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REVIEW

Toxoplasma gondii infection and behaviour – location, location, location?

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Summary

Parasite location has been proposed as an important factor in the behavioural changes observed in rodents infected with the protozoan *Toxoplasma gondii*. During the chronic stages of infection, encysted parasites are found in the brain but it remains unclear whether the parasite has tropism for specific brain regions. Parasite tissue cysts are found in all brain areas with some, but not all, prior studies reporting higher numbers located in the amygdala and frontal cortex. A stochastic process of parasite location does not, however, seem to explain the distinct and often subtle changes observed in rodent behaviour. One factor that could contribute to the specific changes is increased dopamine production by *T. gondii*. Recently, it was found that cells encysted with parasites in the brains of experimentally infected rodents have high levels of dopamine and that the parasite encodes a tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of this neurotransmitter. A mechanism is proposed that could explain the behaviour changes due to parasite regulation of dopamine. This could have important implications for *T. gondii* infections in humans.

Key words: brain, dopamine, manipulation, neurotransmitter, parasite.

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Introduction

In Real Estate, the axiom is that ‘location’ is the most important quality for a property or, as the saying goes, it’s ‘the three things that matter’. The potential role of location of parasites in the host behavioural changes induced by infection will be considered in this review. *Toxoplasma gondii* is one amongst several parasites that have been shown to alter the behaviour of their host and have been found in the brain. These, often very distantly related, parasites have separately evolved mechanisms to alter the behaviour of their host organism. Classic examples include those involving the alteration of host behaviour as a means to increase transmission of the parasite, such as that of the trematode *Dicrocoelium dendriticum*, which induces infected ants to ‘freeze’ at the top of a blade of grass at dusk, enhancing their consumption by grazing ruminants, or another trematode, *Euhaplorchis californiensis*, which alters the behaviour of the California killifish, increasing the likelihood of its consumption by birds, the parasite’s definitive host (Moore, 2013; Lafferty and Shaw, 2013). Another example is the amphipod *Gammarus*, which becomes attracted to light when infected with *Polymorphus paradoxus*, an Acanthocephalan, increasing consumption by the parasite’s definitive vertebrate host (Helluy, 2013). Understanding these alterations is complicated by the fact that each parasite exerts multiple effects on it host that may result from multiple parasite mechanisms (Cézilly et al., 2013).

Toxoplasma gondii, as a protozoan parasite that forms tissue cysts in the brains of warm-blooded animals and manipulates the behaviour of infected rodents, could be considered as an example of an extended phenotype; a concept proposed by Dawkins in 1982 (Dawkins, 1999). Central to this concept is the idea that the behaviour of an animal will be inclined to maximize the survival of the genes governing that behaviour, whether or not those genes belong to the animal performing the behaviour. The first clear example of a gene fitting this requirement was recently identified in the baculovirus *Lymantria dispar* nucleopolyhedrovirus, which infects European gypsy moth *L. dispar* caterpillars, causing them to climb to the tree-tops where they die and liquefy, releasing thousands of viral particles to infect other naive caterpillars (Hoover et al., 2011). Applying the concept of the extended phenotype to *T. gondii* and rodents means *T. gondii* would be expected to possess a gene or genes that increase predation of the intermediate host by the parasite’s definitive host, the cat, favouring the survival of these genes and *T. gondii* itself. These genes may affect *T. gondii* localization in the brain and/or other aspects of its physiology to achieve the behavioural manipulation of rodents that is to its advantage. This thereby raises the question of whether there is any tropism of *T. gondii* for a specific location in the brain that could mediate the distinct behavioural alterations observed in infected rodents? This paper reviews findings on the location of *T. gondii* cysts in the brain and their possible correlation with observed behavioural changes during infection, and discusses how dopamine could affect host behaviour.

