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Toxoplasma infection and response to novelty in mice

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Abstract. Three groups of mice were infected with *Toxoplasma* and used for behavioral testing using a Y-maze. One group was infected when adult and two groups congenitally, one of these born to dams infected during gestation, the other to dams chronically infected prior to mating. In an initial habituation period each mouse was exposed to a black arm and stem of the maze, entrance to a white arm being blocked by a transparent door. In a subsequent free-choice trial both arms were black and the mouse was free to explore all parts of the maze. During both periods infected mice were more active than controls. Infected mice engaged in less grooming behaviour indicative of less approach-avoidance conflict than controls prior to entry into a choice arm at the beginning of the free-choice trial. Infected mice spent more time in the familiar than in the novel (previously blocked) arm during the free-choice trial; conversely, uninfected mice spent more time in the novel than in the familiar arm. It is suggested that the reported behavioural changes would lead to dissemination of the infection in the environment by ultimately making infected mouse intermediate hosts more susceptible to predation by domestic cats, the definitive hosts of *Toxoplasma*.

Introduction

Studies of the behavioral effects of *Toxoplasma* infections indicate that infected mice: (1) have impaired performance in negotiating complex mazes (Piekarski et al. 1978; Witting 1979); (2) have impaired motor performance, as measured by the number of falls from a rotating cylinder (Hutchison et al. 1980a; Hay et al. 1983a); (3) have greater activity levels when exposed to a novel environment presented in an 'open box' (Hay et al. 1983b, 1984a); (4) show a smaller relative preference for the central, more exposed areas of the 'open box' apparatus (Hay et al. 1983b, 1984a); and (5) are less responsive to novel stimulation (Hutchison et al. 1980b).

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The generally lower level of responsiveness to novel stimuli shown by *Toxoplasma*-infected mice was first demonstrated by the use of a simple Y-maze apparatus (Hutchison et al. 1980c). In this experiment, mice infected in adulthood and their controls were individually exposed, for an 'habituation period', to a black arm and stem of the Y-maze; entrance to a white arm was blocked by a transparent door. In a subsequent 'free-choice trial', in which both arms were black, the uninfected mice spent more time in the novel (previously blocked) arm, whereas the infected mice showed no preference for either arm.

The primary purpose of the study reported here was to discover whether or not mice with congenital *Toxoplasma* infections would show a decreased response to novel stimulation presented in the Y-maze. Given the findings of a previous study which used entirely different procedures, and which indicated that congenitally infected mice do appear to be less responsive to novel stimulation, (Hay et al. 1983b) we hypothesized that they would.

In addition, we hypothesized that if *Toxoplasma*-infected mice are less responsive to novel stimulation, then it might be expected that such mice would engage in lower levels of 'displacement activities' (Tinbergen 1952) indicative of less approach-avoidance conflict at the choice-point of the maze prior to exploring the novel and familiar arms at the beginning of the free-choice trial. Animals exposed to situations which appear to induce approach-avoidance conflict, often engage in activities which appear to the observer to be irrelevant.

In the case of rats, it has been demonstrated that when they vacillate between approaching and withdrawing from novel objects they often engage in bouts of grooming, with higher levels of grooming in situations which present higher levels of novelty (Bindra and Spinner 1958). Furthermore, rats exposed to maze situations similar to that described above tend to show increased levels of grooming behaviour in the area between the novel and familiar arms – that is, in the area where it might be expected to observe higher levels of approach-avoidance conflict (Aitken 1971). For these reasons, we hypothesized that if infected mice are less responsive to novel stimuli, then it might be expected that they would show lower levels of grooming (relative to uninfected controls) before exploring the novel and familiar arms of the maze at the beginning of the free-choice trial.

The present investigation also allowed further examination of apparently conflicting results reported in previous investigations. First, Hutchison et al. (1980b) and Hay et al. (1983b, 1984a), found that *Toxoplasma*-infected mice were more active than uninfected controls when exposed to novel stimulation. However, in the earlier study using the Y-maze apparatus, Hutchison et al. (1980c) found that the infected mice were less active than uninfected controls during the initial relatively brief habituation period.

