Toxoplasma gondii Actively Inhibits Neuronal Function in Chronically Infected Mice

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Abstract

Upon infection with the obligate intracellular parasite Toxoplasma gondii, fast replicating tachyzoites infect a broad spectrum of host cells including neurons. Under the pressure of the immune response, tachyzoites convert into slow-replicating bradyzoites, which persist as cysts in neurons. Currently, it is unclear whether T. gondii alters the functional activity of neurons, which may contribute to altered behaviour of T. gondii-infected mice and men. In the present study we demonstrate that upon oral infection with T. gondii cysts, chronically infected BALB/c mice lost over time their natural fear against cat urine which was paralleled by the persistence of the parasite in brain regions affecting behaviour and odor perception. Detailed immunohistochemistry showed that in infected neurons not only parasitic cysts but also the host cell cytoplasm and some axons stained positive for Toxoplasma antigen suggesting that parasitic proteins might directly interfere with neuronal function. In fact, in vitro live cell calcium (Ca²⁺) imaging studies revealed that tachyzoites actively manipulated Ca²⁺ signalling upon glutamate stimulation leading either to hyper- or hypo-responsive neurons. Experiments with the endoplasmatic reticulum Ca²⁺ uptake inhibitor thapsigargin indicate that tachyzoites deplete Ca²⁺ stores in the endoplasmatic reticulum. Furthermore in vivo studies revealed that the activity-dependent uptake of the potassium analogue thallium was reduced in cyst harbouring neurons indicating their functional impairment. The percentage of non-functional neurons increased over time. In conclusion, both bradyzoites and tachyzoites functionally silence infected neurons, which may significantly contribute to the altered behaviour of the host.

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Introduction

Toxoplasma gondii is an obligate intracellular protozoal parasite which replicates sexually in the intestine of its specific hosts, i.e. cats and other felidae. The parasite infects a broad spectrum of intermediate hosts including mice and men. Upon oral infection with oocysts or cysts, T. gondii transforms into fast replicating tachyzoites in the intestine of intermediate hosts, disseminates throughout the body, and infects numerous organs including the central nervous system (CNS). In the CNS, tachyzoites infect microglia, astrocytes and neurons. Under pressure of the immune system, intra-neuronal tachyzoites transform into slow-replicating bradyzoites, which form cysts [1]. Electron microscopic studies demonstrated T. gondii cysts within neurons but not in other cell types of the CNS [2]. However, in up to 50% the definite target cell of the cysts could not be identified. It is thought that the cyst wall separates the parasite from the cytoplasm of the host cell, which in combination with the major histocompatibility complex antigen negativity of neurons results in an escape of the parasite from detection by the immune system [3]. Immune evasion of T. gondii is very successful and the parasite persists unlimited in the CNS of its host.

Up to 30% of humans are chronically infected with T. gondii and it is generally believed that persistence of T. gondii is clinically asymptomatic [4]. However, recent studies suggest that T. gondii may alter the behaviour of humans or even increase the risk for neurological diseases including headache, epilepsies, and schizophrenia [5–8]. Furthermore, several experimental studies have demonstrated that T. gondii manipulates the behaviour of rodents. Importantly, it has been reported that toxoplasmosis converts the natural fear of mice against cat urine into attraction, which may greatly facilitate the transmission of the parasite from mice to its specific host, the cat [9–11].

At present it is unclear, how the parasite may alter the behaviour of its host. Since T. gondii infects and persists in neurons, the parasite may directly modulate neuronal function. To address this question, we performed combined in vivo and in vitro studies on the influence of bradyzoites and tachyzoites on neuronal function in T. gondii-infected BALB/c mice. In fact, in vitro experiments, demonstrated that live but not heat-killed tachyzoites actively prevented normal Ca²⁺ responses of glutamate-stimulated neurons. In good agreement, in vivo experiments showed that neurons harbouring T. gondii cysts became functionally impaired as...
indicated by a reduction of neuronal activity-dependent thallium uptake, a potassium analogue. In addition, immunohistochemistry demonstrated that not only cysts but also the cytoplasm of many infected neurons stained positive for *Toxoplasma* antigen indicating that bradyzoite antigens are not sequestered in cysts. Collectively, these findings establish that *T. gondii* alters actively neuronal function and can no longer be regarded as a silent passenger of these findings.