Behavioural alterations in infected animals and humans

Striking changes in behaviour have been observed in rodents infected with *T. gondii*. Infected rodents show a reduction in their innate aversion to cat odour, and though both infected and uninfected rats preferred an area that contained their own scent, the infected rats showed a preference for the cat odour area over an area with rabbit scent (a non-predator), while the opposite was true for the uninfected rats (Berdoy et al., 2000). The innate aversion to cat odour appears to become a potentially fatal feline attraction in *T. gondii*-infected rats and mice (Berdoy et al., 2000; Vyas et al.,

2007). As well as this 'fatal feline attraction', *T. gondii* infection also leads to increased activity (Hay et al., 1985; Webster, 1994) and decreased neophobic behaviour in rats (Berdoy et al., 1995; Webster et al., 1994a). The changes in host behaviour appear specific, as described in the accompanying article in this issue (Webster and McConkey, 2013) (see also Webster and McConkey, 2010). It has been reported that in rats with *T. gondii* infection, the attraction to cats is potentially modulated, at least in part, through the sexual arousal pathways, with activation of the posterodorsal medial amygdala to a level comparable to that observed during exposure to an oestrous female rat, indicating that the parasite may hijack this sexual attraction mechanism in order to override the rat's innate aversion to cat odour (House et al., 2011).

To understand the importance and the selective advantages of the host behavioural changes that may have contributed to its evolution in *T. gondii*, the parasite's life cycle must be considered. *Toxoplasma gondii* is an intracellular protozoan parasite and its definitive host is the cat family (Felidae), being the sole animal in which *T. gondii* can undergo sexual reproduction (Hutchinson, 1966). *Toxoplasma gondii* can infect many warm-blooded animals as intermediate or secondary hosts, including humans in the latter case. In these intermediate/secondary hosts, *T. gondii* replicates rapidly in tissues (as the tachyzoite form) until suppressed by the host's immune response. Some parasites differentiate into slowly replicating bradyzoite forms as tissue cysts. The formation of such tissue cysts, predominantly in the brain and muscles, permits *T. gondii* to generate a long-standing infection until the point of predation by a member of the cat family and the chance to complete its life cycle. With this in mind, it is easy to see why *T. gondii* manipulation of rodent behaviour is advantageous to the transmission of the parasites, increasing the chances of predation and completion of the parasite life cycle.

Although most infections in humans are considered asymptomatic, changes in human behaviour have been associated with *T. gondii* infection in some studies (Flegr, 2013). It should be noted that chronic infection is based on seroprevalence as it is not possible to detect encysted *T. gondii* in humans, except post-mortem. Correlations have been observed between seroprevalence for *T. gondii* and increased risk of traffic accidents as well as extraversion and less conscientiousness ($P<0.03$) in a recent study of 47 infected and 276 uninfected students (see Flegr, 2013).

Toxoplasma and neurological disorders

Toxoplasma gondii has been correlated with several neurological disorders. Meta-analysis of 38 studies of *T. gondii* seroprevalence and schizophrenia found the likelihood of infection in schizophrenia patients to be 2.7 times greater than that in the general population [odds ratio OR=2.7, $P=0.005$ (Arias et al., 2011); OR=2.73, $P<0.000001$ (Torrey et al., 2007)]. The impact of prenatal infection on later development of schizophrenia is unclear although high maternal levels of *T. gondii* antibodies during pregnancy have been associated with schizophrenia in the offspring, while there was no significant effect found for moderate levels of antibodies (Brown, 2011; Brown et al., 2005). Intriguingly, there are several areas of similarity between the epidemiology of toxoplasmosis and schizophrenia, with the two having comparable ages of onset and socioeconomic demographics (Yolken et al., 2009).

