Spatial and discrimination learning in rodents infected with the nematode *Strongyloides ratti*

V. A. BRAITHWAITE,* D. J. SALKELD, H. M. McADAM, C. G. HOCKINGS, A. M. LUDLOW *and* A. F. READ

Institute of Cell, Animal and Population Biology, Division of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

(*Received 8 December 1997; revised 10 March 1998; accepted 10 March 1998*)

SUMMARY

Recent work has shown that mice with subclinical parasitic infections suffer impaired spatial learning and memory, as assayed in an open-field water maze. Although the mechanism underlying this effect is not clear, the phenomenon has been reported following infection with both a protozoan parasite (*Eimeria vermiformis*) and a gastrointestinal nematode (*Heligmosomoides polygyrus*). In a variety of experiments, we examined the effects of a different gastrointestinal nematode, *Strongyloides ratti*, on the ability of rats and mice to learn a spatial or a discrimination task. Animals were tested at various stages post-infection, with different levels of infection, using different lines of *S. ratti* and with varying experimental protocols. All animals learned the tasks, but we found no evidence of an effect of *S. ratti* infection on learning or memory. Even rats infected with approximately 5000 *S. ratti* larvae, a dose which has an impact on rat body size, showed no deficit in learning ability. Various reasons for the conflict between our results and those previously reported for *E. vermiformis* and *H. polygyrus* are discussed. Our results show that impaired learning and memory following parasitic infection is not a ubiquitous or at least easily replicated phenomenon.

Key words: spatial memory, discrimination learning, host behaviour, water maze, *Strongyloides ratti*.

INTRODUCTION

Parasites can have profound effects on host behaviour (Barnard & Behnke, 1990; Thompson & Kavaliers, 1994). These effects range from alterations in host behaviour which enhance parasite transmission to changes which are more easily interpretable as non-adaptive pathology (Read, 1990). In either case, host fitness can be substantially impaired. Cognitive function, which is presumably closely correlated with host fitness, is an important example. Numerous studies with animal parasites have demonstrated detrimental effects of parasitic infection on cognition (Olsen & Rose, 1966; Dolinsky, Burright & Donovick, 1981; Sei *et al*. 1992; Gibertini *et al*. 1995; Brot *et al*. 1997; Adams & Fell, 1997). Moreover, correlations between parasitic infection and cognitive ability in children have been reported most of this century (see Nokes & Bundy, 1994; Watkins & Pollit, 1997). Explanations of these patterns have proved controversial, but there is at least some experimental evidence from double-blind placebo trials on children that parasites can reduce cognitive performance (Nokes *et al*. 1992*a*, *b*, but see Meeks Gardner, Grantham-McGregor & Baddeley, 1996). Animal models could make it possible to investigate the underlying mechanisms using laboratory host–parasite systems.

Recently, 2 experiments revealed impaired spatial learning and memory in laboratory mice after subclinical infection with the protozoan parasite *Eimeria vermiformis* (Kavaliers, Colwell & Galea, 1995) and the nematode *Heligosomoides polygyrus* (Kavaliers & Colwell, 1995). Importantly, the effect of *H. polygyrus* was larval dose dependent. Spatial learning and memory was assessed using the open-field water maze ('Morris' water maze; Morris, 1984; Stewart & Morris, 1993). Mice exposed to parasites learnt the task more slowly and performed less well in the retention trials than did controls (Kavaliers & Colwell, 1995; Kavaliers *et al*. 1995).

Kavaliers & Colwell (1995) speculated that the impaired spatial performance in *H. polygyrus*-infected mice may be a general phenomenon arising as a result of host neuromodulatory responses to parasitic infection such as altered opioid activity. Opioid systems are implicated in the mediation of spatial learning and memory. Endogenous opioid peptides and opiate agonists can impair spatial learning and memory while opiate antagonists act to increase it (McGaugh, Introini-Collison & Castellano, 1993). Impaired spatial ability arising through such a mechanism has several potentially interesting consequences. First, it should be possible to use laboratory models to investigate the mechanisms involved in parasite-induced cognitive

* Corresponding author: Tel: +44 131 650 5448. Fax: +44 131 667 3210. E-mail: vab@holyrood.ed.ac.uk

impairment in children. Secondly, in the wild, animals may face a trade-off between immunocompetence and cognitive ability. Reduced cognitive capacity may therefore be a direct cost of mounting responses against infection. Thirdly, impaired cognitive ability is frequently interpreted as adaptive modification of host behaviour by parasites to enhance their own transmission, particularly when the parasite has direct access to host central nervous system (e.g. Rau, 1984; Berdoy, Webster & Macdonald, 1995). Kavaliers & Colwell's (1995) finding that a directly transmitted gastrointestinal nematode can also induce such effects implies that these effects could be very general, emphasizing the need for caution before invoking parasite adaptation.

Here we report our attempts to generalize the findings of Kavaliers & Colwell (1995) that a nematode with a direct life-cycle impairs spatial learning and memory, by using a different host–parasite system and a different learning task. We used the gastrointestinal nematode *Strongyloides ratti* in laboratory rats and mice, and tested them in water mazes on the hidden platform (spatial) task and on a discrimination task, at varying stages following infection with varying numbers of larvae.

MATERIALS AND METHODS

Hosts

Rats (Lister hooded, Harlan Olac, UK), 2–3 months old (180–250 g), and mice (C57/BL/6J, Harlan Olac, UK), 2–3 months old (approximately 25 g) were used. All animals were males, and were caged in pairs with wood shavings for bedding at 21 ± 1 °C under a 12 h:12 h light:dark cycle (light 05.30–17.30). Food (41B, Harlan Olac, UK) and water were available *ad libitum*. Animals were age- and size-matched within experiments.

