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REVIEW

Toxoplasma gondii infection, from predation to schizophrenia: can animal behaviour help us understand human behaviour?

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Summary

We examine the role of the protozoan *Toxoplasma gondii* as a manipulatory parasite and question what role study of infections in its natural intermediate rodent hosts and other secondary hosts, including humans, may elucidate in terms of the epidemiology, evolution and clinical applications of infection. In particular, we focus on the potential association between *T. gondii* and schizophrenia. We introduce the novel term '*T. gondii*–rat manipulation–schizophrenia model' and propose how future behavioural research on this model should be performed from a biological, clinical and ethically appropriate perspective.

Key words: *Toxoplasma gondii*, rat, mouse, behaviour, manipulation, by-product, pathology, schizophrenia, intermediate host, secondary host, 3Rs.

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Introduction

The ability of parasites to alter host cognition and behaviour captivates the interest of both the scientific and lay communities, partly because it raises novel questions about longstanding philosophical issues such as the existence of free will. 'If the mind is a machine, then anything can control it – anything, that is, that understands the code and has access to the machinery' (Adamo, 2013). Certain parasites, including a notable minority of vertebrates, appear to possess the mechanisms required to manipulate the central nervous system (CNS) of their hosts. Whereas some of the behavioural changes associated with infection are quite general, such as depressed feeding or general malaise/'sickness', others appear remarkable in their specificity, subtly altering only a limited repertoire of host behavioural traits for apparent selective benefit to the parasite.

What happens, however, when a 'manipulatory' parasite has a broad host range, able to infect species both part of and independent to its own life cycle, relevant or not to its own transmission potential? Altering host behaviour in 'inappropriate' hosts is plausibly an unavoidable consequence of parasite-altered behaviour. This raises questions as to whether one would predict similar behavioural alterations to be mirrored across contrasting host organisms, such as that between a rat and a human, despite often markedly divergent life histories, behavioural repertoires, infection exposure histories and natural longevities. If we are to fully elucidate the range of behavioural alterations evoked and the mechanisms employed, between and within species and life stages, how should, therefore, future studies test for these in the most biologically and ethically appropriate manner?

Here, we examine the role of the protozoan *Toxoplasma gondii* as a manipulatory parasite and question what role study of infections in its natural intermediate rodent hosts and other secondary hosts, including humans, may elucidate in terms of the epidemiology, evolution and clinical applications of infection. In particular, we question whether studying the impact of infection on rodent behaviour may help us predict what behavioural changes may be observed amongst infected humans, from subtle to severe. We focus on the potential association between *T. gondii* and schizophrenia, and how future animal behaviour studies should be performed from a biological, clinical and ethically appropriate perspective.

We define and categorize behavioural alterations in terms of whether the behavioural changes observed in an infected host are indicative of: (1) active manipulation for the selective benefit of the parasite; (2) active manipulation for the selective benefit of the host to ameliorate the impact of infection; (3) a generalized pathological response in the host of no obvious selective benefit to the parasite or host; or, finally, a subtle distinction of the latter grouping we refer to here as (4) 'by-product pathology' as a consequence of accidental infection with a parasite selected to manipulate behaviour in an alternative host species and/or life-cycle stage. Furthermore, as this perspective article assesses the applicability of studying *T. gondii* in rat (and/or mice) intermediate hosts as a model for helping us understand both the evolution of and mechanisms underpinning parasite-altered behaviour (ranging from rodent predation to some cases of human schizophrenia), we introduce, for the first time here, the novel term of the '*T. gondii*–rat manipulation–schizophrenia model' (see Appendix 1).

Toxoplasma gondii

Until relatively recently, latent adult-acquired toxoplasmosis in immunocompetent humans and animals was generally considered to be asymptomatic. In contrast, the causative agent, *Toxoplasma gondii*, represents perhaps one of the most convincing examples of a manipulative parasite of vertebrates. *T. gondii* is a highly successful apicomplexan protozoan capable of infecting all warm-blooded animals worldwide, often at extremely high prevalence levels. Members of the cat family (Felidae) are the only definitive hosts, within which the parasite undergoes full gametogenesis and mating within the intestinal epithelium, culminating in the

generation of oocysts containing sporozoites that are shed in the cat's faeces (Hutchison et al., 1969). Infection of intermediate (such as rodents and birds) or other secondary (such as humans and domestic livestock) hosts can occur following ingestion of oocysts (*via* contaminated soil, water or food) or tissue cysts (through raw/undercooked infected meat, including *via* cannibalism), congenital transmission and also potentially, under certain conditions, by sexual transmission (Vyas, 2013). Within intermediate secondary hosts, the parasite undergoes asexual reproduction, characterized by rapidly dividing tachyzoites and the more slowly dividing bradyzoites. Bradyzoites encyst in the brain, heart and other tissues, where they remain potentially for the host's lifetime. Transmission to the feline definitive host occurs when an immunologically naive cat ingests an infected intermediate host through predation (and/or consumes contaminated meat). Since sexual reproduction of *T. gondii* can be accomplished only in felines, there are likely to be strong selective pressures on the parasite to evolve mechanisms to enhance transmission from the intermediate host to the definitive feline host.

The predilection of *T. gondii* for the CNS places it in a privileged position to manipulate host behaviour. Potential mechanisms of action have recently been described elsewhere (Kaushik et al., 2012; McConkey et al., 2013; Vyas and Sapolsky, 2010; Webster and McConkey, 2010) and thus need not be repeated in detail here. However, as similar mechanisms are likely to be involved in both rodent hosts as in latently infected human hosts, irrespective of the manipulatory or pathological outcomes (Appendix 1), and as this raises important implications for the direction of future research in relation to the *T. gondii*–rat manipulation–schizophrenia model (Appendix 1), we will briefly focus here on the proposed mechanistic role of dopamine on the expression of altered host behaviour.

Raised or disrupted dopamine levels have been reported in both rodent and human *T. gondii* infection and within human patients with schizophrenia (Howes and Kapur, 2009; Prandovszky et al., 2011; Stibbs, 1985; Torrey and Yolken, 2003), together with other affective disorders such as obsessive compulsive disorder (OCD) and bipolar disorder and amongst those with suicide attempts (Berk et al., 2007; Denys et al., 2004; Diehl and Gershon, 1992; Roy et al., 1992). Furthermore, recent research indicates that the parasite itself may actually be a source of this dopamine (Gaskell et al., 2009; Prandovszky et al., 2011). Dopamine is synthesised in two steps from its precursor amino acid tyrosine: (1) tyrosine hydroxylase metabolism to produce L-DOPA; then (2) decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase to dopamine. In some cells, dopamine is further metabolized to norepinephrine by dopamine β-hydroxylase. *T. gondii* was recently found to encode a protein with high homology and showing similar catalytic properties to the tyrosine hydroxylases found in mammals. This *T. gondii* ortholog synthesises L-DOPA, precursor to dopamine, as well as tyrosine, and has been demonstrated to result in increased dopamine levels associated with *T. gondii* cysts in the rodent brain (Prandovszky et al., 2011). These mechanistic studies thereby provide both a potential explanation for previous, and a driving force for further, empirical research into behavioural manipulation in intermediate hosts as well as a plausible explanation for by-product pathological changes in behaviour in all infected secondary hosts.

