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Review Article

Toxoplasma gondii-induced neuronal alterationsA. PARLOG,¹ D. SCHLÜTER^{1,2} & I. R. DUNAY¹¹Institute of Medical Microbiology and Hospital Hygiene, Otto-von-Guericke University, Magdeburg, Germany, ²Helmholtz-Centre for Infection Research, Braunschweig, Germany

SUMMARY

The zoonotic pathogen *Toxoplasma gondii* infects over 30% of the human population. The intracellular parasite can persist lifelong in the CNS within neurons modifying their function and structure, thus leading to specific behavioural changes of the host. In recent years, several *in vitro* studies and murine models have focused on the elucidation of these modifications. Furthermore, investigations of the human population have correlated *Toxoplasma* seropositivity with changes in neurological functions; however, the complex underlying mechanisms of the subtle behavioural alteration are still not fully understood. The parasites are able to induce direct modifications in the infected cells, for example by altering dopamine metabolism, by functionally silencing neurons as well as by hindering apoptosis. Moreover, indirect effects of the peripheral immune system and alterations of the immune status of the CNS, observed during chronic infection, might also contribute to changes in neuronal connectivity and synaptic plasticity. In this review, we will provide an overview and highlight recent advances, which describe changes in the neuronal function and morphology upon *T. gondii* infection.

Keywords behavioural manipulation, chronic CNS infection, neuronal alteration, *Toxoplasma gondii*

INTRODUCTION

Toxoplasma gondii (*T. gondii*) is a common zoonotic parasite infecting a wide range of animals and humans (1). The worldwide seroprevalence of *T. gondii* infection is estimated between 30 and 70% in humans, although it varies

markedly across different geographical regions (2–4). Transmission incidence in the human population is mostly linked to hygiene and culinary habits as the infection mainly occurs via ingestion of oocysts that have contaminated water or food, or consumption of meat products infected with viable tissue cysts (3, 4). Although toxoplasmosis can be associated with multi-organ involvement (liver, heart, lung, lymph nodes, eye, and muscle tissue), the infection of the immune privileged central nervous system (CNS) is associated with the most complications. It has been proposed that the intracellular parasites migrate to the brain as early as 7 days post-infection, and cross the blood–brain barrier (BBB) by a Trojan horse mechanism (5–8). Life-threatening toxoplasmic encephalitis can develop upon reactivation of the latent infection in immunocompromised hosts (9).

After reaching the CNS, the parasites can invade all nucleated cells and initiate activation of resident microglia and astrocytes. Initial signs of glia activation can be observed between 7 and 10 days post-infection and are followed by the recruitment of peripheral T cells and mononuclear cells (10–12). The proinflammatory cytokine interferon-gamma (IFN- γ) is a major factor in the control and elimination of *T. gondii* in the brain (13–15). The early activation of microglia and astrocytes correlates with the local production of chemokines and cytokines, which contributes to immune cell recruitment from the periphery. The effective control of parasite replication and disruption of the parasitophorous vacuole is dependent on cell extrinsic mechanisms as well as cell intrinsic mechanisms mediated by cytokine signals (16–18).

Toxoplasma gondii displays a differential invasive and developmental preference in neural cells in relation with the course of infection. Several studies have highlighted the ability of the parasite to infect a large number of glia during the acute phase, in contrast to the low numbers of infected neurons (19–24). After the acute infection, the stage conversion takes place predominantly in neurons resulting in bradyzoite filled cyst development (25, 26).

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Although neurons also produce some chemokines and cytokines, they lack specific intracellular mechanism to inhibit parasite growth, which provides an explanation for the relatively high number of infected neurons during the chronic stage (27–32). Moreover, because of the lack of MHC class I, infected neurons are not recognized by invading CD8⁺ T cells, resulting in long-term protection from the immune system (33). In the latent phase, the slowly replicating bradyzoites can persist lifelong inside neurons evading the host's immune defence mechanisms. Hence, the infected neurons interfere directly or indirectly, with noninfected proximate and distant neurons, potentially leading to altered neuronal function and to behavioural and neuropsychiatric diseases.