Parasites

Strongyloides ratti Sandground 1925 (Rhabditida: Strongyloididae) is a skin penetrating gastrointestinal nematode of rats. It has not been reported from mice in the wild, but can successfully infect and reproduce in laboratory mice (Dawkins *et al*. 1980; Dawkins, Mitchell & Grove, 1982; Dawkins, 1989). In laboratory infections in rats, infections become patent 4–5 days post-infection (p.i.), and larval output peaks 2–3 days later before declining. 'Self-cure' typically occurs 3–6 weeks p.i. There is good evidence that this is a consequence of potent immune responses (reviewed by Dawkins, 1989; Nawa *et al*. 1994). In laboratory mice, the pre-patent period is comparable to that in rats but larval output is lower and is minimal by 2 weeks p.i. (Dawkins *et al*. 1980, 1982; A. Gemmill, personal communication).

Worms were maintained by serial passage in female Wistar rats or were from infections maintained in congenitally hypothymic (nude) rats (Gemmill, Viney & Read, 1997). Two lines were used: ED132 Heterogonic (Viney, 1996), and ED200 (Read, Chan, Morris, Gemmill and Viney unpublished observations). Infections were initiated by subcutaneous injection of infectious third-stage larvae (iL3s) in saline; uninfected controls were similarly injected with equal volumes of saline. Inocula of more than 100 iL3s were prepared by dilution of larvae collected from faecal cultures; smaller inocula were prepared by counting iL3s individually under a binocular microscope. Larval output from infected animals was determined using Baermanns apparatus or by counts of 3-day faecal cultures (Gemmill *et al*. 1997).

Watermaze

The watermaze was a white plastic coated circular fibre-glass pool (193 cm diameter and 65 cm high) housed in a room ($4{\cdot}2 \times 2{\cdot}8$ m) that contained a number of geometric cues attached to the walls (e.g. squares, circles and triangles). The edge of the pool was located 33 cm from the back wall, 35 cm from one side wall, 42 cm from the opposite side wall and 194 cm from the front wall. A video camera was suspended over the centre of the pool. The pool was filled with tap water (24 ± 1 °C) made opaque and milky by the addition of 560 ml of latex solution. Animals were always placed into the watermaze with their head facing the wall of the tank. At the end of each day, the pool was cleaned, sterilized with bleach and allowed to dry out to prevent uncontrolled infection.

Experiments

Several experiments were conducted (Table 1). Experiments 1–5 tested spatial learning and memory, the first 4 with rats and the 5th with mice. Experiment 6 tested discrimination learning in rats. In Exp. 4, all animals were weighed daily in the late afternoon to determine whether there was any detectable effect of infection on animal weight. Experiments 3a and 3b were initiated at the same time using the same cohort of hosts and parasites but had different test days p.i. (Table 1).

Spatial task

When being used to test spatial learning and memory of rats, the watermaze was filled to a depth of 46 cm with tap water. A hidden escape platform (11 cm diameter), submerged 1 cm below the surface, was located 30 cm from the edge of the pool in the south quadrant. For spatial tasks with mice, a white plastic, circular insert (110 cm diameter and 30 cm high) with a perforated base made of PVC was positioned in the southern part of the larger watermaze. The base of the insert was raised on blocks by 30 cm so

Table 1. Experimental details

(In Exps 1–5, test day is the day p.i. when acquisition was tested. Retention was tested the following day. In Exp. 6, acquisition often continued for several days after testing began and retention was not tested.)

Experiment	Host species	Experimental task	Treatment groups (larval dose)	Animals/ treatment group	*S. ratti* strain	Test day (post-infection)	Start positions within swimming sessions	Number of swimming sessions
1	Rat	Spatial	Control (0) Low (20) High (1000)	5 5 5	ED200	7 and 8	Fixed	6
2	Rat	Spatial	Control (0) Low (20) High (1000)	6 5 5	ED132ht	8	Random	6
3a	Rat	Spatial	Control (0) High (1000)	6 6	ED200	6	Fixed	4
3b	Rat	Spatial	Control (0) High (1000)	6 6	ED200	12	Fixed	4
4	Rat	Spatial	Control (0) Low (50) High (500) Clinical (5000)	6 6 6 6	ED200	10	Random	6
5	Mouse	Spatial	Control (0) High (500)	10 10	ED200	5	Fixed	6
6	Rat	Discrimination	Control (0) High (500)	6 6	ED200	12	Random	—

that the top of the insert was 16 cm below the top of the main watermaze. The insert was covered with water to a depth of 17 cm and the hidden escape platform, in the south quadrant, was 7 cm diameter, 0·5 cm below the water surface and 15 cm from the edge of the pool. In both cases, 4 starting locations, designated as north, south, west and east, were spaced equidistantly around the edge of the pool.

The protocols used in these experiments were based on those used by Kavaliers & Colwell (1995), and involved 3 phases.

Pre-acquisition. Experiments 2–5 began with a single trial to assess whether there was any bias in where the rats or mice swam in the watermaze. Individual animals were given 60 sec to swim in the watermaze without an escape platform after being released from a randomly chosen starting location. The time spent in each quadrant was determined from video recordings of each trial.

Acquisition. For each animal, the acquisition phase ran over a morning or an afternoon, and consisted of 4 or 6 swimming sessions, where each session was made up of 4 separate swims (= trials). Each animal therefore received a total of 16 or 24 swims during the acquisition phase, depending on the experiment (Table 1). Within swimming sessions, the inter-trial interval was approximately 45 sec, and the sessions were separated by approximately 30 min. During each trial a rat or mouse was given 60 sec to find the platform. If the test animal found the platform it was allowed to remain on it for 15 sec. Animals that failed to find the platform within the 60 sec limit were placed on the platform for 15 sec. After each swim the animals were towel-dried and during the inter-swimming session interval were held in cages under heat lamps with food and water available. The start positions for each trial within a swimming session were randomized and different, or randomly assigned but fixed for the 4 swims within a swimming session (Table 1). The start positions assigned by either method were the same for all animals within a swimming session.

