

REVIEW ARTICLE

Toxoplasma gondii-altered host behaviour: clues as to mechanism of action

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Abstract: A convincing body of evidence now exists, from both human and animal studies, and encompassing epidemiological to experimental, to indicate that the common protozoan *Toxoplasma gondii* can cause specific behavioural changes in its host. Such behavioural alterations are likely to be the product of strong selective pressures for the parasite to enhance transmission from its intermediate host reservoir, primarily rodent, to its feline definitive host, wherein sexual reproduction can occur and the parasite's life cycle completed. Here we consider what the available data to date may reveal about the potential mechanisms involved, the future research that needs to be performed, and the subsequent implications for animal and human health.

Keywords: *Toxoplasma gondii*, rodents, behaviour, schizophrenia, mechanisms, dopamine

Parasite manipulation

As specified by the 'manipulation hypothesis', certain parasites can alter host behaviour for their own selective benefit. Classic examples concern transmission through the food chain, where a parasite is immature in an intermediate host, which must be eaten by a predatory definitive host before the parasite can reach maturity and complete its life cycle. The parasite thus manipulates the behaviour of its intermediate host so as to enhance its transmission to the definitive host. The protozoan *Toxoplasma gondii* provides a convincing example of such a manipulatory parasite. 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 that are shed in the cat's faeces (Hutchison et al. 1969). If oocysts are ingested by an intermediate host, such as a wild rodent, or another secondary host, such as a human or domestic animal, the parasite undergoes asexual reproduction, characterised by rapidly dividing tachyzoites and the more slowly dividing bradyzoites, which can encyst in the brain, heart, and other tissues, where they remain, potentially for the host's lifetime. Transmission back to the feline definitive host occurs when an immunologically naïve cat ingests bradyzoite-infected tissue through predation or consumption of contaminated meat. Since sexual reproduction of *T. gondii* can be accomplished only in felines, there are strong selective pressures on the

parasite to evolve mechanisms to enhance transmission from the intermediate host to the definitive feline host and thereby complete its life cycle. The predilection of *T. gondii* for the brain of its intermediate host places it in a privileged position to enable such manipulation. A convincing body of evidence now exists to indicate that *T. gondii* can achieve such manipulation.

Studies on rats and mice have demonstrated, for example, that *T. gondii* causes an increase in activity and a decrease in neophobic (the innate fear of novelty) and predator vigilance behavioural traits (Hutchison et al. 1980 a,b, Hay et al. 1983, 1984, Webster 1994, 2001, 2007, Webster et al. 1994, 2006, Berdoy et al. 1995, Lamberton et al. 2008), each of which may be proposed to facilitate transmission of the parasite from the infected intermediate host to the feline definitive host. Moreover, whilst uninfected rats show a strong innate aversion to feline predator odour, *T. gondii* appears to subtly and specifically alter the rats' cognitive perception of cat predation risk, turning their innate aversion into a 'suicidal' fatal feline attraction (Berdoy et al. 2000, Webster et al. 2006, Vyas et al. 2007a). (See Webster 2007 for a review of the *T. gondii*-rodent behavioural studies.)

Toxoplasma gondii and schizophrenia?

Humans can also be secondary hosts for *T. gondii* and it is clearly established that congenital infections, especially early in pregnancy, can produce intracranial

calcifications, mental retardation, deafness, seizures, and retinal damage (Jones et al. 2001). Likewise, some cases of acute adult-acquired toxoplasmosis can result in headache, fever, myalgia, lymphadenopathy and occasionally seizures (Carne et al. 2009). Until relatively recently, latent toxoplasmosis in immunocompetent humans (and animals) was, in contrast, generally considered to be asymptomatic. However, a comprehensive series of studies, performed predominantly by Flegr and colleagues, have observed similar, often subtle, alterations in behavioural traits of latently infected humans to those observed in *T. gondii*-infected rodents – such as increased activity, decreased reaction times and altered personality profiles (Flegr and Hrdý 1994, Webster 2001, Flegr et al. 2002, 2003, Flegr 2007). Furthermore, in a small number of cases, latent *T. gondii* infections in humans may have substantial implications for human health. For example, consistent with a possible impairment in psychomotor performance, individuals with latent toxoplasmosis have been reported to be at a 2.65 times increased risk to be involved in car accidents than the general population (Flegr et al. 2002), a result subsequently replicated by two Turkish groups (Yereli et al. 2006, Kocazeybek et al. 2009). Recent research has also reported that suicide attempters had significantly higher IgG antibody levels to *T. gondii* as compared with patients without a suicide attempt (Arling et al. 2009). Likewise, and of perhaps particular relevance to any consideration of the mechanisms of action involved, is a potential relationship linking *T. gondii* with that of schizophrenia (Torrey and Yolken 2003).

Whilst any association between toxoplasmosis and the development of schizophrenia is likely to occur only in a small proportion of infected individuals, and is applicable to only some cases of schizophrenia, there is a gathering body of convincing evidence that link the two. For instance, both schizophrenia (Cichon et al. 2009) and toxoplasmosis (Johnson et al. 2002) have been demonstrated to have strong familial associations, affecting multiple members of the same family. *Toxoplasma gondii* seroprevalence has also been consistently associated with schizophrenia (Torrey et al. 2000, 2007, Torrey and Yolken 2003, Mortensen et al. 2007, Yolken and Torrey 2008). From over 54 studies published to date which examine this potential association, obtained across a range of countries and epidemiological conditions, all except five reports that individuals with schizophrenia and other psychoses had a higher prevalence of antibodies to *T. gondii* than the controls (Stanley Medical Research Institute website 2010, Torrey et al. 2007). Indeed, there remains 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 ($OR \leq 1.40$) (Purcell et al. 2009). Further examples include that from which 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 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 to uninfected control subjects (Yolken et al. 2001). Likewise, in a recent 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 also 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 (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 did not develop the suicidal feline attraction and altered behavioural profile 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 be at least augmented through their inhibition of *T. gondii* replication, invasion and/or subsequent modulatory impact in infected individuals.