Secondly, although Witting (1979) reported a correlation between the number of *Toxoplasma* tissue cysts in the brains of infected mice and deficits in their performance in negotiating a complex maze, the Y-maze study of Hutchison et al. (1980c) is the only one of our behavioral investigations where a significant correlation between brain cyst counts and a 'behavioral

deficit' has been found – in this case, a negative correlation between the number of brain cysts and time spent in the novel arm of the Y-maze during the free-choice trial.

It was hoped that the present investigation would clarify these apparently conflicting results.

Materials and methods

Preparation of subjects. Strain A albino mice were used. Experimental mice comprised one group infected as adults and two groups infected congenitally, one by infecting the dams during gestation and the other by infecting the dams prior to mating. Controls for the adult-infected group comprised one group infected with a *Toxoplasma*-free brain extract, one group injected with sterile isotonic saline, and one group receiving no treatment. Controls for the two congenitally infected groups comprised their uninfected littermates as well as offspring of dams injected with a *Toxoplasma* cyst-free brain extract; a group comprising offspring of dams receiving no treatment was also included.

Mice infected as adults were 22–23 weeks old when tested and had been infected with a brain homogenate containing ten, 17-week-old *Toxoplasma* tissue cysts of the avirulent Rabbit A (i.e. Beverley) strain (Beverley 1959), 14–15 weeks prior to this; their controls were of comparable age and those infected with *Toxoplasma* cyst-free brain extract or isotonic saline had been treated 14–15 weeks prior to testing. All the congenitally infected mice, offspring of dams injected with the same inoculum as above and their controls, were 14–15 weeks old when subjected to behavioral testing. The uninfected littermates could be detected as such only at autopsy; their behavior has been found in previous studies (Hay et al. 1983a, b, 1984a) to be indistinguishable from that of uninfected mice, offspring of dams uninfected with *Toxoplasma*.

Techniques for breeding, selecting and infecting of mice together with reasons for the treatment of the various control groups have been described and discussed by Hay et al. (1983a).

The infected mice used in this experiment, as in previous studies in this series (Hay et al. 1983a, b, 1984a) appeared to be asymptomatic at the time of testing, showing no obvious external manifestations of toxoplasmosis or differences in appearance or health from uninfected mice of similar age.

Apparatus and procedures. The maze was used previously by Hutchison et al. (1980c) to assess familiarity-novelty discrimination in mice which had received their primary infection with *Toxoplasma* in adulthood. As in this previous investigation we exposed mice individually to a black arm and stem of the Y-maze for a 2-min habituation period. Entrance to a white arm was blocked by a transparent door. Data recorded were: (1) position in maze; (2) gross activity; (3) time spent grooming and freezing; and (4) the number of faecal boluses deposited over the 2-min testing period. Mice were then subjected to a 4-min free-choice trial in which both arms of the Y-maze were black. They were free to explore the entire maze. Data recorded were: (1) initial choice (novel versus familiar arm); (2) initial choice latency; (3) position in maze; (4) gross activity; (5) time spent grooming and freezing; and (6) the number of faecal boluses deposited over the 4-min testing period. Two additional measures to those taken in the previous study were included. These were, time spent grooming and time spent freezing at the choice-point area before initial choice – i.e. before the mouse first moved into one of the choice arms at the beginning of the free-choice trial.

Time spent in the various maze sections and the time spent grooming or freezing were recorded using a time-sampling procedure: position and behavior were noted at 5-s intervals cued by signals through an earpiece worn by the observer. The maze was cleaned with industrial methylated spirit after each free-choice trial. All observations were carried out in silence between 1630 and 2230 hours on successive evenings. The order of testing was determined by a quasirandomisation procedure described in Hay et al. (1983a).