Methods

Ethics statement

All animal experiments were approved according to German and European legislation by the Landesverwaltungsamt Halle (Sachsen-Anhalt, Germany; approval number 42502-2-9751IN).

Animals and infections

Six to eight weeks-old female BALB/c and NMRI were obtained from Harlan-Winkelmann (Borchen, Germany) and kept under special-pathogen free conditions in the animal facility of the University of Magdeburg (Germany). For infection, cysts of a type II strain of *T. gondii* (DX strain) were isolated from the brains of chronically infected NMRI mice (>3 months after infection) and, counted under a light microscope and adjusted to a concentration of 8 cysts/ml in 0.1 M phosphate-buffered saline (PBS). Finally, 500 μl of this suspension was administered orally to BALB/c mice by gavage. For *in vitro* infection of neurons, tachyzoites of the DX strain were grown in human foreskin neurons.

Behavioural experiments

Infected (day 30 and 60 p.i.) and uninfected control mice were tested in an open field arena (1×1 m, divided into 16 quadrants). In all behavioural experiments, mice were only used once, i.e. only before infection, at day 30 or at day 60 p.i. One day before the experiment, mice were familiarised with the field arena for 10 min. At that time, glass containers filled with saline solution were placed in two opposite corners of the field. The next day, one container was filled with two drops of cat urine and the other with rabbit urine. Mice were placed in the centre of the arena and were allowed to explore it freely for 10 min. Movement of mice was monitored with a digital camera and analysed by an automated tracking system (TSE Systems, Germany). It was calculated how much time mice spent in each quadrant, how often mice crossed the field, and how far they moved in each quadrant.

Immunohistochemistry

For immunohistochemistry, mice were narcotized with ketamine/xylazine (10/0.5 mg per 100 g body weight, i.p.) and perfused intracardially with 50 ml of PBS (pH 7.4) followed by 200 ml of 4% paraformaldehyde in PBS. Brains were removed, post-fixed overnight in 4% paraformaldehyde at 4°C, cryoprotected in PBS containing 30% sucrose at 4°C for 2 days, and, thereafter, frozen in 2-methylbutan at −50°C. Serial frontal brain sections (50 μm) were cut on a cryostat (Leica C 3050, Wetzlar, Germany) and collected in 0.1 M PBS. After blocking of unspecific peroxidase and antigen reactions, sections were incubated with rabbit polyclonal anti-*T. gondii* antibody (Biogenex, Duiven, Netherlands) in PBS with 0.1% Triton X100 for 48 h followed by incubation with biotinylated anti-rabbit secondary antibody (Sigma, Munich, Germany) which was visualized using the avidin–biotin–peroxidase method (ABC-kit, Vector Laboratories, Burlingame, CA) with diaminobenzidine as chromogen. Tissue was counterstained with Haematoxylin Eosin, cresyl violet and methyl green, respectively. The number and locations of (i) cysts, (ii) infected neurons, (iii) infected glia, and (iv) inflammatory areas were determined microscopically in each brain section of complete brain series. Brain areas were assigned according to a mouse stereotaxic atlas [14]. For immunohistochemical staining, cells were fixed with methanol, blocked against unspecific reactions 15 minutes with a blocking solution (45 ml PBS; 5 ml goat serum, 2.5 g sucrose, 1 g BSA, 150 μl Triton-X 100) and labelled with a rabbit polyclonal anti-*T. gondii* (Biogenex) and anti-rabbit antibody (Sigma) against *Toxoplasma* as well as with mouse anti-class III β-tubulin (BABC Richmond, CA, USA) and anti-mouse antibody (Sigma) to label neurons.

Cyst count

The total number of *T. gondii* cysts was evaluated on complete *T. gondii*-immunostained brain section series covering all major anatomic regions of the brain at days 30 and 60 p.i. At least eight sagittal sections per mouse were analysed. In addition, brains were isolated from infected mice and dispersed in 2 ml by passing the tissue through needles with regressing diameter. Finally, cysts were counted microscopically in 25 μl of the brain suspension and the total number of cysts per brain was calculated.