There is a significant association of *T. gondii* with personality disorders in psychiatric patients (Hinze-Selch et al., 2010). In particular, *T. gondii* seroprevalence has been associated with suicide. In recent studies, *T. gondii* antisera levels were higher in individuals who had attempted suicide than in a control group ($P<0.004$); infection was associated with suicide in post-menopausal women ($P<0.05$); and in patients with recurrent mood disorders, those attempting suicide had higher *T. gondii* antibody titres than others ($P<0.005$) (Arling et al., 2009; Ling et al., 2011; Yagmur et al., 2010). Indeed, suicide rates across 20 European countries correlate with *T. gondii* prevalence (Lester, 2010). A recent large study found an association of *T. gondii* seroprevalence with bipolar disorder (OR=2.4, $P<0.05$) but no correlation between depression and *T. gondii* ($P>0.05$) (Pearce et al., 2012).

Other neurological disorders have been investigated for potential association with *T. gondii*. Increased incidence of seropositivity for *T. gondii* has been reported in cases of obsessive compulsive disorder (OCD) (OR=3.9, $P<0.001$) with 2.4 times higher seroprevalence in patients than in healthy volunteers (Miman et al., 2010b). OCD symptoms in individuals infected with *T. gondii* have been reduced by treatment of the *T. gondii* infection alone (Brynska et al., 2001; Smadja et al., 1995). There are contradictory reports on the association of *T. gondii* with Parkinson's disease as two preliminary studies showed opposing results; Miman and colleagues found that Parkinson's disease patients were significantly more likely to be seropositive for *T. gondii* ($P<0.01$) (Miman et al., 2010a) whilst Celik and colleagues did not find a statistically significant difference (Celik et al., 2010). Parkinsonian symptoms have been reported in patients suffering from both toxoplasmosis and acquired immunodeficiency syndrome (AIDS), and anti-*T. gondii* medication reduced the observed symptoms (Carrazana et al., 1989; Murakami et al., 2000). In a mouse model system, *T. gondii* infection was found to generate sensorimotor deficits in animals without major brain damage or cognitive dysfunction (Gulinello et al., 2010). In addition to these links of *T. gondii* to schizophrenia, Parkinson's disease and OCD, *T. gondii* has been associated with Tourette's syndrome ($P<0.07$) (Krause et al., 2010) and a link with autism spectrum disorders has been speculated (Prandota, 2010). It is intriguing that dysregulation of dopamine has been implicated in all these disorders. Indeed, the 'dopamine hypothesis' is a long-standing paradigm attributing hyperactive dopaminergic signal transduction to the pathology of schizophrenia (Carlsson, 1988; Howes and Kapur, 2009).

These reports of increased proportions of individuals seropositive for *T. gondii* in cases of neurological disorders do not necessarily indicate a causal relationship between the infection and the disorder. This is illustrated by the fact that not all seropositive individuals also have a neurological disorder, and not all cases with a disorder are seropositive. However, the number of reports associating *T. gondii* and disease states combined with the privileged position of *T. gondii* in the central nervous system (CNS) and its ability to modify dopaminergic signalling of host cells as discussed below (Gaskell et al., 2009; Prandovszky et al., 2011) form a strong argument for *T. gondii* at least acting as a contributing factor in any number of these disorders. Several factors may determine whether the infection could lead to a disorder, and indeed which disorder it may lead to, such as combination with other genetic or environmental risk factors, the strain of *T. gondii* parasite with which the individual is infected, the time of the individual's life at which the infection occurs, the severity of the initial acute infection, and the exact number and location of parasite cysts within the brain during the chronic phase of infection. *Toxoplasma gondii* may confer a risk for neurological disorders as a result of damage caused by the initial infection, by the host's immune response to the parasite, or by the continued presence of parasitic cysts in the

CNS; although alleviation of symptoms by treatment of the *T. gondii* infection (Brynska et al., 2001; Murakami et al., 2000; Smadja et al., 1995) implies an ongoing process beyond the initial acute infection.