Retention. 24 h after acquisition, individual rats and mice were given a 60 sec probe trial to assess their retention of the spatial task. During the probe trials, all animals were filmed starting from the east release point, with no platform in the pool. The resulting video tapes were analysed to assess how much time animals spent in the south quadrant, the location of the platform during acquisition.

Experimental design. Up to 4 animals could be put through the acquisition phase over the course of a morning or afternoon. Since a single experiment necessarily consisted of more animals than that, the experiments had to be broken down into experimental blocks, where a single block consisted of the animals undergoing the acquisition phase during the same morning or afternoon experimental session.

Animals in the maximum of 2 blocks that could be tested on the same day were inoculated with saline or worms at the same time, so that within an experiment, infections were initiated over successive days to ensure that all animals were tested on the same day p.i. Animals were randomly assigned to experimental blocks as follows. Experiment 1: 3 blocks of 4 animals and 1 block of 3; Exp. 2: 4 blocks of 4 animals; Exps 3a, b: 4 blocks of 3 animals; Exp. 4: 6 blocks of 4 animals; Exp. 5: 5 blocks of 4 animals. Within each experimental block, each treatment was represented at least once.

Note that to avoid potential ambiguity, we have restricted our use of the term 'block' to its technical meaning in the experimental design literature, so that an 'experimental block' is as defined above. What we call a 'swimming session' is elsewhere called a 'block of trials' or 'blocks' (Kavaliers & Colwell, 1995; Kavaliers *et al*. 1995) and is equivalent to 4 separate swims. We use 'swim' and 'trial' interchangeably.

Discrimination task

The watermaze was filled to a depth of 20 cm. Two visually distinct platforms (500 ml glass conical flasks) inverted on a doweling rod (secured to a weighted base) were submerged in the pool. In both cases, 5 cm of flask was visible above the water. One platform (ESC+), covered with a black and white striped cloth, allowed rats to climb up and escape the water. The second (ESC−), covered in red adhesive tape, allowed no grip and hence no escape from the water. The rats' task was to approach and mount the ESC+ platform irrespective of location. A further start platform (11 cm diameter) 5 cm below the surface provided a starting position but was submerged sufficiently to encourage the rats to swim and locate the escape platform. A random sequence was used to locate the start platform in 1 of 4 potential positions 35 cm from the watermaze wall in the north, south, west and east. The visible platforms (ESC+ and ESC−) were presented in the opposite side of the watermaze from the start platform. The escaped platform positions were selected from 5 possible positions in an arc at 40°, 65°, 90°, 115° and 140° from the start platform. A random sequence determined precise platform location with the constraints that, ESC+ was placed equally often on the left and right of the start platform, and that the 2 platforms could never be located adjacently.

Rats were randomly allocated to 3 groups, each with 4 animals. Each group consisted of 2 uninfected controls and 2 infected rats (infected with 500 larvae), the infections were staggered by 7 days and each group of 4 was tested as separate experimental blocks, beginning 12 days p.i. Acquisition lasted 3–4 days until the discrimination was learned. The criterion for learning was 9 successive trials where a rat approached and mounted the ESC+ platform without approaching ESC−. During the acquisition phase the rats received 3 categories of trial. First, introductory trials in which 4 swims were made with only ESC+ in the pool. Rats were given 120 sec to find the platform. If they failed to find the platform they were guided by the observer and allowed to climb onto it. Two further swims were given where only the ESC− platform was in the pool. After 120 sec of swimming, the ESC+ platform was placed in the pool and the rats climbed onto it. Secondly, 4 choice trials in which the rat was placed on the submerged platform and allowed to swim until it climbed onto the ESC+ platform, regardless of whether or not contact was made with the ESC− platform. Thirdly, the final series of trials in which performance was measured. Both platforms were present but now contact with ESC− by the rat's fore-legs or nose was scored as an error. The rat was then punished by removing the ESC+ platform for 120 sec before replacing ESC+ and allowing the rat to climb out of the water. A rat's leg making brief contact with ESC− as it was swimming away was not regarded as an error and both platforms remained in the watermaze. The rats were given swimming sessions of 8–10 trials with approximately 60 min break between swimming sessions. If the rats did not learn to find ESC+ after 150 trials the acquisition phase ceased.

Experimental observers

During all spatial learning experiments performance was observed on a television monitor, so that the observer remained out of sight once the animals were placed in the watermaze until the end of a swim. During discrimination learning the observer was visible wherever alterations to the platform arrangements were necessary. Except for Expts 1 and 2, observers were blind to which individual animals were infected.

Statistical analysis

Experiments 1, 2, 3a and 3b were unbalanced within blocks. Experiment 4 was a fully balanced design allowing experimental block to be used in the analysis, but there was no within-block treatment replication, so it was not possible to test treatment × experimental block interactions. Experiment 5 was the only experiment with balanced within-block replication of treatments and which could therefore be tested for the treatment × experimental block interactions. For clarity, and following existing literature (Kavaliers & Colwell; 1995; Kavaliers *et al*. 1995), the tabulated statistics and the bulk of those reported in the texts are from statistical models which do not include experimental block as a factor. However, we also investigated the effects of ex-

perimental block by fitting block as a factor in subsequent analyses of Exps 4–6, and made attempts to investigate the effects of experimental block in Exps 1–3 by balancing the designs by randomly excluding data and fitting experimental block. A Kruskal–Wallis test compared the variation between experimental blocks in Exp. 6.

Time to reach the hidden platform during the acquisition phase was analysed using repeated measures ANOVA, with swimming sessions (1–4 or 1–6) as the within-subject factor, and treatment (worm dose) as a between-subject factor. The significance of the between-rat treatment effect shows whether the average time to find the platform across the 16 or 24 swims is affected by worm treatment (average performance); the significance of the within-rat swimming session × treatment interaction determines whether worm treatment has any effect of the rate of improvement in performance (learning). Times spent swimming in the correct quadrant during the retention tests were analysed by factorial ANOVA, with treatment (worm dose) and where possible experimental block as factors. A Mann–Whitney test compared the variation between infected and control animals in Exp. 6.