To investigate the impact of *T. gondii* on host behaviour, various murine models were established. Although several studies highlighted certain behavioural changes in *T. gondii*-infected mice, the precise mechanisms remained unclear (34–41). Early studies suggested altered exploratory behaviour and reduced grooming activity in infected rodents (34, 38, 40). An important study reported for the first time the ability of the parasite to specifically manipulate the behaviour of rodents in relation to predator–prey interaction. Chronic *T. gondii* infection in mice converted the aversion to cat odour into attraction which most probably leads to increased predation (35). These alterations were found to be highly specific as the infection did not alter anxiety-like behaviour, learned fear, and nonaverse learning. The authors concluded that the high tropism of parasite cysts in the amygdala could explain this observation, as this brain structure is involved in fear behaviour. The first study that offered a comprehensive description of the immunopathological changes and full genome microarray of the chronic stage suggested ongoing low-level inflammation associated with behavioural abnormalities (37). Gulinello *et al.* (39) reported motor coordination and sensory deficits, but normal cognition in infected mice which was associated with widespread parasite cysts and no specific tropism to a certain brain region. The authors claim that the chronic infection induces subtle dysregulation of the CNS via neurotransmitters, with widespread destruction. Experiments conducted by Gatkowska *et al.* (42) described reduced exploratory activity in *T. gondii*-infected mice; however, these behavioural changes were mainly pronounced in the acute stage suggesting a transient nonspecific reaction to inflammation. In conclusion, all studies conducted in different murine experimental models indicate that behavioural changes occur upon acute and chronic *T. gondii* infection; nonetheless, the multiple underlying mechanisms still need to be refined.

In humans, latent infection with *T. gondii* has generally been considered of little clinical consequence. However, in

the recent years, new findings raise awareness of the possible roles of chronic toxoplasmosis in the aetiology of certain mental disorders. In 2001, the first report on the potential impact of *T. gondii* on psychomotor performance suggested that individuals with latent infection exhibited increased reaction times, scored more poorly on a standard computerized test, and appeared to lose their concentration more quickly (43). More recently, Beste *et al.* (44) investigated the possible modulatory role of latent *T. gondii* infection on cognitive functions in elderly individuals. When confronted with an auditory deviant stimulus, elderly individuals with positive IgG *Toxoplasma* serum concentrations revealed delaying processes of attentional allocation and disengagement. Moreover, the implications of the subtle alterations in psychomotor performance on human behaviour were assessed. Association of *T. gondii* infection with negative reaction time and the ability for long-term concentration may account for the observations from three independent groups indicating that individuals chronically infected with *T. gondii* are associated with a two times higher risk of traffic accidents (45–49). However, a recent study by Stock *et al.* revealed a paradoxical improvement of cognitive control process in *T. gondii*-infected healthy individuals, arguing therefore for a rather positive effect of infection on cognitive capacities than the commonly held view that latent infection is detrimental for the host (50, 51).

It has also been proposed that chronic toxoplasmosis can alter human personality profiles. Throughout various studies, Flegr *et al.* have compared individuals with or without antibodies to *T. gondii* to identify correlations between chronic infection and personality traits. Interestingly, the results revealed not only personality disturbances but also prominent gender and age differences (45, 52–55). Intriguingly, classical psychiatric diseases such as schizophrenia, mood disorders, psychosis, and self-directed violence were also linked to toxoplasmosis, as *Toxoplasma* seropositivity was elevated in neuropsychiatric patients compared to healthy volunteers in numerous studies (56–62). Although it is difficult to establish a clear mechanistic correlation, the timing (childhood or adulthood onset of the psychiatric disease) and the route of infection (cyst or oocyst) could also vary between the cases and thus influence differently the development of such mental disorders. One possible explanation for the association between infection and psychiatric illness could be the parasite-induced dysregulation of distinct neurotransmitters.