Potential mechanisms of behavioural manipulation

Tissue cysts containing bradyzoite-stage parasites predominate in neural cells (Fig. 1), but are also found in muscle tissue, as well as visceral organs including the lung, kidney and liver, and can form as early as three days post infection inside cells with a relatively thin (i.e. $< 0.5 \mu\text{m}$) wall. Young tissue cysts as small as $5 \mu\text{m}$ may contain as few as two bradyzoites, but as bradyzoites divide by endogeny, cysts enlarge and can contain hundreds of parasites. Whilst it is possible for cysts to remain undetected and without causing obvious harm to the host, a cerebral infection is initiated when tachyzoite-infected macrophages and dendritic cells pass into the brain, permit-

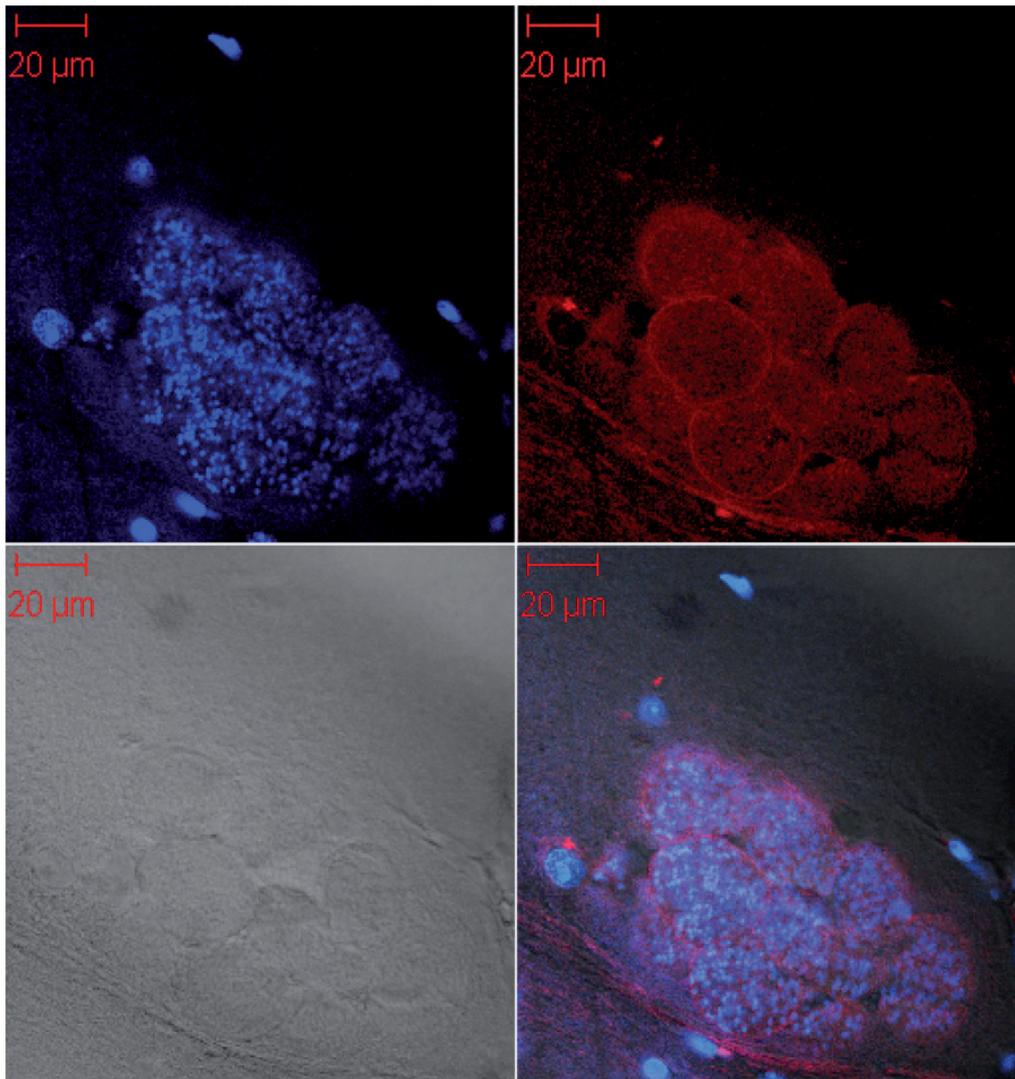


Fig. 1. Brain tissue section from latent *Toxoplasma gondii*-infected mouse (5 weeks post-inoculation with VEG strain) showing neural cell containing multiple cysts. A light image is shown (lower left panel). The nuclei of mouse cells and parasites fluoresce blue with DAPI stain (upper left and lower right) and the cyst walls fluoresce red with lectin-rhodamine stain (upper and lower right panels) with a composite of fluorescence and light (lower right panel). Image produced by Emese Prandovszky, University of Leeds.

ting infection of neurons, and differentiate into encysted bradyzoite stages (Lambert et al. 2009).

The subsequent changes in behaviour observed during latent *T. gondii* infection may be the consequence of a range of indirect and/or direct effects. Whereas indirect effects may involve immune response to infection, direct effects are likely to include the presence of the parasite in the brain or parasite-elicited effects or products. We will consider some of the relative evidence to date for these possibilities below.

Indirect effects: immune response

It is plausible that local immune responses in the brain required to keep *T. gondii* dormant may alter cytokine levels, which could then subsequently influence neuromod-

ulator levels and host behaviour (Novotná et al. 2005). Indeed, a continuous production of proinflammatory cytokines is essential for resistance to acute and chronic infection with *T. gondii* (Aliberti 2005, Miller et al. 2009). Likewise, production of interferon- γ by immune cells is a key component of the body's defences against parasites (Denkers and Gazzinelli 1998), through activation of macrophages and lymphocytes. Simultaneously, the immune response reduces tryptophan levels through the activation of the enzyme indoleamine 2,3-dioxygenase (IDO). Tryptophan starvation is also a key factor in the body's defence against *T. gondii* proliferation (Pfefferkorn et al. 1986, Miller et al. 2009).

It may be of interest to note that, in mice, chronic stimulation of the immune system by inoculation with an at-

tenuated form of another pathogen, that of *Mycobacterium bovis*, induces a sustained elevation in circulating levels of IFN- γ and a chronic activation of IDO, both of which are associated with altered behaviour (Moreau 2005). However, despite its dramatic effects on circulating levels of tryptophan, activation of IDO by cytokines does not appear to modulate host behaviour through alterations in the metabolism of serotonin, at least in terms of the depressive behaviour under investigation in the aforementioned study. Instead it has been proposed that the involvement of IDO in such behavioural alterations involves degradation of tryptophan along the kynurenine pathway, which generates compounds that act as either agonists (for example, quinolinic acid and 3-hydroxy-kynurenine) or antagonists (for example, kynurenic acid) of the NMDA (*N*-methyl-d-aspartate) receptor, the net result from which may be an alteration in glutamatergic neurotransmission (Dantzer et al. 2008). Elevated L-kynurenine, the precursor of kynurenic acid, or inhibition of kynurenine 3-hydroxylase in the brain leads to increased kynurenic acid that directly effects neurotransmitter levels. Kynurenic acid antagonizes $\alpha 7$ nicotinic acetylcholine receptor function and is a NMDA receptor antagonist, and increased kynurenic acid has indeed been found to decrease extracellular dopamine concentration in the rat brain (Wu et al. 2007, Amori et al. 2009).