Within 24 h of testing, individual mice were assessed on a 4-point scale for external signs of disease, weighed, anaesthetized and bled to obtain serum for the detection of *Toxoplasma* antibodies using the Sabin-Feldman dye test as standardized by Aagaard (1960). The brain was removed, weighed and divided into four anatomically well-defined sectors: (1) the olfactory bulbs; (2) the cerebellum; (3) the medulla and pons; (4) the remainder i.e. cerebral hemispheres and upper brain stem. Each sector was weighed and where *Toxoplasma* tissue cysts were observed these were counted in each sector using methods described by Hay et al. (1983a).

Statistical analysis. The data were subjected to a series of Infection Treatment (11) × Sex (2) analyses of variance (Kim and Kohout 1975). As in our previous behavioural studies, the term 'Infection Treatment' as used here refers to the infection (or non-infection) status of the various groups, that is whether the mouse belonged to an infected group (adult-acquired infection or one or the other of the congenitally infected groups) or to any of their respective control groups. Where significant F-ratios were obtained for Infection Treatment effects (i.e. indicating differences between 2 or more of the 11 infected and uninfected groups) *a posteriori* Scheffé comparisons (with $\alpha = 0.05$) were used to determine which groups differed significantly from one another. In order to conserve space, data referring to differences between males and females will only be reported in the tables and figures in cases where there were interactions between Infection Treatment and Sex (i.e. where Infection Treatment had a differential effect on the two sexes).

Results

Ambulatory activity – number of entries into choice arms

Table 1 shows the mean ambulatory activity scores (number of entries into choice arm(s)) over the 2-min habituation and 4-min free-choice trial periods respectively. The Infection Treatment and Sex factors were highly significant for both periods (in both cases, $P < 0.001$). The Infection Treatment × Sex interaction did not approach significance in either analysis. *A posteriori*

Table 1. Means and standard deviations of behavioural measures

	Infected mice from acutely infected dam (60)[a]		Infected mice from chronically infected dam (35)		Acquired infection when adult (64)		All uninfected groups combined (413)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Habituation period								
Activity[b]	15.4	1.2	15.5	1.2	13.4	1.4	11.2	1.6
Free-choice trial								
Activity[b]	38.8	2.9	37.6	2.9	32.9	2.1	30.8	2.3
Time in novel arm[c]	12.4	2.8	12.3	2.6	14.8	2.8	18.2	3.6
Time in familiar arm[c]	18.9	3.4	18.1	3.5	16.6	2.2	13.8	2.7
Grooming pre-free[c] choice	0.7	0.7	0.5	0.6	0.6	0.5	1.3	0.6

[a] Figures in parentheses indicate numbers of mice in each group
[b] Number of arm entrances
[c] 5-s time samples