DIRECT EFFECTS OF *T. GONDII* ON NEURONS

The above-described specific behavioural changes in the experimental murine models as well as in humans chron-

ically infected with *T. gondii* evoke subtle modifications in neuronal function and structure. Because neurons are fundamental elements of the CNS herein, we review the current evidence on the possible direct and indirect mechanisms elicited by *T. gondii* infection to disrupt the activity and morphology of the neurons (Figure 1). One of the most ‘convenient’ explanations for the behavioural and neuropsychological deficits could be that the parasite directly infects neurons interfering with their survival and function. The direct influence of *T. gondii* on the host cells is discussed in many *in vitro* and *in vivo* studies proposing various mechanisms. In the following section, some components of this complex direct interplay between *T. gondii* and neurons will be considered.

Cyst location in the CNS

The location of the cysts has been suggested to play an important role in the behavioural changes of the host. Parasite cysts persist in many areas of the CNS, and some studies proposed specific tropism to certain regions (35, 36, 63–66). In one report, an increased density of cysts was detected in the amygdala of infected mice, providing a possible explanation for the fear loss from felines (35). Others described increased cyst numbers in the amygdala, olfactory bulb, cerebellum, and the cortical regions (36, 63, 67). However, in all studies where cyst numbers were determined, consistently low total parasite numbers were detected (2–500 cysts/mice brain), which makes it difficult to believe as a single explanation for all the changes. In