We report results from many statistical tests. Some may therefore be significant at the 5% level by chance alone (Type 1 error). There are various ways of attempting to control for this (e.g. Rice, 1989), none of which are very satisfactory (Rothman, 1990), so we report uncorrected P values. This allows readers to make any corrections they feel are justified. Any such corrections only reinforce our conclusions. Unless otherwise stated, two-tailed P values are reported throughout.

One uninfected rat in Exp. 1 and 1 infected rat in Exp. 3a failed to complete the acquisition swimming; both of these were excluded from analyses. Weight measurements from 2 of the 28 days of Exp. 4 were missed; for these, we used the average of the weights the day before and after the missing value.

RESULTS

Parasitology

In Exps 1–6 larval counts were made from faeces collected on days 5 and 24 p.i., day 6 p.i., days 14 or 15 p.i., day 13 p.i., day 6 p.i., and days 9 and 15 p.i. respectively. There was no evidence of exposure to larvae in our watermaze: at a time when controls would have been patent had they been infected during testing, worm output was still zero (days 24 p.i. and 14 or 15 p.i. in Exps 1 and 3a respectively). All animals experimentally infected became patent. Within and across all experiments, larval output correlated with inoculating dose (data not shown), except in Exp. 4 where output was highest from infections inoculated with 500 iL3s; output from those infected with 5000 iL3s was approximately 20% of that, which may represent extreme density dependence. All mice infected with *S. ratti* became patent; after the experiment was finished, adult worms were seen in the gastrointestinal tract of dissected mice.

Spatial task (Exps 1–5): learning

In all experiments, animals learnt the task: time taken to find the hidden platform improved significantly during the acquisition phase (within-subject improvement in performance across swimming sessions, $P < 0{\cdot}0001$ in all cases). However, there was little evidence that infection with *S. ratti* interfered with learning.

In Exps 1 and 2, there was some evidence ($P < 0{\cdot}02$, $P < 0{\cdot}09$ respectively) that rats with worms took longer to find the hidden platform, but there was no evidence that this was because they learned the task more slowly: rates of acquisition were not significantly different (Table 2). In Exps 3a, 3b, 4 and 5, there was no evidence of any effect of *S. ratti* infection on average performance or rate of acquisition (Table 2).

Further analysis of Exp. 4, where some rats were inoculated with very large doses of worms reinforces this picture (Fig. 1). A two-group comparison of animals exposed to 5000 larvae or none failed to reveal any effect of worms on average performance or rate of learning (average performance: $F_{1,10} = 1{\cdot}04$, $P = 0{\cdot}33$; rate of acquisition: $F_{5,50} = 0{\cdot}42$, $P = 0{\cdot}83$). However, large larval dose did have an impact on body size (Fig. 2). The growth of rats given 5000 larvae was initially checked, and those rats subsequently grew more slowly. By the time the rats started the acquisition phase of the experiment (day 11 p.i.), rat weight differed significantly between treatment groups ($F_{3,15} = 10{\cdot}4$, $P < 0{\cdot}0001$); *post hoc* comparisons revealed that rats given 5000 worms had put on just 60% of the weight put on by the other rats; there were no significant differences between the other treatment groups. Thus, even when *S. ratti* infection was sufficiently severe to affect body growth, we were unable detect any effects on learning.

The preceding analyses do not incorporate experimental block as a factor in the statistical models. However, there is no suggestion that this would make a difference. In both Exps 4 and 5, where block can be fitted (see Materials and Methods section), there were no significant main effects of block, and still no evidence of any effects of the worms on learning when block was added to the model (average performance: $F_{3,15} = 1{\cdot}0$, $F_{1,10} = 0{\cdot}12$; rate of acquisition $F_{15,75} = 1{\cdot}42$, $F_{5,50} = 1{\cdot}65$ respectively, $P > 0{\cdot}15$ in all cases). In Exp. 5, there was no evidence that infection had different effects in different experimental blocks (treatment × experimental block, $F_{4,10} = 0{\cdot}56$, $P = 0{\cdot}69$ and swimming session

Table 2. Effects of infection with *Strongyloides ratti* on time to find hidden platform in a Morris maze during the learning phase of the experiment

(Tabulated values are F-ratios (with associated degrees of freedom) from ANOVA models without experimental block fitted as a factor (see text). Worm effects are analysed with treatment considered as either presence or absence of worms or, for Exps 1 and 2, as a 3-category factor (0, 20, 1000) and for Exp. 4 as a 4-category factor (0, 50, 500, 5000). Repeated measure analysis of variance was used (see Materials and Methods section); effect on average time is the main between-rat effect of treatment; effect on rate of improvement is the within-rat swimming session × worm treatment interaction.)

	Effect of presence of worms on		Effect of worm dose on	
Exp.	Average time	Rate of improvement	Average time	Rate of improvement
1	$F_{1,12} = 7{\cdot}11$ $P = 0{\cdot}02$	$F_{5,60} = 0{\cdot}77$ $P = 0{\cdot}58$	$F_{2,11} = 3{\cdot}53$ $P = 0{\cdot}065$	$F_{10,55} = 1{\cdot}02$ $P = 0{\cdot}44$
2	$F_{1,14} = 3{\cdot}33$ $P = 0{\cdot}09$	$F_{5,70} = 0{\cdot}17$ $P = 0{\cdot}97$	$F_{2,13} = 1{\cdot}58$ $P = 0{\cdot}97$	$F_{10,65} = 0{\cdot}25$ $P = 0{\cdot}99$
3a	$F_{1,09} = 1{\cdot}68$ $P = 0{\cdot}23$	$F_{3,27} = 1{\cdot}40$ $P = 0{\cdot}26$	—	—
3b	$F_{1,10} = 0{\cdot}04$ $P = 0{\cdot}84$	$F_{3,30} = 0{\cdot}23$ $P = 0{\cdot}88$	—	—
4	$F_{1,22} = 0{\cdot}47$ $P = 0{\cdot}50$	$F_{5,110} = 1{\cdot}02$ $P = 0{\cdot}41$	$F_{3,20} = 0{\cdot}89$ $P = 0{\cdot}46$	$F_{15,100} = 1{\cdot}01$ $P = 0{\cdot}39$
5	$F_{1,18} = 0{\cdot}12$ $P = 0{\cdot}74$	$F_{5,90} = 1{\cdot}13$ $P = 0{\cdot}35$	—	—