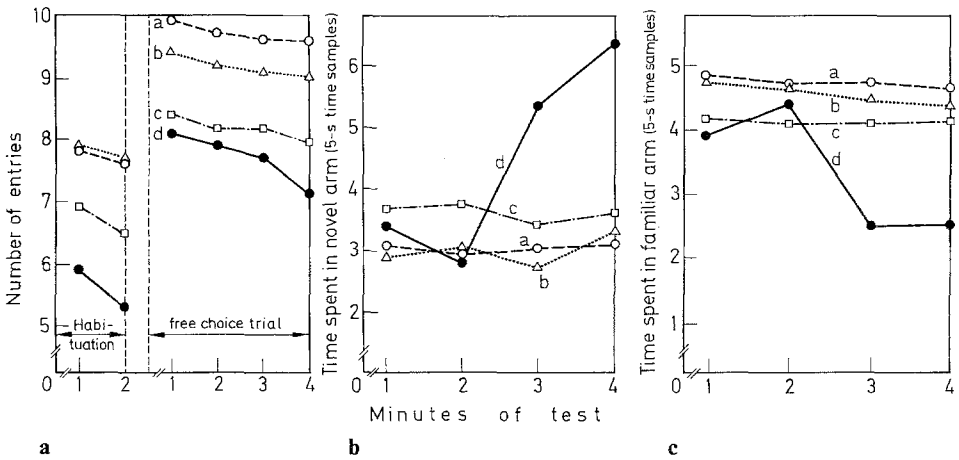

Fig. 1 a–c. Activity of experimental and control mice during the habituation and free-choice trial periods, measured as the mean number of entries into the open maze arm and the two choice arms respectively (**a**) and preference of experimental and control mice for the familiar arm (**b**) and novel arm (**c**) as measured by the mean time spent in the arm during the free-choice trial period. **a** --o---o-- by congenitally infected mice from acutely infected dams; **b** ··△···△·· by congenitally infected mice from chronically infected dams; **c** ··□···□·· by group infected as adults; **d** —●——●— by *all uninfected* groups as controls, combined

Scheffé comparisons showed that in both testing periods the three infected groups were significantly more active than their appropriate uninfected control groups. There were no significant differences between any of the eight control groups (including uninfected littermates of congenitally infected mice); therefore these were combined in Table 1.

Separate comparisons between the three infected groups showed that the two congenitally infected groups were significantly more active than the adult-acquired-infection group; there were no significant differences in ambulatory activity between the two congenitally infected groups. These similarities and differences applied to both periods of testing.

Although not shown in Table 1, as in our previous studies (Hay et al. 1983b, 1984a), females, whether infected or not, tended to be more active than the males in their respective groups.

Figure 1a indicates that the infected groups tended to maintain higher levels of activity over the whole 2-min habituation period and the whole 4-min free choice trial. A series of analyses of variance and *a posteriori* Scheffé comparisons showed that the three infected groups were significantly more active than their respective control groups for each separate minute of the two periods of testing. In each case the Infection Treatment factor was highly significant ($P<0.001$).

Finally analysis of the decrease in activity between the first and second half of the habituation period and the first and fourth quarters of the free-choice trial showed that in both cases this was significantly greater for uninfected than infected mice ($P<0.001$, in both cases). The decrease was also significantly greater for females than for males ($P<0.05$, for the habitu-

ation period; $P<0.01$, for the free-choice trial period) irrespective of whether or not they were infected. The Infection Treatment × Sex interaction did not approach significance.

Preference for choice arms of the Y-maze and time spent in familiar and novel arms during the free choice trial

Approximately half of the mice in each group initially entered the novel arm and approximately half entered the familiar arm. However, initial choice is a crude measure of preference; time measures provide a more sensitive index of preference in this kind of situation (Aitken and Sheldon 1970; Aitken 1972, 1974).

Table 1 shows the mean times spent in the familiar and novel arms of the Y-maze during the 4-min free-choice trial period. The Infection Treatment and Sex factors were significant for both measures (Infection Treatment, $P<0.001$ in both cases; Sex, $P<0.05$, for time spent in the familiar arm and $P<0.001$ for time spent in the novel arm). In neither case did the Infection Treatment × Sex interaction approach significance. A posteriori Scheffé comparisons showed that the three infected groups spent more time in the familiar arm and less time in the novel arm than their respective control groups. Because there were no significant differences between any of the eight control groups (including uninfected littermates of congenitally infected mice), with respect to both measures, the controls were combined in Table 1.

Separate comparisons between the three infected groups indicated that the two congenitally infected groups spent significantly more time in the familiar arm and significantly less time in the novel arm than the adult-acquired-infection group; there was no significant difference between the two congenitally infected groups with respect to either measure.

Females, whether infected or not, tended to spend less time in the familiar arm, and more time in the novel arm, than the males in their respective groups.

Further inspection of Table 1 indicates that *within* each group there were differences between time spent in the familiar and novel arms of the maze. A series of Wilcoxon matched pairs signed-ranks tests showed that infected mice spent significantly more time in the familiar arm than in the novel arm over the 4-min testing period ($P<0.001$, for both congenitally infected groups; $P<0.01$, for the adult-acquired infection group). In contrast, uninfected mice spent significantly more time in the novel arm than in the familar arm ($P<0.001$).