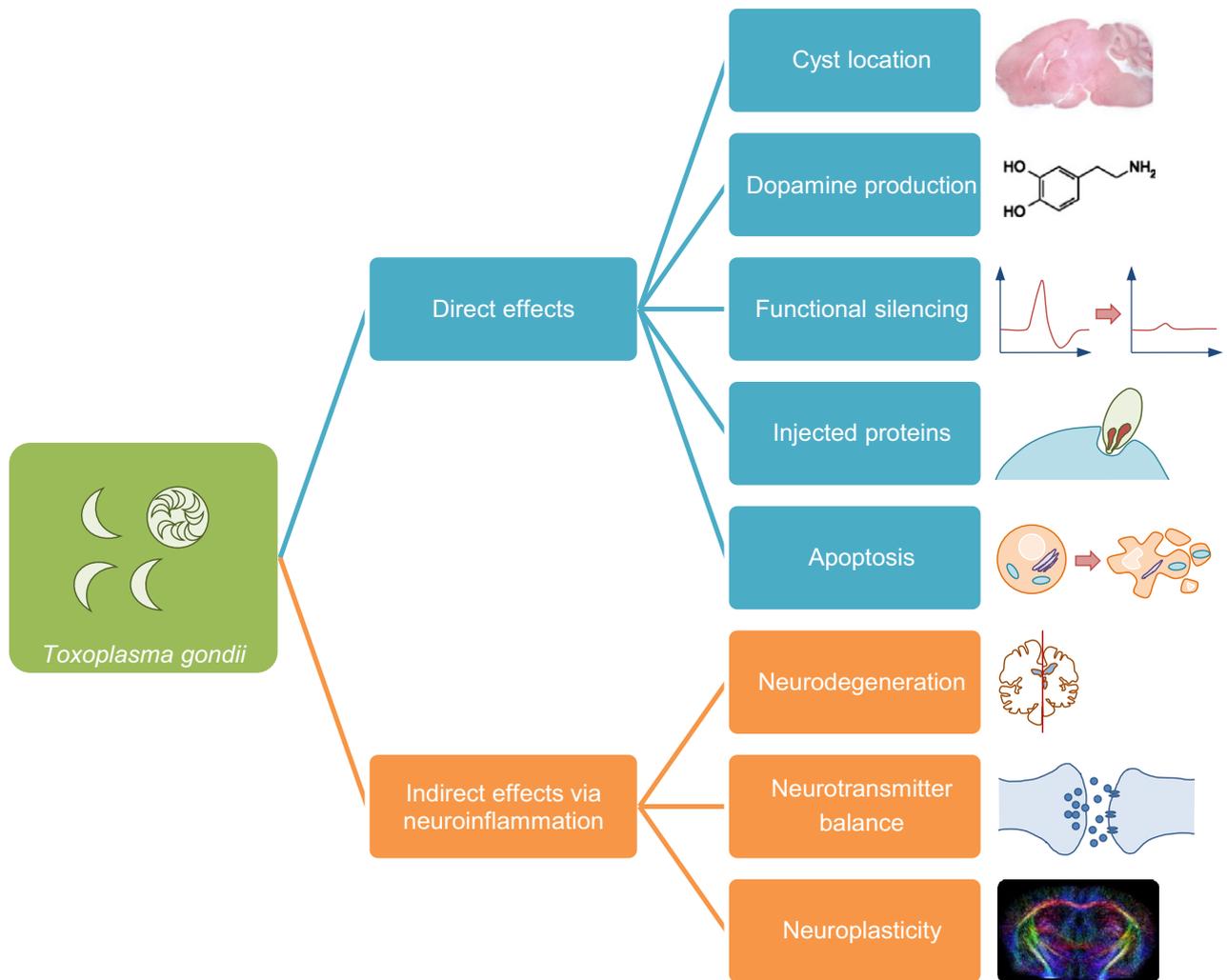


Figure 1 Advised mechanisms how *T. gondii* might alter neuronal function.

our studies, we observed random cyst distribution with no preference to any brain region (65). Importantly, a high density of hypo-intense lesions was detected predominantly in the somatosensory cortex, motor cortices, and hippocampus of infected mice measured by a T2*-weighted magnetic resonance imaging (MRI) sequence, suggesting the presence of parasites in those regions (65). In this context, it is important to mention that parasite-induced local ongoing basal inflammation in the latent stage could also contribute to the disturbed neuronal function (37, 41, 65).

Few studies have described the pathological processes and the location of cysts in human brain samples, due to the complications of accessing post-mortem material (68, 69). These studies mainly focused on immunocompromised hosts that suffered from reactivated toxoplasmosis (68, 69). However, MRI studies in patients with cerebral toxoplasmosis detected lesion mainly in the cortex and cerebellum (70), consistent with several murine studies.

Role of dopamine

One of the most appealing hypotheses to explain neuro-modulatory effects of *T. gondii* infection is based on the capacity of the parasite cysts to disturb dopamine metabolism. In 2009, Gaskell *et al.* (71) found in the genome of *T. gondii* two genes, which encode tyrosine hydroxylase, the rate-limiting molecule for dopamine synthesis. Interestingly, in 2011, Prandovszky *et al.* (72) reported, using *in vitro* as well as *in vivo* approaches, that parasite cysts harboured inside neurons express tyrosine hydroxylase as well as dopamine. Moreover, there was a threefold increase in the amount of dopamine released in infected cells upon stimulation compared to uninfected cells (72). These results lead to the hypothesis that an excess of dopamine produced by the parasite could interfere with crucial brain functions (i.e. locomotion, cognition, memory, mood, learning, reward), directly influencing the behaviour of the intermediary host (73). In humans, dopamine and dopamine signalling pathway dysregulations have been associated with several neurodegenerative and psychiatric disorders (74–77). However, the information provided by the limited number of studies on this topic, in animal models, is not conclusive and even contradictory (78, 79).

Functional silencing of neurons

Recently, Haroon *et al.* (36) reported that *Toxoplasma* tachyzoites directly modulate neuronal function by actively manipulating Ca(2+) signalling upon glutamate stimulation, thus leading to either hyper- or hyporesponsive neurons. Moreover, activity-dependent uptake of the potassium analogue thallium was decreased in neurons

containing the cysts, implying an inhibited function. All major structures of the neurons contained parasite antigens; thus, the enlargement of the neurons itself may further contribute to the impaired neuronal activity. Importantly, both bradyzoites and tachyzoites functionally silenced infected neurons, which may affect the host's behaviour.

