationship between numbers of larvae recovered from the brain of *Toxocara canis*-infected mice and social behaviour and anxiety in the host

D. M. COX* *and* C. V. HOLLAND

Department of Zoology, Trinity College, Dublin 2, Ireland

(*Received 16 September 1997; revised 15 January 1998; accepted 15 January 1998*)

SUMMARY

The effect of the nematode *Toxocara canis* on social behaviour and anxiety levels of adult male outbred (LACA) mice was examined following infection with a single dose of 2000 ova. The actual number of larvae recovered from the brain of each individual mouse was determined after behavioural testing. The effect of the parasite on mouse behaviour was analysed by both the initial dose administered (i.e. infected versus control) and the degree of infection in the brain. There was substantial variation in the number of larvae recovered from the brains of the individual mice and the magnitude of behavioural change was associated with the level of infection. Examination of social behaviour for both analyses revealed that the infection reduced levels of aggressive behaviour and increased levels of flight and defensive behaviours. High infection in the brain induced the greatest degree of behavioural change which decreased in mice with lower infections. In contrast the analysis of anxiety levels in mice by initial dose administered revealed no difference between infected and control mice. Mice with low infection in the brain, however, displayed a greater level of risk behaviour by spending more time in the vicinity of a predator odour and in the light area of a light/dark paradigm than control or high infection mice. The results suggest that the behaviour of mice infected with *T. canis* is influenced by the number of larvae accumulated in the brain. This may have important consequences for the conclusions drawn on the effect of this parasite on murine behaviour.

Key words: *Toxocara canis*, outbred mice, social behaviour, anxiety, brain, predation.

INTRODUCTION

The possibility that parasite transmission might be facilitated as a consequence of alteration in host behaviour has been the focus of much attention over the past 2 decades, culminating in a review by Moore & Gotelli (1990). From these studies it is evident that parasite infection can engender a wide range of behavioural alterations in numerous host–parasite systems. In many instances, the observed change which occurs in the behaviour of the infected host has been regarded as an adaptive 'strategy' by which parasites with indirect life-cycles select for certain behavioural traits in their intermediate hosts which if altered will increase the hosts susceptibility to capture by the parasites final host (Bethel & Holmes, 1973, 1974; Moore & Gotelli, 1990; Poulin, Curtis & Rau, 1992). It has also been suggested that the observed changes in behaviour, may be a strategy developed by the infected host wishing to eliminate the parasite or compensate for its effects (Hart, 1990; Milinski, 1990).

In addition, however, parasites with direct life-cycles or parasites which find themselves in accidental or paratenic hosts have also produced behavioural changes within their hosts (Freeland, 1981; Dolinsky *et al*. 1981, 1985; Kavaliers & Colwell, 1995*a*; Lefcroft & Blaustein, 1995). In order to decipher whether parasites select for behavioural traits to increase their chances of survival or whether the changes are side-effects in response to infection (e.g. Kavaliers & Colwell, 1995*a*) it is necessary to investigate this phenomenon in a large number of host–parasite systems. More studies are required to investigate the mechanism by which the behavioural change is manifest within these hosts. The majority of investigations to date have focussed largely on indirect life-cycle systems with the intermediate host being the main focus of research. The effect of parasite infection on normal or paratenic host behaviour has received little attention despite the obvious importance in answering questions on whether host–parasite manipulation is an adaptive strategy or a pathological side-effect. In many paratenic hosts, the life-cycle of the parasite halts at an immature stage which can migrate aimlessly through the hosts tissues contributing to pathology. Immature stages of parasites which show a predilection for certain tissues in the body may therefore be more appropriately monitored behaviourally in relation to parasite burdens within

* Corresponding author. Tel: +1 6081096. Fax: +1 6778094. E-mail: dmcox@mailtcd.ie

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

that specific area as opposed to simply monitoring changes in behaviour in relation to the dose of infection administered.

Toxocara canis has a direct life-cycle which involves an external egg stage that embryonates in the environment (Glickman, 1993). Within the paratenic host *T. canis* is neurotropic and accumulates in the brain approximately 14 days after the initial infection (Dunsmore, Thompson & Bates, 1983). The implications for studying the behavioural effects of this infection in paratenic hosts such as small rodents, are thus obvious from the point of view of both the transmission of the parasite to the final host and the possible consequences for human behaviour (Holland, 1997). In the wild, mice have been shown to act as a reservoir for *T. canis* (Dubinsky *et al*. 1995) and may therefore serve as an important factor in the circulation and maintenance of the parasite through predation by canids.

Previous studies of altered behaviour on paratenic hosts infected with *T. canis* have shown that rats and mice tend to display a reduction in the ability to learn (Olson & Rose, 1966; Dolinsky *et al*. 1981, 1985). Investigations on exploration, response to novelty and general activity in mice produce conflicting results. Some researches conclude that mice infected with this parasite are less exploratory, less neophilic and less active than control mice (Dolinsky *et al*. 1981; Burright *et al*. 1982) while others demonstrate a reversal of these behaviours in *T. canis*-infected mice even when the same testing procedures were utilized (Hay & Aitken, 1984; Hay, Aitken & Arnott, 1985; Hay *et al*. 1986). Despite the affinity of this parasite for the brain there have been few attempts to associate parasite burden in this area with observed changes in host behaviour as opposed to the relationship with dose of parasite ova given. Furthermore, there is now evidence for significant variation in the numbers of *T. canis* larvae recovered from the brain of individual mice (Skerrett & Holland, 1997).

The aim of the present study was to investigate behavioural changes in mice infected with *T. canis* and to consider the effect of these changes in relation to the manipulation hypothesis. We also aimed to determine whether the magnitude of the behavioural change was related to the level of infection in the brain of the mice. Controlled experimental conditions were employed in order to decipher whether such an association exists. Once this baseline information is established the effects of the parasite in the wild/normal situation can be investigated.

MATERIALS AND METHODS

Mouse maintenance

A total of 100 outbred (LACA) male mice aged between 7 and 8 weeks was used in the experiment. The mice were assigned to 2 groups of 50 for each experiment. Within each group 25 mice were controls and 25 were infected with 2000 embryonated ova of *T. canis*. The mice were housed in an animal maintenance room 2 to a cage for the social experiment (Exp. 1) or in groups of 10 for the anxiety experiment (Exp. 2). The single cages housing 2 mice measured $35 \times 15 \times 13$ cm and the larger cages housing a maximum of 10 mice measured $41 \times 24 \times 13 \times 20$ cm. The maintenance room provide a stable and soundproof environment in which to house the animals. The room was illuminated by a 200 Lux bulb which operated on a 12 h daily cycle (8.00 a.m. lights on – 8.00 p.m. lights off). The room was kept at a constant temperature of $22\,^{\circ}\mathrm{C} \pm 2\,^{\circ}\mathrm{C}$ and humidity of $50 \pm 10\,\%$ RH which was monitored daily. An automatic fan circulated a continuous stream of warm air around the room. The mice were fed Redmills Commercial Rodent Nuts and free access to food and water was available at all times. The mice were habituated to these conditions for 2 weeks before any infection or behavioural tests were carried out.

Infection of mice

Fifteen days after their initial arrival 25 mice from Exp. 1 and 25 mice from Exp. 2 were orally infected by stomach intubation randomly and independent of body weight a dose of 2000 infective ova suspended in 0·2 ml of distilled water. Control mice were sham-inoculated with distilled water. The infection was allowed to establish for 30 days before testing began. Previous research has demonstrated that in mice infected with *T. canis* ova the larvae reach the brain within 2 days and stabilize at this site between days 35 and 45 (Burren, 1971). It was considered that behavioural effects due to the presence of the parasites in the brain would be most conspicuous during this time. Three mice died shortly after infection. However, there was no evidence of malaise in any of the remaining mice throughout the course of the experiment.

Behavioural testing

Behavioural assessment was carried out within the animal maintenance room where the animals were housed. The experiments were carried out over 7 consecutive days between 11.00 a.m. and 6.00 p.m. Each individual experiment was recorded on videotape using a Sony Camcorder Recorder® attached to a video monitor which allowed the animals behaviour to be observed from a position in which the animal could not see the experimenter. After each mouse had been tested, faecal pellets were removed and the whole apparatus was swabbed down with dilute alcohol solution before the next mouse was exposed to the apparatus. The data were collected and recorded by analysing the pre-recorded videos, in

which the infection status of each group was unknown.

Experiment 1 : isolation-induced social interactions

The social interactions of male mice infected with *T. canis* and their uninfected male counterparts was assessed using methods employed by Arnott *et al.* (1990). The behaviour of the mice was subdivided into a number of broad categories. The categories comprised non-social, social and agonistic behaviour which was further divided into 3 subgroups (a) aggressive behaviour, (b) ambivalent behaviour and (c) flight behaviour. A description of the social postures and individual elements of these broad categories of behaviour in laboratory mice has previously been detailed in the work of Grant & Mackintosh (1963) and Mackintosh (1981).

