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Fatal attraction in rats infected with *Toxoplasma gondii*

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We tested the hypothesis that the parasite *Toxoplasma gondii* manipulates the behaviour of its intermediate rat host in order to increase its chance of being predated by cats, its feline definitive host, thereby ensuring the completion of its life cycle. Here we report that, although rats have evolved anti-predator avoidance of areas with signs of cat presence, *T. gondii*'s manipulation appears to alter the rat's perception of cat predation risk, in some cases turning their innate aversion into an imprudent attraction. The selectivity of such behavioural changes suggests that this ubiquitous parasite subtly alters the brain of its intermediate host to enhance predation rate whilst leaving other behavioural categories and general health intact. This is in contrast to the gross impediments frequently characteristic of many other host–parasite systems. We discuss our results in terms of their potential implications both for the epidemiology of toxoplasmosis and the neurological basis of anxiety and cognitive processes in humans and other mammals.

Keywords: *Rattus norvegicus*; *Toxoplasma gondii*; parasite manipulation; cat odours; anxiety; predation

1. INTRODUCTION

According to the manipulation hypothesis, a parasite may alter the behaviour of its host for its own benefit, usually by enhancing its transmission rate. The hypothesis implies that such host behaviour modification represents a sophisticated product of parasite evolution aimed at host manipulation, rather than an accidental side-effect of infection (Barnard & Behnke 1990; Poulin 1994). Parasites that are transmitted through the food chain constitute classic examples of such manipulation: the parasite is immature in the intermediate host and must be eaten by a predatory definitive host before it can reach maturity and complete its life cycle. Unfortunately, however, many studies have either attached little importance as to whether the host in question normally carries the parasite and/or studied hosts maintained under highly unnatural laboratory conditions. The transferability of such studies and their applicability to the epidemiology and evolution of disease in the wild may thus be open to question (Moore & Gotelli 1990; Webster *et al.* 2000).

The host–parasite system *Rattus norvegicus–Toxoplasma gondii* provides a convenient model in which to examine such questions. *T. gondii* is an intracellular protozoan (Beverley 1976) capable of infecting all mammals. Its associate disease, toxoplasmosis, is of significant economic, veterinary and medical importance (Luft & Remington 1986; Schmidt & Roberts 1989) and has sparked renewed interest due to its debilitating reactivation in AIDS and other immunosuppressed patients (Luft & Remington 1986). *T. gondii* has an indirect life cycle, where members of the cat family are the definitive hosts of the parasites and the only mammals known to shed *T. gondii* oocysts with their faeces (Hutchinson *et al.* 1969). If the oocysts are ingested by another mammal such as a wild rodent (the intermediate host) small thin-walled cysts form in various tissues, most commonly the brain. Such cysts remain viable for the life of the host (Remington & Krahenbuhl 1982). A cat can therefore become infected by either of two routes: it may directly ingest oocysts shed from another cat in the environment, or it may ingest cysts when eating infected intermediate-host prey (Hutchinson *et al.* 1969).

Previous field and experimental studies demonstrated that wild rats represent a significant and persistent intermediate-host reservoir for *T. gondii*, with a mean prevalence of 35% across all populations irrespective of environmental conditions and maintained, at least in part, through congenital transmission (Webster 1994*a*). It may thus be feasibly expected to benefit the *T. gondii* parasite if it could somehow enhance the transmission rate from this large intermediate-host reservoir to the cat definitive host, and so complete its life cycle. Moreover, since sexual reproduction of *T. gondii* can be accomplished only in the feline, there might be strong selective pressure on the parasite to evolve such a mechanism.

Indeed, there are several reasons to predict that the *T. gondii* parasite may be able to achieve this. Principally, the formation of parasitic cysts in the brain of its host places *T. gondii* in a privileged position to manipulate behaviour (Werner *et al.* 1981). Accordingly, recent studies on both wild and wild–laboratory hybrid rats have demonstrated that *T. gondii* causes an increase in activity (Webster 1994*b*) and a decrease in neophobic (fear of novelty) behaviour (Webster *et al.* 1994; Berdoy *et al.* 1995*b*), both of which can be argued to facilitate transmission to the felid definitive host. In contrast, other costly behavioural patterns such as competition for mates and social status (Berdoy *et al.* 1995*a*), which do not have any obvious impact upon cat predation rate, are left unaltered by the parasite (Berdoy *et al.* 1995*b*).