Direct effects: localisation in the brain

Cysts containing *T. gondii* appear to be distributed across an extremely wide variety of brain regions, with a high frequency in neuronal cells (Webster et al. 2006, Gonzalez et al. 2007, Vyas et al. 2007a). Multifocal lesions and histopathological changes in these cyst-containing regions of the brain have been observed amongst infected humans and rodents. These include inflammatory granulomatous changes of perivascular areas, progressive deposition of necrotic material, and subsequent vesicular occlusion and sclerosis (Werner et al. 1981). Cerebral cysts may cause neurodegeneration in one of several pathogenic mechanisms, such as blockage or interference with the functions of infected neurons, although it has been observed that infected neuronal cells can continue to form synapses. The effects may also be dependent on the cyst burden in the infected animals.

Gross pathology alone is, however, unlikely to account for the observed behavioural changes in the majority of cases, since other important behavioural characteristics in rodents, such as social status and mating success, are left intact (Berdoy et al. 1995, Webster 2007). Indeed, such apparent specificity of the impact of *T. gondii* on the behaviour of its rodent intermediate host at least, towards traits likely to increase predation by a feline definitive host, may provide us with key clues in terms of possible neurobiological mechanistic explanations.

An alternative direct mechanistic explanation for the observed specific behavioural alterations would therefore relate to (despite the widespread distribution of the parasite) potential preferential localisation of *T. gondii* to specific brain areas. Several different areas of the brain have been described as the predominant cyst location including the olfactory bulbs, amygdala, nucleus accumbens, cerebral cortex, cerebellum, medulla oblongata, basal ganglia, septohippocampal and perihippocampal regions (Gonzalez et al. 2007, Vyas et al. 2007a, Di Cristina et al. 2008, Unno et al. 2008). We will consider aspects of the first three here.

The apparent subtle ability of these rats to distinguish between (a) a predatory cat and a non-predatory mammal such as rabbit odours (Berdoy et al. 2000, Webster et al. 2006, Vyas et al. 2007a), and (b) even between contrasting potential predator host species odours, where infected rats have been shown to be attracted to/differentiate between a cat definitive host odour from that of a non-definitive host predator such as mink (Lamberton et al. 2008) or dog (Kannan et al. 2010, this volume), (c) that the specificity of the response appears to be restricted to the middle ranges of cat odour strength (Vyas et al. 2007b), and finally (d) that the observed effects appear to represent an apparent change in the cognitive perception of the host to such cat odour, a 'fatal feline attraction', rather than a destruction of that behavioural trait (Berdoy et al. 2000), all suggests this is not a simple disruption or reduction of, for example, olfaction in general, nor likely to indicate any sweeping changes in the neural substrates involved in bringing about these effects (Lamberton et al. 2008). One could thus speculate, therefore, that *T. gondii* could induce the inactivation of a few olfactory receptors important specifically for cat, but not other species. Indeed, recent studies have shown that, for example, cat odour but not fox odour (trimethylthiazoline) activates accessory specific olfactory and defence-related brain regions in rats (Staples et al. 2008), although the apparent dose sensitivity of the cat odour response may mitigate against this potential explanation (Vyas et al. 2007b). Alternatively one may speculate some form of *T. gondii*-induced alteration to the emotional 'valence' (how negative or positive an emotional response) of cat odour by brain rewiring (Lamberton et al. 2008). Indeed, it is known that each glomerulus in the olfactory bulb represents a single species of odorant receptor (Buck and Axel 1991). Recent studies, aimed to elucidate how odour maps are interpreted in the brain, found that mutant dorsal-zone-depleted mice with the dorsal domain of their olfactory bulb devoid of glomerular structures (although second-order neurons were present in the vacant areas), generated by ablating olfactory sensory neurons, lacked innate responses to the aversive odorant of feline (snow leopard)-urine-treated stimuli, even though they were capable of detecting them.

Furthermore, these mutant mice could be conditioned for aversion to the feline urine with their remaining glomeruli, which indicates that, at least in these rodents, aversive information is received by the olfactory bulb through separate sets of glomeruli, those dedicated for innate and those for learned responses (Kobayakawa et al. 2007).

Areas of the limbic regions of the brain also appear plausible candidates for the impact of *T. gondii* through preferential localisation. Indeed, it has been suggested that activation of the central amygdala may mediate species specific stimuli-specific fear, whereas somewhat less explicit information, such as that produced by exposure to a threatening environment for several minutes, may activate the bed nucleus of the stria terminalis (Davies 1998). Although in those studies standard psychometric behavioural assays, such as through the use of swim/learned helplessness and/or electric shock behavioural assays for example, may have evoked generalized anxiety, the handling of the rats in stress-minimized environments in the aforementioned *T. gondii* studies (Webster 1994, Webster et al. 1994, 2006, Berdoy et al. 1995, 2000, Lamberton et al. 2008), in contrast, may be more highly specific, and may thereby suggest activation of the amygdala is more likely than the bed nucleus of the stria terminalis (Davies 1998). Likewise, other studies have shown that the administration of corticotropin-releasing factor (CRF) receptor antagonists into the central amygdala of rats reduces several fear-related behavioural responses (Koob and Heinrichs 1999). Furthermore, it has been shown that 'risk' seeking rats, which are less anxious in black-white box and elevated-plus maze behavioural trials, have lower basal levels of CRF mRNA in the central amygdala in contrast to their counterparts (Kabbaj et al. 2000). Oppositely, fawn-hooded rats have been shown to exhibit more stress-induced fear (freezing) than Wistar rats and they have higher levels of CRF mRNA in their central amygdala (Altemus et al. 1995). One could therefore speculate that a predilection of *T. gondii* for the amygdala could influence these behavioural traits, in part through lowering of an individual's CRF levels. Indeed, the reaction by potential prey to cat stimuli is used to study the neurological basis of anxiety and the mechanisms of anxiolytic (anxiety relieving) drugs, and such studies have found that also blocking the normally anxiogenic NMDA receptors in the amygdala causes laboratory rats to 'fearlessly' approach areas treated with cat urine (Adamec et al. 1999), lending further support to the potential involvement of this brain locality in the alteration of host behaviour by *T. gondii*.