Figure 1 b and c indicates that the marked differences between the infected and uninfected mice in the time spent in the familiar and novel arms of the maze occurred over the last 2 min of the free-choice trial. A series of analyses of variance and *a posteriori* Scheffé comparisons showed no consistent differences in either measure for the first 2 min of testing. However, in the 3rd and 4th min of testing, the three infected groups spent more time in the familiar arm and less time in the novel arm than their

respective controls. For each measure, during these latter two testing periods, the Infection Treatment factor was highly significant ($P<0.001$). All groups, irrespective of Infection Treatment, showed an increase in time spent in the novel arm and a decrease in time spent in the familiar arm. However, and not surprisingly, analysis of the decrease in time spent in the familiar arm and increase in time spent in the novel arm from the first to the fourth quarter of the free-choice trial showed that in both cases differences were significantly greater for the uninfected than for the infected mice ($P<0.001$, in both cases). Thus, the overall difference between the uninfected groups was mainly due to a marked decrease in time spent in the familiar arm (Fig. 1 b) and a corresponding increase in time spent in the novel arm (Fig. 1 c) by the uninfected groups.

There was no significant difference between the sexes with respect to the decrease in time spent in the familiar arm. However, the increase in time spent in the novel arm was significantly greater for females than for males ($P<0.05$). In neither case did the Infection Treatment × Sex interaction approach significance.

Finally, a series of Wilcoxon matched pairs signed-ranks tests showed that the infected mice spent significantly more time in the familiar arm than in the novel arm for each of the 4 min of testing ($P<0.001$, for each 1-min period). The uninfected mice also tended to spend more time in the familiar arm than in the novel arm during the 1st and 2nd min of testing ($P<0.001$, for each 1-min period); during the 3rd and 4th-min of testing, however, the uninfected mice spent significantly more time in the novel than in the familiar arm ($P<0.001$, for each 1-min period).

Measures of fearfulness and displacement behaviour

There were no significant differences between the infected and uninfected groups with respect to time spent freezing and grooming during the habituation and free-choice trial periods. There were no significant differences between any of the groups with respect to the amount of time spent in the stem and choice point area prior to initial choice. There was also no significant difference between any of the groups with respect to time spent freezing at the choice-point-area, prior to entry into one or other of the choice arms.

Grooming at choice-point area prior to entry into a choice arm. Table 1 shows the mean times spent grooming in the choice point area prior to entry into a choice arm. Of the two main effects, only the Infection Treatment factor was significant ($P<0.001$). *A posteriori* Scheffé comparisons showed that the three infected groups spent significantly less time grooming than did their appropriate control groups. There were no significant differences between any of the eight control groups (including uninfected littermates of congenitally infected mice); therefore these have been combined in Table 1. Separate comparisons showed that there were no significant differences in this behaviour between the three infected groups.

Table 2. Means and standard deviations of the number of faecal boluses deposited during the 2-min habituation period and the 4-min free-choice trial

	Habituation				Free-choice			
	Males No. boluses		Females No. boluses		Males No. boluses		Females No. boluses	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1. *Infected* mice from *acutely* infected dam	0.9	0.6	1.5	0.6	1.8	0.7	3.1	0.9
2. *Infected* mice from *chronically* infected dam	1.2	0.7	2.0	0.6	2.0	0.6	3.5	0.7
3. *Acquired* infection when adult	1.1	0.7	2.1	0.7	2.5	0.8	3.3	0.7
4. *All* uninfected groups *combined*	1.5	0.6	1.0	1.0	3.7	0.7	2.6	0.6

Defaecation – number of faecal boluses deposited during the habituation and free-choice trial periods. Table 2 shows the mean number of faecal boluses deposited during the 2-min habituation period and 4-min free-choice trial period. For both measures, the differences with respect to Infection Treatment and Sex were significant (Infection Treatment, $P<0.001$, in both cases; Sex, $P<0.01$, for the habituation period, $P<0.001$, for the free-choice trial period). In both cases, the Infection Treatment × Sex interaction was highly significant ($P<0.001$). The nature of this interaction is clearly evident in Table 2. *A posteriori* Scheffé comparisons showed that uninfected male mice in each group deposited significantly more faecal boluses than did uninfected female mice in each group. However, the reverse was true for infected mice; infected female mice in each group deposited significantly more faecal boluses than did the infected male mice in each group. These findings were significant for each period of testing.

