

# Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on *Toxoplasma gondii*'s ability to alter host behaviour

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With increasing pressure to understand transmissible agents, renewed recognition of infectious causation of both acute and chronic diseases is occurring. Epidemiological and neuropathological studies indicate that some cases of schizophrenia may be associated with environmental factors, such as exposure to the ubiquitous protozoan *Toxoplasma gondii*. Reasons for this include *T. gondii*'s ability to establish persistent infection within the central nervous system, its ability to manipulate intermediate host behaviour, the occurrence of neurological and psychiatric symptoms in some infected individuals, and an association between infection with increased incidence of schizophrenia. Moreover, several of the medications used to treat schizophrenia and other psychiatric disease have recently been demonstrated *in vitro* to possess anti-parasitic, and in particular anti-*T. gondii*, properties. Our aim here was thus to test the hypothesis that the anti-psychotic and mood stabilizing activity of some medications may be achieved, or at least augmented, through their *in vivo* inhibition of *T. gondii* replication and invasion in infected individuals. In particular we predicted, using the epidemiologically and clinically applicable rat-*T. gondii* model system, and following a previously described and neurologically characterized 'feline attraction' protocol that haloperidol (an anti-psychotic used in the treatment of mental illnesses including schizophrenia) and/or valproic acid (a mood stabilizer used in the treatment of mental illnesses including schizophrenia), would be, at least, as effective in preventing the development of *T. gondii*-associated behavioural and cognitive alterations as the standard anti-*T. gondii* chemotherapeutics pyrimethamine with Dapsone. We demonstrate that, while *T. gondii* appears to alter the rats' perception of predation risk turning their innate aversion into a 'suicidal' feline attraction, anti-psychotic drugs prove as efficient as anti-*T. gondii* drugs in preventing such behavioural alterations. Our results have important implications regarding the aetiology and treatment of such disorders.

**Keywords:** *Toxoplasma gondii*; parasite-altered behaviour; schizophrenia; medication

## 1. INTRODUCTION

Certain parasites selectively alter host behaviour to enhance their transmission (Barnard 1990). The protozoan *Toxoplasma gondii* provides a convincing example of such manipulation. *T. gondii* has an indirect life cycle, in which members of the cat family are definitive hosts (Hutchison *et al.* 1969). If oocysts shed with the faeces of an infected cat are ingested by an intermediate host such as a wild rodent, or another secondary host such as a human or domestic livestock, schizogony occurs and small thin-walled cysts form in various tissues and organs, most commonly the brain, where they remain potentially for the host's lifetime (Remington & Krahenbuhl 1982). The parasite completes its life cycle when a cat consumes an infected intermediate host (Hutchison *et al.* 1969).

In addition to that inherent for all indirectly transmitted parasites, since sexual reproduction of *T. gondii* can be accomplished only in the feline, there are likely to be particularly strong selective pressures on the parasite to evolve mechanisms to enhance the transmission rate from the intermediate to the definitive host. The predilection of the parasite for the brain of its intermediate host places *T. gondii* in a privileged position to cause such a manipulation (Werner *et al.* 1981). Accordingly, studies on rats have demonstrated that *T. gondii* causes an increase in activity (Webster 1994) and a decrease in neophobic (fear of novelty) behaviour (Webster *et al.* 1994), both of which may facilitate transmission to the feline definitive host. Moreover, while rats have innate defensive reaction to predator odours, in particular feline (Blanchard *et al.* 1990), *T. gondii* appears to alter the rats' cognitive perception of cat predation risk, turning their innate aversion into a 'suicidal' feline attraction (Berday *et al.* 2000).

*T. gondii*'s ability to infect all mammals, often at very high prevalence, with for example, 20–80% of humans

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infected (Desmonts & Couvreur 1974), makes the implications of its behaviour-altering activity of significant theoretical and clinical importance. In humans, while the often severe sequelae of congenitally acquired toxoplasmosis are well known, most infections are acquired postnatally (Webster 2001). Latent toxoplasmosis results when the, usually mild, symptoms of the acute stage disappear after a few weeks (Remington & Krahenbuhl 1982). While cases of psychiatric complications such as disorientation, anxiety, depression and even psychoses with schizophreniform characters are well recognized within the immunosuppressed, due to either acute infection or secondary reactivation of the disease (Arendt *et al.* 1999), latent toxoplasmosis has been traditionally viewed as asymptomatic within immunocompetent humans and animals (Roberts & Janovy 2000). This view, however, appears to be largely based on a lack of research into the later stages of the disease and, more specifically, possible associated diseases. Indeed, similar psychiatric complications and meningoencephalitis can also occur within *T. gondii*-infected immunocompetent human hosts (Couverur & Thulliez 1996; Carme *et al.* 2002; Kaushik *et al.* 2005), and recent human studies have revealed that latent toxoplasmosis may even cause personality changes (Flegr *et al.* 1996), decreased IQ (Flegr *et al.* 2003) and psychomotor performance (Havlicek *et al.* 2001). *T. gondii* has also been demonstrated to contribute to the formation of certain types of brain tumours (Ryan *et al.* 1993), and a potential link between *T. gondii* and neuropsychiatric disorders, in particular schizophrenia, has been proposed.