The reports of high *T. gondii* concentrations in the nucleus accumbens of infected rats (Gonzalez et al. 2007) may also be of key interest here, in terms of both elucidating the potential mechanism of action and its subsequent impact upon the behaviour of both the parasite's rodent and indeed human hosts (see also Vyas and Sapolsky

2010, this volume). The nucleus accumbens is a collection on neurons within the striatum which is thought to play an important role in, amongst other traits, reward, pleasure and fear (Schwienbacher et al. 2004). Furthermore, there is a well-described projection from the retrohippocampus subiculum and entorhinal cortex to the nucleus accumbens that is involved in the control of psychomotor behaviour, and is implicated in the aetiology of schizophrenia (Gray et al. 1991). Studies using rats have reported increases in extracellular dopamine in the nucleus accumbens following infusion of the excitotoxin *N*-methyl-D-aspartate (NMDA) into the retrohippocampus projection. Likewise, inhibition of γ -aminobutyric acid (GABA) receptors following similar administration of bicuculline was reported to increase dopamine in the nucleus accumbens, accompanied by a long-lasting increase in dopamine metabolism. Such results have been proposed to provide a mechanistic explanation whereby a primary insult in the temporal cortex can produce a hyperdopaminergic state (Mitchell et al. 2000), and thus one could perhaps speculate a similar hyperdopaminergic state may be produced as a consequence of *T. gondii* cysts presence/insult in this region.

Nevertheless, despite all such plausible explanations, unless some form of differential localisation specificity or 'seeking' has evolved on the part of the parasite, and/or a total 'saturation' of all brain regions with invading *T. gondii* occurs, one would suspect that such a direct effect would require serendipitous infection of a particular brain area, such as the amygdala, to induce the behavioural change, which may be difficult to resolve with the observed specificity of manipulation observed. Furthermore, the very consistency in the specific behavioural responses evoked between individuals, at least between infected rodents, and between different studies, would similarly appear to necessitate a high degree of serendipitous specific localisation of parasite cysts by seeking or saturation. Therefore one may suspect that any such localisation may work in conjunction with other direct effects on the neural system as described below.

Direct effects: neurotransmitter modulation

Neuromodulation may represent an ideal mechanism whereby *T. gondii* can influence, at least in part, the expression of host behaviour (Blanchard et al. 1990, Berdoy et al. 2000, Torrey and Yolken 2003, Webster et al. 2006). Under healthy conditions, corticosteroids, for example, mediate behavioural adaptation via central mineralocorticoid receptor and glucocorticoid receptor mechanisms (Korte 2001). It is important to realize that such corticosteroids do not, however, specifically regulate emotional behaviour and physiology. Rather they induce chemical changes in particular sets of neurons, making certain behavioural and physiological outcomes more likely in

a certain context in time, as a result of the strengthening or weakening of a particular neural pathway (Korte 2001).

Exposure to cat and rat odour has been demonstrated to cause long-term changes in rodent behaviour that can be observed in the absence of subsequent exposures (Blanchard et al. 2001, Adamec et al. 2005, Staples et al. 2005). For example, exposure to cat odour produces a long-term increase in anxiety when measured in elevated plus-maze (Adamec et al. 2005). This increase in unconditioned fear is associated with, not only lasting changes in amygdalar neurotransmission, but also in stress hormone secretion in brain regions related to regulation of stress (Adamec et al. 2006). The feline attraction in *T. gondii*-infected rodents when similarly exposed to cat odours suggests that the parasite may be able to somehow alter such neurotransmission and neuromodulatory pathways (Berdoy et al. 2000, Webster et al. 2006).

The first study that specifically examined the potential impact of *T. gondii* on neurotransmitter levels, in laboratory mice, found no changes in serotonin or 5-hydroxyindoleacetic acid as a result of infection. Norepinephrine, however, showed a 28% decrease in acute but not in chronically infected mice, whilst Homovanillic acid (HVA) showed a 40% increase in acute but not chronic infection. Dopamine was normal in acute infection but showed a 14% rise above uninfected mice in sulfadiazine-treated mice with chronic infection (Stibbs 1985). Raised or disrupted dopamine levels in particular have also been reported in both human *T. gondii* infection (Flegr et al. 2003) and within patients with schizophrenia (Torrey and Yolken 2003, Howes and Kapur 2009).

High levels of horizontal activity have also been associated with increased levels of dopamine in the mesolimbic and nigrostriatal regions. Therefore the hyperactivity of rodents recorded due to *T. gondii* infection (Hutchison et al. 1980b, Webster 1994, 2007) could suggest high dopaminergic activity. Likewise, altered novelty seeking behaviour, as observed in *T. gondii*-infected rodents (Hutchison et al. 1980b, Hay et al. 1983, Webster 1994, Berdoy et al. 1995, Webster 2001), has also been associated with mesolimbic dopaminergic activity and prefrontal cortex activity, which contains a high concentration of D4 dopamine receptors (Kabbaj and Akil 2001, Powell et al. 2003). Recent evidence for this was shown in a Hole board test which measured the length of time mice spent sniffing and head dipping at holes in order to test levels of novelty seeking. Infected mice were recorded to have spent a significantly greater proportion of time head dipping and sniffing at holes compared to uninfected controls (Skallová et al. 2006). Treating rodents with a dopamine selective uptake inhibitor (GBR 12909 1-(2-(bis(4-fluorophenyl)methoxy)-ethyl)-4-(3-phenylpropyl)piperazin) altered the behaviour of mice, suggesting that the effects of *T. gondii* on host behaviour may be directly related to an increase in dopamine (Skallová et al. 2006). Likewise, the

potential role of dopamine was further supported by tests of the effect antipsychotics have on the behavioural effects of *T. gondii* in rats. Without drug treatment, infected rats demonstrated the suicidal feline attraction and altered behaviour described above. Following treatment with either antiparasitic, antipsychotic, or mood stabilisers medications, however, such behaviour was significantly reduced, with the greatest impact produced by the antipsychotic haloperidol (Webster et al. 2006). As haloperidol is a known dopamine D2 antagonist, its superior therapeutic impact in normalizing the behaviour of infected individuals may be speculated to occur through, in part, its ability to directly and indirectly alter dopamine levels. There may also, however, be a contributory antiparasitic component as this antipsychotic has been observed to inhibit *T. gondii* growth or establishment *in vitro* (Jones-Brando et al. 2003) and *in vivo* (Webster et al. 2006).