Thallium application and autometallography

Uninfected control mice as well as at day 30 and 60 p.i. injected mice were used for thallium (Tl) experiments. Intravenous Tl+ application and staining were performed according to previously published protocols [15,16]. In brief, catheters were placed into the right external jugular vein of mice and two days after catheterization mice were injected with 200 μl of 0.05% thallium diethylidithiocarbamate in 0.9% NaCl. Immediately thereafter, mice were anaesthetized and transcardially perfused with freshly prepared 0.325% Na2S in 100 mM PBS to fix intra-neuronal Tl+ by sulfide precipitation, followed by perfusion with 100 mM PBS containing 3% glutaraldehyde and 0.16% Na2S. Finally, brains were isolated, cryoprotected in PBS containing 30% sucrose at 4°C for 2 days, and, thereafter, frozen in 2-methylbutane at −50°C and post fixed with glutaraldehyde. Brain sections were cut in 25 μm thick slices and developed for visualization of Tl+. Every third section was counterstained with hemalum or Nissl. Tl+ staining was studied microscopically and Tl+ positive as well as Tl+ negative cysts were count in 50 frontal sections (approx. every third section).

Live cell Ca2+ imaging

Cortical cultures from BALB/c mice embryos (E18) were prepared for *in vitro* calcium imaging. Neurons were cultivated in Neurobasal Medium as described by Xie et al. [17]. Live cell imaging was performed as previously published [18]. Intracellular Ca2+ responses were recorded by live cell imaging using cortical cultures grown on glass coverslips for 10–14 days. The cultures were infected with tachyzoites or heat-killed *Toxoplasma* of the DX strain at an multiplicity of infection of 1 for 24, 48, and 72 hours, respectively. On the day of experiment, coverslips were incubated with 2.5 μM flou-4 pentaacetoxy-methylster (Invitrogen, Darmstadt, Germany) for 30 min. Thereafter, coverslips were placed in a stainless steel chamber, mounted on a thermocastically controlled stage (37°C) of an inverted confocal fluorescence microscope (AXIOVERT 100M, LSM PASCAL, Zeiss, Jena, Darmstadt, Germany).
Germany) and superfused (1 ml/min) with HEPES-buffer (140 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM glucose, 10 mM HEPES; pH 7.47) for at least 2 min to obtain a baseline. Thereafter, the neurons were stimulated with 50 µM glutamate for 5 seconds. In some experiments, thapsigargin (2 µM, Sigma, Deisenhofen, Germany) was used to stimulate neurons. Fluorescent images (excitation 488 nm, emission >505 nm) were captured sequentially (10 s intervals). Using Zeiss LSM software, the fluorescence intensity was quantified as average intensity within a region of interest after the subtraction of the background values.

Statistics

Graphpad Prism (Graphpad Software, USA) was used to perform statistical analysis. Behaviour data were analysed by Mann-Whitney U-test for inter-group data comparison and Wilcoxon-test for intra-group data comparison. Students t-test was used for all other set of data. All data are shown as mean value ± SEM.

Results

Altered behaviour of T. gondii-infected mice in chronic but not acute TE

It has been previously shown that infection of BALB/c mice with a type II strain (ME49) of T. gondii abolished the fear of mice against cat urine. Therefore, we used this model of Toxoplasma encephalitis (TE) to further study the interaction of T. gondii with the CNS and in particular with neurons. In good agreement, we also identified neurons as the only cyst harbouring cell population in the CNS. To analyse which parts of T. gondii infected the soma, dendrites and axons (Fig. 3 A–F). This intraneuronal distribution of cysts was detectable both at day 30 and 60 p.i. Importantly, parasitic antigen was also detected outside cysts in a large number of T. gondii-infected neurons (Fig. 3A–F) as well as glia cells (not shown). Some neurons were Golgi-like stained by the T. gondii antigen and their axons and dendrites could be followed over long distances (Fig. 3G). Thus, T. gondii antigen was not absolutely sequestered in cysts but was also present in the host cell cytoplasm. In neurons, cysts but also T. gondii antigens might impact on the function of the host cells to the advantage of the parasite.