While any potential association between toxoplasmosis and the development of schizophrenia is likely to occur only in a small proportion of infected individuals, neuropathological studies have reported that glial cells, especially astrocytes, are selectively affected in both toxoplasmosis and schizophrenia (Halonen *et al.* 1996; Cotter *et al.* 2001), as are levels of dopamine, serotonin, norepinephrine and other neurotransmitters (Torrey & Yolken 2003). Likewise, epidemiological studies have demonstrated an association between *T. gondii* infection with increased incidence of schizophrenia (Torrey *et al.* 2000; Torrey & Yolken 2003). For example, analyses of serum samples obtained from mothers shortly before or after giving birth revealed a significantly raised proportion of IgM antibodies to *T. gondii* in those whose children subsequently develop schizophrenia in later life (Torrey & Yolken 2003), and individuals suffering from first-episode schizophrenia have significantly elevated levels of IgG, IgM and/or IgA class antibodies to *T. gondii* antibodies, within both serum and cerebral spinal fluid (CSF), compared to uninfected control subjects (Yolken *et al.* 2001). Indeed, meta-analysis here of all reported studies testing for an association between *T. gondii* antibody titres and first incidence schizophrenia status (reviewed in Torrey & Yolken (2003)) revealed that 18 of the 19 studies showed higher (11 significantly higher) *T. gondii* prevalence within schizophrenia patients than controls (table 1). Of particular interest here are studies that have demonstrated that *T. gondii* antibodies of schizophrenia patients treated with anti-psychotic drugs are intermediate between those of patients never treated and those of control groups, with a significant further reduction in those patients undergoing current drug treatment,

suggesting that anti-psychotic treatment may affect *T. gondii* levels (Leweke *et al.* 2004). This is supported by the observation that many anti-psychotic drugs commonly used in the treatment of schizophrenia inhibit the replication of *T. gondii* tachyzoites in cell culture (Jones-Brando *et al.* 2003). One could, therefore, conclude that the anti-psychotic and mood stabilizing activity of some medications may be achieved, or at least augmented, through their inhibition of *T. gondii* replication and invasion in infected individuals.

The aim of the current study was thus to test the hypothesis that anti-psychotic drugs commonly used in the treatment of human affective disorders such as schizophrenia inhibit *T. gondii* replication *in vivo* and thereby help alleviate *T. gondii*-induced cognitive and behavioural alterations. In particular we predicted, using the epidemiologically and clinically applicable rat-*T. gondii* model system, and following a previously described and neurologically characterized 'feline attraction' protocol (File *et al.* 1993; Hogg & File 1994; Adamec *et al.* 1999; Berdoy *et al.* 2000) that haloperidol (HAL, an anti-psychotic used in the treatment of mental illnesses including schizophrenia) and/or valproic acid (VAL, a mood stabilizer used in the treatment of mental illnesses including schizophrenia), would be, at least as, effective in preventing the development of *T. gondii*-associated behavioural and cognitive alterations as the standard anti-*T. gondii* chemotherapeutics pyrimethamine with Dapsone (PD). HAL and VAL were chosen as, in a battery of tests across 12 neuroleptic compounds, both were demonstrated to be most effective in inhibiting *T. gondii* replication *in vitro* (Jones-Brando *et al.* 2003), although no *in vivo* test has yet been performed. Moreover, HAL's mode of action is thought to involve acting as a dopamine D2 antagonist, with dopamine having been proposed as one of the 'missing links' in elucidation of the potential association between schizophrenia and toxoplasmosis (Flegr *et al.* 2003), particularly since both disorders are characterized by raised levels of this neurotransmitter (Stibbs 1985; Torrey *et al.* 2000; Torrey & Yolken 2003). The results obtained should have important clinical and theoretical implications.

## 2. MATERIAL AND METHODS

Observations were carried out using adult Lister-hooded laboratory rats. The rat provides a useful model here as the chronic impact of *T. gondii* within the CNS of rats has been demonstrated to be more applicable to human latent toxoplasmosis, relative to that of the more acute infections in animals such as mice (Hrda *et al.* 2000; Webster 2001). Moreover, while as yet there are no specific neuropsychiatric disorder rodent models available (Anon. 2003), to test the prediction here that some anti-psychotics can alleviate the impact of *T. gondii* on host behaviour, through their direct or indirect impact upon the parasite, it is imperative to use a model system with a well characterized *T. gondii*-altered behavioural repertoire (Crabbe & Morrise 2004).