Current evidence for *T. gondii* manipulation of intermediate host behaviour

Though behavioural studies of wild animals with naturally occurring infections remain rare, wild brown rats (*Rattus norvegicus*) on farmlands exhibit higher activity levels and, at least under certain situations, an increased propensity to be trapped in cages, amongst *T. gondii*-infected individuals relative to their uninfected counterparts (Webster, 1994; Webster et al., 1994). Laboratory and/or experimentally controlled naturalistic studies on rats and mice have also demonstrated that *T. gondii* infection is associated with a range of subtle behavioural alterations, many of which would facilitate parasite transmission from the infected intermediate host to the feline definitive host [detailed reviews of which may be found elsewhere (Webster, 2001; Webster, 2007)]. For example, *T. gondii*-infected rodents exhibit an increase in activity and a decrease in predator vigilance behavioural traits (Berdoy et al., 1995; Hay et al., 1983; Hay et al., 1984; Hutchison et al., 1980a; Hutchison et al., 1980b; Lamberton et al., 2008; Webster, 1994; Webster, 2001; Webster, 2007; Webster et al., 1994; Webster et al., 2006). Moreover, whilst uninfected rats show a strong innate aversion to predator odour, *T. gondii* infection appears to subtly alter the rats' cognitive perception of cat predation risk, turning their innate aversion into a 'suicidal' 'fatal feline attraction' (Berdoy et al., 2000; Vyas et al., 2007c; Webster et al., 2006). Such fatal feline attraction appears specific towards a response to cat (urine) odour, with no difference observed between infected and uninfected rats in their responses to odours of non-predatory mammals such as rabbit (Berdoy et al., 2000; Vyas et al., 2007c; Webster et al., 2006) nor contrasting potential predatory species odours such as mink (Lamberton et al., 2008) or dog (Kannan et al., 2010). Furthermore, other key health and behavioural traits, such as social status and mating success, remain intact and indistinguishable between infected and uninfected rats (Berdoy et al., 1995) (but see Vyas, 2013). Overall, such rodent studies are consistent with the hypothesis that *T. gondii* specifically manipulates the behaviour of its rodent (rat at least) intermediate host rather than simply causing a broad pathology or destruction of particular behavioural traits (Appendix 1).

Current evidence for *T. gondii* 'general pathology' and 'by-product' alterations of secondary host behaviour

Although empirical studies beyond those focused upon rodents are rare, *T. gondii* appears to cause a range of behavioural alterations across host species. Within the feline definitive host itself, some potential 'general pathology' (Appendix 1) neurological signs have been reported, such as circling, head bobbing, atypical crying and increased affectionate behaviour, even though CNS toxoplasmosis in felines is uncommon (Bowman, 2002; Dubey and Carpenter, 1993). California sea otters with moderate to severe toxoplasmic encephalitis have been observed to be 3.7 times more likely to be attacked by sharks than their uninfected counterparts (Miller et al., 2004), suggesting that they may exhibit aberrant behaviour similar to that displayed in infected intermediate host rodents, although as a 'by-product' (Appendix 1) of infection of no current adaptive advantage to the parasite in this host–predator combination. Physical and behavioural changes in *T. gondii*-infected human secondary hosts may also be viewed as either 'general pathology' or 'by-product' (Appendix 1) and/or a potential spectrum between the two, depending on the particular characteristics, severity and trait altered. For instance, one may suspect that *T. gondii*-induced meningoencephalitis [including within immunocompetent adult hosts (Kaushik et al., 2005)] and brain cancer (Thomas et al., 2012) may be indicative of 'general pathology', perhaps simply as a consequence of the extended duration of infection within the CNS of the relatively longer-lived human host as compared with the short lifespans of rodent or bird intermediate hosts. Others,

however, appear more indicative of 'by-product' pathology, where we see similar physiological or behavioural alterations in human secondary hosts to that observed in rodent intermediate hosts, even if humans are not (currently) under selective pressure to influence transmission potential. Indeed, human studies have revealed a range of subtle behavioural alterations associated with *T. gondii* infection, many of which may be comparable to those observed amongst infected rodent intermediate hosts – such as increased activity, decreased reaction times and altered personality profiles (Flegr, 2007; Flegr, 2013; Flegr et al., 2002; Flegr and Hrdy, 1994; Flegr et al., 2003; Webster, 2001). Even an equivalent of the fatal feline attraction phenomenon observed in infected rats (Berdoy et al., 2000) has been identified in humans, where *T. gondii*-positive humans showed altered questionnaire responses to the odours of the domestic cat (and of the brown hyena) (Flegr et al., 2011). Such subtle changes in human behaviour and personality in response to latent *T. gondii* infection at the individual level have even been proposed to alter the human aggregate personality at the population level (Lafferty, 2006).

Latent *T. gondii* infection in the human host may, furthermore, in a small number of cases, have substantial health implications. Consistent with a possible impairment in psychomotor performance and/or enhanced risk-taking personality profiles, individuals with latent toxoplasmosis have been reported to be at a 2.65 times increased risk to be involved in a traffic accident relative to the general population (Flegr et al., 2002), a result subsequently replicated by other groups (Flegr et al., 2009; Kocazeybeka et al., 2009; Yereli et al., 2006). Another recent study, albeit significant only in a subset with lower socioeconomic status, linked *T. gondii* seropositivity with workplace accidents (Alvarado-Esquivel et al., 2012). There is also the ever growing and convincing body of evidence concerning a potential relationship linking *T. gondii* with that of some forms of affective and neurological disorders in humans. Correlations have been found for OCD (Miman et al., 2010b), Parkinson's disease (Miman et al., 2010a), Alzheimer's disease (Kusbeci et al., 2011), suicide (Arling et al., 2009) and bipolar disorder (Pearce et al., 2012). The most substantial body of empirical evidence gathered to date relates to the potential association between *T. gondii* and some cases of schizophrenia in humans. *T. gondii* seroprevalence has been associated with schizophrenia in at least 38 studies to date (Mortensen et al., 2007; Torrey et al., 2007; Torrey et al., 2012; Torrey et al., 2000; Torrey and Yolken, 2003; Yolken and Torrey, 2008). Recent meta-analyses assessing potential associations between different infectious agents and schizophrenia found a highly significant association with *T. gondii* (OR=2.70; CI 95%: 1.34–4.42; P=0.005) (Arias et al., 2012) and a stronger association between schizophrenia and detection of *T. gondii* antibodies (combined odds ratio 2.73) than for any human gene in a genome-wide linkage analysis study (OR≤1.40) (Purcell et al., 2009). There are similarities in the epidemiology of schizophrenia (Cichon et al., 2009) and toxoplasmosis (Johnson et al., 2002), where, for instance, both have been demonstrated to have strong familial associations, affecting multiple members of the same family. Further support for an association includes analyses of serum samples obtained from mothers shortly before or after giving birth that revealed a significantly raised proportion of immunoglobulin M (IgM) antibodies to *T. gondii* in those whose children developed schizophrenia in later life (Torrey and Yolken, 2003), and individuals suffering from first-episode schizophrenia have significantly elevated levels of IgG, IgM and/or IgA class antibodies to *T. gondii*, within both serum and cerebral spinal fluid (CSF), compared with uninfected control subjects (Yolken et al., 2001). Likewise, in a study of military personnel from whom serum specimens were available from periods of up to 11 years prior to the onset of their schizophrenia (180 individuals with schizophrenia and 532 matched controls), significantly increased levels of IgG antibodies to *T. gondii* were observed prior to the onset of illness (hazard ratio=1.24, P<0.01), with a peak in the six months prior to onset but seen as early as three years prior to the onset (Niebuhr et al., 2008). Studies have even demonstrated that *T. gondii* antibodies in patients with schizophrenia treated with antipsychotic drugs are intermediate between those of patients never treated and those of control groups, with a significant reduction in those patients undergoing current drug treatment, thereby suggestive that antipsychotic treatment may affect *T. gondii* infection levels (Leweke et al., 2004). Indeed, antipsychotic drugs used in the treatment of schizophrenia have been observed to inhibit the replication of *T. gondii* tachyzoites in cell culture (Goodwin et al., 2011; Jones-Brando et al., 2003). Likewise, *T. gondii*-infected/exposed rats treated with the same key antipsychotic or mood stabiliser drugs during the tachyzoite replicative stage of infection, in particular that of the dopamine D2 antagonist haloperidol (Webster et al., 2006) or the dopamine selective uptake inhibitor GBR 12909 {1-[2-[bis(4-fluorofenyl)metoxy]-etyl]-4-[3-fenylpropyl]piperazin} (Skallova et al., 2006), did not develop the potentially suicidal feline attraction or other predation-specific altered behavioural profiles displayed by their untreated but infected counterparts, nor was there the same level of parasite establishment within the brains of these drug-treated infected rats relative to their untreated infected counterparts (Webster et al., 2006). Such results therefore raise the hypothesis that the antipsychotic and mood-stabilizing activity of some medications may at least be augmented through their inhibition of *T. gondii* replication, invasion and/or subsequent modulatory impact in infected individuals.

What animal models should future research incorporate to elucidate the impact of *T. gondii* on host behaviour?