Apparatus. The testing apparatus consisted simply of a neutral standard home cage which was divided into 2 equal portions by a wooden barrier and covered with moss peat bedding.

Methods. For identification purposes 25 of the 50 male mice were randomly chosen and marked with picric acid. The mice were housed in pairs in standard Type 1 NKP cages. One marked mouse was housed with 1 unmarked mouse and assigned a labelled code of A1A2, BIB2 etc. One mouse from each cage was then randomly selected (marked or unmarked) and infected with a single dose of 2000 infective ova as described previously. The 25 groups of mice were maintained in these groupings for 25 days. Five days prior to the commencement of behavioural testing the mice were removed into individual cages. Behavioural testing began by placing the mice in the test cage on either side of the barrier. The pairings of mice at this stage were different to the pairings in the original housing arrangements. The mice were left in the apparatus for 1 min. The barrier was then removed allowing the mice access to the whole cage and each other. A video recorder was immediately activated which monitored the mice over a 10 min period. This procedure was repeated for all the pairings of mice. Fresh bedding was applied before each new pair of mice were introduced to the apparatus. Playback of the tape allowed the behaviour of each mouse to be categorized according to the descriptions reported by Mackintosh (1981). The total attacking time (cumulative time spent in fighting) and the number of first time attacks were also recorded as further indicators of aggressive behaviour. Both were scored to the nearest second using a digital stopwatch.

Experiment 2 : fear-induced exploration using a light/dark apparatus and novel odours

The 50 mice were housed randomly in 5 groups of 10 and each cage was assigned a letter. The 10 mice within each cage were then coded with picric acid and within these groups 5 mice were randomly infected with *T. canis* and 5 remained as controls.

(*i*) *Light/dark box*. Investigation of exploration in a novel environment and reaction to open and exposed areas was investigated in the standard light/dark apparatus. The apparatus is made of Plexiglas and measures $40 \times 40 \times 9{\cdot}9$ cm and is divided into 2 areas, a light area of dimensions $25 \times 40 \times 9{\cdot}9$ cm which was coloured white and illuminated using a 60 watt lamp which was centred over the light area. The dark area measured $15 \times 40 \times 9{\cdot}9$ cm and was coloured black. Each area was accessible to the mice by a square door positioned in the centre of the barrier separating the light and dark compartments. A large pane of glass covered the whole apparatus during the testing procedure and prevented the escape of the mice (Crawley & Goodwin, 1980).

Testing began by placing a single mouse into the centre of the light area of the apparatus. The video camera was then activated and murine behaviour was recorded over a 10 min period. The following measurements were made. (1) The latency to enter the dark side of the box, after initial introduction to the test apparatus; (2) the latency to re-enter the light area of the box; (3) the duration of time spent within the light area; (4) the number of transitions performed from the light to dark area; (5) number of rears performed in the light area.

(*ii*) *Predator odour*. The response to predator and non-predator odours by *T. canis*-infected and uninfected mice was investigated by subjecting the mice to the odours in a restricted environment, the latter in this case being the Y maze (Kavaliers & Colwell, 1995*b*).

The odour preferences of the individual male mice were investigated in a wooden Y maze apparatus. The arms were 8 cm in width and 30 cm in length. Two stimulus compartments were situated at the end of the two arms of the Y in which the odour cues were placed. The stimulus compartments and the start box measured 14 cm in length. A solid Plexiglas barrier restricted the mouse to the start box, while perforated Plexiglas barriers at the ends of the 2 stimulus arms prevented contact with the odour sources. Removable solid Plexiglas barriers were also present at 'seams' 8 cm into each of the stimulus arms. These barriers prevented exposure of the mice to the odour cues until the designated test times.

Three odour sources were utilized in the experiment. These consisted of a predator, non-predator and a control odour. The source of the predator odour came from the litter tray of a feral cat. The non-predator odour was obtained form the litter trays of laboratory bred rabbits which was mixed with unused cat litter. The control odour source was unused cat litter alone. The experiment

was run for 3 days for each mouse using the following odour combinations. (1) Predator and control odour (Day 1). (2) Predator and non-predator odour (Day 2). (3) Non-predator and control odour (Day 3). The same amount of litter was introduced into an open-topped plastic container which was placed in the relevant stimulus arm. The experiment began by placing a mouse in the start box of the apparatus for 2 min after which the solid barrier was removed allowing the mouse access to the 2 arms of the maze, 2 min later the solid Plexiglas barriers were removed, exposing the mouse to the odours. The video camera was then activated and murine behaviour was recorded over a 5 min period.

The following measurements were made. (1) The duration of time spent by a mouse in each arm within 8 cm of an odour source. (2) The number of bouts of grooming performed in either arm. The 'preference' of the mice for an odour source was defined as the duration of time spent in 1 arm divided by the total time spent in the 2 stimulus arms.

Larval counts

The mice in each experiment were killed by cervical dislocation on the day following the last behavioural experiment. The brain was removed using fine scissors and forceps. The Baermann procedure was carried out in order to count the number of larvae recovered from the whole brain of individual mice. Thus for each mouse, the individual behaviour and corresponding numbers of larvae retrieved from the brain were known. Following processing by the Baermann technique, the fluid in each tube was reduced to a volume of 5 ml. The pellet was thoroughly mixed by being vortexed for 15–20 sec at high speed. A volume of 0·2 ml was removed, placed on a glass slide and a 20×50 mm cover-slip was placed on top. In some instances, it was necessary to add extra fluid to the tube to evenly disperse the brain tissue when it was too dense. The slide was then examined under the light microscope under $\times 10$ objective and systematically scanned for larvae. The procedure was repeated until all the brain material was examined. When all the fluid was examined, the tube was rinsed with a small volume of fresh saline in order to locate any larvae which may have adhered to the bottom or sides of the tube. The total number of larvae from all the fluid was then recorded.

Statistical methods

All statistical tests were carried out at the 95% confidence limit. Data from the predator odour and light/dark tests were subjected to a log transformation and analysed using a Student's *t*-test. In the predator experiment *t*-tests were used to investigate differences between control and infected groups and also for intra-group comparisons of the preference of either control or infected mice for a particular odour arm. The data relating to the social interactions in infected and uninfected mice were not normally distributed, thus a non-parametric Mann Whitney U-test was required.

RESULTS

Larval burdens in the brains of mice

The individual variation in larval recoveries was large among the mice despite the fact that they received a single dose of 2000 ova each (Figs 1 and 2). This large variation resulted in some mice carrying very few larvae in the brain while others had relatively heavy burdens and the effect of this variation was reflected in the resulting behaviour of the mice. Due to the deaths of 3 mice shortly after infection, 47 brains were sampled and all were positive for *T. canis* larvae. The percentage of larvae recovered from the brain as a percentage of the total dose administered was 2·2 and 3·3% respectively for each experiment. In the investigation of social interactions the mean number of larvae recovered was $54{\cdot}3 \pm 42{\cdot}8$; range 7 to 146. In the investigation of anxiety the mean number of larvae recovered was $66{\cdot}2 \pm 33{\cdot}1$; range 10 to 129. On the basis of the findings obtained from the investigation of larval recoveries in the brains of the infected mice each infected group was classified into low and high *T. canis* larval recovery. The cut-off level for the classification of the mice into low and high infection for the social interaction experiment was 50. Individual mice with 50 or less larvae recovered from the brain were classified as low infection ($n = 10$; mean $= 26{\cdot}2 \pm 12{\cdot}2$; range 7 to 45) and individual mice with greater than 50 larvae were classified as high infection ($n = 15$; mean $= 96{\cdot}5 \pm 36{\cdot}9$; range 54 to 146). The cut off level for the classification of the mice into low and high infection for the predator odour and light/dark experiment was 70. Individual mice with 70 or less larvae recovered from the brain were classified as low infection ($n = 11$; mean $= 40{\cdot}2 \pm 20{\cdot}1$; range 10 to 66) and individual mice with greater than 70 larvae were classified as high infection ($n = 11$; mean $= 92{\cdot}2 \pm 20{\cdot}1$; range 68 to 129).

Individual larval accumulation and behavioural response

The behavioural manifestations observed in the infected mice were found to be associated with the level of infection in the brain of the mice and were somewhat dependent on the behaviour which was under investigation. In the non-specific test of social behaviour high infection induced a greater degree of behavioural change, which decreased in mice with lower infections. In the specific tests of anxiety and

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

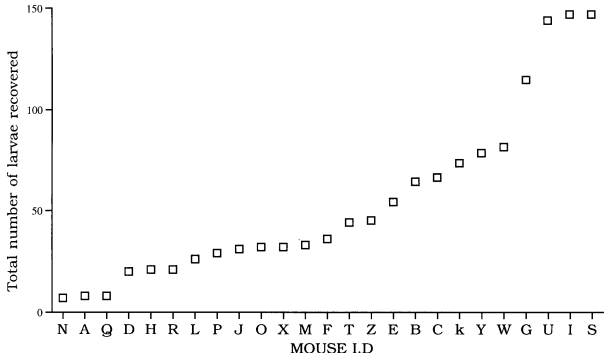

Fig. 1. Larval recoveries from the brains of mice infected with 2000 *Toxocara canis* ova used in the investigation of social interactions ($n = 25$).