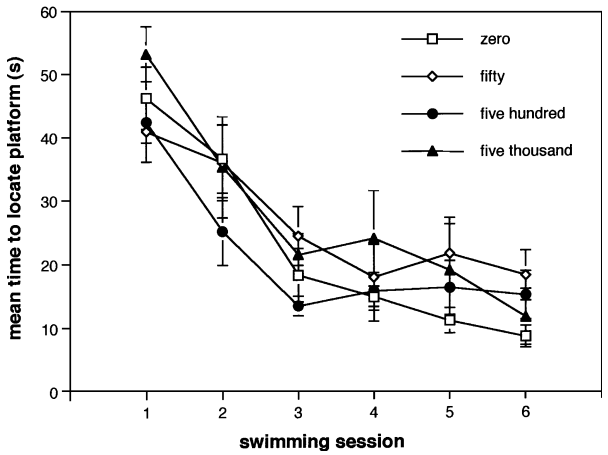

Fig. 1. Acquisition of the spatial watermaze task in Exp. 4, as measured by time to find a submerged hidden platform by rats infected with 0, 50, 500 or 5000 larvae of *Strongyloides ratti* 10 days earlier (Exp. 4). Over the course of a morning or afternoon, rats received 6 swimming sessions each consisting of 4 swims. Plotted points are the mean across 6 animals showing the average time taken to reach the platform during the 4 trials in a swimming session; vertical lines denote ±1 S.E. (based on $n = 6$ animals not $n = 24$ swims). There were no significant differences in performance between groups.

× treatment × experimental block, $F_{20,50} = 0{\cdot}67$, $P = 0{\cdot}83$). In Exps 1–3, there were no significant differences between experimental blocks in average performance of rate of acquisition. When those experiments were balanced by randomly excluding data and block was fitted to the models, there was still no evidence that treatment had any effect on learning.

Spatial task (Exps 1–5) : retention

During the retention phase of the spatial experiments, rats spent significantly more time than expected by chance alone in the quadrant which had contained the hidden platform in the learning phase of the experiments (Table 3; Fig. 3). In Exps 2–5, time spent in that quadrant prior to learning was measured. In all those experiments, rats spent significantly more time in that quadrant than they had in pre-acquisition trials (paired t-tests, $P < 0{\cdot}01$ in all cases). In Exp. 1, they spent longer in the correct quadrant than the 15 sec expected by chance alone. Thus, rats remembered the location of the platform the day after the acquisition phase. However, the time spent in the correct quadrant was unrelated to worm treatment in any of the experiments (Table 3). In all but 1 experiment, infected animals performed significantly better than they had done before the acquisition phase (Exp. 2: $t_9 = 3{\cdot}7$, $P = 0{\cdot}005$; Exp. 3a: $t_4 = 1{\cdot}6$, $P = 0{\cdot}18$; Exp. 3b: $t_5 = 2{\cdot}6$, $P = 0{\cdot}046$; Expt. 4: $t_{17} = 7{\cdot}2$, $P < 0{\cdot}0001$; Exp. 5: $t_9 = 2{\cdot}3$, $P = 0{\cdot}046$). The one exception was the experiment with the smallest sample size (Exp. 3a, $n = 5$ infected animals), but in that experiment there was, nevertheless, a significant improvement across all animals and the performance of infected and uninfected animals did not differ significantly (Table 3).

In only 1 of the experiments was the time spent in the correct quadrant during the retention phase significantly different between experimental blocks (Exp. 5) with mice; when block was added to the analysis of the data from that experiment, treatment effect was still not significant ($F_{1,10} = 4{\cdot}0$, $P = 0{\cdot}073$) and no treatment × experimental block interaction ($F_{4,10} = 1{\cdot}36$, $P = 0{\cdot}32$). In Exp. 4, the treatment effect remained non-significant even after controlling for experimental block ($F_{2,10} = 0{\cdot}12$). Thus, there was no evidence from any of the experiments that *S. ratti* infection interfered with memory.

Even after pooling the data from all the spatial experiments (to give a grand total of 97 animals) and fitting experiment and worm treatment (infected or not) as factors there was still no evidence of a