Additional analyses were conducted to discover whether or not the behavioral differences between the infected and uninfected groups were independent of defaecation scores. All of the behavioral measures found significant were subjected to a series of analyses of covariance, with Infection Treatment and Sex as non-metric factors and bolus counts as a covariate. In all analyses, the major differences between the infected and uninfected groups remained as before, showing them to be independent of defaecation.

Measures of health

Additional analyses were conducted to discover whether or not the behavioral differences between the infected and uninfected groups were independent of measures which have been used as indices of health. Again all of the behavioral measures found significant were subjected to a series of analyses of covariance, with Infection Treatment and Sex as non-metric factors and

Table 3. Measures relating to the number, central tendency and variability of *Toxoplasma* tissue crysts in the brain of infected mice used for the Y-maze test

		Olfactory bulbs	Cere-bellum	Medulla and pons	Cerebral hemispheres and upper brain stem	Whole brain
Range	A	0–44	4–163	0–101	36–2688	40–2996
	B	0–38	5–161	6–83	88–3008	99–3290
	C	0–84	26–156	21–158	488–6329	535–6727
Median	A	13	31	19	411	483
	B	12	22	23	503	576
	C	37	72	53	1261	1442
Mean	A	15	42	24	566	648
	B	14	33	28	585	659
	C	38	74	61	1823	1996
Standard deviation	A	10	35	19	489	538
	B	8	30	19	491	517
	C	18	30	34	1250	1280

A = infected offspring from dams infected with *Toxoplasma* during pregnancy. B = infected offspring from dams chronically infected with *Toxoplasma*. C = mice with chronic acquired *Toxoplasma* infections

body weight and a subjective health rating (rated on a 4-point scale at the time of behavioral testing) as covariates. In all analyses the major differences between the infected and uninfected groups remained as before, showing them to be independent of measures of health.

Correlations between behavioral measures and brain cyst counts

Measures describing the number, variability and central tendency (mean and median) of *Toxoplasma* tissue cysts in the four brain sectors and the brain as a whole, of the infected mice used in this study are given in Table 3. We wished to discover whether there were any associations between these and the behavioral measures found significant in the analyses described above. Thus correlations were computed between the behavioural measures and: (1) the number of cysts in the whole brain; (2) the number of cysts in the whole brain per gram of brain tissue; (3) the number of cysts in each of the four brain sectors; (4) the number of cysts in each of the four brain sectors per gram of brain tissue; and (5) the number of cysts in each of the four brain sectors as a fraction of the total brain weight. Correlations were computed for each sex and for each of the infected groups. None of the correlations was significant.

Discussion

The infected mice irrespective of sex and category of infection, spent significantly less time in the novel arm and significantly more time in the familiar arm of the Y-maze than did their appropriate controls. In addition, compari-