T. gondii can be successfully maintained *in vitro* and hence many studies on the parasite, such as those examining gene expression, can be performed without the need for animal infections. However, to fully elucidate the impact upon behaviour, and the mechanisms involved, future animal (and human) studies, across a range of different host species and individuals, will remain essential. Host factors undoubtedly contribute to differences in behavioural and clinical outcomes of infection, even when the parasite genotypes remain constant. For example, inherent differences in genetic predisposition at the genera, species and individual level, the state of the immune system, the time of *T. gondii* exposure (e.g. prenatal pregnancy trimester, neonate, juvenile or adult), the duration of infection (e.g. humans live longer than the average rodent intermediate host) and past behavioural and parasitic histories are all likely to be important. Host gender is also extremely important in determining the behavioural outcome of *T. gondii* infection in both humans and rodents (Flegr, 2007; Webster, 2007). For instance, recent studies have revealed sex-specific changes in gene expression and behaviour induced by chronic *T. gondii* infection in mice (Xiao et al., 2012). Similarly, *T. gondii* has been reported to increase testosterone levels in men but decrease levels in women (Flegr et al., 2008).

In terms of which host species to examine to further understand the evolution and epidemiology of this parasite, it seems intuitive that studies should ideally incorporate its major intermediate host

species, especially those maintained under natural or semi-naturalistic conditions. Likewise, correlations between apparent altered behaviour and *T. gondii* infection amongst other wildlife secondary hosts with 'naturally' occurring infections, such as seen for the sea otters (Miller et al., 2004), are also extremely useful. Such studies, however, remain rare, and further investigations are warranted. For instance, minimally invasive studies on wild bird populations could be fascinating, particularly as birds, common in the diet of predatory felines, are plausible major intermediate hosts and hence likely to remain under strong selective pressures. Furthermore, as birds detect and subsequently avoid predatory cats primarily *via* their visual system (Cuthill et al., 2000), in contrast to olfactory perception by rodent intermediate hosts (Dielenberg et al., 2001), this may be pertinent to further elucidating mechanism(s) of action, particularly since *T. gondii* is known to frequently infect and interfere with its host's visual system. Moreover, the avian archistriatum is believed to be homologous to parts of the mammalian amygdala, with a role in assigning an emotional value to new stimuli by means of multimodal association of temporally coincidental stimuli and to elaborate complex responses (Medina and Reiner, 2000; Puelles et al., 2000). However, whilst there are reports on the prevalence of *T. gondii* infection amongst wild bird populations [e.g. wild fowl and pigeons (Tenter et al., 2000)], there are, unfortunately, to the authors knowledge, no *T. gondii*–bird behavioural studies published. (One study did attempt to associate *T. gondii* infection status with potential predation risk in 115 wild nesting mountain bluebirds (*Sialia currucoides*) – however, the majority of birds sampled were juveniles and all but two individuals had *T. gondii* titres of ≥1:32, and thus no significant association could be detected; J.P.W. and A. Moehrenschlager, unpublished observations.)

Rats and/or mice are the obvious continued model system for *T. gondii* research. In addition to being key intermediate host species, we know a great deal about the behaviour and physiology of these creatures. The rodent CNS and immune system, sharing many structural and neurochemical similarities, are often used as an experimental model for humans, they are generally straightforward to maintain, and specific genetic models are being developed. Nevertheless, rather than simply replicate earlier research, future studies should consider carefully what precise animal models should be used and why, particularly within the ethical remit of today's Replacement, Refinement and Reduction of animals in research (3Rs) environment. Furthermore, it must be emphasized that rats and mice are not the same in terms of their susceptibility, behaviour, morbidity and overall response to *T. gondii*. For instance, whilst some *T. gondii*-altered behavioural traits, such as increased activity levels (Hay et al., 1984; Webster, 1994; Webster, 2001), appear to be similar in rats and mice, other behavioural traits appear to show contrasting results. One example is the observed decreased ability of *T. gondii*-infected hosts to recognize and/or respond to novel stimuli; this can result in either a decrease of neophobia in neophobic hosts such as rats (Webster et al., 1994) or an increase of neophobia in neophilic hosts such as mice or indeed humans (Hodková et al., 2007). Likewise, early studies found that whilst learning capacity was reduced in some laboratory rats, this was much milder and rarer than that observed for laboratory mice (Piekarski et al., 1978; Witting, 1979). Potential explanations for these differences related to the higher infection rate of *T. gondii* in the brains of mice than rats during latent toxoplasmosis and the former's increased potential for severe morbidity during the acute phase of infection. Indeed, whilst the general health and behaviour of laboratory rats usually appears unaffected by infection, laboratory mice often show high parasite-induced mortality, even with Type II *T. gondii* strains, and/or signs of acute infection such as running in circles with their heads bent to one side (Piekarski et al., 1978; Witting, 1979). (Indeed, it may be noted that, in contrast, in all the rat studies performed by the current authors over the years, not a single rat has demonstrated any such overt pathology or mortality following *T. gondii* exposure; J.P.W., unpublished observations.) It is thus strongly proposed that experiments with more resistant animals, such as rats, provide a far superior model in which to study the behaviour changes induced by *T. gondii*, from both an ethical and biological perspective in terms of their generalizability to humans, relative to the mouse model (Hrda et al., 2000; Webster, 2007).

Even within rat species, the strain or line of rat to be used may have important implications in terms of understanding this host–parasite interaction. For instance, Lister-hooded rats are believed to be the most behaviourally similar to wild rats, at least in terms of food-related neophobia (Barnett, 1958; Webster, 2007), whilst Fawn-hooded rats have been shown to exhibit more stress-induced fear (freezing) than Wistar rats and have higher levels of corticotropin-releasing factor (CRF) mRNA in their central amygdala (Altemus et al., 1995). One could perhaps also propose that future *T. gondii* studies could incorporate the spontaneously hypertensive rat (SHR) model of anxiety, to help further elucidate host from parasite associations in relation to generalized anxiety profiles.

In terms of future research into the '*T. gondii*–rat manipulation–schizophrenia' model, a number of potentially useful rat *in vivo* models are already available that could plausibly provide valuable experimental tools to further test the hypothesis of causality. Prenatal exposure to various infectious agents has been linked to increased risk of neurodevelopmental brain disorders, and specifically those associated with altered dopaminergic development. For example, there is recent evidence that rats born to mothers exposed to the viral mimic polyriboinosinic-polyribocytidylic acid (poly-I:C) in pregnancy provide a useful rodent model for human affective disorders such as schizophrenia (Vuillermot et al., 2012). Admittedly, whilst previous studies found no significant difference in the hyperactivity profiles between adult-acquired and congenitally acquired *T. gondii* infections amongst rats (Webster, 1994), it may be fascinating to examine the impact of *T. gondii* on the resulting developmental, behavioural and neurophysiological phenotype amongst such poly-I:C offspring – hence a 'two-step environmental–environmental (i.e. infection:infection) hit'. Likewise, although there are no rodent models available yet for hallucinations (see below), *N*-methyl-δ-aspartate receptor (NMDAR) antagonists, such as MK-801, can induce schizophrenia-like psychoses, together with other 'positive symptoms', in rats (Brigman et al., 2010). The mechanism involves hyperactivity of mesolimbic dopamine neurons, although multiple brain regions and multiple neurotransmitter systems are affected (Seeman, 1987). Hence, one may predict that future studies incorporating the additional exposure of *T. gondii* in such rats may further provide valuable (environmental–environmental interaction) insights into the mechanisms involved and phenotypic outcomes.

Genetically engineered and naturally occurring mutant rodent models can also be particularly useful in providing requisite information about the neurodevelopmental, behavioural and molecular consequences of dysregulation in specific genes, and hence the potential gene–environmental interactions of infection with *T. gondii* here on the subsequent outcome. At present, there

are several useful and relevant mouse models available that may be particularly useful for future *T. gondii* research, in particular those using knock-out (KO) mice involving dopamine (dopamine synthesis and/or regulation genes) (Kirby et al., 2010). Unfortunately, there are few KO rat models yet available, although several are underway and their development is likely to prove extremely valuable for examining gene–environmental interactions. In particular, we may predict that, for example, the combination of such genetic (KO line) and environmental (*T. gondii* infection) factors not only exerts additive effects on behavioural traits such as locomotor hyperactivity and fatal feline attraction but also produces synergistic effects in the development of, for instance, impaired attentional shifting and sustained attention. Furthermore, we may predict that the combination of both these gene–environmental factors is necessary to trigger maldevelopment of the host dopamine system in these aetio-pathological processes.