Experimental rats ( $n=49$ ) were orally exposed with 20 cysts of the low virulence cyst-forming ME-49 strain. This strain had been maintained by continuous passage of infective brain homogenate in mice from the University of Leeds. Since it has been demonstrated that the route of infection (horizontal versus congenital) has no significant effect on the

Table 1. Meta-analysis of *T. gondii* prevalence in persons with severe psychiatric disorder. (Nineteen studies testing for a potential association between *T. gondii* antibodies in persons with schizophrenia and other severe psychotic disorders versus controls have been performed since 1953 (all studies reviewed and cited in Torrey & Yolken (2003)). Eighteen of the 19 studies included complete sample size information to allow meta-analysis, three of which gave results for two types of tests (a 'colour change in fish' study, was excluded here due to any lack of consensus for this as a sensitive or specific anti-*T. gondii* antibody test). The remaining data were divided into four test types. Meta-analysis of all studies examined, and across all diagnostic tests, showed a significant positive association between the presence of anti-*T. gondii* antibodies within patients suffering from schizophrenia or related severe affective disorders. \*The heterogeneity in the Dye results was due to a single Mexican study with OR=14.22 (exact 95% CI: 11.81–17.22). If this study is excluded then the remaining four studies are similar and yield the following results: dye (four studies): OR, 3.00 (LR 95% CI: 2.28–3.95), test for homogeneity,  $p=0.20$ .)

| test                | number of studies | odds ratio                                 | test for homogeneity |
|---------------------|-------------------|--|----------------------|
| enzyme immunoassay  | 6                 | 2.79 (LR 95% CI: 2.07–3.79, $p<0.001$ )    | $p=0.08$             |
| skin                | 5                 | 3.79 (LR 95% CI: 3.34–4.31, $p<0.001$ )    | $p=0.07$             |
| complement fixation | 4                 | 1.81 (LR 95% CI: 1.18–2.74, $p=0.007$ )    | $p=0.27$             |
| dye                 | 5                 | 12.63 (LR 95% CI: 10.98–14.58, $p<0.001$ ) | $p<0.001^*$          |

characteristics of *T. gondii*-altered behaviour in rats (Webster 1994) and adult-acquired infection is more applicable to human latent infection (because most human congenital infection results in death or severe symptoms), adult-acquired infection was used throughout this study. Uninfected control rats ( $n=39$ ) were sham orally exposed with an equal quantity of isotonic saline. Rats were divided into eight groups each containing 8–12 rats (four drug treatment groups, each subdivided into an infected and uninfected control group).

The treatments were either: (i) water control; (ii) pyrimethamine ( $3\text{ mg kg}^{-1}\text{ d}^{-1}$ ) with Dapsone (PD,  $5\text{ mg kg}^{-1}\text{ d}^{-1}$ ), an anti-*T. gondii* treatment which has previously been shown to prevent latent toxoplasmosis both in rodents (Derouin *et al.* 1991; Brun-Pascaud *et al.* 1996) and humans (Girard *et al.* 1993); (iii) haloperidol (HAL,  $1.5\text{ mg kg}^{-1}\text{ d}^{-1}$ ), an anti-psychotic used in the treatment of mental illnesses including schizophrenia (Jones-Brando *et al.* 2003); or (iv) valproic acid (VAL,  $40\text{ mg kg}^{-1}\text{ d}^{-1}$ ) a mood stabilizer used in the treatment of mental illnesses including schizophrenia (Jones-Brando *et al.* 2003). HAL and VAL were dissolved in distilled water, while PD was dissolved in ethanol. Despite the highly variable doses, which are administered to human patients, and because of the half-life differences of these anti-psychotics between humans and rodents, the doses of each drug administered here were those specifically recommended for rodent models (Kapur *et al.* 2003). Daily oral drug (or water placebo) administration commenced 14 days after *T. gondii* exposure, and continued for a further 14 days in all groups. Thus, our aim here was to examine the impact of the drugs primarily on the replicating tachyzoite *T. gondii* stage, prior to the development of (the relatively more drug-insensitive) bradyzoite cysts.

Drugs were administered via the oral route as this most accurately mimics the most common method by which humans receive the equivalent drug treatments, and also minimizes the amount of stress which drug administration causes to the rats, which was deemed especially important in the current investigation. Likewise for ethical reasons, all drugs (or placebos) were provided contained within fruit-flavoured jelly cubes (Flecknell *et al.* 1999), to which all rats had been previously trained (habituated) to eat daily. All rats were housed 2–4 per large ( $0.6\text{ (L)}\times 0.6\text{ (W)}\times 0.3\text{ (H)}\text{ m}$ ) (hence 4–9 times larger than the required Home Office minimum specifications for grouped rats (HMSO 1989), extensively environmentally enriched cage, randomly mixed between experimental groups, with food and water available

*ad libitum*. All rats were monitored closely and none displayed any symptoms of illness or experimentally induced stress at any point during the study. The work was performed under Home office project licenses PPL 30/2032 and PPL 30/1805, and all procedures were classed as 'mild'.

