Involvement of host defense mechanisms against *Toxoplasma gondii* infection in anhedonic and despair-like behaviors in mice

Motamed E. Mahmoud [a,b], Ragab Fereig [a], Yoshifumi Nishikawa [a*]

[a] National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan

[b] Department of Animal Behavior, Management, Genetics and Breeding, Faculty of Veterinary Medicine, Sohag University, Sohag, 82524, Egypt

Running Head: Depression-like behaviors in *Toxoplasma*-infected mice

* Corresponding author at: National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan.

E-mail address: nisikawa@obihiro.ac.jp (Y. Nishikawa).

ABSTRACT

Toxoplasma gondii is a pathogen relevant to psychiatric disorders. We recently showed that reactivation of chronic *T. gondii* induced depressive-like behaviors in mice. Further, it has been hypothesized that depression-like behaviors are mediated via a host defense mechanism against invading pathogens; proximate mechanisms of this behavioral hypothesis remain unclear. In the present study, we investigate the contribution of indoleamine 2, 3-dioxygenase (IDO), inflammation and interferon-gamma (IFN-γ) on anhedonic- and despair-related behaviors in *T. gondii*-infected mice using sucrose preference and forced swim tests, respectively. First, we confirmed BALB/c mice exhibited both sickness and depression-like behaviors during acute infection. Treatment of infected wild-type mice with minocycline (anti-inflammatory drug) abated sickness and anhedonic- and despair-like behaviors; whereas in *T. gondii*-infected mice, treatment normalized kynurenine/tryptophan (Kyn/Trp) ratios in both plasma and brain tissue. Additionally, *T. gondii* infection failed to induce anhedonic and despair-like behaviors or increase Kyn/Trp ratio in immunocompromised (IFN-$\gamma^{-/-}$) mice; whereas, sickness behavior was observed in both immunocompetent and IFN-$\gamma^{-/-}$ mice following infection. Furthermore, treatment with 1-methyl tryptophan (an IDO inhibitor) did not affect locomotor activity, attenuated clinical score and anhedonic- and despair-like behaviors, and resulted in normal Kyn/Trp ratios in *T. gondii*-infected wild-type mice. Although low levels of serotonin and dopamine were observed in the brain during acute and chronic infection, anhedonic- and despair-like behaviors were not detected in the chronic stage of infection. Collectively, our results demonstrated that immune enhancement in response to infection with *T. gondii* resulted in IFN-γ production, IDO activation, and inflammation associated with anhedonic- and despair-like behaviors.

Key words:

Toxoplasma gondii, 1-methyl tryptophan, Minocycline, Interferon-gamma, sickness behavior, anhedonic-like behavior, despair-like behavior

Introduction

Toxoplasma gondii infection is linked to some mood and psychiatric disorders (1, 2). Furthermore, recent evidence indicated reactivation of chronic *T. gondii* infection induce depression-related behaviors in mice (3). As the pathogenesis of infection largely relies upon host immunity, after *T. gondii* infection, peripheral macrophages and lymphocytes quickly become activated to kill intracellular tachyzoites (4). Sickness and depression-related behaviors are mediated by pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) (5–7). However, the distinction between sickness and depressive-symptoms in *T. gondii* infection remain unclear. Experimental immune challenge with bacterial lipopolysaccharides (LPS) or BCG vaccines exhibited behaviors specific for sickness and depressive-like behaviors. For example, mice exhibited sickness symptoms in the form of reduced body weight and locomotor activity, and depressive symptoms such as reduced preference to sucrose and lower motility in forced swim and tails suspension tests (8, 9).

Genes shown to promote major depressive disorder in humans were hypothesized to be associated with successful immune responses, protection from microbes and enhanced survival in the ancestral environment. Therefore, specific depressive symptoms have been suggested to play roles in pathogen host defense (7). IFN-γ has been linked to depressive symptoms by inducing indoleamine-2, 3-dioxygenase (IDO) activation and depleting tryptophan (Trp), the only known precursor of serotonin. Increased serum IFN-γ levels in response to peripheral immune stimulation enhanced cerebral IDO activity, which may reduce Trp levels in the brain of mice (10, 11). In addition, Trp depletion is caused by stress-induced activation of tryptophan 2,