Immune responses to *T. gondii* infection may affect neurotransmission

Infection with *T. gondii* initiates a strong TH_1 immune response in which interferon-γ (IFN-γ), interleukin-12 (IL-12) and CD8+ T-cells predominate (Munoz et al., 2011). The induction of IFN-γ production is dependent on CD8+ intrinsic IL-12 signalling (Wilson et al., 2008). This induction of IFN-γ is critical in controlling *T. gondii* infection in the CNS, limiting tachyzoite replication and preventing toxoplasmic encephalitis, whilst favouring bradyzoite and cyst formation. To enhance this immune response cascade, *T. gondii* possesses a gene, *profilin*, which induces IL-12 expression (Plattner et al., 2008). It is thought that IFN-γ maintains the latent infection by limiting tachyzoite growth and hence would serve in preventing reactivation of tissue cysts to rapidly replicating stages of infection. IFN-γ mediates cognitive effects as sickness behaviour and has been associated with clinical depression (Maes et al., 2012; McCusker and Kelley, 2013), as exemplified by interferon therapy treatment of cancer and the resultant depressive side effects (Trask et al., 2000; Valentine and Meyers, 2005). These side effects are postulated to arise from IFN-γ-mediated induction of indoleamine-2,3-dioxygenase (IDO) expression (Meyers, 1999) as IDO degrades tryptophan (Saito et al., 1991), the essential precursor of serotonin, and serotonin is involved in depressive disorders. The degradation increases the metabolite kynurenic acid, whose levels have been linked to altered glutamergic transmission *via* NMDA receptor hypo-functioning; such alterations are also observed in schizophrenia (Müller et al., 2011). Thus, alterations induced by *T. gondii*-stimulated IFN-γ may indirectly affect cognitive changes with infection. Indeed, the work of Graeff indicating that serotonin may have a role in innate fear behaviour, such as rodent avoidance of cat odour, supports IFN-γ as potentially affecting the fatal feline attraction in rodents (Graeff et al., 1997). This hypothesis supposes that serotonin levels in infected animals will be lower than those in uninfected animals, but decreases in serotonin have not yet been documented and levels were unchanged in infected mice (Stibbs, 1985) (G.M. and H.L.M., unpublished observations). The lack of impact on serotonin levels may be explained by the opposing effects of other cytokines produced in response to *T. gondii* infection, namely IL-1 and IL-6, which can increase tryptophan levels in all brain regions (Dunn, 2006) or alternatively there are only proximate effects on serotonin in the specific area surrounding the cysts.

The immune responses stimulated by *T. gondii* infection do not present an obvious mechanism that will elicit the highly selective behavioural changes seen in intermediate hosts and disorders in humans, but this does not preclude a contribution to the observed behavioural changes. The contribution could be stochastic and dependent on the cell type in which *T. gondii* localizes.

Localization of *T. gondii* in the CNS

Toxoplasma gondii manipulation of intermediate host behaviour could be due to the parasite localizing in specific brain regions and the most likely brain regions are those associated with fear processing. The primary brain region involved in fear processing is the amygdala (Misslin, 2003) and recent work provides a potential process for fatal feline attraction mediated by pathways that activate the amygdala (House et al., 2011). The amygdala receives sensory inputs, in part, from the thalamus and sensory cortical areas, and has outputs to the hypothalamus, periaquaducatal grey and brainstem areas, triggering classical fear response behaviours such as the fight-or-flight response and freezing behaviours. The importance of the amygdala in fear responses can be seen in rats with amygdalar lesions that are exposed to sedated cats, where instead of avoiding the cat these rats actually approach the cat and physically explore it (Blanchard and Blanchard, 1972). Such responses are very similar to those described above for *T. gondii*-infected rats and attraction to cat odour (Berdoy et al., 2000; Vyas et al., 2007). This behavioural manipulation could be explained if *T. gondii* showed tropism for the amygdalar areas of the brain, which would be evidenced by an increased density of *T. gondii* tissue cysts in this region. Indeed, a study that assessed the tissue cyst density systematically in brain regions found an increased cyst density in the amygdalar regions based on haematoxylin and eosin staining (Vyas et al., 2007); although, in this study, *T. gondii* tissue cysts were found in most brain regions and observations in our laboratory and others have found cysts in numerous brain regions in infected mice. Bioluminescent imaging of *T. gondii* that express luciferase under the control of a bradyzoite-specific gene promoter found the principal luminescence to be in the cerebral cortex, colliculi, cerebellum and olfactory bulbs (Di Cristina et al., 2008). McLeod and colleagues (Hermes et al., 2008) used a similar approach to Vyas and colleagues (Vyas et al., 2007), but did not specifically focus on the amygdala, and found increased cyst numbers in both the cortex and the diencephalon, the region containing the thalamus (Hermes et al., 2008). Recently, a study directly assessed tropism in *T. gondii* tissue cyst location and found the amygdala, hippocampus, olfactory bulbs and a number of cortical regions, including the entorhinal, somatosensory and motor cortices, had higher cyst numbers proportional to volume in five mice analysed (Berenreiterova et al., 2011). However, the majority of brain regions consisting of grey matter showed some level of infection, suggesting that *T. gondii* does not have a highly specific tropism for distinct brain regions.