#### (a) Feline attraction, activity and behaviour

To test the potential effect of *T. gondii* and drug treatments on the rat's perception of predation risk, activity levels and behavioural repertoire, we observed the continuous exploratory behaviour of rats in  $1\times 1\text{ m}$  pens (figure 1). Following our previously published protocol (Berdoy *et al.* 2000), the base was covered with a layer of woodchips to provide a homogeneous surface that could be cleaned between each test. Each corner contained 15 drops of one of four distinct odours deposited within plastic nest-boxes: the rat's own smell (own urine-soiled woodchip bedding), neutral smell (fresh woodchips treated with water), cat odour (fresh woodchips treated with undiluted cat urine) and rabbit odour (fresh woodchips treated with undiluted rabbit urine). Rabbit odour was used as a control for a mammalian non-predator. A food bowl and water bowl were placed on either side of each of the nest-box entrances, each of which was treated to three drops of their respective odour. The position of the four smells was changed between each test to avoid positional biases. The positioning and behaviour displayed within each  $50\times 50\text{ cm}$  area was recorded individually by continuous 10 s interval sampling for a period of four hours per rat, judged to be a sufficient test period based on preliminary trials which revealed that the majority of test subjects, irrespective of infection status or drug treatment, remained in a single nest-box after approximately 2.5 h of a trial. Behavioural traits recorded focused on those likely to increase feline predation rate and hence completion of the parasites life cycle, either through time spent 'still' for greater than 3 s while exposed (conspicuousness), or increased activity, either measured as proportion of time spent 'out' versus inside a nest-box, or 'active' versus inactive, when a rat has already been within a nest-box for greater than 5 min with no nose poking out/movement inside the nest-box); or 'grooming in exposed areas' (decreased attention to predator avoidance). Additional behavioural traits recorded included 'water consumption' (drinking from water bowl), 'feed' (eating from food bowl or spilt pellets); rearing; mount (rat on top of nest-box); or HDB (on the next box with its head down the back). The pen was located in a test room, which

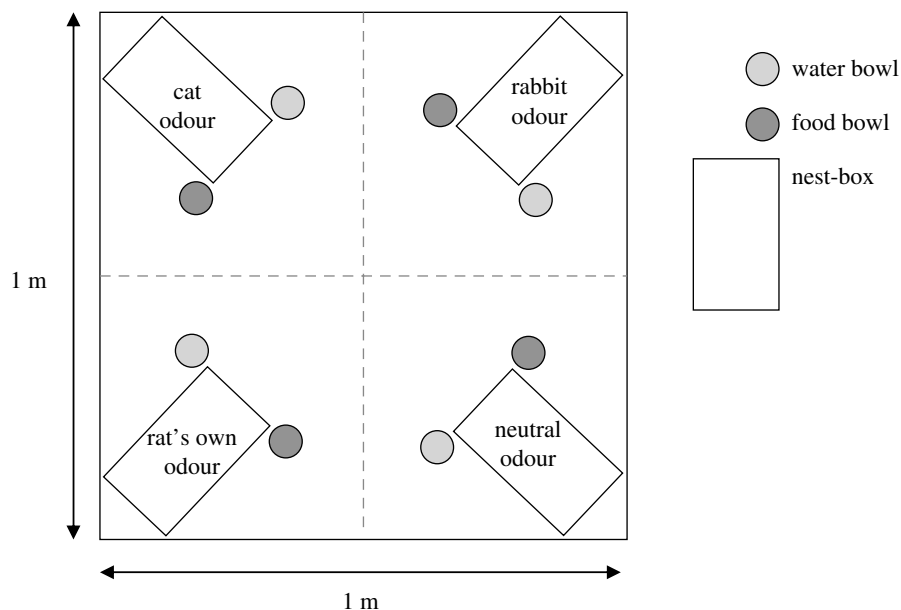


Figure 1. Schematic diagram of 1×1 m test pen.

did not contain any other rats or potential disturbances during each trial, adjoining the area in which the rats were housed to minimize the stress induced by relocation to the test pen. During each test period, the test room remained fully lit (to facilitate visual clarity of the videos), and the test subject was left undisturbed for the duration of the trial. Prior to infection with *T. gondii* or administration of drugs, 4 h videotaped trials of a limited number of randomly selected rats (3–4) from each treatment group were recorded (analysis of this pre-infection group (104 rat-hours of observation) revealed as expected, no significant differences between groups). Six to eight weeks after infection and drug administration, each rat underwent a single 4 h videotaped trial (representing the post-infection trials). Six-eight weeks post *T. gondii* exposure was chosen here as this is when avirulent cyst-forming *T. gondii* strains tend to form bradyzoites and hence represent the latent state of infection (Hutchison *et al.* 1969).