Furthermore, a recent study indicates that the parasite itself may actually be a source of the neurotransmitter dopamine (Gaskell et al. 2009). Dopamine is synthesised in two steps from its precursor amino acid tyrosine: tyrosine hydroxylase metabolism to produce L-DOPA then decarboxylation of L-DOPA by aromatic L-amino acid decarboxylase to dopamine. In some cells, dopamine is further metabolised to norepinephrine by dopamine beta-hydroxylase. Through screening of the parasite's genome, *T. gondii* was found to encode a protein with high homology to the tyrosine hydroxylases found in mammals and other neural-containing multicellular organisms. This enzyme, not previously described in protozoa, represents the rate-limiting step in dopamine synthesis and is a member of the highly conserved family of aromatic amino acid hydroxylases whose members include enzymes for synthesis of tyrosine and 5-hydroxytryptophan, from phenylalanine and tryptophan, respectively. Recombinant expression of the catalytic domain of the parasite enzyme showed catalytic activities similar to mammalian enzymes. The *T. gondii* ortholog was found to synthesise L-DOPA, precursor to dopamine, as well as tyrosine. Hence the *T. gondii* is unique in possessing both tyrosine hydroxylase and phenylalanine hydroxylase activity with a bias towards L-DOPA synthesis and can metabolise phenylalanine to L-DOPA. L-DOPA is known to be rapidly metabolised to dopamine by aromatic L-amino acid decarboxylase in dopaminergic neurons and packaged into vesicles. Importantly, the parasite enzyme cannot metabolise tryptophan to the serotonin precursor 5-hydroxytryptophan and is not a tryptophan hydroxylase. Moreover, *T. gondii* was found to possess two nearly identical genes encoding this tyrosine hydroxylase, with the same kinetic properties yet differing in their timing of expression. One copy of the gene is constitutively expressed across the parasite life cycle and one copy is expressed at high levels during the brain and muscle cyst forming stages. A putative signal sequence and immunohistochemistry suggest the enzyme

is secreted out of parasites (McConkey, G., unpublished observations). One scenario to explain the differential expression and two catalytic activities of the two genes is that this enzyme may fulfil multiple biological functions through the parasite life cycle. Hence the enzyme may supply the parasite with tyrosine as a nutrient (although the reason for its extracellular location is unclear) during periods of growth (i.e. tachyzoite stages) whereas the enzyme may serve to synthesise L-DOPA during quiescent (i.e. bradyzoite stages). These possible roles and how they would be regulated will be interesting to resolve through future research.

Aromatic amino acid hydroxylases have not been found in any other apicomplexan, except a gene with homology to the *T. gondii* genes is found in bioinformatic searches of the closely related *Neospora caninum*. Indeed, this too is fascinating as the indirectly-transmitted life cycle of *N. caninum*, just like *T. gondii*, includes intermediate and definitive hosts and consists of an asexual and a sexual cycle – in the case of *N. caninum*, the asexual reproduction occurs in intermediate hosts such as cattle, sheep, goats and horses whilst the sexual cycle only occurs in the dog or coyote definitive hosts. Whilst a canine definitive host may, potentially, place less selective pressures on a parasite to increase predation rates in its intermediate host, relative to a feline definitive host, it may thus be very interesting to also contrast and compare potential neurological and behavioural changes in *Neospora*-infected individuals with that of *T. gondii*-infected individuals. Whatever the case, it is possible that this enzyme represents a unique feature of the tissue cyst forming apicomplexan parasites. Such results thereby provide a highly promising potential mechanistic explanation for the behavioural observations observed and provide a significant contribution to the literature on parasite manipulation of their intermediate hosts.

Conclusions and next steps

In summary, extensive studies carried out under different experimental conditions suggest that *T. gondii* changes the behaviour of rodents so as to make them more likely to be predated on by cats, the parasite's definitive host. Additional studies have demonstrated that the behavioural change induced by *T. gondii* can be partially reversed, or at least prevented, by treatment with some antipsychotic medications, in particular that of haloperidol. From a mechanistic perspective, a prime candidate by which the parasite evokes such behavioural change appears to be through altered dopamine levels – either by, for instance, 'insult' to (via, in part, chronic inflammation), the brain from the presence of *T. gondii* in specific brain regions or networks of regions, and/or through the parasites up-regulating their own dopamine. Any one of these

avenues could involve a subtle tropism of the parasites for structures involved in the neurocircuitry of fear and anxiety, such as the amygdala and/or nucleus accumbens.

As approximately 30–50% of the human population across the world is *T. gondii* seropositive, these findings may have both fundamental and applied implications. Whilst much further research into elucidating the potential mechanisms involved within and between host species is certainly required, the next steps may be to assess any direct association between levels of neurotransmitters and cyst stages of the parasite. For instance, future tests may be able to identify whether the parasite produces dopamine in cyst stages, and under what conditions/brain localisations, and whether this is indeed subsequently influencing the host dopaminergic system, in particular whether this is a contributory factor or sufficient to explain the behavioural alterations observed (Webster and McConkey 2009). A more complicated picture would involve roles of parasite product combined with host response factors. The results obtained should have direct implications for the potential understanding of the mechanisms involved in *T. gondii*-induced behavioural alterations and hence also the role of the parasite in some cases of human schizophrenia and affective disorder. There have been, for example, more than 6,700 articles and 181,000 citations on the topic of 'dopamine and schizophrenia' between 1991–2009 (Howes and Kapur 2009). Likewise, new developments, such as the recently proposed version III of the dopamine hypothesis of schizophrenia, provide comprehensive frameworks encompassing a broad range of environmental and genetic risk factors that may converge neurochemically (Howes and Kapur 2009), although *T. gondii* (and other potential neurotropic agents) may surely still need to be incorporated into this framework. Similarly, we may expect to see exciting new developments as a result of advancing genome-wide association studies (GWAS) aimed to identify common genetic and epigenetic risk factors that influence health and disease, in this case in terms of identifying potential predisposing genes that interact with *T. gondii* to determine the outcome of the infection (Jamieson et al. 2009). As a consequence of such further interdisciplinary research, a comprehensive understanding of the impact *T. gondii* exerts on host neurology, and the mechanisms involved, should raise both theoretical and applied implications in terms of toxoplasmosis as well as potentially providing further understanding of the neurobiology associated with behaviour in general.