What parasite strains should future research use and why?

The choice of animal host to study – down to species, strain, gender and past parasitic and behavioural histories – clearly will have an effect on the outcome of any behavioural assay used and, in doing so, should provide further key information regarding the evolution, mechanisms involved and behavioural outcome of infection. In a similar manner, so will that of the parasite used within such studies. Key parasite-associated factors may plausibly relate to the route of infection, such as oocyst or tissue cyst (Webster, 2001; Webster and McConkey, 2010) or perhaps even a sexually transmitted route (Vyas, 2013). Timing of initial infection may also be important, whether congenital (and at which trimester), neonatal or adult acquired (Webster, 1994; Webster, 2001). The strain of *T. gondii* is also undoubtedly of paramount importance in terms of the clinical, behavioural and ethical outcome of infection. *T. gondii* is composed of three major genotypes, Types I, II and III (previously estimated to encompass 94% of all isolates), which have emerged as the dominant strains worldwide (Howe et al., 1997). Whilst Type I strains tend to be fatal in mice, Type II and III parasites are relatively avirulent and more readily form cysts and thereby establish chronic infections (Howe et al., 1996; Sibley and Boothroyd, 1992; Sibley et al., 2002), which makes the latter two strains more suitable for the study of behavioural changes with chronic infection. Type II and III strains also show higher expression of the parasite's tyrosine hydroxylase genes, proposed to be involved in behavioural changes, relative to Type I (Gaskell et al., 2009; Prandovszky et al., 2011). Infection with Type II also accounts for most human cases (60–80%) in Europe and North America (Ajzenberg et al., 2002; Ajzenberg et al., 2009; Peyron et al., 2006), although both Types I and III are found in Colombia (Peyron et al., 2006). However, it is critical to clarify that sampling has been largely biased towards parasites recovered from symptomatic humans and domestic animals, and hence relatively little is known about the majority of wild animal infections or, potentially, even those human infections with no apparent disease. A role for atypical genotypes in cases with severe host morbidity has, however, been indicated by the Californian sea otter populations suffering increased mortality – whilst 40% were infected with the common zoonotic Type II strain, 60% were infected with a genotype that possessed novel alleles at three genetic loci different from the alleles found in Types I–III (Miller et al., 2004). Furthermore, recent research suggests that such atypical strains, previously referred to as A and X, may designate together as 'Type 12' (Khan et al., 2011), and this Type 12 lineage may actually account for 46.7% (79/169) of isolates and dominate amongst wildlife of North America (Dubey et al., 2011). One could thus perhaps postulate that future molecular typing studies could reveal different clinical and behavioural outcomes in human or other *T. gondii* infections in relation to whether the zoonotic infection route may be wildlife or domestic, typical or atypical genotype. Even within *T. gondii* type, however, different clinical and behavioural outcomes may be predicted. Within Type II alone, variation in host cell gene expression (Xiao et al., 2011; Hill et al., 2012) and development (Diana et al., 2004), host immune and encephalitic response (Araujo and Slifer, 2003; Hill et al., 2012), parasite dissemination, reactivation and recrudescence (Saeij et al., 2005), and impact on host behaviour (Kannan et al., 2010) have all been reported between different strains. For example, whilst both Prugniaud (Pru) and ME49 have been reported to increase attraction to cat odour in mice at 2 months post-infection, in at least one study this behaviour was no longer present with the ME49 strain at 7 months (Kannan et al., 2010). Pru-infected mice in this study were also reported to have greater hyperactivity than their ME49-infected counterparts, whilst only the ME49-infected group showed impaired spatial working memory (Kannan et al., 2010).

Future studies aimed at elucidating mechanisms of action may also benefit from recent advancements in mutant *T. gondii* strain development. Within Type I, for instance, new conditional mutant RH lines have recently been constructed *via* promoter replacement strategies that target genes encoding proteins that are conserved and unique to these apicomplexan parasites (Sheiner et al., 2011). Whilst no longer photosynthetic, the apicoplast, a red algal endosymbiont, is a centre of metabolic activity harbouring several major anabolic pathways, and these studies have revealed new apicoplast proteins, currently with no assigned function (Sheiner et al., 2011) and which, hypothetically at least, could perhaps be relevant to behavioural alterations.

RHΔhxgprt and PruΔhxgprt strains, for example, have also been produced to provide parasites in which the selectable marker HXGPRT could be used for gene insertion/replacement (Donald et al., 1996), although targeting specific *T. gondii* genes for knockout has previously proved difficult due to a high frequency of nonhomologous recombination in the parasite. The production of RHΔ*ku80*Δ*hxgprt* (Fox et al., 2009) and also notably the Type II PruΔ*ku80*Δ*hxgprt* (Fox et al., 2011) has, however, greatly improved the efficiency of gene replacement by homologous recombination in *T. gondii* strains, thereby increasing the opportunity to investigate the contributions of individual *T. gondii* genes to behavioural modifications of the host. Indeed, within Type II, and hence those lines particularly applicable for the '*T. gondii*–rat manipulation–schizophrenia' model, key areas of interest for the '*T. gondii*–rat manipulation–schizophrenia' model will be parasites such as PRU lines with differential levels of expression and/or KO of the tyrosine hydroxylase gene shown to be associated with dopamine metabolism (Gaskell et al., 2009; Prandovszky et al., 2011; Webster and McConkey, 2010).

What behavioural assays should future research incorporate to assess the impact of *T. gondii* on animal host behaviour?

In addition to replicating prior observations, novel approaches and tests are required, and there is no doubt that animal models can play an essential role in furthering this theoretical and applied area of research. However, if such animal studies are to be performed, it is imperative that they are performed appropriately, testing biologically and evolutionary-applicable hypotheses, and, equally importantly, in the most ethical and non-invasive manner possible,

particularly considering today's 3Rs environment. Once again, this relates to the precise hypotheses to be tested. We know, to date, that a range of behavioural alterations are observed in animals, particularly rodents, infected with *T. gondii*. Further studies deciding which tests to use should thus consider whether the aim is to simply further elucidate the behavioural repertoire impacted by this parasite – which may serve the purpose of helping us understand further the potential evolution, transmission potential and mechanisms involved directly, particularly as new molecular tools and techniques are developed. If the aim is to specifically test hypotheses relating to the role of *T. gondii* in human affective disorders such as schizophrenia, however, the behavioural assays to be used are not necessarily the same as those for either studying schizophrenia alone or the impact of *T. gondii* on host behaviour alone. Furthermore, when choosing such assays, one must consider that, in the absence of any true 'schizophrenia rodent model', the behavioural repertoire of a rodent infected with *T. gondii* may well be altered but not necessarily in the same way as that of a human with schizophrenia. For instance, if considering selective benefits to the parasite of *T. gondii* in rodents, one should focus on behavioural traits specifically associated with enhanced predation rate (such as altered activity, feline attraction and altered neophobia profiles). On the other hand, if explicitly testing for the spectrum of traits relevant to schizophrenia, a different set of behavioural assays (such as working memory, selective attention, set shifting, social interaction and psychophysiological measures) may be more appropriate. Nevertheless, there may well be certain circumstances where specific behavioural assays may be applicable to both and hence highly suitable for further study on the *T. gondii*–rat manipulation–schizophrenia model.

There are generally considered to be three groups of major symptoms of schizophrenia in humans: positive, negative and cognitive/executive (Carter et al., 2008). 'Positive symptoms' are so-called because they add to the normal behavioural repertoire. While there are no rodent models available yet for hallucinations, other positive symptoms, such as psychomotor agitation and hyper-responsivity to psychotomimetic drugs, are modelled in rodents by testing locomotor responses and hyperactivity-inducing effects of psychostimulants (e.g. amphetamine) and other psychotomimetics [e.g. the NMDAR antagonists dizocilpine/MK-801 or PCP (Brigman et al., 2010)]. Thus, one could perhaps propose that the increased activity observed in *T. gondii*-infected rat studies (Webster, 1994; Webster, 2001b) is also useful as a 'positive symptom' assay. Likewise, as the fatal feline attraction invoked by this parasite is not simply a reduction or removal of a behavioural trait, but instead a positive reversal of an innate behaviour, an apparent 'alteration of the mind of the rat in the face of predation' (Berdoy et al., 2000; Webster et al., 2006), one could perhaps also propose that altered fatal feline attraction behavioural assays may be another valuable behavioural assay relevant to both *T. gondii* epidemiology and evolution but also as a potential 'positive symptom' indictor for further '*T. gondii*–rat manipulation–schizophrenia' models (Appendix 2).