In terms of human infections, there have been very few studies of *T. gondii* localization and the majority of these studies have focused on the sites of infection/reactivation in AIDS patients who are immunocompromised and have rampant active infections. These studies identified a variety of brain regions by MRI, including the frontal and parietal lobes amongst other areas of the cerebral cortex, the basal ganglia and the cerebellum (Brightbill et al., 1996; Maeda et al., 2006; Schroeder et al., 2006; Suzuki et al., 2010). In a single study focusing on psychiatric patients, 14 of whom had a diagnosis of schizophrenia, an attempt to amplify parasite DNA using nested PCR from post-mortem tissue from the orbital frontal cortex was unable to detect *T. gondii* DNA (Conejero-Goldberg et al., 2003). This result is probably due to the low number of parasites relative to host material (i.e. relative amount of DNA) and the apparent stochastic nature of *T. gondii* localization in the brain.

Based on the studies described above, it does not appear that *T. gondii* parasites have tropism for specific brain regions in their secondary hosts, although the amygdalar regions may be more consistently infected, and so further explanation for the highly specific mechanism by which *T. gondii* influences rodent and potentially human behaviour needs to be sought. Possibilities include *T. gondii* manipulation of immune responses and/or specific neurones or glia based on their biochemical composition.

Cellular location of *T. gondii* encysted in brain

A number of *in vitro* studies have indicated that cysts can be found in astrocytes; however, *in vivo* studies in mice found cysts nearly exclusively in neurones in chronically infected animals, albeit with some evidence of an interaction between astrocytes and *T. gondii* cysts (Hermes et al., 2008; Melzer et al., 2010). In a time course of chronic infection, the majority of infected host cells in mouse brains were identified as neurones based on electron microscopy analysis (Ferguson and Hutchinson, 1987). Another study also found encysted parasites in neurones, but not in glia (Sims et al., 1989). In a study observing astrocytes in brains infected with virulent Type I *T. gondii* strain, parasites were seen to be colocalized with astrocytes and were found beside neuronal nuclei (Gonzalez et al., 2007). Hence, encysted *T. gondii* are ideally positioned for neuromodulation with infection of neurones.

Proximate changes in dopamine during *T. gondii* infection

One mechanism of neuromodulation could be through alterations in neurotransmitter levels. Indeed, in a well-quoted study, Stibbs found that total brain dopamine in chronically infected mice was elevated to 114% of the level in uninfected mice, whilst other neurotransmitters remained unchanged (Stibbs, 1985). In contrast, in acute infections homovanillic acid (HVA), a degradation product of dopamine, was elevated to 140% of uninfected levels in the brain whilst other neurotransmitters remained unchanged (Stibbs, 1985). In this experiment, chronic infections were established in 10 mice infected with a virulent Type I *T. gondii* strain and drug cured with sulphadiazine. Similar changes in total dopamine levels were not found in mice chronically infected with a less virulent Type III strain or in congenitally infected mice (Goodwin et al., 2012).