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REFERENCES

- ADAMEC R.E., BLUNDELL J., BURTON P. 2005: Neural circuit changes mediating lasting brain and behavioral response to predator stress. *Neurosci. Biobehav. Rev.* 29: 1225–1241.
- ADAMEC R.E., BLUNDELL J., BURTON P. 2006: Relationship of the predatory attack experience to neural plasticity, pCREB expression and neuroendocrine response. *Neurosci. Biobehav. Rev.* 30: 356–375.
- ADAMEC R.E., BURTON P., SHALLOW T., BUDGELL J. 1999: NMDA receptors mediate lasting increases in anxiety-like behaviour produced by the stress of predator exposure – implications for anxiety associated with post-traumatic stress disorder. *Physiol. Behav.* 65: 723–737.
- ALIBERTI J. 2005: Host persistence: exploitation of anti-inflammatory pathways by *T. gondii*. *Nat. Rev. Immunol.* 5: 162–170.
- ALTEMUS M., SMITH M.A., DIEP V., AULAKH C.S., MURPHY D.L. 1995: Increased mRNA for corticotrophin releasing hormone in the amygdala of fawn-hooded rats, a potential animal model of anxiety. *Anxiety* 1: 252–257.
- AMORI L., WU H.Q., MARINOZZI M., PELLICCIARI R., GUIDETTI P., SCHWARCZ R. 2009: Specific inhibition of kynurenate synthesis enhances extracellular dopamine levels in the rodent striatum. *Neuroscience* 159: 196–203.
- ARLING T.A., YOLKEN R.H., LAPIDUS M., LANGENBERG P., DICKERSON F.B., ZIMMERMAN B.S., BALIS T., CABASSA J.A., SCRANDIS D.A., TONELLI L.H., POSTALOACHE T.T. 2009: *Toxoplasma gondii* antibody titers and history of suicide attempts in patients with recurrent mood disorders. *J. Nerv. Ment. Dis.* 197: 905–908.
- BERDOY M., WEBSTER J.P., MACDONALD D.W. 1995: Parasite altered behaviour: is the effect of *Toxoplasma gondii* on *Rattus norvegicus* specific? *Parasitology* 111: 403–409.
- BERDOY M., WEBSTER J.P., MACDONALD D.W. 2000: Fatal attraction in *Toxoplasma*-infected rats: a case of parasite manipulation of its mammalian host. *Proc. R. Soc. Lond., Ser. B*, 267: 1591–1594.
- BLANCHARD R.J., BLANCHARD D.C., RODGERS J., WEISS S.M. 1990: The characterisation and modelling of antipredator defensive behavior. *Neurosci. Biobehav. Rev.* 14: 463–472.
- BLANCHARD R.J., YANG M., LI C.I., GERVAO A., BLANCHARD D.C. 2001: Cue and context conditioning of defensive behaviors to cat odor stimuli. *Neurosci. Biobehav. Rev.* 25: 587–595.
- BUCK L., AXEL R. 1991: A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65: 175–187.
- CARME B., DEMAR M., AJZENBERG D., DARDE M.L. 2009: Severe acquired toxoplasmosis caused by wild cycle of *Toxoplasma gondii*, French Guiana. *Emerg. Infect. Dis.* 15: 656–658.
- CICHON S., CRADDOCK N., DALY M., FARAONE S.V., GEJMAN P.V., KELSOE J., LEHNER T., LEVINSON D.F., MORAN A., SKLAR P., SULLIVAN P.F. (PSYCHIATRIC GWAS CONSORTIUM COORDINATING COMMITTEE) 2009: Genomewide association studies: history, rationale, and prospects for psychiatric disorders. *Am. J. Psychiatr.* 166: 540–556.
- DANTZER R., O'CONNOR J.C., FREUND G.G., JOHNSON R.W., KELLEY K.W. 2008: From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9: 46–56.
- DAVIES M. 1998: Are different parts of the extended amygdala involved in fear versus anxiety? *Biol. Psychiatr.* 44: 1239–1247.
- DENKERS E.Y., GAZZINELLI R.T. 1998: Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin. Microbiol. Rev.* 11: 569–588.
- DI CRISTINA M., MAROCCO M., GALIZI R., PROIETTI C., SPACCAPELO R., CRISANTI A. 2008: Temporal and spatial distribution of *Toxoplasma gondii* differentiation into bradyzoites and tissue cyst formation *in vivo*. *Infect. Immun.* 76: 3491–3501.
- FLEGR J. 2007: Effects of *Toxoplasma gondii* on human behaviour. *Schizophr. Bull.* 33: 757–760.
- FLEGR J., HAVLÍČEK J., KODYM P., MALÝ M., ŠMAHEL Z. 2002: Increased risk of traffic accidents in subjects with latent toxoplasmosis: a retrospective case-control study. *BMC Infect. Dis.* 2: 1–6.
- FLEGR J., HRDÝ I. 1994: Influence of chronic toxoplasmosis on some human personality factors. *Folia Parasitol.* 41: 121–126.
- FLEGR J., PREISS M., KLOSE J., HAVLÍČEK J., VITÁKOVÁ M., KODYM P. 2003: Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite *Toxoplasma gondii*. *Biol. Psychol.* 63: 253–268.
- GASKELL E.A., SMITH J.E., PINNEY J.W., WESTHEAD D.R., MCCONKEY G.A. 2009: A unique dual activity amino acid hydroxylase in *Toxoplasma gondii*. *PLoS ONE* 4: e4801.
- GONZALEZ L.E., ROJNIK B., URREA F., URDANETA H., PETROSINO P., COLASANTE C., PINO S., HERNANDEZ L. 2007: *Toxoplasma gondii* infection lower anxiety as measured in the plus-maze and social interaction tests in rats: a behavioral analysis. *Behav. Brain Res.* 177: 70–79.
- GRAY J.A., FELDON J., RAWLINS J.N.P., HEMSLEY D.R., SMITH A.D. 1991: The neuropsychology of schizophrenia. *Behav. Brain Sci.* 14: 1–84.
- HAY J., AITKEN P.P., HAIR D.M., HUTCHISON W.M., GRAHAM D.I. 1984: The effect of congenital *Toxoplasma* infection on mouse activity and relative preference for exposed areas over a series of trials. *Ann. Trop. Med. Parasitol.* 78: 611–618.
- HAY J., AITKEN P.P., HUTCHISON W.M., GRAHAM D.I. 1983: The effect of congenital and adult-acquired *Toxoplasma* infections on activity and responsiveness to novel stimulation in mice. *Ann. Trop. Med. Parasitol.* 77: 483–495.
- HOWES O.D., KAPUR S. 2009: The dopamine hypothesis of schizophrenia: version III – the final common pathway. *Schizophr. Bull.* 35: 549–562.
- HUTCHISON W.M., AITKEN P.P., WELLS B.W.P. 1980a: Chronic *Toxoplasma* infections and familiarity-novelty discrimination in the mouse. *Ann. Trop. Med. Parasitol.* 74: 145–150.
- HUTCHISON W.M., BRADLEY M., CHEYNE W.M., WELLS B.W.P., HAY J. 1980b: Behavioural abnormalities in *Toxoplasma*-infected mice. *Ann. Trop. Med. Parasitol.* 74: 507–510.
- HUTCHISON W.M., DUNACHIE J.F., SHIM J., WORK K. 1969: The life cycle of *Toxoplasma gondii*. *Br. Med. J.* 4: 806.
- JAMIESON S.E., CORDE H., PETERSEN E., MCLEOD V.R., GILBERT R.E., BLACKWELL J.M. 2009: Host genetic and epigenetic factors in toxoplasmosis. *Mem. Inst. Oswaldo Cruz* 104: (online ahead of print).
- JOHNSON J., SUZUKI Y., MACK D. 2002: Genetic analysis of influences on survival following *Toxoplasma gondii* infection. *Int. J. Parasitol.* 32: 179–185.
- JONES-BRANDO L., TORREY F., YOLKEN R. 2003: Drugs used in the treatment of schizophrenia and bipolar disorder inhibit the replication of *Toxoplasma gondii*. *Schizophr. Res.* 62: 237–244.
- JONES J.L., LOPEZ A., WILSON M., SCHULKIN J., GIBBS R. 2001: Congenital toxoplasmosis: a review. *Obstet. Gynecol. Surv.* 56: 296–305.
- KABBAJ M., AKIL H. 2001: Individual differences in novelty-seeking behavior in rats, a *c-fos* study. *Neuroscience* 106: 535–545.