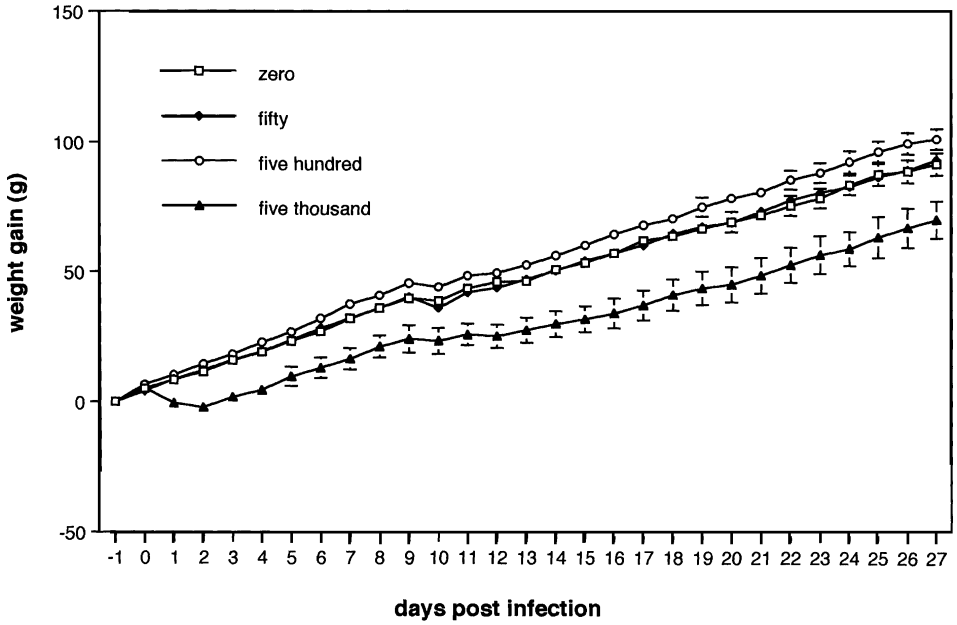

Fig. 2. Weight gain from the day of infection for animals infected with 0, 50, 500 or 5000 larvae of *Strongyloides ratti* (Exp. 4). Plotted points are the mean of 6 animals (±1 S.E.). The acquisition phase (which consisted of 24 swims/animals) occurred on day 10 p.i. Note that in many cases, the standard errors are less than the point size.

Table 3. Time spent in the quadrant of spatial watermaze which contained the hidden platform during the acquisition phase

(Rats were given a 60 sec swim the day before or after the acquisition phase. Tabulated values are mean (±S.E.) times for all animals (columns 2 and 3) and those which were uninfected (column 4) or infected (column 5) within an experiment, and F-ratios for the effects of worm treatment on retention (in ANOVA models without experimental block fitted as a factor).)

	All animals		Uninfected	Infected	
Exp.	Before Acquisition	After Acquisition	After Acquisition	After Acquisition	Worm effect† After Acquisition
1	—	27·5±2·2 ($P = 0{\cdot}001$)*	31·5±3·2	25·9±2·7	$F_{2,11} = 0{\cdot}90$, $P = 0{\cdot}43$
2	13·9±1·2	27·3±2·6 ($P = 0{\cdot}0009$)	22·9±3·2	30·0±3·9	$F_{2,11} = 1{\cdot}21$, $P = 0{\cdot}33$
3a	12·9±1·2	22·1±2·9 ($P = 0{\cdot}009$)	21·8±1·5	22·6±6·6	$F_{1,9} = 0{\cdot}02$, $P > 0{\cdot}50$
3b	12·9±0·7	24·2±3·2 ($P = 0{\cdot}004$)	21·6±3·8	26·8±5·2	$F_{1,10} = 0{\cdot}66$, $P = 0{\cdot}43$
4	14·2±1·1	33·5±2·4 ($P < 0{\cdot}0001$)	35·0±7·0	33·1±2·3	$F_{3,20} = 0{\cdot}11$, $P > 0{\cdot}50$
5	10·9±1·0	20·4±2·4 ($P = 0{\cdot}002$)	22·9±3·7	17·9±2·9	$F_{1,18} = 1{\cdot}12$, $P = 0{\cdot}30$

* P values are the probability that the times before and after acquisition differed by chance alone (from two-tailed t-test), except for Exp. 1, where no pre-acquisition test was done, and the P value is from a one-sample t-test against the expected mean time of 15 sec.

† Exps 1, 2 and 4 included different larval doses; the conclusions are unaltered if these are reanalysed as 2 group comparisons (infected or not), or if the group receiving the largest larval dose is compared with the uninfected controls ($P > 0{\cdot}2$ in all cases).

difference in the time infected and uninfected animals spent in the quadrant which had contained the platform the previous day (uninfected animals, 25·3±1·8 sec ($n = 38$), infected animals, 27·2±1·5 sec ($n = 59$); main effect of infection status, $F_{1,85} = 0{\cdot}002$, $P = 0{\cdot}97$; infection status × experiment interaction, $F_{5,85} = 0{\cdot}91$, $P = 0{\cdot}48$). This conclusion is unaltered if analysis is confined to rats only, or to a comparison of uninfected animals and the most heavily infected animals within each experiment ($P > 0{\cdot}5$ in all cases).

Power calculations estimate the probability of detecting, at a specified significance level, a difference of specified magnitude given observed variability in the data (Armitage & Berry, 1987). To measure within-group variability in our experiments, we separately calculated the residual variance in retention performance for wormy and control animals after fitting experiment as a factor. Calculations using these values (infected: $\sigma^2 = 109$, $n = 59$; uninfected: $\sigma^2 = 111$, $n = 38$), one-tailed expectations and equation 6·8 of Armitage & Berry (1987,

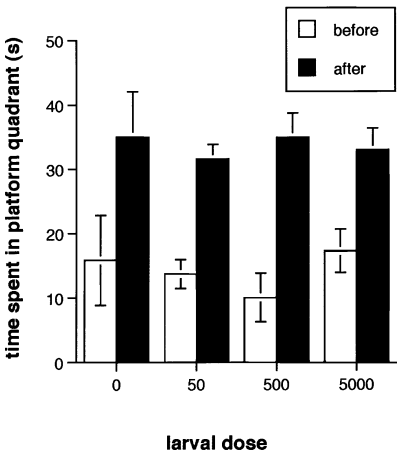

Fig. 3. Performance during the pre-acquisition (before) and retention (after) phases of the spatial water maze task in Exp. 4, as measured in a 1 min trial by the time spent swimming in the quadrant which held the hidden platform during the acquisition phase. No platform was available during these trials. Bars are means of 6 animals (± 1 S.E.).