For any small mammal under heavy predation pressure, the capacity to detect and avoid areas associated with high predation risk is likely to be of strong selective advantage. Rats have evolved an innate and pronounced defensive reaction to predator odours, including cat

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(Vernet-Maury *et al*. 1984; Blanchard *et al*. 1990; Berdoy & Macdonald 1991; Klein *et al*. 1994). Even naive laboratory rats that have not been in contact with cats for several hundred generations still show strong aversive reactions when confronted with cat odours. Such innate anti-predator behaviour and the inherent anxiety that signs of cat presence seem to engender (Blanchard *et al*. 1990) is, from the parasite's point of view, an obvious obstacle militating against its successful transmission to its cat definitive host. Here we investigate whether the parasite is able to interfere with the rat's innate reaction to potential predation risk by cats.

2. MATERIAL AND METHODS

Observations were carried out on adult Lister–hooded laboratory rats, which were outbred four generations previously with male rats trapped from rural UK farms. Laboratory–wild hybrids, rather than pure wild rats, were used so as to ensure known parasitic and social histories of individuals, whilst still obtaining behavioural patterns comparable to those of their wild counterparts. The Lister–hooded laboratory strain was chosen because of its reported behavioural similarity to wild rats (Mitchell 1976). The laboratory rat population was serologically and parasitologically *T. gondii* negative. All rats were also treated with ivermectin anthelmintic (MSD-Agvet Ltd, Hoddesdon, UK) in order to ensure freedom from helminthic or ectoparasitic infections that could bias the data (Ostlind *et al*. 1985).

Experimental rats ($n = 32$) were orally infected with 20 cysts of the low-virulence cyst-forming RRA (Beverley) strain in isotonic saline. This strain had been maintained by continuous passage of infective brain homogenate in outbred AA strain mice bred in house at the University of Strathclyde (precise details are published in Webster 1994*b*). Control rats ($n = 32$) were sham inoculated with isotonic saline. At the end of the study the rats were killed with carbon dioxide. *T. gondii* antibodies were determined by the IgG indirect latex agglutination test (Toxoreagent; Eiken, Tokyo, Japan; Tsubota *et al*. 1977). Titres $\geqslant$ 1:32 were considered positive (Webster 1994*a*,*b*; Webster *et al*. 1994). *T. gondii* brain cysts were determined by microscopic examination of macerated brains in phosphate-buffered saline. Data from any exposed rat found to be serologically or parasitologically *T. gondii* negative at the end of the study were excluded from analysis. Thus the final sample size for analysis consisted of 23 infected rats and 32 uninfected rats.

To test the potential effect of *T. gondii* on the rat's perception of predation risk we observed the nocturnal exploratory behaviour of rats in outdoor pens (2 m × 2 m). The ground was covered with a layer of white sand to provide a homogeneous and neutral surface that could be cleaned between each test. The pens were enriched with a labyrinth of bricks dividing the area into an array of 16 cells. Each corner contained seven drops of one of four distinct odours deposited on and within wooden nest-boxes: the rat's own smell (own straw bedding), neutral smell (fresh straw bedding treated with water), cat odour (fresh bedding treated with undiluted cat urine) and rabbit odour (fresh bedding treated with undiluted rabbit urine). Rabbit odour served as a control for a mammalian non-predator. The position of the four smells (own, water, rabbit and cat) was changed between each test in order to avoid positional biases. Each of the scented areas also contained a water and food bowl covered by a transparent plastic cover.

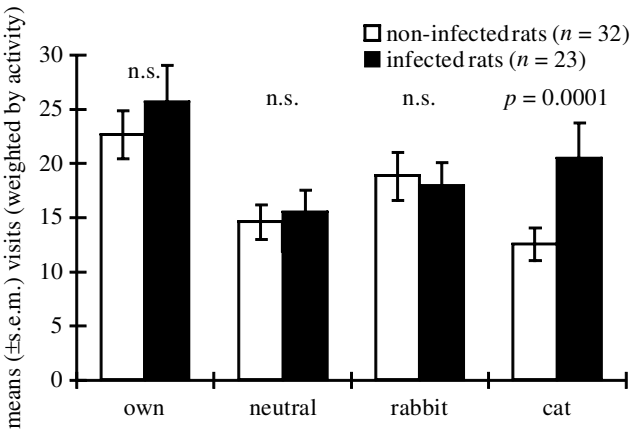

Figure 1. Mean (± s.e.m.) numbers of visits (weighted by overall rat activity) to the four scented areas in the outdoor pens over one night. Uninfected and *T. gondii*-infected rats differ only in their response to areas associated with high predation risk ($F_{1,54} = 22.03$, $p = 0.0001$).