At the end of the investigation, all experimental rats were euthanased by rising concentration of carbon dioxide and cervical dislocation. *T. gondii* antibodies were determined by the IgG indirect latex agglutination test (ILAT: Toxoreagent; Eiken) and IgM Direct agglutination test (BioMerieux, UK). Titres greater than 1 : 16 were considered positive (Webster 1994; Webster *et al.* 1994), and data from any exposed rat found to be serologically negative in both tests ( $n=2$ ) were excluded from the analysis. Thus, the final sample size (and median IgG titre among the *T. gondii* exposed/positive groups) per group was: positive control,  $n=12$  (1 : 32); positive HAL,  $n=11$  (1 : 16); positive PD,  $n=11$  (1 : 32); positive VAL,  $n=12$  (1 : 32); negative control,  $n=8$ ; negative HAL,  $n=11$ ; negative PD,  $n=10$ ; negative VAL,  $n=10$ . Notably, those rats within the positive HAL group which failed to seroconvert on the IgG ILAT, were however IgM seropositive on the Direct agglutination test. (Only *T. gondii* sero-positive rats showed immunohistochemically *T. gondii*-positive neurons and glial cells; data to be published separately.) Those rats unexposed to *T. gondii* remained seronegative.

#### (b) Data analyses

Data collected in 10 s continuous-sampling blocks over the course of the 4 h observation periods (444 h and 260 642

lines of observational data) were analysed using the SAS 8.02 statistical package (SAS Institute, Cary, NC). The data (either in the form of binomial data, for the proportion of 10 s blocks of time (or proportion of entries) of a particular type, or in the form of (logged) durations) were analysed using generalized estimating equations (GEE, Liang & Zeger 1986; Hanley *et al.* 2003) to allow for correlation between observations on the same test subject (rat). We focused on three sets of comparisons: (i) the effect of *T. gondii* infection among untreated rats (comparing infected untreated rats with uninfected untreated rats); (ii) the effect of drugs on infected rats (comparing infected drug-treated rats with infected untreated rats); and (iii) the effect of drugs on uninfected rats (comparing uninfected drug-treated rats with uninfected untreated rats).

### 3. RESULTS

#### (a) Feline attraction

Among untreated rats, those infected spent ‘proportionately’ much more time in the ‘cat’ area than uninfected rats (OR=19.81, 95% CI: 10.49–37.41,  $p<0.001$ ). This difference was a combination of the untreated infected being both more likely to enter the cat area (OR=1.63, 95% CI: 1.37–1.95,  $p<0.001$  comparing cat with non-cat entries; OR=1.71, 95% CI: 1.38–2.12,  $p<0.001$  comparing cat with rabbit entries) and, having entered, stayed 175% longer on average in the cat area than untreated uninfected rats (95% CI: 110–261% longer,  $p<0.001$ , figure 2a).

The effects on the drug treatments differed significantly between infected and uninfected rats ( $p<0.01$  for each measure of feline attraction). Among infected rats, all three drug treatments reduced their likelihood to enter the cat area, although each failed to reach significance (HAL,  $p=0.12$ ; PD  $p=0.11$ ; VAL,  $p=0.74$  comparing cat with non-cat; HAL,  $p=0.22$ ; PD,  $p=0.13$ ; VAL,  $p=0.65$  comparing cat with rabbit). Both HAL and PD significantly reduced the duration of their stays in the cat area ( $p=0.02$  and  $0.01$ , respectively), while a similar non-significant trend ( $p=0.11$ ) was observed for VAL (figure 2b). Among uninfected rats, all three drug