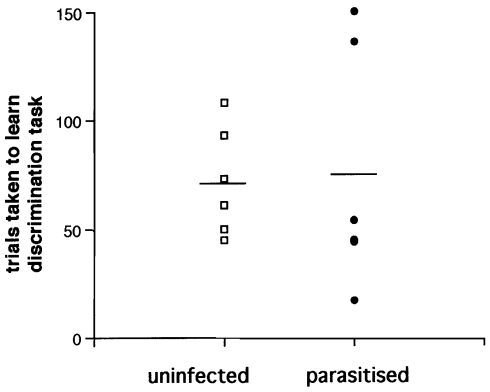

Fig. 4. Number of trials (swims) taken by 6 uninfected and 6 parasitized rats before they learned the discrimination task. The lines represent the mean number of trials taken to learn the task by each group.

p. 182), show that we had a 90% chance of detecting an average difference of at least 6 sec in the time spent in the correct quadrant by infected and uninfected animals, if such a difference existed. In Kavaliers & Colwell's (1995) *H. polygyrus* experiments, infected animals performed no better than they did prior to acquisition, which corresponded to a difference of about 16 sec between infected and uninfected animals in the post-acquisition retention trial. The corresponding figure across all our experiments is 12·9 sec; we had a probability in excess of 99% of detecting a difference of that magnitude. Even if we reduce sample size drastically by excluding all the data from Exp. 5 (mice) and including only data from uninfected animals and those given the largest worm dose in each experiment (i.e. $\geqslant 1000$ larvae) we had a 90% chance of detecting a difference of 7·9 sec if it was present (infected: $\sigma^2 = 148$, $n = 27$; uninfected: $\sigma^2 = 104$, $n = 28$). We also note that infected animals do better in 3 of the experiments but worse in the remaining 3, as expected by chance if there were no effects of worms on spatial memory.

When experimental block was taken into account in the analysis of Exp. 5 (mice), there was a weak but not significant effect of infection on retention ($P = 0{\cdot}073$). In 4 of the 5 experimental blocks, uninfected animals spent longer in the correct quadrant. Power calculations show that we had an 80% chance of detecting that pattern as significant, assuming that the 7 sec average difference between treatment groups within blocks is a good estimate of the magnitude of any difference ($n = 10$ mice/group, within-block variances of 44 and 17·6 for uninfected and infected animals respectively).

Discrimination task (*Exp. 6*)

The number of swims made before 9 consecutive correct trials did not differ between infected and uninfected rats (Fig. 4, Mann–Whitney U-test, $z = 0{\cdot}56$, $P = 0{\cdot}57$). All but 1 of the rats had achieved 9 consecutive correct trials before the 150 swim cut-off; the exception was included in the analysis by giving it a score of 150. There was no effect of experimental block ($P = 0{\cdot}94$, 2 D.F.).

DISCUSSION

We examined the effect of parasitic infection on animals given variable numbers of *S. ratti* larvae, at different numbers of days post-infection, on different learning tasks (spatial and discrimination) and with various alterations to the experimental protocols. Acquisition occurred irrespective of worm treatments, and infected animals were no slower to learn the tasks than were uninfected animals. In the spatial task, wormy and uninfected animals were equally good at retention the day after acquisition. There was no effect of worms on spatial learning even when infection was impairing weight gain.

This lack of effect is in marked contrast to the effects of the endoparasites *H. polygyrus* (another gastrointestinal nematode) and *E. vermiformis* on the performance of mice in the same spatial task (Kavaliers & Colwell, 1995; Kavaliers *et al.* 1995). The effects of those parasites were each easily detected in a single experiment with sample sizes comparable to just 1 of our experiments, yet all 6 of our spatial experiments failed to reveal any effects of *S. ratti* on learning or memory. In the *H. polygyrus* experiments, infected animals did not remember the task at all (Kavaliers & Colwell, 1995); in all our experiments, they did. Our power calculations showed that if *S. ratti* had an effect on retention of comparable magnitude we had probability of $> 99\%$ of detecting it when pooling the data from all our experiments.

The most interesting interpretation of the contrasting results is that *H. polygyrus* and *S. ratti* differ in their ability to impair learning and memory. Kavaliers & Colwell (1995) suggested the impaired learning effect might be mediated by an opioid-immune interaction and it is possible that such an interaction does not occur in rats and mice infected with *S. ratti*. There are also a potentially large number of other explanations for the contrasting conclusions. As far as possible we followed the experimental protocols described by Kavaliers & Colwell (1995) but, inevitably, there will have been minor differences. For instance, they used a strain of mouse we were unable to obtain. We note that when experimental block was taken into account in the 1 experiment in which we did use mice (Exp. 5), there was a weak but non-significant effect ($P = 0{\cdot}073$) of infection on retention of the spatial task. This pattern may be close to significance by chance alone; we have reported many statistical tests. It is, however, formally possible that *S. ratti* does have an effect on memory in mice, and our conclusion that it does not is an erroneous acceptance of the null hypothesis (Type II error). Our calculations showed that given the variability in that experiment, and our sample sizes, such an error would occur about 20% of the time. But even if it has, the almost significant 'pattern' we found contrasts in 2 ways with the previous work on mice with *H. polygyrus*. First, the time our *S. ratti*-infected mice spent in the correct quadrant was significantly greater after acquisition than it was before, indicating that they remembered the task. *H. polygyrus*-infected mice apparently did not (Kavaliers & Colwell, 1995). Secondly, *H. polygyrus* has a strong effect during acquisition (Kavaliers & Colwell, 1995); we found no such effect of *S. ratti*.

The experiments reported here tested for impaired cognition in both rats and mice infected with *S. ratti*. Whishaw (1995) has reported that laboratory mice generally perform less well in watermaze tasks compared to rats. A suggested outcome of this inferior performance is that mice may be more susceptible to disturbances and stressors. We found no effect of infection with *S. ratti* in either rats or mice and both rats and mice were capable of learning the position of the hidden platform in the watermaze. We are currently exploring the effects of *S. ratti* and *H. polygyrus* on spatial learning and memory in different strains of mice. Whatever the outcome of these new experiments, the results reported here show that the observation of impaired spatial learning ability due to parasitic infection is not a ubiquitous, or at least easily replicated, phenomenon.