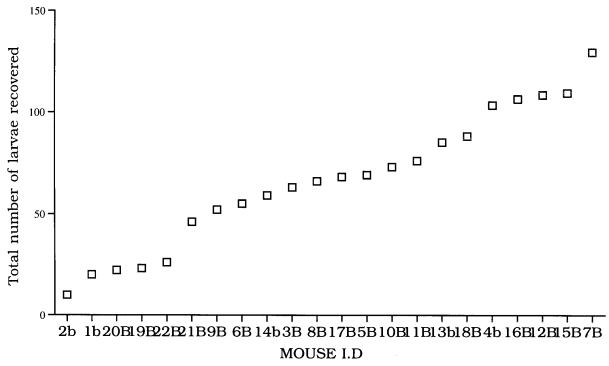

Fig. 2. Larval recoveries from the brains of mice infected with 2000 *Toxocara canis* ova used in the investigation of anxiety ($n = 22$).

exploration mice with lower infections produced a greater alteration from normal behaviour than mice with high infections.

Social behaviour

The social behaviour of the 3 groups of mice are shown in Tables 1–6 and Fig. 3. Changes in social behaviour were most pronounced in mice with high numbers of larvae in the brain, which decreased in mice with lighter infections. Heavily infected mice displayed lower levels of non-social behaviour, reductions in aggressive behaviour combined with high levels of flight and defensive behaviours compared with control and more lightly infected groups. Social and sexual behaviour was the only category which did not show the association between the level of infection and behaviour. The total frequency for each category of social behaviour is shown in Table 1.

Individual elements of the 5 categories of behaviour performed by the control, low and high dose groups

(*i*) *Non-social*. There was 1 significant difference between the control and high infection group, which occurred for the element 'dig' (Table 2). There was an infection response for the majority of non-social elements investigated, by which the frequency decreased as the infection increased. However, the low infection groups displayed higher frequencies of the specific elements of 'wash', 'attend' and 'leave', while the high infection group engaged more in the element of 'stretch attend'.

(*ii*) *Social and sexual*. The control mice engaged more frequently in elements of 'investigate', 'crawl over' and 'follow' behaviour (Table 3). For the individual element of 'investigate' the high infection group investigated their control counterparts less frequently. Mice harbouring high larval numbers were shown to engage more frequently in the element 'sniff'. This was not seen in the low infection group.

(*iii*) *Aggressive*. The number of attacks initiated in the neutral cage was found to be significantly lower in infected mice. This was most pronounced in the mice harbouring high numbers of larvae in the brain (Z statistic $= -2{\cdot}423$; $P \leqslant 0{\cdot}015$) than the mice harbouring low numbers (Z statistic $= -1{\cdot}556$; $P \leqslant 0{\cdot}03$; $P \leqslant 0{\cdot}051$) (Fig. 3A). Similarly, both the infected groups spent significantly less time attacking than control mice and again this was most pronounced in the high infection group (Z statistic $= -1{\cdot}556$; $P \leqslant 0{\cdot}029$) (Fig. 3B). The frequency of the individual elements of aggressive behaviour performed by the 3 groups of mice are shown in Table 4. The control mice performed the most elements of aggressive behaviour as compared with the infected groups.

(*iv*) *Flight*. The high infection group performed the greatest number of elements of flight behaviour compared with the control mice Table 5. This trend was similar for the low infection group with the exception of the individual elements of 'on bars' and 'off bars' in which mice with lower infections engaged in fewer of these elements than the controls. The individual elements of 'evade', 'flee' and 'freeze' were most frequent in mice with high infections.

(*v*) *Ambivalent*. The control group engaged in significantly more offensive behaviour than the infected groups Table 6. The individual elements of 'offensive sideways' and 'offensive upright' became less frequent as the infection increased and high infection group showed a significant reduction in the element of offensive sideways behaviour. Both elements of defensive behaviour were significantly increased in the infected mice although the increase in the defensive upright posture was only significant for mice with high infection.

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

Table 1. Means and medians of the numbers of individual categories of behaviour for the control, low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Non-social	52·0 ± 13·1	51	46·9 ± 12·9	48	42·7 ± 20·7	46	0·262†
							0·339*
Social and sexual	25·5 ± 14·4	21	19·3 ± 22·1	13	21·9 ± 15·5	18	0·464†
							0·046*
Aggressive	40·4 ± 28·1	33	31·6 ± 22·1	28	18·7 ± 21·5	10·5	0·017†
							0·423*
Flight	11·7 ± 14·1	6	14·8 ± 13·9	13	23·8 ± 16·7	20	0·018†
							0·293*
Ambivalent off	7·4 ± 5·1	8	6·1 ± 4·5	5	4·7 ± 3·2	5	0·093†
							0·370*
Ambivalent def	2·5 ± 4·6	1	4·1 ± 3·9	2	6·9 ± 6·3	5	0·037†
							0·120*

* Control and low dose.
† Control and high dose.

Table 2. Means and medians of the numbers of individual elements of non-social behaviour for the control low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Explore	18·9 ± 6·5	19	16·4 ± 5·7	16	15·4 ± 5·9	15·5	0·177†
							0·241*
Wash	1·7 ± 1·2	2	2·2 ± 2·3	1	1·2 ± 1·1	1	0·275†
							0·936*
Self groom	1·8 ± 2·3	2	1·5 ± 1·4	1	1·0 ± 1·4	0	0·215†
							0·936*
Dig	1·9 ± 2·9	1	1·1 ± 2·2	0	0·3 ± 0·6	0	0·048†
							0·282*
Push dig	0·2 ± 0·7	0	0·07 ± 0·2	0	0·08 ± 0·3	0	0·718†
							0·664*
Displacement groom	1·6 ± 1·6	1	1·5 ± 1·3	2	0·8 ± 0·9	1	0·160†
							1·000*
Displacement dig	0·2 ± 1·1	0	0 ± 0	0	0·2 ± 0·5	0	1·000†
							0·320*
Leave	3·2 ± 3·4	2	4·3 ± 2·6	4	2·5 ± 2·7	2	0·604†
							0·128*
Attend	8·7 ± 5·4	10	10·4 ± 5·4	11	7·9 ± 7·3	8·5	0·536†
							0·587*
Stretch attend	0·4 ± 0·9	0	0·2 ± 0·6	0	2·3 ± 5·1	0	0·301†
							0·665*
Approach	7·8 ± 4·6	8	5·0 ± 3·5	4	4·3 ± 3·6	3	0·07†
							0·122*
Explore approach	1·2 ± 1·9	0	0·7 ± 1·1	3	1·2 ± 2·3	0	0·841†
							0·841*
Explore leave	3·8 ± 4·1	3	3·0 ± 4·1	1	3·0 ± 2·6	2·5	0·771†
							0·441*

* Control and low dose.
† Control and high dose.

Exploration and aversiveness carried out for control, low and high infection groups of mice in the light/dark box and predator odour experiments

(*i*) *Light/dark box*. The results for the behaviour of the 3 groups of mice are shown in Fig. 4 A–F. Mice with low infection displayed qualitative differences in their behaviour to both control and high infection groups who displayed similar type behaviour in the the light/dark box. Mice with low infection spent significantly more time in the light area of the apparatus (t statistic = 2·318; $P \leqslant 0·037$).

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

Table 3. Means and medians of the numbers of individual elements of social investigation/sexual behaviour for the control, low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Investigate	5·6 ± 4·2	4	4·5 ± 7·9	4·5	4·2 ± 3·1	2	0·406† 0·057*
Nose	7·9 ± 4·4	7	7·9 ± 6·8	6	6·8 ± 3·6	6	0·414† 0·566*
Sniff	5·7 ± 5·2	4	4·4 ± 5·4	2	6·5 ± 7·1	5	0·922† 0·284*
Follow	4·6 ± 5·2	3	2·3 ± 3·3	1	2·6 ± 3·1	1·5	0·258† 0·098*
Attempt mount	1·2 ± 2·3	0	0 ± 0	0	1·6 ± 3·1	0	0·673† 0·041*
Genital groom	0 ± 0	0	0 ± 0	0	0·08 ± 0·3	0	0·165† –
Push under	0·1 ± 0·3	0	0 ± 0	0	0·1 ± 0·4	0	0·229† 0·792*
Crawl over	0·4 ± 1·1	0	0·08 ± 0·3	0	0·07 ± 0·3	0	0·512† 0·463*

* Control and low dose.
† Control and high dose.