sons *within* groups showed the uninfected mice spent significantly more time in the novel arm than in the familiar arm. These two sets of findings replicate and extend similar findings in the previous study (Hutchison et al. 1980c) in which male mice with *Toxoplasma*-infections acquired in adulthood and their saline-injected controls were examined in the same Y-maze apparatus as used in the present investigation. Given these findings, together with additional findings reported by Hay et al. (1983b, 1984a), using a different apparatus, it now seems clear that *Toxoplasma*-infected mice are less likely to explore novel stimuli than are uninfected mice. Moreover, there were distinct and highly significant differences between the patterns of exploratory behaviour of the infected and uninfected mice. As predicted, the infected mice engaged in significantly *less* grooming behaviour, indicative of *less* approach-avoidance conflict, at the beginning of the free-choice trial period when they were first exposed to the novel and familiar arms of the maze. This is consistent with the hypothesis that *Toxoplasma*-infected mice are less responsive to novel stimulation (Hutchison et al. 1980b, c; Hay et al. 1983b, 1984a). During the first 2 min of the free-choice trial, the uninfected mice spent more time in the familiar arm than in the novel arm (Fig. 1b and c). However, thereafter, there was a marked increase in the amount of time spent in the novel arm with a corresponding decrease in the amount of time spent in the familiar arm. During the 3rd and 4th min of testing, the uninfected mice spent considerably more time in the novel than in the familiar arm. This kind of behaviour – initial approach-avoidance conflict, followed first by cautious exploratory behaviour from a familiar 'base' (analogous to the familiar arm in our study), followed by increasing amounts of time in more novel areas – is remarkably similar to the patterns of exploratory behaviour of captured wild house-mice (*Mus musculus* L.) released in large enclosures as described by Crowcroft and Rowe (1963) and Crowcroft (1966).

All categories of the *Toxoplasma*-infected mice showed a very different pattern of behaviour. Figure 1b and c show that, like the uninfected controls, the infected mice tended to spend more time in the familiar arm than in the novel arm, during the first 2 min of the free-choice trial; thereafter they likewise tended to spend increasing amounts of time in the novel arm. However, this change with time was far less marked than that shown by the uninfected controls. In fact, the infected mice actually still spent more time in the familiar arm than in the novel arm, over the *last* 2 min of testing. Indeed, mere inspection of Fig. 1b and c immediately suggests that the infected mice showed an almost qualitatively different pattern of behaviour to that shown by the uninfected mice.

As stated above, the behaviour observed in the uninfected mice appears analogous to that of captured wild house-mice released into large novel enclosures. It appears that such mice explore novel areas in bouts, presumably making full use of exteroceptive and proprioceptive cues. They continually return via a previously explored route to a 'safe area' with which they have become familiarized or habituated (Crowcroft 1955; Crowcroft and Jeffers 1961). Such ordered movement has also been observed in the

field with the wood or field-mouse *Apodemus sylvaticus* L. which displays regular patrolling of its home range (Brown 1969). It seems that odours from urine, preputial glands, coagulating glands and plantar glands, transported on the bodies of mice, particularly in the anogenital region may be deposited as scent marks, and urine especially may be placed as spots around the edges of enclosures or on conspicuous objects (Wolfe 1969; Barnett and Smart 1970; Smith 1981). House mice and rats mark their paths with urine and follow these trails (Eibl-Eibesfeld 1950, 1953). The following of such olfactory cues presumably enables the mouse to return continually to its 'base area' before it commences on further and longer bouts of exploration. It may be that in some way a mouse's 'awareness' of its having previously been in an area stimulates it to undertake further exploration. Thus, perhaps olfactory and other sensory cues, processed by integrated brain networks initiate further and longer bouts of exploratory behaviour. This might explain why the uninfected mice continued to return to the familiar arm of the maze right to the end of the experimental period. It is worthy of note that Maruniak et al. (1974) found novel stimuli to be the most effective cause of marking with urine by male laboratory mice. In *Toxoplasma*-infected mice, however, brain damage, caused either directly or indirectly by the parasite, may disturb this processing of sensory cues. If our hypothesis is correct, this may be one reason why *Toxoplasma*-infected mice fail to explore novel stimuli to the same extent as uninfected mice. Other possible explanations to account for these marked differences in behaviour have been discussed at considerable length elsewhere (Hay et al. 1983a, b). Statistical comparisons made between the three infected groups showed that both congenitally infected groups were more active than the adult-acquired infection group, during both periods of testing, while not being significantly different from each other. The same was the case with respect to time spent in the maze arms during the free-choice trial: the congenitally infected groups spent more time in the familiar arm and less time in the novel arm than did the adult acquired infection group; again there were no significant differences between the two congenitally infected groups. As we have suggested previously such differences may result from the effect of congenital toxoplasmosis upon critical brain cell development periods during embryogenesis and early postnatal development. Such cellular development in the mouse brain has been documented by Korr (1980) and Rodier (1980). Another possible cause of the differences in behaviour could be that ocular impairments (Hay et al. 1981; Lee et al. 1983; Hay et al. 1984b) found so far to occur only in congenitally infected mice, are making such mice less sensitive to illumination and thus more active, and also less responsive to maze stimuli. Irrespective of the mode of acquisition of infection the results suggest two ways in which *Toxoplasma* infections might be disseminated by wild mice. First, it is possible that anything which affects the finely balanced patterns of exploratory behaviour in small rodents would be maladaptive and would render them more liable to predation by cats, the definitive hosts of *Toxoplasma*. As mentioned above, there appears to be a qualitative difference between the patterns of exploratory