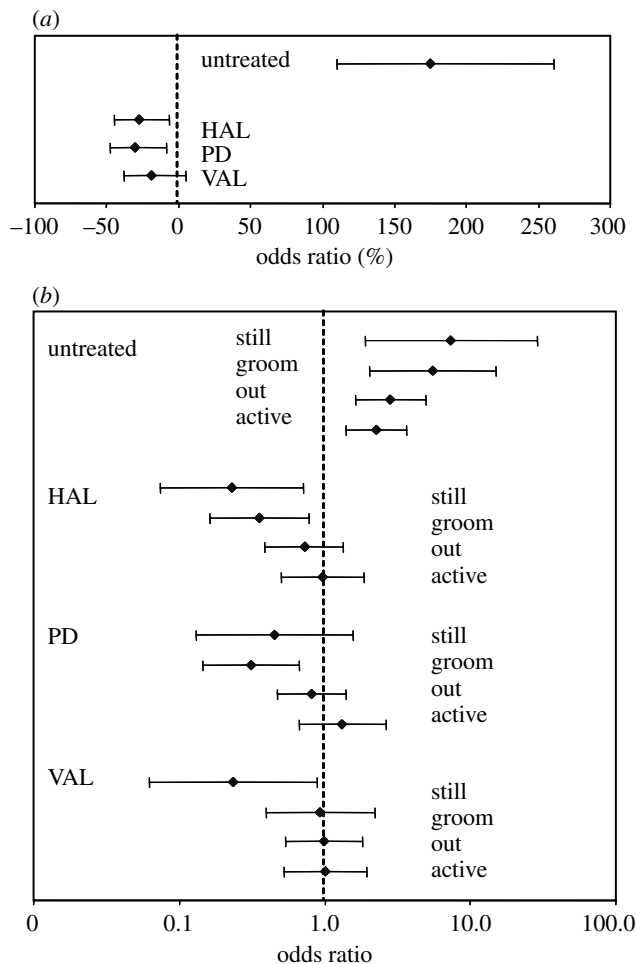


Figure 2. Impact of *T. gondii* and drug treatments on (a) feline attraction (measured here as duration spent in cat-scented areas) and (b) activity and behaviour. The 'untreated' comparison demonstrates the effect of untreated infection (specifically comparing untreated infected rats with untreated uninfected rats). The 'HAL', 'PD', and 'VAL' comparisons demonstrate the effect of drug treatment on infected rats (specifically comparing treated infected rats with untreated infected rats). An apparent suicidal feline attraction and risk behavioural profile was clearly associated with untreated infection. Behavioural traits likely to increase feline predation rate, and hence completion of the parasites life cycle, were all increased relative to their untreated uninfected counterparts. These included: an increased proportion of time spent in areas with evidence of cat presence (entrances and duration of time spent in cat areas); conspicuousness (still for greater than or equal to 3 s, while exposed, or increased activity, either measured as proportion of time spent 'out' versus inside a nest-box, or 'active' versus inactive, when a rat has already been within a nest-box for greater than 5 min with no nose poking out/movement inside the nest-box); and decreased attention on predator avoidance (grooming in exposed areas). Treatment of infected rats with both the anti-*T. gondii* PD and the anti-psychotic HAL reduced feline attraction, and reduced several predation-risk behavioural traits, with a similar non-significant trend for the mood-stabilizer VAL.

treatments significantly increased both their likelihood to enter the cat area and the duration of their stays in the cat area (HAL,  $p=0.004$ ; PD,  $p<0.001$ ; VAL,  $p<0.001$  comparing cat with non-cat; HAL,  $p=0.02$ ; PD,  $p=0.002$ ; VAL,  $p<0.001$  comparing cat with rabbit; HAL,  $p=0.002$ ; PD,  $p=0.001$ ; VAL,  $p<0.001$  for

duration of stays in cat). However, these increases were in every case less than those associated with untreated infection.

No significant effect of infection was observed on proportion of time spent within the non-predatory control 'rabbit' area, indicative of the specificity of the *T. gondii* feline attraction response (untreated infected versus treated infected,  $p=0.20$ ). Likewise, there was no overall significant effect of treatment on the proportion of time spent within the rabbit area (effect of drugs among infected: HAL,  $p=0.39$ ; PD,  $p=0.64$ ; VAL,  $p=0.63$  and among uninfected: HAL,  $p=0.93$ ; PD,  $p=0.20$ ; VAL,  $p=0.64$ ).

#### (b) Activity and behaviour

Analysis of rat activity (active variable) revealed that untreated infected rats were significantly more active than their untreated uninfected counterparts ( $p<0.001$ ; figure 2b, table 2), as has been reported previously (Webster 1994, 2001). The effects on the drug treatments on activity levels differed significantly between infected and uninfected rats ( $p<0.001$ ). While activity levels among infected rats were non-significantly affected by medication (HAL,  $p=0.94$ ; PD,  $p=0.43$ ; VAL,  $p=0.98$ ), all three drug treatments significantly increased activity among uninfected rats (HAL,  $p=0.02$ ; PD,  $p=0.001$ ; VAL,  $p=0.02$ ). Similar effects of infection ( $p<0.001$ ) and drug treatments on uninfected rats (HAL,  $p=0.11$ ; PD,  $p=0.006$ ; VAL,  $p=0.02$ ) were observed on the proportion of time spent outside of the nest-boxes during the trial.

Infected untreated rats were much more likely to be either still ( $p=0.004$ ) or grooming ( $p=0.001$ ), while exposed than their uninfected untreated counterparts, which may have displayed these behaviours within the relatively 'safe'/less exposed confines of a nest-box instead (figure 2b, table 2). Untreated infected rats were also significantly more likely to drink than their untreated uninfected counterparts (OR=2.05,  $p=0.016$ ), which may be here potentially reflective of their increased activity. *T. gondii* infection had little impact on any other behavioural trait measured (Rear,  $p=0.10$ ; HDB,  $p=0.50$ ; mount,  $p=0.30$ ; feed,  $p=0.56$ ).