(*ii*) *Predator odour test predator's control*. The Day 1 results for the reaction of the 3 infection groups to both the predator and control odour are shown in (Fig. 5 A). The low infection group spent more time in the predator arm than the control group and this difference was approaching significance (t statistic = −1·964; $P \leqslant 0{\cdot}075$). There was no difference for control and high infection groups. The time spent in the control arm of the maze for the groups of mice was found to be greater in the high infection group compared with the controls (t statistic = −2·443; $P \leqslant 0{\cdot}035$), there was no difference observed between the control and low infection group. Intra-group comparisons of the time spent in the 2 odour arms revealed that the control group showed no preference for either arm, while the low infection group showed a preference for the predator odour (t statistic = 2·478; $P \leqslant 0{\cdot}026$). In contrast, the high infection group preferred the control arm (t statistic = −2·538; $P \leqslant 0{\cdot}026$). The low infection group performed more bouts of grooming in the predator arm than the controls (t statistic = 1·766; $P \leqslant 0{\cdot}026$). The low infection group performed more bouts of grooming in the predator arm than the controls (t statistic = 1·766; $P \leqslant 0{\cdot}099$), while the high infection group performed less numbers of grooms than the control group (t statistic = −1·888; $P \leqslant 0{\cdot}069$). There was no significant difference for the number of grooms carried out in the control arm. The low infection group was the only group which showed a significant difference in the amount of grooms performed in the 2 arms, grooming significantly more when in the predator odour arm than the control arm (t statistic = 2·700; $P \leqslant 0{\cdot}015$).

Predator versus non-predator. The Day 2 reaction of the 3 infection groups to both the predator and non-predator odour are shown in Fig. 5 B. The response of the mice to this odour combination revealed that there was no significant difference between the 3 groups for the time spent in the predator arm on Day 2. The time spent in the non-predator arm was found to be significantly less in the low infection group as compared with the controls (t statistic = 2·349; $P \leqslant 0{\cdot}028$), the control group spent the greatest amount of time in the new odour arm. Intra-group comparisons revealed the control group showed no preference for either arm, while low and high groups showed a greater preference for the predator odour arm (t statistic = −2·802; $P \leqslant 0{\cdot}01$ and t statistic = −2·231; $P \leqslant 0{\cdot}04$ respectively). Grooming in the predator arm was found to be significantly greater in the high infection group (t statistic = −2·088; $P \leqslant 0{\cdot}05$), while no significant difference was observed between the low infection group and controls. The control mice did not differ significantly in the number of grooms carried out in either arm. The high infection group, however, groomed significantly more in the predator arm than in the non-predator arm (t statistic = 2·910; $P \leqslant 0{\cdot}009$). The low infection group also groomed more when in the predator arm and this difference was approaching significance (t statistic = 2·00; $P \leqslant 0{\cdot}063$).

Non-predator versus control. For Day 3 the time spent in the non-predator arm and control arm of the maze did not differ significantly between the 3 groups of mice (Fig. 5 C). Intra-group comparisons

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

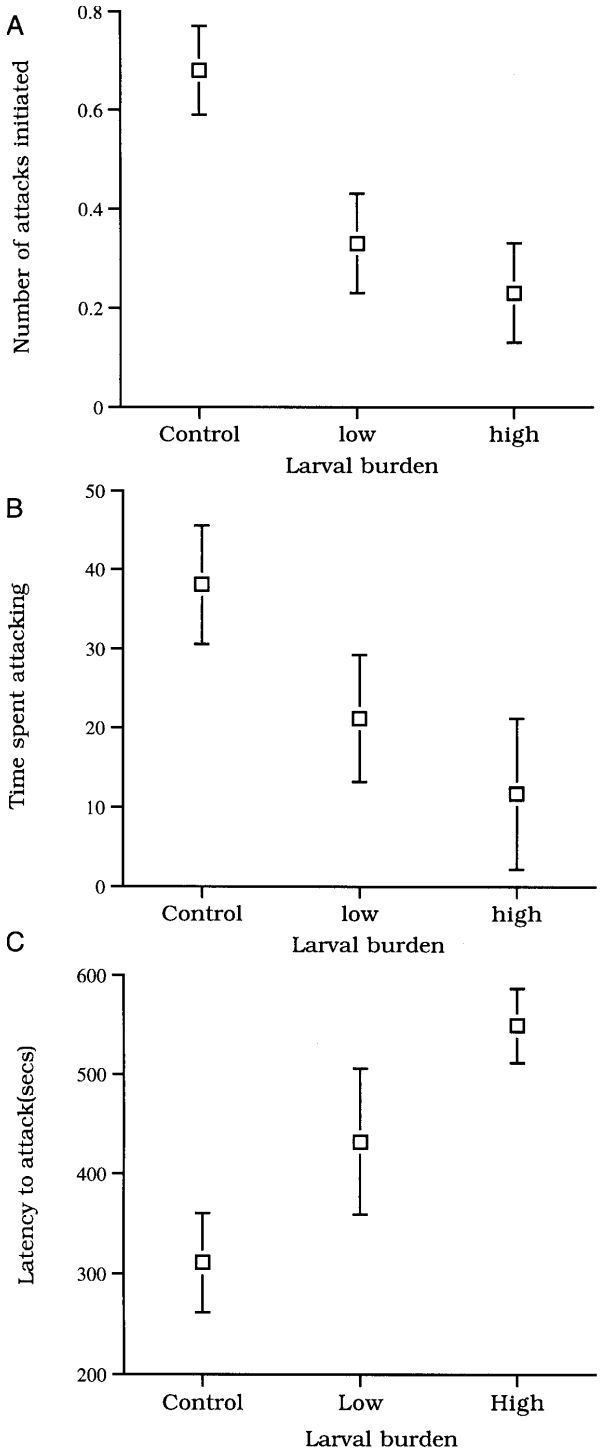

Fig. 3. The mean number of first time attacks performed, the mean time spent attacking and the latency to attack by control, low and high infected groups.

revealed that the control and low infection groups showed a greater preference for the non-predator arm (t statistic = 8·320; $P \leqslant 0{\cdot}0001$; t statistic = −4·787; $P \leqslant 0{\cdot}0004$). There were no significant differences between the number of grooms performed in the control arm and non-predator arm between the 3 groups of mice.

The results for the preference ratio are shown in Fig. 6. On Day 1, the low infection group displayed the greatest preference for the predator odour, although this difference was not found to be statistically significant. The high infection group showed the least preference for the odour arm and this was found to be significantly different when compared with the controls (t statistic = 2·655; $P \leqslant 0{\cdot}016$) which showed no particular preference for either arm. On Day 2 the infected groups both showed a greater preference for the predator odour arm, this preference was found to be significantly different for the low infection group as compared with the control group (t statistic = −2·193; $P \leqslant 0{\cdot}037$). The control group again showed no particular preference for either odour arm. On Day 3 the low infection group showed the greatest preference for the non-predator arm, while the high infection group showed the least preference. The preference for the non-predator odour arm was not found to be statistically significant between the control and infected groups of mice. Over the 3 days of testing the trend for the low dose group and the control group was similar, in that the preference ratio remained constant for Days 1 and 2 and then increased substantially by Day 3. The opposite trend was observed in the high infection group.

DISCUSSION

The results presented in this paper raise 3 essential points with respect to the effect of *T. canis* on murine behaviour, namely, (i) the manipulation hypothesis versus natural side-effects, (ii) the use of analysis by larval burden as opposed to traditional analysis (infected versus control) and (iii) the implications of the behavioural changes observed in the murine model with respect to potential effects on human behaviour.

The present investigation highlighted that the nematode *T. canis* altered the behaviour of infected mice in a manner likely to increase the possibility of detection by and transmission to a definitive caniid host. This was true in the investigation of social behaviour for both analyses, while only the analysis by larval burden revealed a decrease in risk assessment in the novel/anxiety paradigms. A decrease in aggression coupled with a decrease in anxiety level and lack of inhibition to open, bright areas could create a situation in which the risk element of being located by a potential predator is increased in the mouse displaying the behavioural alteration. The investigation of social interactions between mice suggests that those with heavy burdens are more likely to be exposed to a predator, due to a significant reduction in the overall category of aggressive behaviour with a corresponding increase in flight behaviour, mice with lower burdens may have a greater chance of survival due to less dramatic effects on aggressive behaviour.

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

Table 4. Means and medians of the numbers of individual elements of aggressive behaviour for the control, low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Threat	2·5 ± 3·2	2	2·7 ± 3·3	1	1·2 ± 1·3	1	0·313† 0·212*
Aggressive groom	3·8 ± 5·3	1	2·0 ± 4·9	0	0·7 ± 2·4	0	0·002† 0·043*
Attack	8·1 ± 7·1	6	6·4 ± 5·3	9	3·5 ± 4·9	1·5	0·098† 0·576*
Bite	7·8 ± 7·2	6	6·6 ± 4·9	7	3·1 ± 4·9	2	0·051† 0·840*
Chase	4·4 ± 5·6	2	2·6 ± 3·2	1	1·6 ± 3·3	0	0·122† 0·603*
Rattle	9·6 ± 8·6	8	8·5 ± 11·2	5	5·0 ± 4·8	4	0·087† 0·293*
Circle	0·04 ± 0·2	0	0 ± 0	0	0 ± 0	0	0·525† 0·505*
Zig Zag	0·04 ± 0·2	0	0 ± 0	0	0 ± 0	0	0·525† 0·505*
Walk around	1·5 ± 2·6	0	0·6 ± 1·3	0	0·7 ± 1·2	0	0·628† 0·299*
Over	2·5 ± 3·9	1	3·4 ± 3·2	3	1·5 ± 3·4	0	0·345† 0·335*

* Control and low dose.
† Control and high dose.