Each rat was tested singly and videotaped from dusk to dawn with a low-intensity camera fixed on a scaffolding 3 m above the test pens. The pens were illuminated from above with two 1 kw halogen lamps to which the rats had completely habituated (Berdoy 1994).

The effect of infection status on visits to the four scented areas was tested using a profile analysis in the General Linear Model procedure in SAS (SAS 1988) to take into account the fact that responses to the four areas are linked. Since the number of cells visited is proportional to rat activity (only rats who emerge from their nest-boxes will show a preference or avoidance to smells) the test of parallelism was carried out on means weighed by overall cell use after checking that there was no difference between infected and uninfected rats ($F_{1,54} = 0.85$, $p = 0.4$). Residuals were tested for normality. The level of aversion or preference to cat areas was tested by comparing (*t*-test) the relative visits to cat versus rabbit areas (cat minus rabbit).

3. RESULTS

The rats' nocturnal behaviour in the outdoor pens (total of 670 rat-hours of observation) revealed a significant divergence between infected and uninfected rats in their overall response to the smells (GLM repeated measures, $F_{3,159} = 9.19$, $p = 0.0001$), which was caused by a differential response to cat odours ($F_{1,54} = 22.03$, $p = 0.0001$; figure 1). Uninfected rats exhibited a healthy aversion of cat-scented areas ($n = 32$, $t = -3.33$, $p = 0.002$). Infected rats, however, were significantly less averse ($n = 23$, $t = 2.36$, $p = 0.002$) and showed no overall avoidance of areas with signs of cat presence ($t = 0.21$, $p = 0.8$). Alterations induced by *T. gondii* infection were confined to the predator's odour, as both types of rats behaved similarly with respect to areas containing their own smell (which was preferred by both), neutral smell and rabbit odour (figure 1).

Since the number of cells visited is proportional to exploratory activity, the impact of *T. gondii* was predictably more visible amongst rats who explored the pen more intensively ($n = 55$, $F_{1,54} = 27.38$, $p = 0.0001$). Thus, amongst the most active animals (top 25%, $n = 14/55$; seven infected and seven uninfected), control rats

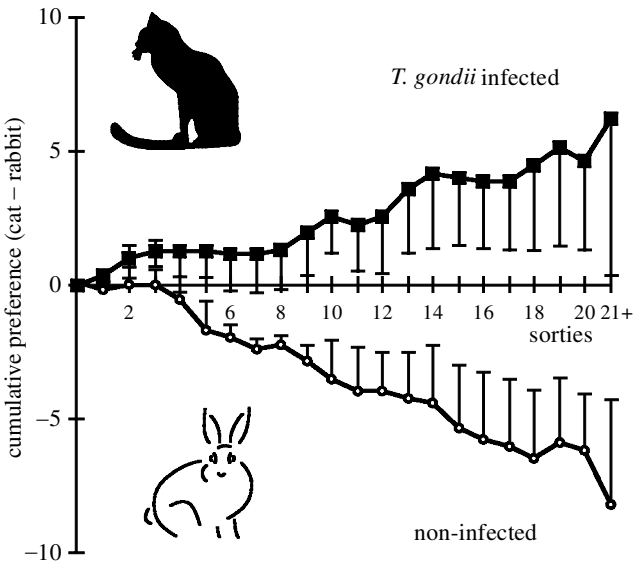

Figure 2. Development of preference or avoidance throughout the night exhibited by the 25% most active rats ($n = 14$, seven infected rats, seven uninfected). Results are shown as the mean cumulative number of cat cells minus the number of rabbit cells visited during each sortie. The data above the x-axis therefore represent a relative preference for the cat areas whilst data below the x-axis indicate avoidance. Vertical bars describe 95% confidence intervals. Time on the x-axis is represented in terms of sorties within the night. Sorties are characterized by bursts of rat activity separated by intervals when the rats shelter into a nest-box for a minimum of 1 min. The rising line for uninfected rats indicates a prolonged, and sensible, avoidance of cat-scented area that is essentially maintained throughout the night. In contrast, *T. gondii*-infected rats tend to exhibit a preference for predator-scented areas. The difference between uninfected and *T. gondii*-infected rats is significant from the third sortie onwards.

continued to exhibit a stable avoidance of cat-scented areas throughout the night, whereas *T. gondii*-infected rats showed a preference for areas with signs of cat presence (figure 2).