3-dioxygenase (TDO), a hepatic enzyme, and/or the ubiquitous enzyme, IDO (1, 2, 4, 5). IDO catabolizes Trp into neurotoxic metabolites kynurenine (Kyn) and kynurenic acid (12, 13). Although the catabolism of Trp is stimulated by induction of TDO and IDO, it is still argued that a reduction in Trp blood levels under conditions of stress and inflammation decreased the formation of cerebral serotonin (13). Furthermore, circulating Kyn crosses the blood-brain barrier (BBB), whereby it elevates cerebral Kyn levels (14). Hence, circulating Trp circulation can cross the BBB by competing with other amino acids (13). Minocycline (Mino; 2nd generation tetracycline) is widely used to block the expression of pro-inflammatory cytokines in peripheral and central organs (15–17), and prevent ischemic neuronal death (18, 19). 1-methyl DL-tryptophan (1-DL-MT) has become a reference drug for blocking IDO by competing with Trp. However, only 1-L-MT inhibits enzyme activity of IDO, while 1-D-MT does not (23). Unlike Mino, 1-DL-MT blocks IDO-mediated immune events in rheumatoid arthritis (20–22), and inhibits *T. gondii* multiplication *in vivo* (23, 24). IDO activation in the brain has been proposed to induce Kyn, which may contribute to the depressive symptoms of epilepsy, Alzheimer's disease and cerebral malaria (25–27).

We selected BALB/c mice to examine immune enhancement, as C57BL/6 mice have an insufficient intracerebral immune response (28, 29). BALB/c mice are considered genetically resistant to *T. gondii* infection and, instead of developing acute fatal toxoplasmic encephalitis, establish a chronic latent infection (30–32). Therefore, we predict BALB/c mice will exhibit higher depressive-like behaviors compared with sickness symptoms. We hypothesized that *T. gondii*-induced depressive behaviors were based on immune enhancement in terms of IFN-γ production, activation of IDO and disruption of serotonergic neurotransmission. To test this hypothesis, we used different

approaches to investigate sickness (clinical score and locomotor activity) and depressive (anhedonic- and despair-like) behaviors during the acute stage of *T. gondii* infection in wild-type and IFN-γ-deficient mice (BALB/c background) in the context of treatment with Mino or 1-DL-MT to inhibit inflammation or block IDO functions, respectively. Our findings provide insight into immune enhancement associated with the development of anhedonic- and despair-like behaviors during the acute stage of *T. gondii* infection.

Methods

Ethics statement

This study was performed in strict accordance with recommendations from the Guide for the Care and Use of Laboratory Animals published by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Obihiro University of Agriculture and Veterinary Medicine (Permit numbers 24-13, 24-15, 25-59, 25-61). All injections were performed under isoflurane anesthesia and every effort was made to minimize animal suffering.

Animals

According to our established experimental model (3, 33), experiments were performed using wild-type female BALB/c mice (7 weeks old). Female IFN-$\gamma^{-/-}$ mice (BALB/c background) were obtained from Clea Japan (Tokyo, Japan) and maintained at the National Research Center for Protozoan Diseases (Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan). Animals were examined after one-week accommodation in specific pathogen free-conditions under stable conditions (12-h light/dark cycles; light on from 07:00 h to 19:00 h). Food and water were administered *ad libitum* and all behavioral experiments commenced at 09:00 h.

Toxoplasma gondii culture and infection

Type II PLK strain of *T. gondii* was utilized; the parasite was maintained as tachyzoites in Vero cell culture. After syringe lysis purification, wild-type and IFN-$\gamma^{-/-}$ mice were either intraperitoneally (ip) infected with 10^3 tachyzoites or injected with sterile PBS, as

previously described (33, 34). At designated time points, mice were decapitated without anesthesia, blood was collected in heparinized tubes to obtain plasma, and organs were instantly frozen in liquid nitrogen for storage at −80°C until further analyses.

Treatments and experimental groups

Four days post-infection (dpi), groups of *T. gondii*-infected and PBS-injected mice were treated with either Mino (Sigma-Aldrich, St. Louis, MO; 10 mg/kg, ip) or PBS once a day for 4 days (16). Similarly, groups of *T. gondii*-infected and PBS-injected mice were subcutaneously treated with 50 mg/kg 1-methyl-DL-tryptophan (1-DL-MT; Sigma) or its vehicle once a day for 4 days starting 4 dpi. Injections of 1- DL-MT were prepared in 0.1 N HCl, neutralized with an equal volume of 0.1 M NaOH, buffered with 2× PBS and filtered through a 0.2-μm syringe filter.

Cell culture

Murine microglia cell line (MG6) was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Sigma) containing 10% heat-inactivated fetal bovine serum (FBS; Nichirei Biosciences, Tokyo, Japan), 10 μg/ml bovine insulin (Sigma), and 100 μM 2-Mercaptoethanol (Sigma) (35, 36). Astrocyte cell line (OS3) was cultured in Modified Eagle's Medium (MEM; Sigma) containing 10% heat-inactivated FBS and 5 μg/ml bovine insulin (37). Both MG6 and OS3 cell lines were provided by RIKEN BRC through the National Bio-Resource Project of MEXT, Japan.