Table 5. Means and medians of the numbers of individual elements of flight behaviour for control, low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Evade	1·0 ± 1·7	0	2·2 ± 1·6	3	1·7 ± 4·5	0	0·878† 0·018*
Retreat	0·8 ± 1·6	0	1·3 ± 2·1	0	1·1 ± 2·8	0	0·848† 0·655*
Flee	1·7 ± 2·7	0	2·0 ± 3·3	1	6·6 ± 7·7	5·5	0·051† 0·611*
On back	2·7 ± 3·9	0	2·9 ± 4·3	1	4·2 ± 5·1	2·5	0·138† 0·732*
Kick	2·6 ± 4·2	1	4·8 ± 6·4	3	3·8 ± 5·0	2·5	0·396† 0·076*
On bars	1·1 ± 3·4	0	0·4 ± 0·7	0	2·4 ± 4·7	0	0·177† 0·649*
Freeze	0·4 ± 1·1	0	0·4 ± 0·9	0	1·1 ± 1·7	0·5	0·041† 1·000*
Off bars	1·1 ± 3·9	0	0·6 ± 1·1	0	2·2 ± 4·8	0	0·365† 0·591*

* Control and low dose.
† Control and high dose.

Previous studies of social interactions among mice (Freeland, 1981; Rau, 1983, 1984; Edwards, 1988; Arnott *et al*. 1990) and rats (Berdoy, Webster & Macdonald, 1995) infected with different parasites reveal that parasitism does not always affect the host in the same way. Rau (1983) found that 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. These results are in agreement with the present investigation in that

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

Table 6. Means and medians of the numbers of individual elements of ambivalent behaviour for the control, low and high dose groups over the 10 min observation period

	Control		Low		High		
Behaviour	Mean ± S.E.	Median	Mean ± S.E.	Median	Mean ± S.E.	Median	*P* value
Offensive sideways	1·7 ± 2·1	1	1·0 ± 2·5	0	0·2 ± 0·4	0	0·028† 0·170*
Offensive upright	2·6 ± 4·1	1	1·4 ± 2·9	0	1·2 ± 2·3	0	0·285† 0·229*
Sideways posture	2·9 ± 2·6	2	3·3 ± 2·6	3	3·2 ± 3·1	3	0·862† 0·569*
Upright posture	0·2 ± 0·6	0	0·3 ± 0·6	0	0 ± 0	0	0·175† 0·615*
Defensive sideways	0·08 ± 0·3	0	1·3 ± 1·3	1	0·7 ± 1·2	0	0·031† 0·003*
Defensive upright	2·4 ± 4·5	1	2·7 ± 2·8	1	6·1 ± 5·4	5	0·041† 0·337*

* Control and low dose.
† Control and high dose.

parasite infection suppresses aggressive behaviour. The parasite *Toxoplasma gondii* has been shown to affect the social behaviour of mice and rats in different ways which may be due to the fact that this parasite also infects the brain of these rodents and that the number of cysts present was a determining factor with respect to the altered behaviour. Arnott *et al*. (1990) found that laboratory mice infected with *T. gondii* were more likely to be territorially aggressive when paired with an uninfected and previously unencountered mouse, whereas Berdoy *et al*. (1995) reported that *T. gondii* had no significant effect on the establishment or maintenance of social status or mating success in laboratory/wild hybrid rats during competitive mating situations. However, these authors used different methods which incorporated a naturalistic design to measure social interaction between rats.

Aggression in wild rodents is responsible for determining populations, as dominant males are generally more successful at mating and maintaining territories (Southwick, 1958) which in turn is essential to survival. Similarly defensive behaviours are strongly associated with flight behaviour in the mouse (Mackintosh, 1981) and this type of behaviour was most frequently observed in heavily infected mice. The subordination induced in these mice may lead to the expulsion from an established habitat, forcing the more subordinate males into subquality habitats or peripheral territories, leaving them more vulnerable to predation. Previous work investigating the behaviour of populations of house mice has demonstrated that 97% of emigrating animals were socially subordinate (Butler, 1980), which suggests that infected mice in the present study especially those with high infection in the brain may be driven out by dominants and become transient. In addition, mice with no fixed territory have been shown to be taken by predators more often than territorial mice (Metzgar, 1967) and those with higher activities, larger home ranges and greater exposure are captured more frequently than mice which suppress this behaviour (Haukisalmi, Henntonnen & Pietiannen, 1994). This suggests that infected mice displaying timid behaviour in the present study will increase their exposure to predation.

While the investigation of social behaviour suggested that infected mice displayed alterations in behaviour due to parasite infection using both analyses, we cannot conclude the same for the investigation of risk behaviour in the novel/anxiety tests. The analysis of the mice by traditional (infected versus control) methods, suggested that infection had no effect on this specific element of behaviour in mice. This finding highlights the importance of categorizing mice as low or high infection status in relation to risk behaviour in rodents. However, we must also consider the unusual reversal of low larval burden in the brain producing a greater shift in normal behaviour than high larval number with respect to anxiety and risk assessment.

Exploration and anxiety are generally examined using behavioural conflict paradigms by which the apparatus is novel but contains aversive properties (e.g. Pellow *et al*. 1985; Lister, 1987; Crawley & Goodwin, 1980; Montgomery, 1958). In such tests, behaviour is influenced by 2 opposing motivational forces by which the natural drive to explore versus the negative drive to avoid open, exposed and threatening areas. This type of behaviour may be affected to a greater extent as a result of damage caused to certain areas of the brain by the migrating larvae compared to social behaviour. The initial analysis of infected versus control mice in the 2 aversive tests revealed that both groups did not exhibit a particularly strong aversion towards the

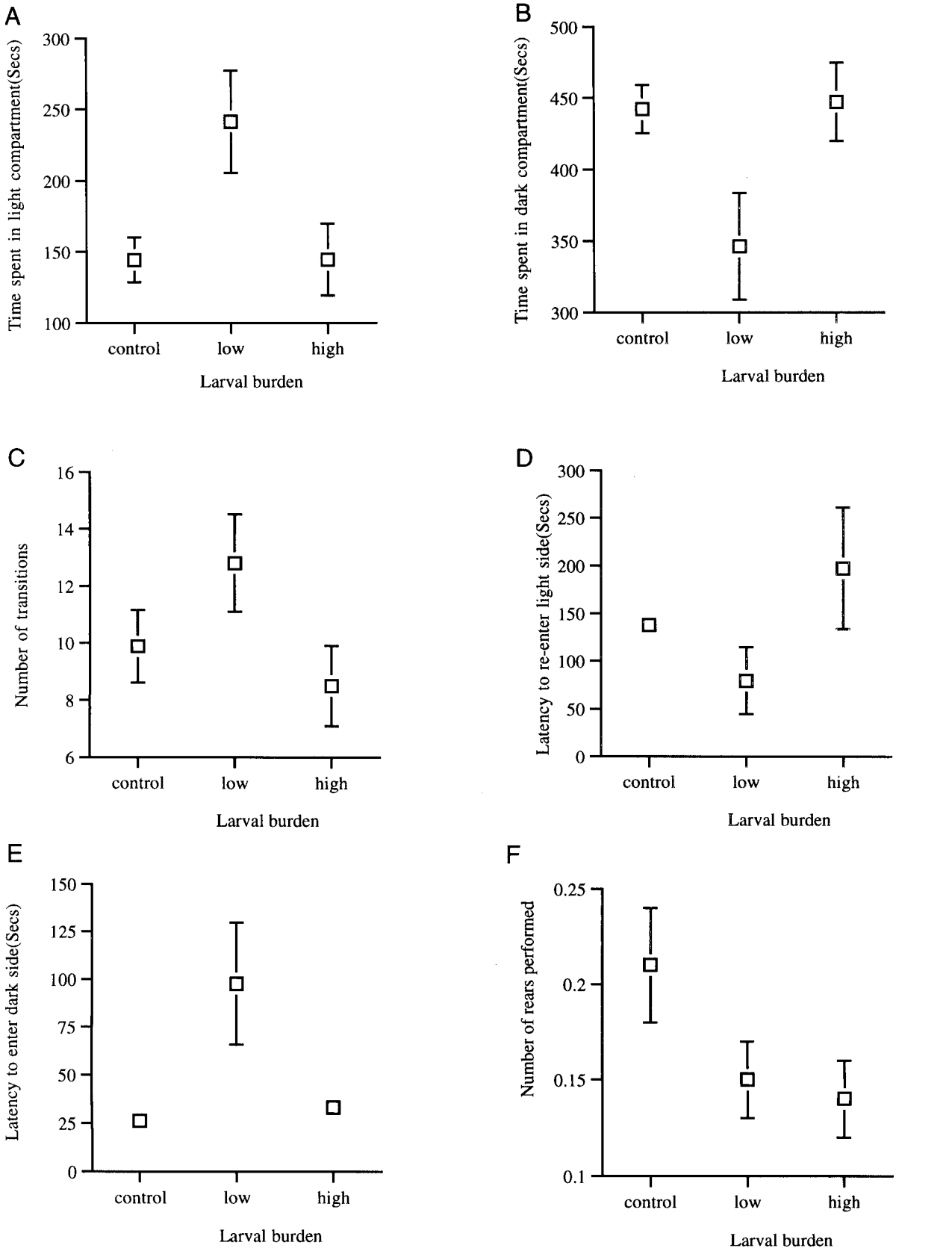

Fig. 4. The exploratory behaviour of control mice and mice with low and high brain burdens in the light/dark box.