4. DISCUSSION

Inherent within the parasite manipulation hypothesis is the premise that behavioural modification represents a sophisticated product of parasite evolution rather than an accidental side-effect of infection (Barnard & Behnke 1990). However, in the few cases where the relationship between physiology and behaviour has been investigated, clinical parasitism is usually evident and has caused the complete loss of a particular behaviour rather than a modification of a specific complex behavioural pattern as illustrated here (e.g. Rau 1983, 1984). Even studies indicating that parasites can affect host learning and spatial performance (e.g. Stretch *et al.* 1960; Kvalsvig 1988; Nokes *et al.* 1992) have been confounded by parasite-induced disruptions of overall host health status (Thompson & Kavaliers 1994). The same does not appear to be true of subclinical *T. gondii* infection. We found that infected individuals show no difference from uninfected individuals in terms of general health status (Webster 1994*b*; Berdoy *et al.* 1995*b*), and behavioural categories unlikely to influence predation rate, even when energetically costly, appear unaltered (Berdoy *et al.* 1995*a*). Moreover, we found here that the alterations induced by *T. gondii* infection were confined to the predator's odour, as both types of rats behaved similarly with respect to areas containing their own smell (which was preferred by both), neutral smell and rabbit odour (figure 1). This suggests that the potentially fatal attraction exhibited by infected rats was not caused by a gross impairment of olfactory faculties. Instead, manipulation by *T. gondii* appears to alter subtly the cognitive perception of the host in the face of predation risk. As with any evidence of host behavioural alterations, further investigations should now ideally incorporate the outcome of real predation rates by the appropriate definitive host as the yardstick of advantage to the parasite (Webster *et al.* 1994, 2000; Poulin 1992; Moore & Gotelli 1990). Nevertheless, whilst direct predation studies are fraught with practical as well as some ethical difficulties, we have shown previously that *T. gondii*-infected rats are indeed more likely to be caught by traps in the wild (Webster *et al.* 1994).

In addition to the implications raised here for the epidemiology of *T. gondii* in the wild in terms of increased transmission rates, the results of this study may also have causal and functional implications.

From a causal view point, our findings may have implications for the study of the neurological basis of behaviour. Indeed, the reaction by potential prey to cat stimuli is used to study the neurological basis of anxiety and the mechanisms of anxiolytic (anxiety relieving) drugs. Such studies have found, for example, that blocking the normally anxiogenic NMDA receptors in the amygdala causes rats to approach cats 'fearlessly' (Adamec *et al.* 1999) in much the same way as our infected rats approached the areas treated with cat urine. One could speculate that such an effect might imply an anxiolytic action of *T. gondii*. Likewise, exposure of laboratory rats to predator odours, but not other noxious odours, induces fast wave activity in the dentate gyrus of the hippocampus (File *et al.* 1993; Hogg & File 1994). Such a response can be blocked by serotonin (5-HT) antagonists (Blanchard *et al.* 1990; Kavaliers & Colwell 1991) or even by the presence in these mice of another protozoan, *Eimeria vermiformins* (Kavaliers & Colwell 1994). Such observations could suggest that some parasitic infections, such as *T. gondii* and *E. vermiformins*, may be able to attenuate the 5-HT-sensitive predator-induced response, thereby reducing the accompanying anxiety-related anticipatory defence reactions of a host to a predator.

Finally, we believe that these results may also provide a functional explanation of the altered brain function in infected humans, where *T. gondii* prevalence has been found to range from 22% in the UK to 84% in France (Desmonts & Couvreur 1974). Although humans represent a dead-end host for the parasite, our results could suggest that the reports of altered personality and IQ levels in *T. gondii*-infected patients (Burkinshaw *et al.* 1953; Flegr & Hrdy 1994) represent the outcome of a parasite evolved to manipulate the behaviour of another mammal. It is noteworthy that rat behaviour is often viewed as the outcome of a conflict between pronounced neophobic reactions and strong exploration tendencies characteristic

of opportunistic omnivores. The uneasy balance between these conflicting motivations, very pronounced in rats but also visible in humans ('the omnivores paradox', Rozin 1976), may thus provide a particularly fertile ground for manipulation by *T. gondii*.

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