Injected parasite proteins manipulate the host cell

Recently, a new theory emerged presenting evidence that *T. gondii* can deliver certain effector proteins without invading the host cell (80, 81). This evokes the idea that the parasites might influence their host cells without even infecting them. The parasite proteins could remain and manipulate neurons lifelong, or other affected cells could serve as antigen decoys and protect cysts containing neurons. However, it is not known yet how long these proteins remain within the injected neurons and how they modify their activity. This study opens a new avenue and supports the idea that cyst persistence is not absolutely necessary to interfere with the host neuronal function.

Blocked apoptosis

Programmed cell death is an important mechanism of the host to fight invading pathogens and to regulate immune responses. Importantly, *T. gondii* has evolved strategies to directly inhibit host cell apoptosis, presumably to hamper elimination and to survive the host's immune response (82–84). The proposed intracellular mechanisms include the upregulation of the anti-apoptotic genes in the host cell, or the modulation of the apoptotic signalling cascades, for example reduced activation of caspase molecules, and downregulation of poly ADP-ribose polymerase expression (85, 86). Interestingly, infected cells as well as bystander cells can be deactivated by the parasite by thwarting apoptosis (87, 88). Therefore, the inhibition of apoptosis in neuronal cells can be a straightforward survival method to overcome the host's immune response; however, additional experiments are needed to address this hypothesis.

INDIRECT EFFECTS OF *T. GONDII* ON NEURONS

In addition to the local, relatively limited functional effects of *T. gondii* cysts on neurons, as discussed above, the hallmark of chronic infection is the permanent resident glia cell activation throughout the whole CNS and the recruitment of peripheral immune cells (12, 89, 90). This prolonged neuroinflammation is able to directly influence a

large number of neurons located in various neuroanatomical areas (65, 91–93).

Neuroinflammation via peripheral mediators

Neuroinflammation can be induced not only following the invasion of the neural parenchyma by the parasite but also by peripheral immune stimuli. Before reaching the CNS, the parasite triggers systemic inflammation, which is initiated in the intestine following oral ingestion. Within the first 2–3 days post-infection, in response to parasite recognition and invasion, enterocytes produce a set of chemoattractant molecules and cytokines (16, 94–97). Up to 3–5 days post-infection, a massive infiltration of proinflammatory immune cells, such as neutrophils, monocytes, macrophages, dendritic cells, T lymphocytes, as well as parasites, can be observed in the gut and other organs (9, 98–102). Cell-mediated immunity confers protection against *T. gondii* through the production of inflammatory molecules such as interleukin-1 (IL-1), IL-4, IL-5, IL-6, IL-8, IL-12, IL-15, IL-18, IL-22, tumour necrosis factor (TNF), IFN- γ , nitric oxide (NO), and others which engage distinct pathways and specific effector mechanisms required for the appropriate control of the infection (6, 16, 89, 103).

The importance of peripheral immune mediators in the pathophysiology of brain disorders has been documented for schizophrenia, major depression, Alzheimer's disease, Huntington's disease, stroke, and traumatic brain injury (104–106). Immune signals from the systemic milieu are able to initiate neuroinflammatory processes, which in turn can cause or accelerate dysfunctions in synaptic plasticity, neurotransmitters activity, and neuronal circuitry (105–107). Interestingly, behaviour changes were also detected in response to other parasitic infections, such as *Heligmosomoides polygyrus*, *Toxocara canis*, or *Eimeria vermiformis* (108–111). These parasites do not directly infect the CNS, suggesting an even more complex peripheral or apparently global indirect neuromodulatory effects rather than direct alterations of neuronal function. Therefore, it is likely that following infection with *T. gondii*, even before the parasite reaches the CNS, certain circulating immune mediators create local or global disruptions of neuronal physiology. The mechanisms responsible for the initiation of neuroinflammation by the peripheral molecules involve changes in the BBB permeability, trafficking of mediators and effector cells across the BBB and choroid plexus (100, 112–115).