predator odour or the light area of the light/dark box, which was indicated by the similarity in the time spent and entries into the aversive areas for both groups. 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 this stimulus; however, this was not observed. The subjects used in

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

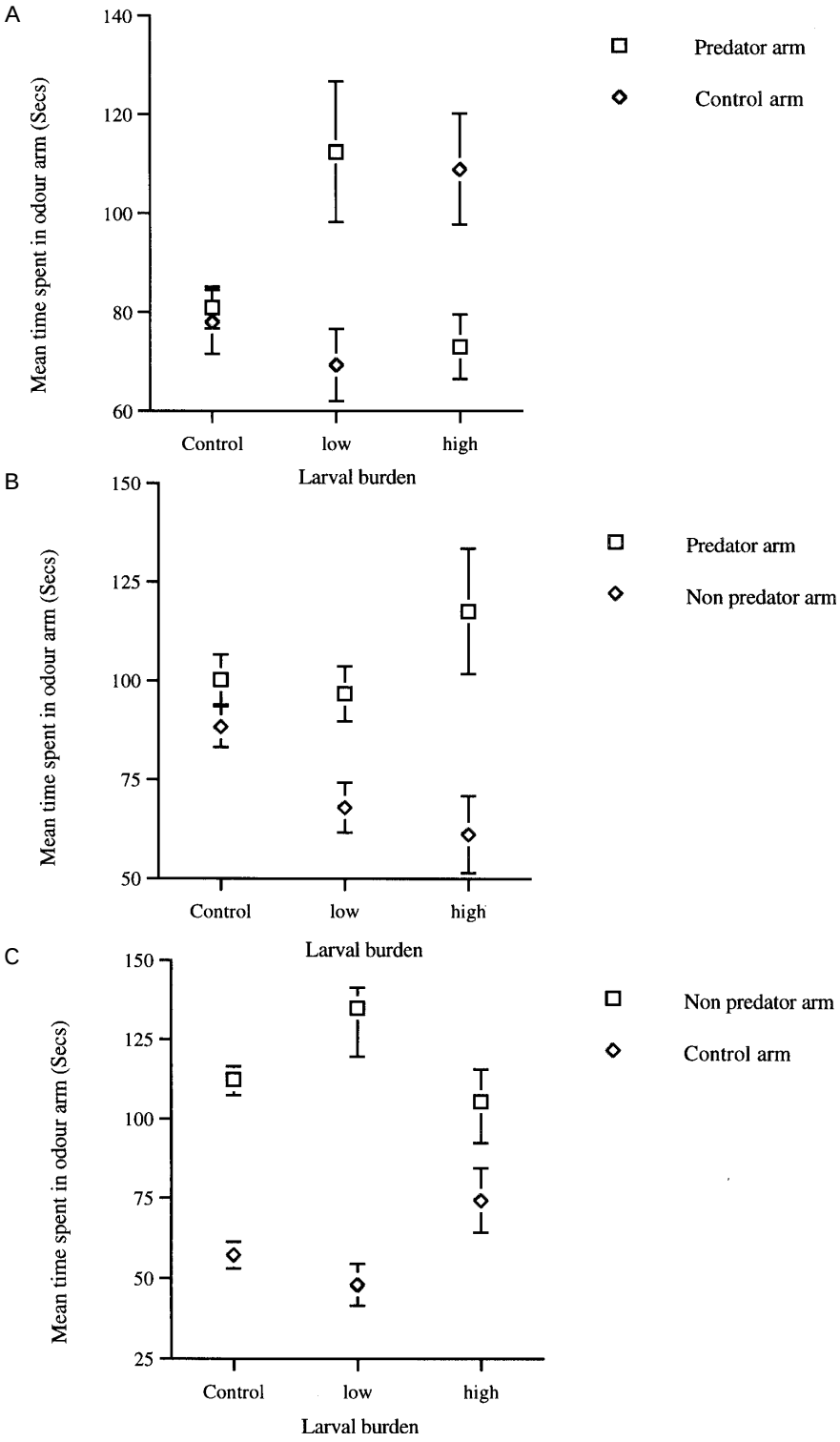

Fig. 5. The mean time spent in each odour arm for each group on test Days 1, 2 and 3.

this experiment had no previous exposure to either cat or rabbit odour, thus it was assumed that possible risk assessment evoked by the odours may result from an innate recognition of the cat odour as a danger signal. The failure for this response to manifest itself in the control mice led us to the conclusion that either the mice had lost their response to predators, as suggested by Antalfi (1963) or that the chosen predator odour was not strong enough to elicit a fear response.

Behavioural differences were observed, however, between the control, low and high infection groups in the anxiety experiments which led to the conclusion that the infection did reduce anxiety or cautiousness in the mice. The differences between the groups on initial analysis may have been undetectable due to the failure of the high infection group to respond in a similar manner to the low infection group in the manifestation of risk behaviours in the novel/anxiety experiments. *T. canis-*

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

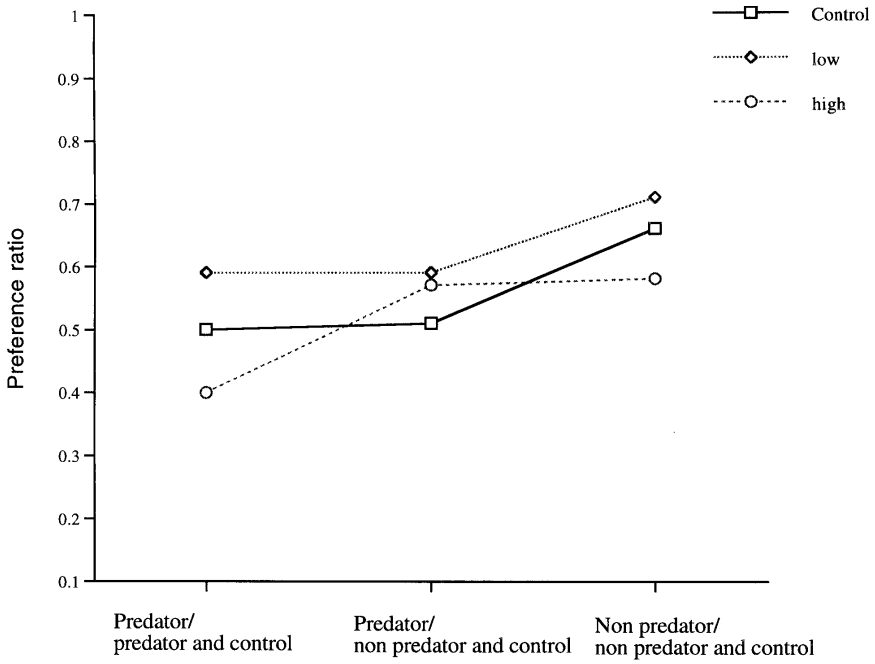

Fig. 6. The response of control, low and high dose groups of mice in the Y maze odour preference apparatus to the 3 odour combinations for Days 1, 2 and 3.

infected mice have previously been shown 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 apparatus (Hay & Aitken, 1984). This is similar to the response behaviour of infected mice especially those with low infection in the present study. Mice with low infection displayed a preference for the predator odour on Day 1, this was followed by a significant predator preference by high and low infection groups on Day 2. The tendency to explore the aversive areas of the novel environments was greatest in the mice with low infection on initial exposure, suggesting these mice were displaying inappropriate defence behaviour.

Mice will exhibit both curiosity and fear on exposure to a new environment and we would expect to observe a reduction in fear and exploratory response on repeated exposures (Harro, 1993). The fact that control mice explored the 2 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 more familiar would indicate that these mice were more cautious. This suggests that these mice explored the environment more effectively and may therefore be quicker at seeking out potentially safe areas if confronted by a predator. The results from these tests would suggest that mice infected with *T. canis* can display lower levels of anxiety in a threatening situation which in turn is dependent on the larval number in the brain. Thus the parasite may reduce the first line of defence in the mice, which is to avoid detection (King, 1985). The protozoan *Eimeria vermiformis* has also been shown to reduce predator-induced fear or anxiety in mice (Kavaliers & Colwell, 1995*b*).