- KABBAJ M., DEVINE D.P., SAVAGE V.R., AKIL H.J. 2000: Neurobiological correlates of individual differences in novelty-seeking behaviour in the rat, differential expression of stress-related molecules. *Neuroscience* 20: 6983–6988.
- KANNAN G., MOLDOVAN K., XIAO J.-C., YOLKEN R.H., JONES-BRANDO L., PLETNIKOV M.V. 2010: Parasite strain-dependent effects of *Toxoplasma gondii* on mouse behaviour. *Folia Parasitol.* 57: 151–155.
- KOBAYAKAWA K., KOBAYAKAWA R., MATSUMOTO H., OKA Y., IMAI T., IKAWA M., OKABE M., IKEDA T., ITOHARA S., KIKUSUI T., MORI K., SAKANO H. 2007: Innate versus learned odour processing in the mouse olfactory bulb. *Nature* 450: 503–510.
- KOCAZEYBEK B., ONER Y.A., TURKSOY R., BABUR C., CAKAN H., SAHIP N., UNAL A., OZASLAN A., KILIC S., SARIBAS S., ASLAN M., TAYLAN A., KOC S., DIRICAN A., UNER H.B., OZ V., ERTEKIN C., KUCUKBASMACI O., TORUN M.M. 2009: Higher prevalence of toxoplasmosis in victims of traffic accidents suggest increased risk of traffic accident in *Toxoplasma*-infected inhabitants of Istanbul and its suburbs. *Forensic Sci. Int.* 187: 103–108.
- KOOB G.F., HEINRICH S. 1999: A role for corticotropin-releasing factor and urocortin in behavioural responses to stressors. *Brain Res.* 848: 141–145.
- KORTE S.M. 2001: Corticosteroids in relation to fear, anxiety and psychopathology. *Neurosci. Biobehav. Rev.* 25: 117–142.
- LAMBERT H., VUTOVA P.P., ADAMS W.C., LORÉ K., BARRAGAN A. 2009: The *Toxoplasma gondii*-shuttling function of dendritic cells is linked to the parasite genotype. *Infect. Immun.* 77: 1679–1688.
- LAMBERTON P.H.L., DONNELLY C.A., WEBSTER J.P. 2008: Specificity of the *Toxoplasma gondii*-altered behaviour to definitive versus non-definitive host predation risk. *Parasitology* 135: 1143–1150.
- LEWEKE F.M., GERTH C.W., KOETHE D., KLOSTERKÖTTER J., RUSLANOVA I., KRIVOGORSKY B., TORREY E.F., YOLKEN R.H. 2004: Antibodies to infectious agents in individuals with recent onset schizophrenia. *Eur. Arch. Psychiat. Clin. Neurosci.* 254: 4–8.
- MILLER C.M., BOULTER N.R., IKIN R.J., SMITH N.C. 2009: The immunobiology of the innate response to *Toxoplasma gondii*. *Int. J. Parasitol.* 39: 23–39.
- MITCHELL S.N., YEE B.K., FELDON J., GRAY J.A., RAWLINS J.N.P. 2000: Activation of the retrohippocampal region in the rat causes dopamine release in the nucleus accumbens: disruption by fornix section. *Eur. J. Pharmacol.* 407: 131–139.
- MOREAU M. 2005: Bacille Calmette-Guerin inoculation induces chronic activation of peripheral and brain indoleamine 2, 3-dioxygenase in mice. *J. Infect. Dis.* 192: 537–544.
- MORTENSEN P.B., NØRGAARD-PEDERSEN B., WALTOFT B.L., SØRENSEN T.L., HOUGAARD D., TORREY E.F., YOLKEN R.H. 2007: *Toxoplasma gondii* as a risk factor for early-onset schizophrenia: analysis of filter paper blood samples obtained at birth. *Biol. Psychiat.* 61: 688–693.
- NIEBUHR D.W., MILLIKAN A.M., COWAN D.N., YOLKEN R., LI Y.Z., WEBER N.S. 2008: Selected infectious agents and risk of schizophrenia among U.S. military personnel. *Am. J. Psychiat.* 165: 99–106.
- NOVOTNÁ M., HANUSOVA J., KLOSE J., PREISS M., HAVLICEK J., ROUBALOVÁ K., FLEGR J. 2005: Probable neuroimmunological link between *Toxoplasma* and cytomegalovirus infections and personality changes in the human host. *BMC Infect. Dis.* 5: e54.
- PFEFFERKORN E., ECKEL M., REBHUN S. 1986: Interferon-gamma suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. *Mol. Biochem. Parasitol.* 20: 215–224.
- POWELL S.B., PAULUS M.P., HARTMAN D.S., GODEL T., GEYER M.A. 2003: RO-10-5824 is a selective dopamine D4 receptor agonist that increases novel object exploration in C57 mice. *Neuropharmacology* 44: 473–481.
- PURCELL S., WRAY N., STONE J., VISSCHER P., O'DONOVAN M., SULLIVAN P., SKLAR P. (INTERNATIONAL SCHIZOPHRENIA CONSORTIUM) 2009: Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460: 784–752.
- SCHWIENBACHER I., FENDT M., RICHARDSON R., SCHNITZLER H.-U. 2004: Temporary inactivation of the nucleus accumbens disrupts acquisition and expression of fear-potentiated startle in rats. *Brain Res.* 1027: 87–93.
- SKALLOVÁ A., KODYM P., FRYNTA D., FLEGR J. 2006: The role of dopamine in *Toxoplasma*-induced behavioural alterations in mice: an ethological and ethopharmacological study. *Parasitology* 133: 1–11.
- STAPLES L.G., HUNT G.E., CORNISH J.L., MCGREGOR I.S. 2005: Neural activation during cat odor-induced conditioned fear and “trial 2” fear in rats. *Neurosci. Biobehav. Rev.* 29: 1265–1277.
- STAPLES L.G., MCGREGOR I.S., APPELBACH R., HUNT G.E. 2008: Cat odor, but not trimethylthiazoline (fox odor), activates accessory olfactory and defense-related brain regions in rats. *Neuroscience* 151: 937–947.
- STIBBS H.H. 1985: Changes in brain concentrations of catecholamines and indoleamines in *Toxoplasma gondii* infected mice. *Ann. Trop. Med. Parasitol.* 79: 153–157.
- TORREY E.F., BARTKO J.J., LUN Z.R., YOLKEN R.H. 2007: Antibodies to *Toxoplasma gondii* in patients with schizophrenia: a meta-analysis. *Schizophr. Bull.* 33: 729–736.
- TORREY E.F., RAWLINGS R., YOLKEN R.H. 2000: The antecedents of psychoses: a case-control study of selected risk factors. *Schizophr. Res.* 46: 17–23.
- TORREY E.F., YOLKEN R.H. 2003: *Toxoplasma gondii* and schizophrenia. *Emerg. Infect. Dis.* 9: 1375–1380.
- UNNO A., SUZUKI K., XUAN X., NISHIKAWA Y., KITO H., TAKASHIMA Y. 2008: Dissemination of extracellular and intracellular *Toxoplasma gondii* tachyzoites in the blood flow. *Parasitol. Int.* 57: 515–518.
- VYAS A., KIM S.K., SAPOLSKY R.M. 2007b: The effects of toxoplasma infection on rodent behavior are dependent on dose of the stimulus. *Neuroscience* 148: 342–348.
- VYAS A., SAPOLSKY R.M. 2010: Manipulation of host behaviour by *Toxoplasma gondii*: what is the minimum a proposed proximate mechanism should explain? *Folia Parasitol.* 57: 88–94.
- VYAS A., SEON-KYEONG K., GIACOMINI N., BOOTHROYD J.C., SAPOLSKY R.M. 2007a: Behavioural changes induced by *Toxoplasma* infection of rodents are highly specific to aversion of cat odours. *Proc. Natl. Acad. Sci. U. S. A.* 104: 6442–6447.
- WEBSTER J.P. 1994: The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology* 109: 583–589.
- WEBSTER J.P. 2001: Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbes Infect.* 3: 1–9.
- WEBSTER J.P. 2007: The effect of *Toxoplasma gondii* on animal behavior: playing cat and mouse. *Schizophr. Bull.* 33: 752–756.
- WEBSTER J.P., BRUNTON C.F.A., MACDONALD D.W. 1994: Effect of *Toxoplasma gondii* on neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology* 109: 37–43.
- WEBSTER J.P., LAMBERTON P.H.L., DONNELLY C.A., TORREY E.F. 2006: Parasites as causative agents of human affective