Central neuroinflammation

As briefly mentioned above, data obtained from animal studies indicate that, once the CNS is invaded by *T. gondii*,

resident cells as well as recruited immune cells induce a chronic neuroinflammatory milieu characterized by the predominance of Th1-type (IFN- γ , IL-12, IL-1, IL-6, TNF) immune responses (15, 116, 117). In the last years, research focused on the impact of the broad range of immune responses of the CNS on human behaviour and neuropsychiatric morbi has received increasing attention (105, 118, 119). These studies have revealed that patients diagnosed with major depression, obsessive-compulsive disorders, and schizophrenia show increased biomarkers of inflammation such as IFN- γ , IL-12, IL-1, IL-6, and TNF (119–122). Chronic neuroinflammation following infection with *T. gondii* in immunocompetent hosts can potentially elicit behavioural alterations and neurological disorders either by causing neurodegeneration, neurotransmitter abnormalities, and/or by triggering subtle alterations in the morphology and functionality of neurons.

Neuroinflammation – neurodegeneration

Most of the inflammatory mediators are potentially toxic for neurons (123–125). For example, neurons are highly susceptible to NO, and the toxic effects of this molecule have a central role in the pathophysiology of several neurodegenerative and demyelinating disorders (126, 127). Therefore, one would expect a high rate of degenerating neurons associated with the presence of *T. gondii* in the brain. Interestingly, using specific markers for neuronal death (i.e. anti-NeuN antibody and FluoroJadeB), we observed only limited neurodegeneration in the CNS of chronically infected mice (65, 66). Moreover, another study performed in a murine model of Alzheimer's disease suggests that infection with *T. gondii* inhibits neuronal degeneration (128). A possible explanation is provided by a study of Rozenfeld *et al.* (129) where they demonstrate that IFN- γ -activated microglia have an active role in neuronal protection by stimulating the production of transforming growth factor beta-1, which inhibits inducible nitric oxide synthase. These results suggest that neurodegeneration is, at most, a marginal factor in the aetiology of behavioural and neurological changes associated with chronic toxoplasmosis.

Neuroinflammation – neurotransmitters

Fine-tuning of the activity of neurotransmitters is critical for the proper development and integration of physiological brain processes. Thus, dysregulation in the metabolism of neurotransmitters, their transport, and receptors have been linked to different types of pathological manifestations (i.e. dopamine is linked to schizophrenia and Parkinson's disease, serotonin is implicated in depression,

glutamate is related to schizophrenia, Alzheimer's disease, Huntington's disease) (74–77, 130, 131). Evidence from several human and animal studies has demonstrated that acute or chronic administration of cytokines interferes with several neurotransmitter systems involving glutamate, serotonin, acetylcholine, and dopamine leading to classical psychiatric illness (132–134). Cytokines and inflammatory mediators such as IFN- γ , TNF, NO, IL-1, and IL-6 commonly present during toxoplasmic encephalitis may affect neurotransmitters via (1) activation of indoleamine-2,3-dioxygenase enzyme (IDO), (2) activation of mitogen-activated protein kinase pathways (MAPK), (3) alteration in tetrahydropterin (BH4) enzyme activity, and (4) excitotoxicity and oxidative stress (105). IDO-increased expression elicits the degradation of tryptophan, the essential amino acid, which is the precursor of serotonin (135, 136). Additionally, stimulation of MAPK pathways that control a wide spectrum of cellular processes, including growth, differentiation, and stress responses, can have dual effects on two crucial neurotransmitters relevant for behaviour (serotonin and dopamine). For example, it can augment the function of serotonin transporter, thus lowering the availability of the serotonin at the level of the synapse (137, 138). On the other hand, MAPK signalling was found to decrease dopamine recycling, leading to excessive stimulation of dopamine receptors (139). BH4 is involved in the synthesis of monoamine neurotransmitters serotonin, dopamine, and norepinephrine (140). Cytokines can reduce monoamine availability in relevant brain areas by decreasing the concentrations of BH4 (141).