Negative symptoms of schizophrenia are so-called because they subtract from the normal behavioural repertoire, and include blunted affect, social withdrawal and loss of pleasure in normally rewarding activities (anhedonia). Various rodent assays for social behaviour and anhedonia have been typically used to model other disorders such as anxiety (File and Seth, 2003), autism (Crawley, 2004) and depression (Strekalova et al., 2004), but also lend themselves well to the study of abnormalities in these behaviours in models of schizophrenia. However, whilst *T. gondii*-infected mice may suffer severe morbidity and hence may show equivalent 'negative symptoms' as a result of generalized pathology (Appendix 1), *T. gondii* does not generally induce any specific negative symptoms in rats, as can be illustrated by the normal social behaviour and mating success between infected and uninfected rats maintained under naturalistic conditions (Berdoy et al., 1995). Nevertheless, the fact that the 'pleasure/reward' system does appear to be altered in *T. gondii*-infected rodents, even if in perhaps an opposite direction to those related to schizophrenia in humans – i.e. some evidence of increased pleasure/fatal feline attraction through increased sex drive (enhanced pleasure rather than loss of pleasure, again potentially associated with increased dopamine levels) – there is an argument for use of these behavioural assays when examining the *T. gondii*–rat manipulation–schizophrenia model. For instance, in relation to modelling 'negative symptoms', one could perhaps test for alterations between infected and uninfected rodents in terms of their preference or motivation to obtain rewarding substances, such as sucrose, even if the direction of response predicted may be different between rats and humans (Appendix 2).

Abnormalities in cognition and executive functions are also a prominent feature of schizophrenia in humans and range from deficits in episodic memory, impaired attention and sensorimotor gating to impaired reversal learning and set-shifting. Learning and memory can certainly be, and frequently are, assessed in rodents using a range of standard behavioural assays, such as that of the reference memory version of the Morris water maze (see Appendix 2). However, although impaired episodic memory is one of the strongest features of the cognitive profile of schizophrenia (Ranganath et al., 2008), rodent models of this disease have generally not relied upon such measures – and indeed do not distinguish a model of schizophrenia from other conditions that are also characterized by memory deficits, e.g. Alzheimer's disease (Brigman et al., 2010). Furthermore, one could perhaps particularly question their utility here for any *T. gondii*–rat manipulation–schizophrenia model, and perhaps even the potential applicability of such cognition, learning and/or memory behavioural assays in the *T. gondii*–rodent system in general. This may be explained as, if *T. gondii* is selectively altering intermediate host behaviour to alter predation rate, one may predict there to be no selective advantage for this parasite to alter such traits – as with a cat, the change of avoiding predation on encounter is likely to be all or none – and hence such assays are less relevant to understanding the epidemiology and evolution of such manipulation. Indeed, although there are some conflicting results, particularly in the early literature, indicating potential cognitive deficits in mice after infection with *T. gondii* (Piekarski et al., 1978; Witting, 1979), our own earlier behavioural assays of short- and long-term memory, as assessed using different maze combinations (J.P.W. and P. H. L. Lamberton, unpublished observations) using Lister-hooded laboratory rats with adult-acquired infections with the avirulent (Beverley) strain, found no difference between infected and uninfected individuals. Likewise, recent research using C57BL/6 mice chronically infected with the avirulent *T. gondii* (ME49, a Type II strain) has found no impact of *T. gondii* infection on cognition (Gulinello et al., 2010).

Prepulse inhibition (PPI) of the startle response provides a measure of selective attention, as well as sensorimotor gating, and has been widely used in schizophrenia research (Swerdlow et al., 2008; Van den Buuse et al., 2003; Weiss and Feldon, 2001). Whilst one could thus propose incorporation of this test into *T. gondii*-altered behavioural studies to allow direct comparison with pharmacological and genetic studies, this assay may not, however, be sufficiently sensitive to assess the very subtle behavioural

alterations observed between *T. gondii*-infected and uninfected rats. Indeed, recently it was found that general olfaction and sensorimotor gating as assessed using the PPI acoustic startle were normal in both male and female *T. gondii*-infected mice (Xiao et al., 2012). Furthermore, as the acoustic/auditory startle or foot shock often used in the PPI test may be stressful for the animal, it may be a less than ethically ideal assay for this particular system, and more biologically and ethically appropriate alternative behavioural assays are available. These include, for example, the five-choice serial reaction time task (5-CSRTT), which also tests for the attentional dysfunction observed in human schizophrenia [as well as attention deficit hyperactivity disorder (ADHD), Alzheimer's, Parkinson's, aging and addiction] (Bari et al., 2008). The T-maze test of impulsivity also tests for attention deficit and involves the dopaminergic and serotonergic systems (Denk et al., 2005) (Appendix 2).

Another potential behavioural assay for both *T. gondii* and schizophrenia concerns assays incorporating odour detection. Studies to date suggest that rodents infected with *T. gondii* do not appear to have any general disruption of their odour/olfaction system – as they can, for instance, discriminate between feline and other predator and non-predator odours and even between different concentrations of odour presented – and hence it appears that their perception of cat odour is affected (Berdoy et al., 2000; Kannan et al., 2010; Lamberton et al., 2008; Vyas et al., 2007b). Olfaction dysfunction is frequently observed in patients with schizophrenia, with the greatest impact on odour identification (Cohen et al., 2012), which may thereby present a parallel to that observed in *T. gondii*-infected rodents. Thus, further examination of the subtle changes in odour-specific threshold and identification deficits observed is warranted as part of the *T. gondii*–rat manipulation–schizophrenia model, and a range of standardized behavioural assays are available (Appendix 2).

Cognitive flexibility is a critical executive function that can be broadly defined as the ability to adapt behaviours in response to changes in the environment. There are, furthermore, potentially useful non-invasive behavioural assays for these deficits in rodents available that could well be highly applicable for a *T. gondii*–rat manipulation–schizophrenia model. These include, amongst others, intra-dimensional/extra-dimensional digging tasks. During such assays, rodents are trained to dig for food reward using either olfactory (digging medium odour) or tactile (digging medium texture) cues. The rewarded cue in the same dimension is switched to test for intra-dimensional shifting. The rewarded cue is changed to the different dimension to test for extra-dimensional shifting. Likewise, the Wisconsin Card Sorting Task (WCST) (Grant and Berg, 1948) has been one of the more commonly employed assays for impaired cognitive flexibility in schizophrenic patients, and analogous versions have been developed for use in rodents. In essence, these tasks involve the subject selecting between stimuli, which vary from one another in more than one perceptual dimension, and being reinforced for choosing a stimulus based upon one specific dimension alone, e.g. odour. During an 'intra-dimensional shift' (IDS), the form of the dimension the subject must choose is changed by the experimenter, e.g. from cinnamon to chocolate odour. In an 'extra-dimensional shift' (EDS), the correct dimension is changed altogether, such that choices must be guided by the new dimension (texture) while ignoring the previously rewarded dimension. In a rodent IDS/EDS analog of the WCST, rats (Birrell and Brown, 2000) dig in sand to make choices based on the dimension of texture or smell, thereby providing another example of a potentially useful, biologically and ethically behavioural assay available and able to be incorporated into future research in this field (a non-exclusive list being provided in Appendix 2).

General discussion

Can *T. gondii* infections in animal models really help us understand behavioural changes in humans?