It is difficult to assess why mice with lower brain infection show a greater deviation from normal behaviour than the high infection group in measures of risk behaviour. The difference may be attributable to brain function and the site which the larvae occupy but we have no evidence as yet to confirm or refute this suggestion. Previous investigations of histopathology in the brains of infected *T. canis* mice have revealed that the variation in the damage observed is partly determined by location, size and the activity of the parasite (Sprent, 1955*a*, *b*; Burren, 1971; Summers *et al.* 1983). Lesions are widely observed and include necrosis, cavitation, macrophage activation and perivascular cuffing, and in cases where the lesions are severe there is a loss of neural parenchyma (Summers *et al.* 1983). The larvae are rarely surrounded by a tissue response and can thus continually migrate through the closed environment of the brain, where they can remain for up to 6 months after infection.

In the investigation of specific behaviours, the actual site of the larvae, as opposed to the parasite load, may be responsible for producing the resulting behavioural alteration in the infected mice. The fact that the low infection group displayed the greatest level of risk assessment in both paradigms may suggest that the parasite affected an area of the brain associated with this type of behaviour. In this context it is of note that, depending on the anatomical system affected in the brain, behaviour may be manifested in different ways (Means, Leander & Isaacson, 1971; Capiobanco & Hamilton, 1976). Similarly, a pre-

https://doi.org/10.1017/S0031182098002649 Published online by Cambridge University Press

vious study which investigated the exposure of rats to a cat odour in a novel environment, found that there was an increase in the release and decrease in the uptake of the neurotransmitter GABA in hippocampal and cortical areas of the brain (File, Zangrossi & Andrews, 1993). It is therefore possible that *T. canis* is capable of affecting chemical functioning within the brain through the release of secretory/excretory products, which ultimately affect the transmission of essential signals related to behaviour. Another possibility is that prior to infection the baseline behaviour of mice with high larval burdens in the brain, was different to that of the mice with low burdens and control mice, in that they had naturally higher levels of neophobic or cautionary behaviour. If the parasite induces a reduction in this element of behaviour, so as to increase risk behaviour, then the effect of the parasite on the behaviour of an individual may actually bring it within the range of normal behaviour displayed by the control mice. We are at present investigating the relationship between the site of the larvae within the brain and the observed behavioural changes in individual mice. Such an investigation may shed more light on the reversal effect observed with the low infection in the investigation of anxiety and fear in these mice. Despite the complexity of this finding it is evident that the degree of infection in the brain is important in the manifestations seen in the resulting behaviour.

On the basis of the finding that *T. canis* and altered behaviour in rodents is associated with the infection in the brain, the immune response in wild mice may be important in the magnitude of the alteration of their behaviour. If the immune response is such that wild mice have a higher liver trapability of *T. canis* larvae then behavioural changes in wild mice may be minimal due to less larvae reaching the brain. Within a new host *T. canis* larvae are rapidly trapped in the liver and the degree of immobilization or retention of larvae in the liver is a reflection of the level of innate resistance (Abo-Shehada & Herbert, 1989). In a natural situation wild rodents are likely to be prone to repeated infections which has been shown previously to result in less accumulation of larvae in the brain (Abo-Shehada, Al-Zubaidy & Herbert, 1991).

Blanchard *et al*. (1990) stated that there is increasing evidence that different defence behaviours are represented by significantly different areas in the brain in terms of neuroanatomy and neurochemistry. This suggests that in mice infected with *T. canis* the differences in aggression and defence behaviour may be profoundly different in mice with high infection as opposed to low infection in this area.

The conclusions from the present investigation are dependent on brain burden and the fact that the mouse is a paratenic host for *T. canis*. The results raise the question are these behavioural changes a result of manipulation by the parasite or a side-effect from the presence of the parasite in the brain? Due to the fact that *T. canis* can be maintained in a multitude of paratenic hosts, all of which may behave quite differently it is unlikely that the parasite could select for a behavioural trait which if altered, would have the same consequence in all these 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 infected host will be taken by the correct predator in every confrontational situation. Parasite manipulation of intermediate host behaviour, as opposed to paratenic host's 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, Huntingford & Crompton, 1993) and is therefore more specific.

We suggest that the alterations observed in the mice are a side-effect to the parasitic infection and that these effects may have important implications regarding human behaviour. The finding from the present study, which indicated that low brain infections had a larger effect on specific cognitive functions may be important to the natural development of young children. Investigations concerning the effect of *T. canis* (Nelson, Greene & Ernhart, 1996; Magnaval *et al*. 1997) and *T. gondii* (Flegr *et al*. 1996) on human behaviour have recently been conducted. Nelson *et al*. (1996) did not find a consistently strong relationship between *T. 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 *T. gondii* immunity and certain personality factors in university students 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 than controls (Hutchison, Aitken & Wells, 1980; Hay *et al*. 1984; Webster, Brunton & Macdonald, 1994).

The conclusions drawn from the present investigation are first, that the behaviour displayed by the mice was altered by *T. canis* and the changes observed were related to parasite burden in the brain of the infected mice. This suggests that in studies involving parasites with a predilection for the CNS, the data may be best interpreted in light of the number of parasites in the brain at the time of testing as opposed to the dose administered.

Secondly, the changes observed in the behaviour of the infected mice would most likely increase their susceptibility to predation in a threatening situation,

although it is unlikely that the changes are a direct result of manipulation, but rather a consequence of the infection.

The authors are extremely grateful to Peter Stafford for his technical assistance and 2 anonymous referees for their helpful comments on earlier drafts. This research was partly funded by the Irish American Partnership.

REFERENCES

ABO-SHEHADA, M. N. & HERBERT, I. V. (1989). Variations in the innate resistance to experimental *Toxocara canis* infection in two strains of mice. *Veterinary Parasitology* **33**, 297–307.

ABO-SHEHADA, M. N., AL-ZUBAIDY, B. A. & HERBERT, I. V. (1991). Acquired immunity to *Toxocara canis* infection in mice. *Veterinary Parasitology* **38**, 289–298.

ANTALFI, S. (1963). Biological determination of the intensity of the alarm reaction in house mice. *Journal of Comparative and Physiological Psychology* **56**, 889–891.

ARNOTT, M. A., CASELLA, J. C. & HAY, J. (1990). Social interactions of mice with congenital *Toxoplasma* infection. *Annals of Tropical Medicine and Parasitology* **84**, 149–156.

BERDOY, M., WEBSTER, J. P. & MACDONALD, D. W. (1995). Parasite-altered behaviour: is the effect of *Toxoplasma gondii* specific? *Parasitology* **111**, 403–409.

BETHEL, W. M. & HOLMES, J. C. (1973). Altered evasive behaviour and responses to light in amphipods harbouring acanthocephalan cystacanths. *Journal of Parasitology* **59**, 945–956.

BETHEL, W. M. & HOLMES, J. C. (1974). Correlation of development of altered evasive behaviour in *Gammarus* (Amphipoda) harbouring cystacanths of *Polymorphus paradoxus* (Acanthocephala) with the infectivity to the definitive host. *Journal of Parasitology* **60**, 272–274.

BLANCHARD, R. J., BLANCHARD, D. C., RODGERS, D. & WEISS, S. M. (1990). The characterization and modelling of anti-predator defensive behaviour. *Neuroscience and Biobehavioural Reviews* **14**, 463–472.

BURREN, C. H. (1971). The distribution of *Toxocara canis* larvae in the central nervous system of the mouse. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **65**, 450–453.

BURRIGHT, R. G., DONOVICK, P. J., DOLINSKY, Z. S., HURD, Y. & CYPESS, R. (1982). Behavioural changes in mice infected with *Toxocara canis*. *Journal of Toxicology and Environmental Health* **10**, 621–626.

BUTLER, R. G. (1980). Population size, social behaviour and dispersal in house mice: a quantitative investigation. *Animal Behaviour* **28**, 78–85.

CAPOBIANCO, S. & HAMILTON, L. W. (1976). Effects of interruption of limbic system pathways on different measures of activity. *Physiology and Behaviour* **17**, 65–72.

CRAWLEY, J. N. & GOODWIN, F. K. (1980). Preliminary report of a simple animal behaviour model for the anxiolytic effects of benzodiazepines. *Pharmacology, Biochemistry and Behaviour* **13**, 167–170.

DOLINSKY, Z. S., BURRIGHT, R. G., DONOVICK, P. J., GLICKMAN, L. T., BABISH, J., SUMMERS, B. & CYPESS, R. H. (1981). Behavioural effects of lead and *Toxocara canis* in mice. *Science* **213**, 1142–1144.

DOLINSKY, Z. S., HARDY, C. A., BURRIGHT, R. G. & DONOVICK, P. J. (1985). The progression of behavioural and pathological effects of the parasite *Toxocara canis* in the mouse. *Physiology and Behaviour* **35**, 33–42.