Treatment with HAL ( $p=0.01$ ) and VAL ( $p=0.03$ ) significantly reduced the time infected rats spent exposed and still, and similarly treatment with HAL ( $p=0.009$ ) and PD ( $p=0.003$ ) reduced the time infected rats spent grooming (figure 2b). PD, however, significantly increased food consumption among infected rats ( $p=0.010$ ), while VAL increased their time spent mounting onto ( $p=0.02$ ), and with their heads 'sniffing' down the back of nest-boxes ( $p=0.01$ ). Among uninfected rats, all three drug treatments significantly increased drinking (HAL: OR=2.54,  $p=0.01$ ; PD: OR=3.32,  $p=0.004$ ; VAL: OR=2.8,  $p=0.002$ ), and these increases were in each case greater than that associated with untreated infection. VAL, as for in the infected treated rats, was again associated with significant increased next box mounting ( $p=0.01$ ), and both PD ( $p=0.002$ ) and VAL ( $p=0.001$ ) was associated with an increase in rearing behaviour.

#### 4. DISCUSSION

An apparent suicidal feline attraction and risk behavioural profile among untreated infected individuals was clearly

Table 2. Impact of *T. gondii* and drug treatments on activity and behaviour: percentage (and SEM) time spent performing each activity, by infected untreated rats, uninfected untreated rats, and infected rats treated with HAL, PD and VAL. (Behavioural traits as described for figure 2.)

|                 | <i>T. gondii</i> infected |             |             |             | uninfected  |             |             |             |
|-----------------|---------------------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                 | untreat.                  | HAL         | PD          | VAL         | untreat.    | HAL         | PD          | VAL         |
| <i>n</i> (rats) | 12                        | 11          | 11          | 12          | 8           | 11          | 10          | 10          |
| still/exposed   | 2.9% (1.4)                | 0.7% (0.2)  | 1.4% (0.6)  | 0.7% (0.4)  | 0.4% (0.2)  | 0.3% (0.2)  | 0.3% (0.1)  | 0.2% (0.1)  |
| groom           | 6.3% (2.0)                | 2.3% (0.5)  | 2.0% (0.4)  | 5.9% (1.7)  | 1.2% (0.5)  | 3.6% (2.7)  | 1.6% (0.5)  | 2.7% (1.9)  |
| active          | 59.3% (5.4)               | 58.7% (6.5) | 65.8% (6.6) | 59.5% (6.5) | 39.1% (3.2) | 50.7% (4.0) | 62.0% (6.1) | 58.5% (7.7) |
| 'out'           | 36.0% (5.1)               | 29.1% (5.1) | 31.5% (4.1) | 35.8% (5.6) | 16.6% (2.8) | 23.5% (3.7) | 28.5% (3.6) | 30.4% (6.1) |

evident here. Under natural conditions, these are all behavioural traits likely to increase predation rate by the definitive host and hence completion of the parasites life cycle (Webster 2001), either through increased risk behaviours (presence in areas with evidence of cat presence), conspicuousness (increased activity or still and exposed), or through decreased attention on predator avoidance (grooming in exposed areas) relative to their untreated uninfected counterparts (figure 2, table 2). Treatment of infected rats with, in order of decreasing efficacy: HAL, PD and VAL, reduced these predator-risk behavioural traits. Therefore, our results lend support to the hypothesis that the anti-psychotic and mood stabilizing activity of some medications used in the treatment of schizophrenia and human affective disorder may be augmented through their inhibitory impact upon *T. gondii* in infected individuals

In terms of potential mechanistic explanations, the treatments, at least PD and HAL, may function by directly minimizing *T. gondii* replication and invasion of host brain cells, as has been demonstrated *in vitro* (Jones-Brando *et al.* 2003). Indeed, our unpublished observations on *T. gondii* immunohistochemical staining of tissue sections throughout the brains of these experimental rats indicate that the frequencies of *T. gondii* exposed animals showing immunohistochemically positive neurons and glial cells was reduced following drug treatment, with a superiority of HAL over PD and VAL (S. Weis & I. C. Llenos 2005, personal communication). Such anti-*T. gondii* activity may be related, at least in part, to the calcium inhibitory properties of these drugs. *T. gondii* tachyzoites require calcium in order to invade host cells, and this invasion is inhibited by calcium channel blockers and calmodulin antagonists, including trifluoperazine, another phenothiazine anti-psychotic (Pezzella *et al.* 1997). Accordingly, HAL and VAL, are each capable of inhibiting calcium transport through cellular ion channels (Itoa *et al.* 1996; Johannessen 2000).