Sucrose preference test

Anhedonic-like behavior is putatively considered as a reduction in sucrose preference (38). First, mice were habituated with two bottles of water for one week, followed by one bottle of 1% sucrose and one bottle of water for two days before treatment. The bottle position was switched every day according to a reward test protocol (3, 38). Total consumption of each fluid was measured daily and sucrose preference was calculated using the following formula: [Sucrose intake / (Water intake + Sucrose intake)] × 100.

Forced swim test (FST)

FST evaluations were performed as described previously (39) and in our study (3). FST was conducted under normal light for 6 min and then immobility time was analyzed. Mice were individually placed in the water-filled FST cylinder (Coulbourn Instruments, White Hall, PA; 12-cm diameter, filled to a 25-cm water depth). Water temperature was adjusted to within a thermoneutral range (31 ± 1°C) for rodents. Immobility was defined as remaining motionless, except for necessary movements to maintain floating. The first two minutes of the test allowed for accommodation. Duration of immobility within a 6-min session was recorded as an immobility score. Analysis was performed offline by an experienced observer blinded to experimental groups. After the testing period, mice were towel dried and returned to their housing conditions.

Clinical score

Ethograms were customized according to the appearance of clinical symptoms during *T. gondii* infection within home and naïve cages, as previously reported (3). In brief, scores varied from 0 (no signs) to 10 (all signs). Recorded signs included hunching, piloerection, warmth-seeking behavior (hiding in corner of the cage and beneath

bedding), ptosis (drooping or falling of the upper eyelids), sunken eyes, ataxia, reluctant movement, deficient evacuation and touch reflexes, and lying on belly. Each symptom was equivalent and worth one point, and there were exactly ten measures.

Footprint test

Each mouse was exposed to the footprint test (40) once a day with some modifications. In brief: the feet of mice were brushed with black and red (right- and left-fore foot, respectively), and green and yellow (right- and left-hind foot, respectively) nontoxic paints. Animals were then allowed to walk along a 50-cm-long and 10-cm-wide enclosed runway with 10-cm-high walls. All mice performed three training runs before being subsequently tested with one run per day. A stride length was measured as the average distance of forward movement between each stride. For each step, three values were measured from one run, excluding footprints made at the beginning and end of the run where the animal was initiating and finishing movement, respectively. The mean value of each set of three values was used in subsequent analysis.

Quantitative reverse transcriptase-PCR (qRT-PCR)

Total RNA was extracted from the left halves of brains using TRI Reagent (Sigma). Reverse transcription was performed using Superscript III™ Reverse Transcriptase (Thermo Fisher Scientific, Waltham, MA), according to manufacturer's instructions. Amplification was performed using a standard protocol recommended by the manufacturer (2 min at 50°C, 10 min at 95°C, 40 cycles at 95°C for 15 s, and 60°C for 1 min). Samples were run in duplicate. Amplification, data acquisition and data analysis were carried out in an ABI Prism 7900HT Sequence Detection System (Thermo Fisher),

198 and calculated cycle threshold (Ct) values were exported to Microsoft Excel for analysis.
199 Expression levels of each gene relative to glyceraldehyde 3-phosphate dehydrogenase
200 (GAPDH) were calculated using the $2^{\Delta Ct}$ method (User Bulletin no. 2; Perkin-Elmer
201 Applied Biosystems, Waltham, MA). Optimal reference gene was selected based on the
202 Cotton EST database (http://www.leonxie.com). Specific primers for each gene were
203 designed using PRIMER EXPRESS software (Perkin-Elmer Applied Biosystems). A
204 list of primer sequences is shown in supplementary Table 1.
205
206 **DNA isolation and qPCR detection of *T. gondii***
207 Quantitative (q) PCR was performed on purified DNA from brain homogenate, cultured
208 cells and egressed parasites in the culture medium using TRI reagent (Sigma), as
209 previously described (34). Briefly, after DNA purification, 50 ng of DNA extracted
210 from brain homogenate or total cellular DNA was used for amplification of parasite
211 DNA with primers specific for the *T. gondii* B1 gene (41). Parasite numbers were
212 calculated by interpolation on a standard curve, with Ct values plotted against a known
213 parasite concentration. MG6 and OS3 cells were pre-treated with 1-DL-MT (0.05–1.0
214 μM), Mino (5.0–40.0 nM), or IFN-γ (250 units/ml) for 24 h. After changing the media,
215 cells were treated again with 1-DL-MT or Mino, and infected with *T. gondii* (PLK
216 strain; with MOI = 0.25) for an additional 48 hr. Data are presented as % of control
217 inhibition in *T. gondii*-infected cells according to the previously described formula (34).
218 The percentage of inhibition = [(mean value of control) − (value of test sample)]/(mean
219 value of control)] × 100.
220
221 **High-performance liquid chromatography (HPLC)**