- disorders?: The impact of anti-psychotic and anti-protozoan medication on *Toxoplasma gondii*'s ability to alter host behaviour. Proc. R. Soc. Lond., Ser. B, 273: 1023–1030.
- WEBSTER J.P., MCCONKEY G.A. 2009: The effect of *Toxoplasma gondii* on host behaviour: studies on mechanism of action using psychotropics, anti-parasite medication and parasite genes. Eur. Neuropsychopharmacol. 19 (Suppl. 3): S179.
- WERNER H., MASIHI K.N., SENK U. 1981: Latent *Toxoplasma* infection as a possible risk factor for CNS disorders. Zentralbl. Bakteriol. Mikrobiol. Hyg. I. Abteilung Originale A, 250: 368–375.
- WU H.Q., RASSOULPOUR A., SCHWARCZ R. 2007: Kynurenic acid leads, dopamine follows: a new case of volume transmission in the brain? J. Neur. Transm. 114: 33–41.
- YERELI K., BALCIOĞLU I., ÖZBILGIN A. 2006: Is *Toxoplasma gondii* a potential risk for traffic accidents in Turkey? Forensic Sci. Int. 163: 34–37.
- YOLKEN R.H., BACHMANN S., ROUSLANOVA I., LILLEHOJ E., FORD G., TORREY E.F., SCHROEDER J. 2001: Antibodies to *Toxoplasma gondii* in individuals with first-episode schizophrenia. Clin. Infect. Dis. 32: 842–844.
- YOLKEN R.H., TORREY E.F. 2008: Are some cases of psychosis caused by microbial agents? A review of the evidence. Mol. Psychiat. 13: 470–479.

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