Recently, extremely high concentrations of dopamine were found to accumulate in cyst-containing brain cells and *in vitro* infection was found to induce high amounts of dopamine in neural cells (Prandovszky et al., 2011) (see Fig. 1). Hence, infection induces a local effect on dopamine levels. In tissue sections of chronically infected mice brains, parasite cysts contained high levels of dopamine based on staining with a commercial antibody specific to dopamine (i.e. one that does not stain other neurotransmitters). Staining could not be disrupted by competition with exogenous serotonin. Infection of a neural cell line that expresses the proteins needed for packaging and release of catecholamines led to the synthesis and release of excessive dopamine. As this cell line synthesizes dopamine, the production of dopamine induced by infection was measured as an increase in dopamine levels. High amounts of dopamine were released from infected neural cells following stimulation. This result indicates that the large amount of dopamine produced is properly packaged for release from infected neurones. The physiological implications are impressive as tissue cysts *in vivo* contain a hundredfold more parasites than *in vitro* cysts and, hence, infected neurones could potentially release several hundred times more dopamine than uninfected dopaminergic neurones. Through this mechanism, *T. gondii* could discreetly alter dopamine levels from specific (i.e. infected) neurones. A link between dopamine and behaviour changes in infected rodents is supported by experiments in which the dopamine antagonists haloperidol and GBR 12909 were found to prevent the behavioural alterations in *T. gondii*-infected rats and suppress changes in hole-board exploration with infection in male mice, respectively (Skallová et al., 2006; Webster et al., 2006).

Toxoplasma gondii can directly increase dopamine production as the parasite synthesizes a tyrosine hydroxylase (Gaskell et al., 2009); in neurones, tyrosine hydroxylase converts the amino acid tyrosine to L-DOPA, the precursor of dopamine. Based on analysis of the genome of *T. gondii* (made publicly available at http://www.toxodb.org), two tyrosine hydroxylase genes were identified using sensitive bioinformatics software that annotates genomic data based on hidden Markov models of enzymes (Whitaker et al., 2009). The expression of one gene, *TgAaaH2*, was found to be upregulated during differentiation of parasites to the bradyzoite tissue cyst form. The other gene, *TgAaaH1*, was constitutively expressed. As tyrosine hydroxylases are members of a family of aromatic amino acid hydroxylases, functional assays were required for accurate assignment. Based on kinetics, recombinant enzymes of both genes were found to preferentially synthesize L-DOPA from tyrosine (and hence they are named tyrosine hydroxylases) but the *TgAaaH2* product has a threefold higher affinity for tyrosine than for phenylalanine whereas the *TgAaaH1* product has only a twofold higher affinity for tyrosine. Importantly, the parasite enzymes were not able to utilize tryptophan and therefore are not tryptophan hydroxylases – a key enzyme for serotonin synthesis. Because of the dual activities of the enzyme, it not only synthesizes L-DOPA from tyrosine but