The very characteristics of precisely those parasites able to manipulate host behaviour, rather than parasitism *per se*, may plausibly make them the prime candidates to be associated with clinical disorders in infected humans, whether it be as a direct or indirect consequence, from specific manipulation, or 'side-effect/by-product' of infection (Appendix 1). Indeed, despite such parasite's often apparently sophisticated mechanisms aimed to achieve manipulation, it seems unlikely that they would also possess, at high cost, yet more sophisticated mechanisms able to distinguish and discriminate against expressing such manipulation to within only those 'correct' hosts intended. Indeed, there would not be selective pressure for this specificity. This may be particularly relevant to those multiple-host parasites transmitted in the food chain, as these may be particularly likely to infect the 'wrong host'. *T. gondii* may well therefore be under, or have been under, strong selective pressures to specifically manipulate its rodent intermediate host in order to enhance predation to the feline definitive host, but it is also a ubiquitous parasite capable of infecting, through a range of transmission routes, all warm-blooded animals (Beverley, 1976). Moreover, several of these species, including humans, have considerably longer life-spans than a rodent, and hence one could reasonably propose may be more susceptible to developing 'unselected' pathological behavioural changes simple as a by-product of their extended durations of infections. One of these pathologies may thereby include the potential association between *T. gondii* and schizophrenia in some humans. Can thus studying the behaviour of small intermediate hosts with natural or experimentally exposed infections really help us understand behavioural changes in *T. gondii*-infected humans? There seems little doubt that the subtle behavioural changes observed in rodents infected with *T. gondii* will be reflected by similar subtle behavioural changes in humans, and a convincing body of empirical evidence now exists in support of this (Flegr, 2013). However, when it comes to considering animal infection models for human severe behavioural alterations, as occur within schizophrenia, the case is undoubtedly more complex. There is no doubt that animal, in particular rodent, models have been and continue to be useful in helping us understand aspects of schizophrenia – such as in terms of elucidating how current and potential future antipsychotic drugs work. Indeed, it was an understanding of the interaction of certain neuroleptics with dopamine receptors that was an instigator in formulating the still-maintained theory that schizophrenia involves some form of dysregulation of brain dopamine function (Creese et al., 1976; Seeman, 1987; Seeman et al., 1976). However, we are unlikely to ever be able to reproduce the full phenotypic spectrum of a human psychiatric disorder such as schizophrenia in a rat or mouse (Arguello and Gogos, 2006). Schizophrenia is a highly heterogeneous disorder of myriad symptoms. The presentation of different symptoms and their severity varies considerably across patients. Nevertheless, while this complexity cannot be fully recapitulated in the rodent models, specific symptom categories can be behaviourally modelled. A constructive starting point has been to demarcate schizophrenia-related phenotypes into the clinical categories of positive, negative and cognitive/executive symptoms (Brigman et al., 2010). Of course, an essential step in clarifying the

aetiology of schizophrenia is understanding the gene–environment interactions contributing to this and associated disorders. In this context in particular too, animal models will have a central and indispensable role in the process of helping elucidate the mechanisms involved and perhaps the epidemiology of certain causes of psychiatric disorders (Arguello and Gogos, 2006). Where such animal models are to be used, it is of course imperative that these must only be performed in a biologically appropriate and ethical manner. It is important to emphasize, however, that mechanistic insights into the nature of the deficit under investigation cannot be achieved by behavioural assessment alone. Rather, an interdisciplinary approach that begins at the behavioural level whilst combining the cellular and molecular levels is essential (Arguello and Gogos, 2006). The hope is that future research will allow us to make some truly novel insights into the epidemiology and mechanisms of *T. gondii*-altered behaviour and, potentially, into the pathophysiology and ultimate prevention or treatment of certain neuropsychiatric diseases such as schizophrenia.

Appendix 1. Glossary of terms relating to parasite-altered behavioural alterations

Sickness behaviour

Morbidity in the host, often displayed as general malaise or 'sickness', that may facilitate transmission of the parasite. Often observed with vector-borne diseases, where a decrease in host energy and activity levels can increase the likelihood of being bitten by an insect vector, *via* an increase in host landing rate and a reduction in fly swatting behaviours (Ewald, 1994; Holmstad et al., 2006). Examples of this can be seen in rodents infected with *Trypanosoma brucei brucei* (Darsaud et al., 2003) and various *Plasmodium* spp. (Day and Edman, 1983; Grau et al., 1987). Another example may be sneezing and coughing in humans, which facilitates the transmission of aerosolised pathogens, *via* increasing the volume and distance travelled of aerosolised particles containing the pathogen (Gralton et al., 2011).

General pathology

Morbidity (or mortality) in the host of no obvious selective benefit to host or parasite. This may be the case with neurosyphilis caused by *Treponima pallidum*, which is associated with cognitive deterioration and neuronal loss long after the period of infectivity (O'Donnell and Emery, 2005). *Schistosoma mansoni* infection has also been associated with reduced exploratory behaviours, cognitive abilities and nociception in the human host (Aloe and Fiore, 1998; Aloe et al., 1996; Fiore et al., 1998; Fiore et al., 2002) that is of no apparent selective benefit to the parasite, which relies on contact with freshwater snails for its transmission.

Parasite manipulation

Behavioural alterations of the host with a specific selective benefit to the parasite. This is the case with *T. gondii*, where a reduction in fear of feline odour and increased activity are thought to be specific selective advantages to the parasite, increasing its chance of transmission by predation *via* the feline definitive host (Berdoy et al., 2000; Webster, 2001). Another example may be the Seoul virus, which, similar to the rabies virus in dogs, is thought to increase aggressive behaviours in rats, where the resulting wounding may facilitate transmission *via* contact with virus present in saliva or excrement (Hinson et al., 2004; Klein et al., 2004). Other heteroxenous parasites that appear to cause their intermediate hosts to be predated upon more than expected by their definitive hosts include *Plagiorynchus cylindraceu* in isopods (Moore, 1983), *Sarcocystis cernae* in voles (Hoogenboom and Dijkstra, 1987) and *Euhaplorchis californiensis* in fish (Lafferty and Morris, 1996) (and see Moore, 2013).

By-product

Behavioural alterations displayed in accidental/dead-end hosts as a side-effect of selective adaptations in the parasite's natural host species. This appears to be the case in *T. gondii* infection of humans, where adaptations designed to facilitate transmission in the rodent intermediate host are also observed in humans: such as lowered reaction times (Novotna et al., 2008), subtle personality alterations and reduced novelty-seeking (Flegr, 2007). This can also be seen in the case of *T. gondii*-infected sea otters showing abnormal behaviour and increased predation by sharks (Miller et al., 2004), despite sharks being a dead-end host to the parasite. Another example of this is perhaps *Lyssavirus* spp., causative agents of rabies. In wild hosts such as dogs and bats, the virus' mechanisms within the central nervous system may facilitate transmission; for example, dogs with furious rabies show higher levels of aggression and biting (Kaplan, 1986) that may increase chance of transmission *via* infected saliva to susceptible hosts' blood and body tissues (Rupprecht et al., 2002). However, in humans, a dead-end host to the virus causes hyperactivity, phobic spasms and severe agitation (Hemachudha et al., 2002) that is of no selective benefit to the virus.

T. gondii–rat manipulation–schizophrenia model

The novel term, first used here, relating to studies of *T. gondii* infection in rats as a model system for examining the evolution and mechanisms of *T. gondii*-altered behaviour in its natural intermediate host species to examine both 'parasite manipulation' and its 'by-product' physiological and behavioural changes in accidental secondary host species such as humans. Specific focus is placed upon the use of *T. gondii* in rats as a biologically and ethically appropriate model for understanding the aetiology, symptoms and mechanisms of action underpinning (some cases of) human schizophrenia and related affective disorders.

Appendix 2. Examples of non-invasive behavioural tests for assessing the impact of *Toxoplasma gondii* on rodent behaviour

Elevated plus maze

What it measures

Generalised anxiety behaviour. This tests a rat's unconditioned fear response to heights/open spaces, as compared to a darker enclosed space, and is based on the classic approach–avoidance theory. The elevated plus maze has been used as a model of state, unconditioned anxiety for over two decades and is well documented and reviewed (Carobrez and Bertoglio, 2005; Hogg, 1996; Walf and Frye, 2007).