Given the ability of the immune response to influence neurotransmitter metabolism, it seems likely that *T. gondii*-induced neuroinflammation triggers neurotransmission alterations, which in turn may explain most of the infection-related changes. For example, in a study from 1985, Stibbs reports no changes in the whole brain levels of serotonin and its metabolite in infected animals (79), a result confirmed by others (64). Norepinephrine levels went down by 28% in acutely infected mice. Moreover, the levels of dopamine remained unchanged during acute infection, but suffered a 14% increase during the chronic infection (79). However, contrary to Stibbs's results, a gender-based comparative study revealed that infection led to an increased serotonergic activity in females and males, irrespective of the timing of infection (78). Additionally, a consistent elevation of the dopaminergic system activity was measured only in male mice during the acute phase, but not the chronic phase of infection (78). Despite the limited number of studies and the apparently conflicting results, which may arise from methodological differences, it is a fact that infection with *T. gondii* can cause neurochemical alterations, which are associated in certain

circumstances with abnormal behaviour. Nevertheless, the role of specific neurotransmitters in normal and pathological behaviour and the complex interactions between the neurotransmitter systems suggest that this hypothesis may need to be refined.

Another possible mechanistic explanation for the association between *T. gondii* infection and psychiatric illness is the cytokine-mediated activation of IDO. Tryptophan degradation by IDO leads to increased concentrations of two neuroactive metabolites, quinolinic acid (QA), and kynurenic acid (KA) (142). A recent study confirmed that 1 month after infection with a type II *T. gondii* strain, there was a dramatically increased concentration of both kynurenic and quinolinic acids in the brains of infected mice (143). In human patients, excessive QA and KA levels have been correlated with a number of neurodegenerative disorders, depression, and schizophrenia (142, 144–146). Produced primarily by microglia, QA binds to glutamate N-methyl-D-aspartate receptors (NMDARs) inducing excitotoxicity and oxidative stress in the brain (142, 147). Kynurenic acid, which is produced primarily in astrocytes, is a potent antagonist of NMDARs, leading to disturbances in glutamatergic and dopaminergic neurotransmission (142, 147). The structure and the role of NMDARs in a variety of normal and pathological brain processes have been reviewed elsewhere (148–150). Interestingly, our ongoing experiments indicate that chronic exposure to *T. gondii* leads to profound alterations in specific subunits of the glutamate receptors (A. Parlog and I. R. Dunay, unpublished observations).

Neuroinflammation – alterations in neuronal morphology and synaptic plasticity

Synapses provide the physical basis for communication within the brain, and the ability for synapses to maintain their integrity and functionality is critical to maintaining proper CNS function. Dysfunction in proteins that regulate synaptic plasticity likely contributes to the development of neuropsychiatric disorders (151–153). A recent study by our group provided new insights into the impact of chronic *T. gondii* infection induced neuroinflammation on the synaptic physiology and morphology of noninfected neurons. We showed for the first time that chronic infection triggers changes in the protein expression of presynaptic and post-synaptic compartments of the mature synapse. Accordingly, we measured reduced levels of synaptophysin and PSD95 in the somatosensory cortex and hippocampus, two key neuroanatomical structures that are relevant to normal behaviour, cognition, and memory (65). Synaptophysin is essential for the proper activity of synaptic vesicles and neurotransmitter release at the level of