T. gondii tachyzoites inhibit Ca²⁺ signalling of neurons

In order to study the influence of T. gondii infections on the neuronal function we performed combined in vitro and in vivo experiments. Since intraneuronal cysts cannot be induced in vitro, we focussed on tachyzoites, which can also infect neurons in vivo, and studied the influence of tachyzoites on neuronal function by live cell calcium imaging of tachyzoite-infected murine primary cortical neurons. These experiments revealed that tachyzoites also infected the soma, dendrites, and cytoplasm of the cultivated cortical neurons (Fig. 4A–C). We used an multiplicity of infection of 1, which resulted in infection of approximately 20% of neurons. In non-infected cultures, short-term glutamate stimulation of viable, propidium-iodide-negative neurons resulted in an increase of cytoplasmic Ca²⁺ levels, which dropped after withdrawal of the stimulus (Fig. 4D, E). In addition, non-infected neurons of cultures infected with the parasite showed the same increase of cytoplasmic Ca²⁺ (data not shown). In sharp contrast, tachyzoite infection dysregulated glutamate-induced increase of intracellular Ca²⁺. Approximately 56% of infected neurons were hyper-responsive to glutamate and did not reduce intracellular Ca²⁺ levels after termination of glutamate stimulation (Fig. 4D, E). Remaining infected neurons showed the opposite phenomenon and were non-responsive without showing any increase in intracellular Ca²⁺ levels upon glutamate stimulation (Fig. 4D, E). In contrast to living parasites, heat-killed T. gondii did not alter glutamate-induced Ca²⁺ responses in neurons illustrating that active parasitic infection of neurons is a prerequisite for the dysregulation of neuronal function (Fig. 4F).

Thapsigargin, an irreversible SERCA (Sarcoplasmic/endoplasmic reticulum calcium ATPase) inhibitor, induced a sustained increase. These findings indicate that T. gondii infection depleted intracellular Ca²⁺ stores, since the major source of Ca²⁺ is analogous to potassium ions and can therefore be used as a tracer for imaging neuronal activity. Generally, there was no global or regional change of activity in T. gondii-infected brains (Fig. 5A–D, overviews). However, at the immunohistochemical investigation. We observed that cysts resided in all major parts of neurons including the neuronal soma, dendrites and axons (Fig. 3 A–F). This intraneuronal distribution of cysts was detectable both at day 30 and 60 p.i. Important, parasitic antigen was also detected outside cysts in a large number of T. gondii-infected neurons (Fig. 3A–F) as well as glia cells (not shown). Some neurons were Golgi-like stained by the T. gondii antigen and their axons and dendrites could be followed over long distances (Fig. 3G). Thus, T. gondii antigen was not absolutely sequestered in cysts but was also present in the host cell cytoplasm. In neurons, cysts but also T. gondii antigens might impact on the function of the host cells to the advantage of the parasite.
cellular level our results showed that already 40% of cyst
harbouring neurons had a relatively reduced or even no Tl
uptake and, thus, became functionally impaired at day 30 p.i.
(Fig. 5B). In chronic TE (day 60 p.i.), the number of Tl
negative cyst harbouring neurons (Fig. 5D) strongly and significantly
increased to 78% (p < 0.05, Fig. 5E). Importantly, cyst harbouring
neurons of all brain regions showed a reduced Tl uptake at day
60 p.i. suggesting that functional inactivation of neurons in chronic
TE is not limited to certain neuronal subtypes. In addition, Tl
negative cysts had normal host cell borders (Fig. 5A, D, insets)
illustrating that Tl negativity was not caused by a rupture of
cysts.

Figure 1. Loss of fear against cat urine in chronically T. gondii-infected mice. (A) Compared to non-infected BALB/c mice, no increase in the
time spent in cat urine corner was evident in mice at day 30 p.i. (control: n = 6, infected: n = 6). In contrast, at day 60 p.i., infected mice spent a
significantly longer time in the cat urine corner as compared to non-infected control mice (p < 0.05; control: n = 8, infected: n = 9). (B) The relative
occupancy of the cat urine corner (ratio of time spent only in the cat urine corner to the total time spent in both cat and rabbit urine corner) was
significantly increased at day 60 (p < 0.05) but not at day 30 p.i. as compared to non-infected control mice. (C, E) Compared to non-infected BALB/c
mice, no difference in the number of visits or the distance travelled within the cat and rabbit urine corner were evident at day 30 p.i. (control: n = 6,
infected: n = 6). However at day 60 p.i., the number of visits to and the distance travelled within the rabbit urine corner were decreased (p < 0.05;
control: n = 8, infected n = 9). (D, F) The relative visits to the cat urine corner and the relative distance travelled within the cat urine corner were
significantly increased in the infected mice at day 60 (p < 0.05 for both parameters) but not at day 30 p.i. as compared to non-infected control mice.