Relevance for *T. gondii* research

Widely used behavioural assay for anxiety-related behaviours in rodents and is therefore replicable and comparable with other related tests. It is an easy to use and easily automated assay. Unconditioned response based on natural behaviours seen in wild rats (Barnett, 1958). Non-aversive and can detect subtle changes such as those predicted to arise from *T. gondii* infection (Webster, 2001).

Light–dark Suok test

What it measures

Based on classic approach–avoidance theory, using an unstable and novel arena, this assay assesses balance and anxiety phenotypes

simultaneously, as well as potential vestibular defects (Kalueff et al., 2008).

Relevance for *T. gondii* research

Certain neural circuits are shared by pathways that mediate autonomic control, vestibular control and anxiety (Balaban, 2002). These are modulated by the monoaminergic system, which directly links balance disorders with emotional dysregulation. As *T. gondii* may influence anxiety behaviours in rats (Kaushik et al., 2012) and has been shown to cause sensorimotor deficits in mice (Gulinello et al., 2010), this test allows for both aspects of behavioural alteration to be studied. Dopamine is involved in the modulation of vestibular pathways (de Waele et al., 1995) and in anxiety-related behaviour (Mallo et al., 2007), and *T. gondii* has been shown to alter host dopamine levels (Prandovszky et al., 2011; Webster and McConkey, 2010).

'Fatal feline attraction' four-choice test

What it measures

Innate physiological and behavioural aversion to cat (urine) odour. Infected and uninfected rats' responses are assessed through the number of entrances into and/or duration spent in zones with different odours – feline odour, own (rat) odour, neutral (water) odour and non-predatory mammal (rabbit) odour (Berdoy et al., 2000; Webster et al., 2006).

Relevance for *T. gondii* research

The innate aversion to cat odour amongst rodents provides a profound obstacle for the parasite against successful predation by the feline definitive host. This assay measures the parasite's ability to manipulate such innate traits. Through providing the test rodent with a number of other odours, this assay may be comparable to a situation in the wild where a rat would encounter a number of other mammalian odours, including its own. This allows for the specificity of the difference in response to feline odour as compared to other odours to be shown. The test is also conducted over a long period of time (2.5–12 h) and therefore assesses a rat's response over time rather than just its initial response (thereby further minimizing risk of Type I or II errors). This again may be more relevant to a situation in the wild where short time constraints may not apply. In terms of the *T. gondii*–rat manipulation–schizophrenia model, as the fatal feline attraction invoked by *T. gondii* is not simply a reduction or removal of a behavioural trait, but instead a positive reversal of an innate behaviour, an apparent 'alteration of the mind of the rat in the face of predation' (Berdoy et al., 2000; Webster et al., 2006), one could propose that this assay may serve as a potential 'schizophrenia positive symptom' indicator.

Activity/velocity

What it measures

Velocity of movement, duration of time spent moving, duration of time spent stationary, frequency of entry into a zone, latency to enter a zone of interest. These data can now be easily obtained simultaneously with other behavioural assays using automated behavioural tracking software.

Relevance for *T. gondii* research

There is a convincing body of evidence that *T. gondii* increases activity levels in both rats (Webster, 1994) and mice (Hutchison et al., 1980; Hodkova et al., 2007), which may be predicted to increase the chance of predation by the feline definitive host (Webster, 2001). In terms of the *T. gondii*–rat manipulation–schizophrenia model, positive symptoms of schizophrenia, such as psychomotor agitation and hyper-responsivity to psychotomimetic drugs, are modelled in rodents by testing locomotor responses and hyperactivity (Brigman et al., 2010). Thus, one could perhaps propose that the increased activity observed in *T. gondii*-infected rat studies is also useful as a 'positive symptom' assay.

Two-choice test

What it measures

Entrance into and/or time spent in zones with different odours, e.g. feline *vs* rabbit, or domestic cat *vs* puma, or domestic cat *vs* cheetah (Lamberton et al., 2008).

Relevance for *T. gondii* research

Related to the 'fatal feline attraction' and 'novel odour assays', this specifically allows preference for, and/or discrimination between, two odours (such as two contrasting predatory odours alone) to directly be tested. This may be particularly pertinent for measuring between *T. gondii* definitive host *vs* non-definitive host predator odours or even between different predatory feline species with different definitive host capacities.

Continuous spontaneous alternation

What it measures

Spontaneous alternation is very sensitive to hippocampal dysfunction. Alternation reflects the animal's motivation to explore its environment. There also appears to be involvement of the vestibular system (Deacon and Rawlins, 2006; Lalonde, 2002). Continuous spontaneous alternation has been widely used as a simple measure of short-term spatial working memory and can reflect responsiveness to novelty as well as sensory and attentional factors (Hughes, 2004). Dopamine in the septum and hippocampus has been implicated in the exploration of novel maze arms (Lalonde, 2002).

Relevance for *T. gondii* research

This test requires no habituation and is based on the animal's natural exploratory behaviour of a novel environment. This may tie in with previous studies that show that *T. gondii*-infected rodents show altered exploration of novel environments (Berdoy et al., 1995). As the assay involves a continuous alternation test, there is no experimenter contact during the trial, the test is non aversive and it is easily automated. This may allow for the more subtle behavioural changes usually seen in *T. gondii* infection (Webster, 2001) to be detected. Tests for hippocampal dysfunction are important in *T. gondii* research as the parasite has been shown to invade hippocampal neurons and glial cells (Creuzet et al., 1998), and the septohippocampal pathway is involved in the innate fear of feline odour (Vyas et al., 2007a). The assay also involves the vestibular system; this may be important as *T. gondii* has been shown to cause sensorimotor deficits in mice (Gulinello et al., 2010). In terms of the *T. gondii*–rat manipulation–schizophrenia model, schizophrenia patients have been shown to display spatial working memory and short-term memory deficits (Aleman et al., 1999; Barch et al., 2002; Park and Holzman, 1992). Spontaneous alternation has also been seen to be affected by other neurotropic diseases such as scrapie (Guenther et al., 2001). Hippocampal dysfunction also plays a role in the neuropathology of schizophrenia (Harrison, 2004). Finally, there is a speculated involvement of dopamine in spontaneous alternation behaviour (Lalonde, 2002).

Burrowing

What it measures

Can be used to sensitively measure a wide range of behavioural abnormalities (Deacon, 2006a). An advantage of this test is that it allows digging behaviour to be directly measured by manual observation and can give a more detailed profile than marble burying.

Relevance for *T. gondii* research

The test is very sensitive and therefore able to detect very subtle behavioural changes. The test is non-aversive and examines a natural behavioural trait whilst mimicking an environmental situation encountered by wild rats, therefore making it more applicable to the natural intermediate host of the parasite in the wild. Burrowing behaviour may, in part, display elements of defensive burying (Deacon, 2006a; Dellomo et al., 1994; Fanselow et al., 1987; Pinel and Treit, 1978), which could be of particular interest for *T. gondii* research if associated to predation-related defence mechanisms. In terms of the *T. gondii*–rat manipulation–schizophrenia model, burrowing is decreased by selective serotonin reuptake inhibitors and by changes in interleukin levels in rats (Deacon, 2006a). The burrowing test has been shown to be a sensitive assay for other neurotropic diseases such as scrapie (Deacon et al., 2001).

Marble burying

What it measures

Marble burying is dependent on hippocampal function and, as a result, is disrupted by agents that affect hippocampal function. Marble burying is inhibited by many compounds that are active on the serotonergic system, many of which attenuate anxiety, depression or obsessive–compulsive disorder (OCD) (Deacon, 2006b).

Relevance for *T. gondii* research

As for burrowing above, marble burying behaviour may, in part, display elements of defensive burying (Dellomo et al., 1994; Fanselow et al., 1987; Pinel and Treit, 1978), which is of particular interest for *T. gondii* as this can be a predation-related defence mechanism. The test is non-aversive and mimics an environmental situation encountered by wild rats, therefore making it more applicable to the natural intermediate host of the parasite in the wild. *T. gondii* infection may affect hippocampal function, as the parasite has been shown to invade hippocampal neurons and glial cells (Creuzet et al., 1998), and the septohippocampal pathway is involved in the innate fear of feline odour (Vyas et al., 2007a). In terms of the *T. gondii*–rat manipulation–schizophrenia model, there is evidence for the hippocampus playing an important role in the neuropathology of schizophrenia (Harrison, 2004). *T. gondii* may also modulate host anxiety levels (Kaushik et al., 2012), and anxiety levels may be disrupted in OCD (Miman et al., 2010b) as well as other human mood disorders (Arling et al., 2009; Jones-Brando et al., 2003; Ling et al., 2011; Yagmur et al., 2010).