behaviour shown by infected and uninfected mice and this in our view makes infected mice more liable to predation by cats.

Secondly, it has been suggested that rodents which explore novel or exposed areas of their environment are more vulnerable to predation by cats (Elton 1953). Thus it is possible that mice with asymptomatic *Toxoplasma* infections – mice which tend to show a smaller preference for novel or exposed areas of their environment – may be less vulnerable to predation. If this occurs in mice which are capable of reproduction, it would be of considerable value in the dissemination of infection among mice as intermediate hosts. Such mice (if female) would produce litters containing congenitally infected individuals.

However, as the infection progresses mice may become less able to discriminate between novel and familiar stimuli, as suggested by findings obtained in a preliminary study (Hutchison et al. 1980c) in which some of the mice were more heavily infected. Consequently, at this later stage in the disease process mice may be more prone to predation.

These hypotheses can perhaps be examined in field surveys such as that reported by Hay et al. (1983c). Briefly, rodent trapping devices are set up in areas where there are known to be cat-rodent interactions. Such interactions can be detected due to the presence of *Cysticercus fasciolaris* in the rodent liver. The large liver cyst containing this organism which is the intermediate stage in the life cycle of the large tapeworm of the cat (*Taenia taeniaeformis*) can only develop when cat faeces containing the ova of this cestode are ingested by mice and other rodents. Thus it would be certain that mice in such an area had been exposed to pathogens present in cat faeces. Data obtained in this way may provide useful information. For example, if mice with asymptomatic infections are less liable to explore novel stimuli like traps then it might be expected that the majority of infected trapped rodents would be in older age groups.

The uninfected males deposited significantly more faecal boluses than did the uninfected females. This finding is consistent with a theory of sex differences in behaviour (Gray 1971, 1979) which suggests that male rodents tend to show higher levels of defaecation when exposed to mildly stressful stimuli. However, this difference was completely reversed among the infected mice; infected females deposited significantly more faecal boluses than did infected males. It thus seems possible that *Toxoplasma* infection affects mechanisms responsible for controlling defaecation and/or emotional elimination in novel environments.

Finally, it would be interesting to compare patterns of urination in *Toxoplasma*-infected mice with those of controls. Gray (1979) has suggested that urination may be used in scent marking by mice. If *Toxoplasma*-induced brain damage disrupts processing of sensory cues (e.g. odours from mice) in their exploratory activities then we might expect to find differences in scent marking behaviour between infected and uninfected mice exploring novel environments.

In conclusion, if such behavioral alterations occur in wild mouse populations they would certainly ultimately promote the infection of predatory

cats and be of major importance in the continuation of the life-cycle of *Toxoplasma*. In urban areas, free-ranging house-cats, preying on infected mice, would shed oocysts into the soil along with voided faeces. The oocysts could be ingested by mice along with the cat faeces, which they apparently eat quite often (Avery 1974). This would complete a dynamic transmission cycle, the most important side effect of which would be the infection of humans.

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