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Since heat-killed parasites did not alter glutamate-induced Ca\textsuperscript{2+} influx, whereas infected neurons responded either with a short Ca\textsuperscript{2+} response, which dropped within 50 sec to baseline levels, or a sustained calcium response in uninfected neurons, the observation that thapsigargin, an irreversible SERCA inhibitor, induced a sustained calcium response in uninfected neurons, implies that cysts also ensure their intraneuronal survival.

The parasitophorous vacuole of *T. gondii* is in intimate contact with the endoplasmic reticulum of the host cell and interacts with the calcium modulating ligand of the organelle through the parasitic type III trans-membrane protein GRA3 [20,21]. This interaction might modulating ligand of the organelle through the parasitic type III reticulum of the host cell and interacts with the calcium stores in the endoplasmatic reticulum.

Pioneer work of Ferguson and colleagues has identified neurons as the only cyst harbouring cell population in the CNS in 1987 [2]. In extension, we identified that cysts inhibited neuronal activity as revealed by T\textsuperscript{IT}AMG staining. These experiments revealed that T\textsuperscript{IT}AMG-stained brain sections demonstrated strongly reduced potassium influx upon stimulation, implying that cysts also ensure their intraneuronal survival.

In addition, we identified that cysts inhibited neuronal activity as revealed by T\textsuperscript{IT}AMG staining. These experiments revealed that T\textsuperscript{IT}AMG-stained brain sections demonstrated strongly reduced potassium influx upon stimulation, implying that cysts also ensure their intraneuronal survival.
the efficient transmission of the parasite from an intermediate host to its specific host. In the natural life cycle of *T. gondii*, the transmission of the parasite from mice to cats plays a pivotal role and, therefore, the loss of fear against cat urine odor may be specifically induced by the parasite-mediated manipulation of the CNS and the functional impairment of infected neurons. Importantly, (i) the functional impairment of neurons by cysts, (ii) the high number of cysts in behaviourally relevant brain regions, and (iii) the loss of fear against cat urine odor all increased over time. Since the total number of intracerebral *Toxoplasma* cysts declined significantly from acute to chronic TE, factors other than the total intracerebral parasite load were responsible for the loss of fear against cat urine odor. In this context, it is remarkable that the number of cysts did not decline but slightly increased over time in the hypothalamus and the amygdala. This confirms previous findings by Vyas et al. [9,11] which also observed a relative high amount of cysts in the amygdala of *T. gondii*-infected BALB/c mice, which led to the suggestion that the presence of *Toxoplasma* cysts in brain areas important for behaviour might be responsible for the behavioural changes. However, in contrast to Vyas et al. we did not observe an attraction of infected mice towards cat urine and additionally

Figure 3. *T. gondii* cysts reside in all major compartments of neurons. (A–F) *T. gondii* cysts were identified in dendrites (A, B) (striatum, day 30 i.p.), somata (C, D) pyramid cell in cortex, day 30 i.p., and axons (E, F) (striatum, day 60 i.p.) in *T. gondii* immunostained brain sections. *T. gondii* antigen was also located outside intraneuronal cysts in dendrites (A–D, G), axons (E–G), and somata (A–G). In (G) (cortex, day 60 i.p.), the small arrow points to a dendrite and the large arrow to an axon of a pyramidal neuron which was stained Golgi-like by the *T. gondii* antibody. doi:10.1371/journal.pone.0035516.g003
Toxoplasma Inhibits Neuronal Function

Dendritic infection

Somal infection

Axonal infection

E

glutamate (50 µM)

180

160

140

120

100

80

60

40

arbitrary fluorescent intensity

0

200

400

600

800

1000

Time (s)

controls

hyper-responsive

hypo-responsive

F

glutamate (50 µM)

160

140

120

100

80

60

40

arbitrary fluorescent intensity

0

200

400

600

800

1000

Time (s)

control

Heat-killed Toxoplasma

G

thapsigargin (2 µM)

140

120

100

80

60

40

20

0

arbitrary fluorescent intensity

0

200

400

600

800

1000

Time (s)

control

infected

H

thapsigargin (2 µM)

Ca²⁺ (2 mM)