synaptic cleft (154). PSD95 is crucial for the correct assembly of the post-synaptic receptors (i.e. NMDARs) and the regulation of synaptic plasticity (155–159). Additionally, to explore the hypothesis that impaired synaptic trafficking is underlined by morphological changes, we investigated whether dendritic arborization and dendritic spines modifications in noninfected pyramidal neurons located in the cortex and hippocampus were impacted by infection. Dendritic spines are specialized membrane protrusions located on neuronal dendrites, with a very complex structure and fundamental functions for the synaptic physiology and plasticity (160, 161). Of note, reduction in dendritic complexity and dendritic spines abnormalities has been reported to have an essential role in the etiopathology of several neuropsychiatric disorders (161–164). Our analysis indicates that chronic *T. gondii* infection is negatively influencing the structure of neurons by reducing their dendritic complexity and importantly by inducing modifications in the dendritic spines number/distribution and morphology (65). Similar results were reported in other brain structures as well as in the neuronal components of the enteric nervous system. In an attempt to identify the possible mechanisms employed by *T. gondii* to reduce fear response as part of the manipulation of the host, Mitra *et al.* (165) show in a rat model of chronic infection that *T. gondii* causes a retraction of dendritic arborization in basolateral amygdala neurons. The enteric nervous system is a collection of neurons that integrates all aspects of motility, secretion, immune, and inflammatory processes of the gastrointestinal tract (166–168). Several studies provided experimental evidence that chronic infection with *T. gondii* elicits morphological changes of neuronal subpopulations found in the wall of different segments of the gastrointestinal tract (169–173). Any possible role of such neuroplasticity changes of myenteric neurons in the behavioural and psychiatric diseases remains to be clarified. Nevertheless, all of these results suggest that morphological alterations of the neurons during latent infection might not be an epiphenomenon and may be biologically relevant.

Finally, in the same study, using a state-of-the-art imaging technique, we investigated whether the inflammatory lesions and their location have any impact on the whole brain neurocircuitry. *In vivo* qualitative and quantitative analysis by diffusion-tensor MRI (DT-MRI) combined with a fibre-tracking methodology revealed a loss of fibre density in the cortical and subcortical regions. Furthermore, we showed by DT-MRI and electron microscopy a pattern of *T. gondii*-induced white matter pathology that consists of myelin decompaction and degeneration (65). A considerable body of evidence indicates that neuropsychiatric disorders, such as schizophrenia, might not be the

result of focal brain abnormalities, but rather the consequence of pathological interactions between several brain regions leading to abnormal integration of brain functions (174–177). Deficits in inter-regional functional coupling can arise from impairments of neuroconnectivity due to changes in morphology/distribution of dendritic spines, alterations of the synaptic plasticity (i.e. composition of some receptors and their subunits), or white matter structural abnormalities (174, 178–181). Interestingly, in a recent study, Horacek *et al.* (182) have assessed by voxel-based morphometry MRI the changes in the volume of the brain cortex of schizophrenic individuals, with or without IgG against *T. gondii*. They report that patients with schizophrenic chronically infected with *T. gondii* had significant reductions in grey matter volume of several cortical regions as compared with the noninfected patients (182). The anatomic substrates for reported volume changes indicate impairments of structural connectivity. In this context, despite the limitation of the murine model of chronic toxoplasmosis, it can be speculated that these results contribute to a better understanding of the possible association between *T. gondii* infection and behavioural or mental illness.

PERSISTENT BEHAVIOURAL CHANGES AFTER PARASITE CLEARANCE

The hypothesis about parasite persistence, cyst location, and ongoing inflammation recently has been challenged by an interesting study, where the specific behavioural changes remained even after clearance of the parasites (41). As behavioural changes occur already in the acute stage, it is possible that the initial infection is followed by an immediate and permanent neuronal damage. However, these experiments were performed with the attenuated type I *T. gondii* and the majority of the human infections occur upon infection and persistence with the type II and III clonal lineages, where the parasite cysts remain lifelong.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Accumulating evidence supports the idea that chronic *T. gondii* infection affects neuronal function and structure and may, thus, uniquely interfere with the host's behaviour. The profound neuronal alterations that can be elicited by the persisting parasites are investigated and described by several *in vitro* and *in vivo* approaches. Considering the many different aspects involved in the *T. gondii*-neuronal interaction, it is unlikely that a single mechanism would explain the behavioural and neuropsychiatric perturbations associated with *T. gondii* infection.

Future studies should also investigate whether the parasite has a tropism for specific neuronal populations or subpopulations (i.e. GABAergic or dopaminergic), or whether other cells types such as oligodendrocytes are affected by the ongoing infection. Moreover, considering that *T. gondii* infection has high seroprevalence worldwide, more comprehensive studies in the human population need to be completed.

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