We thank R. Morris, M. Ramsay, M. Kavaliers and M. Scott for helpful discussions about this work at various stages; G. MacMillan, P. Grantham, L. Mitchell and A. Harrower for help setting up the watermaze; and B. Chan and A. Gemmill for help with worms; S. Boath, H. Borthwick, S. Fleming and J. Tweedie for animal husbandry. This work was funded by a Royal Society grant to V.B. (574006.G503) and a BBSRC Fellowship support grant to A.R. A.R. is a BBSRC Advanced Research Fellow.

REFERENCES

ADAMS, D. B. & FELL, L. R. (1997). The effect of infection with the abomasal nematode, *Haemonchus contortus*, on the avoidance behaviour of a sheep. *International Journal for Parasitology* **27**, 665–673.

ARMITAGE, P. & BERRY G. (1987). *Statistical Methods in Medical Research*. Blackwell Scientific Publications.

BARNARD, C. J. & BEHNKE, J. M. (1990). *Parasitism and Host Behaviour*. London, Taylor & Francis.

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

BROT, M. D., RALL, G. F., OLDSTONE, M. B. A., KOOB, G. F. & GOLD, L. H. (1997). Deficits in discriminated learning remain despite clearance of long-term persistent viral infection in mice. *Journal of Neurovirology* **3**, 265–273.

DAWKINS, H. J. S. (1989). *Strongyloides ratti* infections in rodents: value and limitations as a model of human strongyloidiasis. In *Strongyloidiasis: a Major Roundworm Infection of Man* (ed. D. I. Groves), pp. 287–332. London: Taylor & Francis.

DAWKINS, H. J. S., GROVE, D. I., DUNSMORE, J. D. & MITCHELL, G. F. (1980). *Strongyloides ratti*: susceptibility to infection and resistance to reinfection in inbred strains of mice as assessed by excretion of larvae. *International Journal for Parasitology* **10**, 125–129.

DAWKINS, H. J. S., MITCHELL, G. F. & GROVE, D. I. (1982). *Strongyloides ratti* infections in congenitally hypothymic (nude) mice. *Australian Journal of Experimental Biology and Medical Science* **60**, 181–186.

DOLINSKY, Z. S., BURRIGHT, R. G. & DONOVICK, P. J. (1981). Behavioural effects of lead and *Toxocara canis* in mice. *Science* **231**, 1142–1144.

GEMMILL, A., VINEY, M. E. & READ, A. F. (1997). Host immune status determines sexuality in a parasitic nematode. *Evolution* **51**, 393–401.

GIBERTINI, M., NEWTON, C., FRIEDMAN, H. & KLEIN, T. W. (1995). Spatial learning impairment in mice infected with *Legionella pneumophila* or administered exogenous interleukin-β. *Brain, Behaviour and Immunity* **9**, 113–128.

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

KAVALIERS, M. & COLWELL, D. D. & GALEA, L. A. M. (1995). Parasitic infection impairs spatial learning in mice. *Animal Behaviour* **50**, 223–229.

MCGAUGH, J. L., INTROINI-COLLISON, I. B. & CASTELLANO, C. (1993). Involvement of opioid peptides in learning and memory. In *Opioids II* (ed. Herz, A.) pp. 429–447. Springer-Verlag, New York.

MEEKS GARDNER, J., GRANTHAM-MCGREGOR, S. & BADDELEY, A. (1996). *Trichuris trichiura* infection and cognitive function in Jamaican school children. *Annals of Tropical Medicine and Parasitology* **90**, 55–63.

MORRIS, R. G. M. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods* **11**, 47–60.

NAWA, Y., ISHIKAWA, N., TSUCHIYA, K., HORII, Y., ABE, T., KHAN, A. I., BING-SHI, ITOH, H., IDE, H. & UCHIYAMA, F. (1994). Selective effector mechanisms for the expulsion of intestinal helminths. *Parasite Immunology* **16**, 333–338.

NOKES, C. & BUNDY, D. A. P. (1994). Does helminth infection affect mental processing and educational achievement? *Parasitology Today* **10**, 14–18.

NOKES, C., GRATHAM-McGREGOR, S. M., SAWYER, A. W., COOPER, E. S. & BUNDY, D. A. P. (1992*a*). Parasitic helminth infection and cognitive function in school children. *Proceedings of the Royal Society of London, Series B* **247**, 77–81.

NOKES, C., GRATHAM-McGREGOR, S. M., SAWYER, A. W., COOPER, E. S., ROBINSON, B. A. & BUNDY, D. A. P. (1992*b*). Moderate to heavy infections of *Trichuris trichiura* affect cognitive function in Jamaican school children. *Parasitology* **104**, 539–547.

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

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

READ, A. F. (1990). Parasites and the evolution of host sexual behaviour. In *Parasitism and Host Behaviour* (ed. C. Barnard & J. M. Behnke), pp. 117–157. London: Taylor and Francis Ltd.

RICE, W. R. (1989). Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

ROTHMAN, K. J. (1990). No adjustments are needed for multiple comparisons. *Epidemiology* **1**, 43–46.

SEI, Y., ARORA, P. K., SKOLNICK, P. & PAUL, I. A. (1992). Spatial-learning impairment in a murine model of AIDS. *FASEB Journal* **6**, 3008–3013.

STEWART, C. A. & MORRIS, R. G. M. (1993). The watermaze. In *Behavioural Neuroscience; A Practical Approach*, (ed. A. Shaga), pp. 107–122. New York: IRL Press.

THOMPSON, S. N. & KAVALIERS, M. (1994). Physiological bases for parasite-induced alterations of host behaviour. *Parasitology* **109**, S119–S138.

VINEY, M. E. (1996). Developmental switching in the parasitic nematode *Strongyloides ratti*. *Proceedings of the Royal Society of London, Series B* **263**, 201–208.

WATKINS, W. E. & POLLIT, E. (1997). ''Stupidity or Worms'': do intestinal worms impair mental performance? *Psychological Bulletin* **121**, 171–191.

WHISHAW, I. Q. (1995). A comparison of rats and mice in a swimming pool place task and matching to place task: some surprising differences. *Physiology and Behaviour* **58**, 687–693.