Another, not mutually exclusive, explanation for the effects of *T. gondii* and drug treatment on feline avoidance behaviour relates to their potentially neuromodulatory impact, either directly or indirectly (Blanchard *et al.* 1990; Berdoy *et al.* 2000; Torrey & Yolken 2003). 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, and such studies have found, for example that blocking the normally anxiogenic NMDA receptors in the amygdala also causes laboratory rats to 'fearlessly' approach areas treated with cat urine (Adamec *et al.* 1999). Likewise, while exposure of rats to

predator 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 antagonists (Blanchard *et al.* 1990), or alternatively by the presence in mice of another protozoan, *Eimeria vermiciformis* (Kavaliers & Colwell 1994), which could suggest a similar neuromodulatory action of certain protozoan parasites including *T. gondii*. Indeed, *T. gondii* infection is known to be associated with raised dopamine levels in particular (Stibbs 1985; Flegr *et al.* 2003). Dopamine levels are also often raised within patients with schizophrenia (Torrey & Yolken 2003). Thus, as HAL is a dopamine D2 antagonist (Seeman 1980), one could propose that its superior therapeutic impact here may be through a combination of both its ability to inhibit *T. gondii* replication and to reduce, directly and indirectly, dopamine levels. This may contrast to the restricted anti-parasitic, rather than neuromodulatory, action of PD. Likewise, while the mechanisms by which VAL may exert mood-stabilizing effects are not yet fully elucidated, recent studies have found little evidence that these are mediated by its effects on serotonin or dopamine receptors (Delva *et al.* 2002). Thus, one may postulate that the relatively lowered success of VAL in decreasing the parasite-induced behavioural alterations observed here may also be restricted to those achieved through its direct inhibition on parasite replication alone, albeit less efficaciously than PD.

Our results raise several important theoretical and applied implications, from, for example providing further support for the theory of *T. gondii* as a causative agent in some cases of schizophrenia, to predicting that such drugs may be expected to be particularly effective in individuals with schizophrenia who are also infected with *T. gondii*. It may thus be worth investigating the *T. gondii* inhibitory effects of other anti-psychotics, especially the second-generation agents (olanzapine, clozapine, quetiapine, ziprasidone, risperidone and aripiprazole), as well as the effectiveness of alternative anti-*T. gondii* treatments, such as pyrimethamine with clindamycin, co-trimoxazole, or ponazuril, as adjunct therapies for schizophrenia. These potential treatments could also prove particularly valuable as prophylactic treatments for groups at serious risk of developing psychiatric disorders in later life as a result of *T. gondii* infection. Moreover, the results here, particularly with HAL, suggest that further research into anti-psychotics may provide potential alternatives for anti-*T. gondii* drug treatment, where new anti-bradyzoite treatments, in particular, are imperative.

However, the behavioural changes observed among the uninfected, but treated, rats indicate that serious consideration must be given to possible side-effects of such treatments on human behaviour: all three drug treatments caused uninfected rats to behave, albeit to a much reduced extent, in a similar way to infected untreated rats, in terms of feline attraction and activity levels, with further mild behavioural alterations, such as increased water and food consumption. Many of these behavioural alternations may also relate to the neuromodulatory action of such chemotherapeutics, as discussed above. Indeed, any anxiolytic drug effects may well be predicted to result in treated, but uninfected, rats being less averse to the cat smell, and also potentially more active, than their untreated uninfected counterparts, as was observed here (Blanchard *et al.* 1990). The potential generalizability of such side-effects is certainly worthy of further inter-host specific research. In terms of the current study, the mild drug-induced feline attraction observed here among uninfected rats may, moreover, detract from the drug-induced reductions in the infection-related increases in entrances and duration spent within cat areas.

Our results, as is always the case in research, raise as many questions as they do answers. What will be important to elucidate now is, for example, why any potential effect of *T. gondii* on host behaviour, in particular in terms of the clinical outcome of human behaviour, may differ between individuals. Potential key factors may relate to inherent differences in individual genetic predisposition, the state of the immune system, the time of exposure (e.g. infections in the first trimester of pregnancy may differ from those in the third trimester; and/or prenatal infection may differ from postnatal), the duration of exposure (e.g. humans live longer than the average rodent intermediate host), and/or the part(s) of the brain affected. From the perspective of the parasite, key factors may relate to the dose, the source of infection (i.e. oocyst or cyst stage consumption), the genotype of the infecting strain, and/or even an interaction with other infectious agents.

In summary, our results to date do demonstrate that the behavioural changes associated with *T. gondii* can be effectively reduced by those anti-psychotic drug treatments previously demonstrated to inhibit parasite replication *in vitro*. This may provide further evidence for a potential role of *T. gondii* in the aetiology of schizophrenia, and specifically here that the actions of anti-psychotics may work in part via parasite inhibition. Clinical trials based on these findings are warranted, including those perhaps of anti-psychotic drugs with patients separated into those with and without additional *T. gondii* infection. Such trials could lead to improved prognosis and potentially new medication combinations and therapeutic modalities for the treatment of both toxoplasmosis and severe psychiatric disorders.

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