200

150

100

50

0

-50

arbitrary fluorescent intensity

0

200

400

600

800

1000

1200

Time (s)

control

infected
Figure 4. Disturbed Ca\(^{2+}\) response in glutamate-stimulated, tachyzoite infected cortical neurons in vitro. (A–C) Tachyzoites infect dendrites (A), soma (B), and axons (C) of cultivated cortical neurons as revealed by double immunofluorescence anti-\(T. gondii\) (red fluorescent) and anti-class III \(\beta\)-tubulin staining (green fluorescent). (D) Live cell Ca\(^{2+}\) imaging of non-infected control and tachyzoite-infected neurons upon stimulation with glutamate. The time course of Ca\(^{2+}\) response is shown as three serial images at 0, 500, and 1000 seconds. Red arrow exhibits a hyper-responsive infected cell while the white arrows demonstrate hypo-responsive infected cells. A counter immunofluorescence staining of the infected culture was done with anti-\(T. gondii\) showing the presence of toxoplasma tachyzoits in the imaged neurons. (E) A graphical representation of calcium responses elicited by controls and infected neurons; control (\( n = 6 \)), infected hyper-responsive (\( n = 7 \)), infected hypo-responsive (\( n = 7 \)). Data are represented as mean ± SEM. (F) Neurons infected with heat killed \(T. gondii\) in-vitro showed no changes in calcium responses upon glutamate stimulation. (G) Thapsigargin induces a huge calcium response in the uninfected controls (\( n = 8 \)) which does not regress due to the inability to reuptake calcium in the endoplasmic stores, but the infected neurons (\( n = 9 \)) show only a weak signalling upon thapsigargin-stimulation indicating a depletion of intracellular calcium stores.

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Figure 5. The majority of cyst-infected neurons are functionally silenced in chronic TE. Thallium uptake in \(T. gondii\) infected mice 30 d p.i. (A, B) and 60 d p.i. (C, D). Ti\(^{+}\)AMG shows Ti\(^{+}\) positive, functionally active, cyst-infected neurons (white arrows) at day 30 p.i. (A) and day 60 p.i. (C) as well as Ti\(^{-}\) negative cyst-infected neurons at day 30 p.i. (B) and 60 p.i. (D). The upper-left insets in (A–D) show an overview of the Ti\(^{+}\) stained sections and the area (rectangle) containing the illustrated cysts. In (A, D), c depicts the cytoplasm of the infected neurons and \(\Phi\) the cysts. In (A), lower-left inset, the tissue section has been stained with Ti\(^{+}\) and thereafter counterstained with hemalum to show the intact cell wall of the neuron and cyst (blue arrow). Similarly, in (D) lower-left inset shows the Nissl-counterstained neuron and cyst (blue arrow). (E) A quantitative analysis of Ti\(^{+}\) positive and negative neurons was performed at day 30 and 60 p.i. from three mice per experimental group. The total number of cysts as well as the number of Ti\(^{+}\) negative cysts per brain were determined on 50 corresponding sections from various brain regions of each mouse. Data show a significant increase of Ti\(^{+}\) negative cyst-infected neurons at day 60 compared to day 30 p.i. (\( p < 0.05 \)).

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could not detect behavioural alterations in acute TE. These differences may be explained by the use of different type II strains as well as different doses of infection in our and the previous study.

It has been reported that *T. gondii*-infected humans suffer significantly more frequently from epilepsy as compared to non-infected persons [5,7,27]. In addition, several studies reported an altered behaviour of *T. gondii* infected humans [28,29]. Although it is tempting to speculate that (1) *T. gondii*-induced hyper-responsiveness to excitatory amino acids, as observed in our in vitro experiments, may contribute to the development of epilepsy and (2) *T. gondii*-mediated hypo-responsiveness of infected neurons may cause behavioural changes, we would like to emphasize that these human disorders are complex and that it is unlikely that *T. gondii* can induce these diseases without other crucial co-factors. Even if *T. gondii* contributes to the development of these disorders, various parameters of TE in addition to direct manipulation of neurons by the parasite could play a role including inflammation and changes in neurotransmitter production [1,29,30].

In conclusion, both tachyzoites and bradyzoites functionally impair murine neurons and, thus, can no longer be regarded as silent passengers persisting in its intra-neuronal niche. An exact identification of the mechanisms how bradyzoites and tachyzoites interfere with neuronal function but still guarantee the survival of the functionally impaired neurons will enable the design of new drugs with the potential to eradicate persisting *Toxoplasma* from the CNS.

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Author Contributions

Conceived and designed the experiments: FH UH FA PK FW DS EB. Performed the experiments: FH UH FA PK FW DS EB. Analyzed the data: FH UH FA PK HS FW DS EB. Contributed reagents/materials/analysis tools: JG PK. Wrote the paper: UH JG HS DS EB.