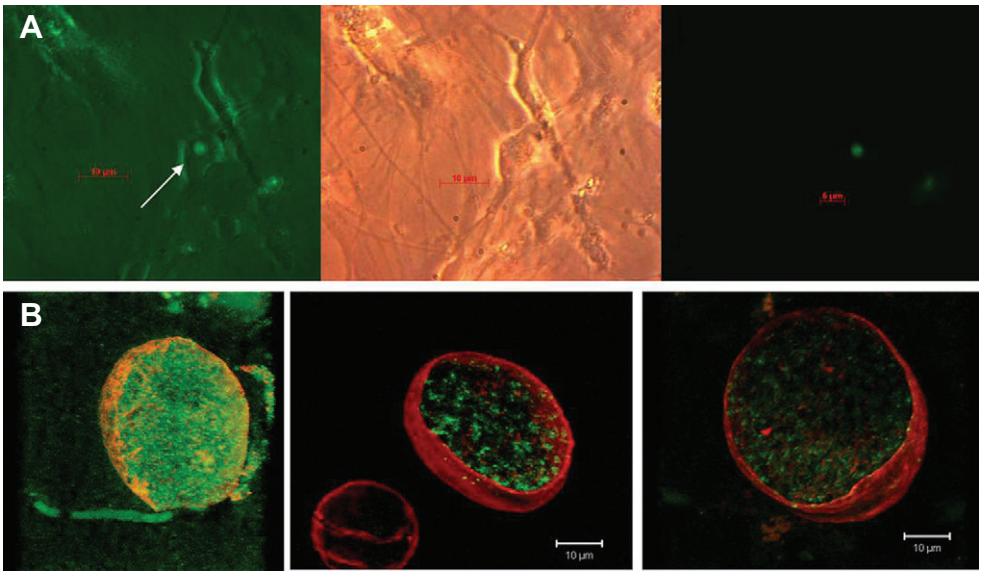

Fig. 1. Bradyzoite (cyst) stage *Toxoplasma gondii*-infected cells and dopamine. (A) Rat primary cortical neuron cultures (11 days old) containing GFP-tagged *T. gondii* bradyzoites (4 days old) viewed by (from left to right) composite, light and fluorescence microscope. (B) Dopamine antibody staining (green, fluorescein secondary antibody) of *T. gondii* tissue cysts (red, lectin-TRITC) in mouse brain (various regions). The two images on the right are 3D projections of a *Z*-stack reconstruction of serial images.

also produces tyrosine from phenylalanine. It is possible that TgAaaH2, expressed in tissue cysts, acts as a tyrosine hydroxylase whereas TgAaaH1 principally serves as a phenylalanine hydroxylase in growing tachyzoites for the synthesis of tyrosine from phenylalanine. Tyrosine hydroxylase was also found to be encoded by genes of the closely related *Neospora caninum* but not the apicomplexans *Plasmodium* or *Eimeria*. Interestingly, for *Neospora*, canines are the definitive host (rather than felines) and they are believed to become infected by ingesting infected meat (similar to *Toxoplasma* in cats). Several secondary hosts have been identified (e.g. cattle) although the intermediate hosts in the wild have yet to be identified. Delineating the evolution of this gene in Apicomplexa awaits genome sequencing of related organisms. The discovery of the tyrosine hydroxylase provided the first substantive evidence of a mechanism for *T. gondii* alteration of behaviour, although definitive experiments to demonstrate a link between *T. gondii* tyrosine hydroxylase and behaviour changes are needed.

For dopamine production, in dopaminergic neurones, L-DOPA synthesized by tyrosine hydroxylase is the rate-limiting step. Hence, the level of dopamine is directly proportional to tyrosine hydroxylase activity. Indeed, exogenous L-DOPA (i.e. levodopa), used for treating Parkinson's disease, causes increased dopamine production in dopaminergic neurones. Dopamine is also further metabolized to noradrenaline (norepinephrine) in some cells. *Toxoplasma gondii* tissue cysts in infected brain cells were found to contain the parasite tyrosine hydroxylase (Prandovszky et al., 2011). This tyrosine hydroxylase appears to contain a signal peptide and is localized to the parasite periphery in intracellular parasites within parasitophorous vacuoles (Gaskell et al., 2009; Prandovszky et al., 2011). Hence, the enzyme may be secreted out of the parasite and into the cyst for catalytic function adjacent to the host cytosol, although this has yet to be tested. Some contribution could, theoretically, be due to the host immune response to infection *via* alteration of other transmitters such as GABA or glutamate and kynurenic acid increases (as discussed above), increasing NMDA antagonistic properties that could alter dopamine metabolism (Mortensen et al., 2007).