DUBINSKY, P., HAVASIOVA-REITEROVA, B., PETKO, I., HOVORKA, R. & TOMASOVICOVA. (1995). Role of small mammals in the epidemiology of toxocariasis. *Parasitology* **110**, 1–7.

DUNSMORE, J. D., THOMPSON, R. C. A. & BATES, I. A. (1983). The accumulation of *Toxocara canis* larvae in the brains of mice. *International Journal for Parasitology* **13**, 517–521.

EDWARDS, J. C. (1988). The effects of *Trichinella spiralis* infection on social interactions in mixed groups of infected and uninfected male mice. *Animal Behaviour* **36**, 529–540.

FILE, S. E., ZANGROSSI, H. & ANDREWS, N. (1993). Novel environment and cat odour change GABA and 5-HT release and uptake in the rat. *Pharmacology, Biochemistry and Behaviour* **45**, 931–934.

FLEGR, J., ZITKOVA, S., KODYM, P. & FRYNTA, D. (1996). Induction of changes in human behaviour by the parasitic protozoan *Toxoplasma gondii*. *Parasitology* **113**, 49–54.

FREELAND, W. J. (1981). Parasitism and behavioural dominance among male mice. *Science* **213**, 461–462.

GLICKMAN, L. T. (1993). The epidemiology of human toxocariasis. In *Toxocara and Toxocariasis Clinical, Epidemiological and Molecular Perspectives* (ed. Lewis, J. W. & Maizels, R. M.), pp. 3–10. Birbeck and Sons Ltd, Birmingham.

GRANT, E. C. & MACKINTOSH, J. H. (1963). A comparison of the social postures of some common laboratory rodents. *Behaviour* **21**, 246–259.

HARRO, J. (1993). Measurement of exploratory behaviour in rodents. *Methods in Neuroscience* **3**, 359–377.

HART, B. L. (1990). *Behavioural Adaptations to Pathogens and Parasites: Five Stages. Neuroscience and Biobehavioural Reviews* **14**, 273–294.

HAUKISALMI, V., HENTTONEN, H. & PIETIAINNEN, H. (1994). Helminth parasitism does not increase the vulnerability of the field vole *Microtus agretis* to predation by the Ural owl *Strix uralensis*. *Annals Zoology Fennici* **31**, 263–269.

HAY, J. & AITKEN, P. P. (1984). Experimental toxocariasis in mice and its effect on their behaviour. *Annals of Tropical Medicine and Parasitology* **78**, 611–618.

HAY, J., AITKEN, P. P. & ARNOTT, M. A. (1985). The effect of *Toxocara canis* on the spontaneous running activity of mice. *Annals of Tropical Medicine and Parasitology* **79**, 221–222.

HAY, J., AITKEN, P. P., HAIR, D. M., HUTCHISON, W. M., AITKEN, P. P. & GRAHAM, D. I. (1984). The effect of congenital *Toxoplasma* infection on mouse activity and relative preference for exposed areas over a series of trials. *Annals of Tropical Medicine and Parasitology* **78**, 611–618.

HAY, J., HUTCHISON, W. M., AITKEN, P. P. & GRAHAM, D. I. (1983). The effect of congenital and adult-acquired *Toxoplasma* infections on activity and responsiveness to novel stimulation in mice. *Annals of Tropical Medicine and Parasitology* **77**, 483–495.

HAY, J., KENDALL, A. T., AITKEN, P. P. & ARNOTT, M. A. (1986). *Toxocara* infection and hyperactivity. *Annals of Tropical Medicine and Parasitology* **80**, 531–533.

HOLLAND, C. H. (1997). Epidemiology of toxocariasis in Ireland: human, animal and environmental aspects. In *Modern Perspectives on Zoonoses*, (ed. Holland, C. V.), pp. 52–65. Royal Irish Academy, Dublin.

HUTCHISON, W. M., AITKEN, P. P. & WELLS, W. P. (1980). Chronic *Toxoplasma* infections and familiarity novel discrimination in the mouse. *Annals of Tropical Medicine and Parasitology* **74**, 145–150.

KAVALIERS, M. & COLWELL, D. D. (1995*a*). Reduced spatial learning in mice infected with the nematode *Heligmosomoides polygyrus*. *Parasitology* **110**, 591–597.

KAVALIERS, M. & COLWELL, D. D. (1995*b*). Decreased predator avoidance in parasitized mice: neuromodulatory correlates. *Parasitology* **111**, 257–263.

KING, C. M. (1985). Interactions between woodland rodents and their predators. In *The Ecology of Woodland Rodents*, *Bankvoles and Woodmice*. (ed. Flowerdew, J. W., Gurnell, J. & Gipps, J. H. W.), pp. 315–332. Oxford University Press, Oxford.

LEFCROFT, H. & BLAUSTEIN, A. R. (1995). Disease, predator avoidance and vulnerability to predation in tadpoles. *Oikos* **74**, 469–474.

LISTER, R. G. (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* **92**, 469–474.

MACKINTOSH, J. M. (1981). Behaviour of the house mouse. *Symposium of the Zoological Society of London* **47**, 337–365.

MAGNAVAL, J. F., GALINDO, V., GLICKMAN, L. T. & CLANET, M. (1997). Human *Toxocara* infection of the central nervous system and neurological disorders: a case-control study. *Parasitology* **115**, 537–543.

MEANS, L. W., LEANDER, D. J. & ISAACSON, R. L. (1971). The effects of hippocampectomy on alternation of behaviour and response to novelty. *Physiology and Behaviour* **6**, 17–22.

METZGAR, L. H. (1967). An experimental comparison of screech owl predation on resident and transient white footed mice (*Peromyscus leucopus*). *Journal of Mammalogy* **48**, 387–391.

MILINSKI, M. (1990). Parasites and host decision making. In *Parasitism and Host Behaviour*, (ed. Barnard, C. J. & Behnke, J. M.), pp. 95–117. Taylor and Francis, London.

MONTGOMERY, K. C. (1958). The relation between fear induced by novelty stimulation and exploratory behaviour. *Journal of Comparative Physiological Psychology* **48**, 254–260.

MOORE, J. & GOTELLI, N. J. (1990). Phylogenetic perspective on evolution of altered host behaviours: a critical look at the manipulation hypothesis. In *Parasitism and Host Behaviour*, (ed. Barnard, C. J. & Behnke, J. M.), pp. 193–230. Taylor and Francis, London.

NELSON, S., GREENE, T. & ERNHART, C. B. (1996). *Toxocara canis* infection in preschool age children: risk factors and the cognitive development of preschool children. *Neurotoxicology and Teratology* **18**, 167–174.

OLSON, L. J. & ROSE, J. E. (1966). Effect of *Toxocara canis* infection on the ability of white rats to solve maze problems. *Experimental Parasitology* **19**, 77–84.

PELLOW, S., CHOPIN, P., FILE, S. E. & BRILEY, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods* **14**, 149–167.

POULIN, R., CURTIS, M. A. & RAU, M. E. (1992). Effects of *Eubothrium salvelini* (Cestoda) on the behaviour of *Cyclops vernalis* (Copepoda) and its susceptibility to fish predators. *Parasitology* **105**, 265–271.

RAU, M. E. (1983). Establishment and maintenance of behavioural dominance in mice infected with *Trichinella spiralis*. *Parasitology* **86**, 319–322.

RAU, M. E. (1984). Loss of behavioural dominance in male mice infected with *Trichinella spiralis*. *Parasitology* **88**, 371–373.

SKERRETT, H. & HOLLAND, C. V. (1997). Variation in the larval recovery of *Toxocara canis* from the murine brain: Implications for behavioural studies. *Journal of Helminthology* **71**, 253–255.

SOUTHWICK, C. H. (1958). Population characteristics of house mice living in English corn ricks: density relationships. *Proceedings of the Zoological Society of London* **131**, 163–175.

SPRENT, J. F. A. (1955*a*). On the invasion of the CNS by nematodes (I) The incidence and pathological significance of nematodes in the CNS. *Parasitology* **45**, 31–40.

SPRENT, J. F. A. (1955*b*). On the invasion of the CNS by nematodes (II) Invasion of the nervous system in ascariasis. *Parasitology* **45**, 41–58.

SUMMERS, B., CYPESS, R., DOLINSKY, Z. S., BURRIGHT, R. G. & DONOVICK, P. J. (1983). Neuropathological studies of experimental toxocariasis in lead exposed mice. *Brain Research Bulletin* **10**, 547–550.

TIERNEY, J. F., HUNTINGFORD, F. A. & CROMPTON, D. W. T. (1993). The relationship between infectivity of *Schistocephalus solidus* (Cestoda) and anti-predator behaviour of its intermediate host the three spined stickleback *Gasterosteus aculeatus*. *Animal Behaviour* **46**, 603–605.

WEBSTER, J. P., BRUNTON, C. F. A. & MACDONALD, D. W. (1994). Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* **109**, 37–43.