Morris water maze

What it measures

Spatial learning and reference memory. It is sensitive to hippocampal synaptic plasticity and NMDAR function (Vorhees and Williams, 2006).

Relevance for *T. gondii* research

Schizophrenia patients show dysfunction in learning and memory (Aleman et al., 1999; Saykin et al., 1991) and therefore using a test that assesses these deficits in *T. gondii*-infected rodents allows us to further understand the link between *T. gondii* infection and schizophrenia observed in humans (Torrey et al., 2007). There is also much evidence that NMDAR hypofunction is involved in schizophrenia (Coyle et al., 2003; Mohn et al., 1999; Olney et al., 1999) and therefore this test provides a relatively non-invasive means of testing this link in rodents.

Five-choice serial reaction time task (5-CSRTT)

What it measures

Impulsivity, attentional processes, accuracy of discrimination, response control (Bari et al., 2008). The test is sensitive to discrete brain lesions and neurotransmitter depletions. The test is particularly used to elucidate neuropsychological mechanisms that are disrupted in pathologies characterised by attentional dysfunction, such as schizophrenia, ADHD, Alzheimer's disease and Parkinson's disease (Bari et al., 2008).

Relevance for *T. gondii* research

Highly relevant for the *T. gondii*–rat manipulation–schizophrenia model, as attentional dysfunction is a pathology found in human sufferers of schizophrenia (Laurent et al., 1999) and other neurological disorders associated with *T. gondii* infection in humans (Kusbeci et al., 2011; Torrey et al., 2000). The test has also been shown to have good translational value, as it was originally adapted from a human task and has been successfully extended to mice and primates (Higgins and Breysse, 2008). This assay provides a useful, less invasive/aversive alternative behavioural assay relevant to the aetiology of schizophrenia compared with that of the more widely used PPI assay.

T-maze test for impulsivity

What it measures

Impulsivity, attention deficits (Bizot et al., 2007; Denk et al., 2005). This test is often used in studies looking at ADHD, as impulsive behaviour (i.e. the selection of small immediate gains in preference to larger delayed gains) is a symptom displayed in ADHD patients (Winstanley et al., 2006).

Relevance for *T. gondii* research

As for the 5-CSRTT assay above, highly relevant for the *T. gondii*–rat manipulation–schizophrenia model, as attentional dysfunction is a pathology found in human sufferers of schizophrenia (Laurent et al., 1999) and other neurological disorders associated with *T. gondii* infection in humans (Kusbeci et al., 2011; Torrey et al., 2000). The test has also been shown to differentially involve the dopimanergic and serotonergic systems in rats (Denk et al., 2005).

Attentional set shifting

What it measures

Impairments in executive processes. This is a characteristic symptom of certain neuropsychiatric conditions where pathophysiology of the prefrontal cortical dopimanergic system is thought to be an underlying cause (Floresco et al., 2006).

Relevance for *T. gondii* research

Impairments in attentional set shifting are observed in patients with schizophrenia and ADHD, due to alterations in dopimanergic functioning within (Floresco et al., 2006). This has been seen in the Wisconsin card sorting task in humans (Lysaker and Bell, 1994; Riccio et al., 1994a; Riccio et al., 1994b; Rybakowski et al., 2005; Tsuchiya et al., 2005). Rodent models of attentional set shifting

may be used to assess mechanisms in the brain involved in such cognitive deficits.

Novel odour tests

What it measures

Novelty-seeking and levels of neophilia/neophobia, as well as olfactory responses and discrimination.

Relevance for *T. gondii* research

Novelty-seeking is an innate characteristic of rodent behaviour, where, in general, mice are neophilic whereas rats are neophobic (Barnett, 1958; Barnett and Cowan, 1976). Infection with *T. gondii* has been shown to decrease neophily in mice and decrease neophobia in rats (Webster, 2001). As rodents rely heavily on their olfactory abilities both for food acquisition and for predator avoidance (Dielenberg et al., 2001), altered neophilia/neophobia in the intermediate host may alter transmission probabilities of the parasite, either by increasing their chance of ingesting the parasite *via* foraging or through increasing their risk of predation by the definitive host. Humans are innately neophilic, and studies have suggested a negative correlation between novelty-seeking and latent toxoplasmosis in humans similar to that observed in rodents (Flegr et al., 2003; Novotná et al., 2005; Skallova et al., 2005). Novel odour assays in rodents may thereby be valuable for evolutionary and mechanistic studies into parasite altered innate behaviour. In terms of the *T. gondii*–rat manipulation–schizophrenia model, *T. gondii*-induced neophobia/neophilia can be ameliorated using D2 antagonists or selective dopamine uptake inhibitors, indicating involvement of the dopimanergic neuromodulatory system on exploratory behaviours (Skallova et al., 2006; Webster et al., 2006). Olfaction dysfunction is also frequently observed in patients with schizophrenia, with the greatest impact on odour identification (Cohen et al., 2012), which may thereby present a parallel to that observed in *T. gondii*-infected rodents.

Gambling test

What it measures

Impulsivity, addiction and risk-taking behaviours (deVisser et al., 2011; Winstanley, 2011; Zeeb et al., 2009).

Relevance for *T. gondii* research

Increased impulsivity and risk-taking are symptoms displayed in several clinical conditions such as schizophrenia and ADHD (Winstanley et al., 2006). Certain drugs that target the dopimanergic (and/or serotonergic) system, in particular that of dopamine agonists, may result in, as a side-effect, increased impulsivity, risk-taking or addictive traits such as gambling in humans (Winstanley, 2011). *T. gondii* infection can increase risk-taking behaviours in rats (Berdoy et al., 1995; Webster, 2001) and in humans (Flegr, 2007), potentially through related alterations of the dopaminergic system (Prandovszky et al., 2011; Webster and McConkey, 2010).

Object recognition test

What it measures

The ability to discriminate between a novel and familiar object, and thereby intact recognition memory of the familiar object, in addition to novelty-seeking behaviour (Bevins and Besheer, 2006). This can test various forms of object recognition, such as the alteration of a particular dimension, recognition of object location or of the environment in which an object was previously encountered.

Relevance to *T. gondii* research

T. gondii alters novelty-seeking behaviours in mice, rats and humans (Webster, 2001), potentially due, in part, to alteration of novelty discrimination mechanisms (Hodkova et al., 2007). As this test is a standardized test used in the field of learning and memory, this may provide a further understanding as to the observed link between *T. gondii* infection and schizophrenia (Torrey et al., 2007; Torrey and Yolken, 2007), as schizophrenia patients display impairments in recognition memory (Danion et al., 1999; Pelletier et al., 2005). Dopamine receptors have been implicated as having a role in functioning of this task (Besheer et al., 1999), which ties in with the potential link between *T. gondii* infection and alterations in the dopimanergic pathways (Prandovszky et al., 2011; Webster and McConkey, 2010). This test does not require exposure to aversive stimuli, or food or water restriction, and has been replicated in many laboratories using both mice and rats (Bevins and Besheer, 2006).

The Holeboard test

What it measures

Anxiety levels (Takeda et al., 1998) and exploration of a novel environment (File and Wardill, 1975a; File and Wardill, 1975b).

Relevance to *T. gondii* research

Widely used behavioural assay for anxiety and exploratory behaviours in rodents and is therefore replicable and comparable with other related tests. Unconditioned response based on natural behaviours seen in wild rats (Barnett, 1958). Non-aversive and can detect subtle changes such as those expected to arise from *T. gondii* infection (Webster, 2001). *T. gondii*-infected mice have been shown to display increased exploratory behaviours in the holeboard test in previous studies (Skallova et al., 2006). This effect has been shown to be suppressed by a selective dopamine uptake inhibitor, suggesting that this test may provide further insights into the association between *T. gondii* infection and changes in the dopaminergic neuromodulatory system (Skallova et al., 2006) and thereby the potential link with schizophrenia in humans (Torrey et al., 2007; Torrey and Yolken, 2007).

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