Hypothesis for specific behaviour changes in intermediate hosts and implications for 'accidental' hosts

The stochastic location of the parasite has important implications for understanding the changes in host behaviour and could suggest host-to-host differences in behaviour effects and an impact of parasite load (i.e. number of brain cysts) with a possible threshold level necessary for behavioural changes. This description does not explain the consistency of behaviour changes between infected rodents within an experiment, such as observed with the fatal feline attraction. Studies have observed different locations for *T. gondii* without agreement on tropism of *T. gondii* for specific brain regions, as reviewed above, so one would expect other mechanisms to be involved in the behavioural changes observed.

Hence, the question remains, how does the parasite promote such defined changes in behaviour? As *T. gondii* is able to synthesize dopamine and high levels of dopamine are associated with infected neural cells (Fig. 1), perhaps a local effect is generated based on where the parasite synthesizes dopamine (Gaskell et al., 2009; Prandovszky et al., 2011). In the published study (Prandovszky et al., 2011), the neural cells with cysts contained high levels of dopamine. We have not noted any differences in the level of dopamine in infected cells between brain regions (G.M. and E. Prandovszky, unpublished observations), although identification of the types of neural cells with cysts and elevated dopamine is needed.

A hypothesis for how dopamine, synthesized *via* the parasite, could result in a very selective behavioural change may be based on the pathway for dopamine biosynthesis. Although tyrosine hydroxylase has been found to be encoded in the genome of *T. gondii*, there is no evidence of a DOPA decarboxylase (aromatic L-amino acid decarboxylase), the second enzyme in the pathway for dopamine biosynthesis. For dopamine to be produced in an infected cell, the host cell would need to supply DOPA decarboxylase for conversion of the parasite-produced L-DOPA to dopamine. DOPA decarboxylase is produced in dopaminergic (mainly neurones and adrenal medulla cells) and serotonergic cells. These include cells involved in noradrenaline synthesis. Therefore, dopamine and the consequences of its overexpression would be restricted to the subset of cells that contain DOPA decarboxylase. It is possible that parasite-mediated L-DOPA exits the infected cell and is converted to dopamine in DOPA decarboxylase-containing cells. Indeed, uptake of exogenous L-DOPA (levodopa) is the mechanism by which this treatment for Parkinson's disease is believed to work. Synthesized dopamine requires packaging, transport and release mechanisms (dopamine is reactive and can be toxic if not properly packaged). Hence, only neurones containing these mechanisms would be affected.

One could envisage a scenario in which L-DOPA is produced within the intracellular tissue cyst and released into the host neurone where it is further metabolized to dopamine and packaged into vesicles for release. This would fit well with the dopamine found in the cell body of infected cells by cytochemical staining and the commercial antibody possibly staining both dopamine and L-DOPA in the cyst-infected neurone (Fig. 1) [see also fig. 2a in Prandovszky et al. (Prandovszky et al., 2011)]. By this mechanism, although the parasite infects many brain regions, only catecholaminergic neurones would be affected and behaviours associated with these neurones. This would provide an elegant yet simple biochemical alteration by which *T. gondii* could invoke distinct behaviour changes but would not require an exclusive targeting of a particular brain region.

Toxoplasma gondii encystment has been found in most brain regions yet tropism for specific brain regions remains controversial in the literature cited above. We propose a mechanism by which distinct localization of parasites to specific brain regions would not be necessary for the distinct changes to behaviour that have been observed. Further studies are needed to test this hypothesis and to ascertain which brain regions are involved in the behaviour changes. Localized host immune response may further contribute to these effects. Hence, the location of infection may be important for the behaviour changes but specific targeting of brain regions in infection does not appear necessary for distinct alterations in behaviour. *Toxoplasma gondii* infections do not appear to rely solely on 'location, location, location' for the behaviour manipulation but could depend on other proximate mechanisms invoked by the parasite.

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