Major monoamines, L-Trp, and their metabolites, were examined in supernatants obtained from the right brain hemispheres, using an SC-5ODS column (Eicompak) and electrochemical detector according to the monoamine analysis application manual (Eicom, Kyoto, Japan) and as previously described (3). Wet samples (100 mg) were homogenized in 0.5 mL of 0.2 M perchloric acid (including 100 μM EDTA-2Na and 1 μg/mL isoproterenol as an internal standard). External calibration was made using freshly prepared standards. Final standard concentrations (100 pg), prepared daily from a stock in 20 mM acetic acid and stored at 4°C until use, were injected into the system. The mobile phase (pH 3.5) consisted of 0.075 M NaH_2PO_4 and 25 mM EDTA in 0.1 M citrate acetate buffer (83%) and methanol (17%). The flow rate was maintained at 0.5 mL/min throughout chromatographic runs. Chromatographs were quantified using PowerChrom software version 2.5 (eDAQ Pty Ltd., Densitone East, Australia).

ELISA

Levels of plasma cytokines (IFN-γ, IL-1β, IL6, and TNF-α) were measured using an ELISA kit (BD Biosciences Pharmingen, Piscataway, NJ) according to the manufacturer's recommendations. Amount of cytokine produced was calculated using a standard cytokine curve performed on each immunoplate. Plasma L-Trp was assayed with a Bridge-It L-Trp Fluorescence Assay (Mediomics, St. Louis, MO) using a fluorescence microplate reader (Trp detection limit was 4.114 μmol as 0.036 at OD value, using 485-nm excitation and 665-nm emission). Plasma L-kynurenine (L-Kyn, Sigma) assayed using a competitive ELISA with mouse L-Kyn monoclonal conjugated antibody (Abcam, Tokyo, Japan; Kyn detection limit was 35.5 nmol as 0.042 at OD value); readings were plotted against an L-Kyn standard curve.

246

247 **Kynurenine production assay**

248 IDO activity is directly correlated with concentration of Kyn, its stable conversion

249 product from Trp, as described previously (3, 34, 42).

250

251 **Statistical Analysis**

252 Statistical analyses were performed using GraphPad Prism 5 software (GraphPad

253 Software, La Jolla, CA). Results are presented as mean ± standard deviation. The

254 significance of differences was evaluated by a two-way analysis of variance (ANOVA)

255 followed by Tukey's multiple comparisons procedure or student's *t*-test. Data for

256 sucrose preference were analyzed by a two-way ANOVA followed by Bonferroni's *post*

257 *hoc* test. Where applicable, data are presented with superscripts to indicate statistically

258 significant differences. A P-value < 0.05 was considered statistically significant.

259

Results

Attenuation of anhedonic-like behavior in *T. gondii*-infected mice with Mino or 1-DL-MT treatment or IFN-γ deficiency

Reduced sucrose preference as a putative indicator of anhedonic-like behavior was measured using a two-bottle preference test (Figure 1). Initially, reduced sucrose consumption appeared at 7 dpi with *T. gondii*, before reaching a peak level at 10 dpi and returning to control levels after 2 weeks (Figure 1A). This result indicates anhedonic-like behavior was induced by acute infection with *T. gondii*. To test the role of inflammatory response in anhedonic-like behavior, treatment of wild-type mice with an anti-inflammatory agent, Mino, were performed (Figure 1B). Compared with the *T. gondii*/PBS-treated group, reduced sucrose consumption during acute infection was prevented by treatment with Mino. This indicates the inflammatory response resulting from infection with *T. gondii* played a role in the anhedonic-like behavior observed in mice. Next, effects of IFN-γ in anhedonic-like behavior were examined using IFN-$\gamma^{-/-}$ mice (Figure 1C), as IFN-γ is an important cytokine for inflammatory responses. Although reduced sucrose consumption was observed in *T. gondii*-infected wild-type mice from 6 to 10 dpi, the infected IFN-$\gamma^{-/-}$ mice showed reduced consumption at similar levels of the infected wild-type animals only at 10 dpi, suggesting a contribution of IFN-γ to anhedonic-like behavior. Since IFN-γ can activate IDO activity, we used IDO inhibitor, 1-DL-MT, to treat anhedonic-like behavior in infected wild-type mice (Figure 1D). Treatment with 1-DL-MT attenuated the reduction in sucrose preference from 9 to 14 dpi in *T. gondii*-infected mice. Collectively, these data suggest anti-inflammatory treatment, deficiency of IFN-γ or inactivation IDO may prevent onset of anhedonic-like behavior.

Attenuation of despair-like behavior in *T. gondii*-infected mice with Mino or 1-DL-MT treatment or IFN-γ deficiency

To test the effect of *T. gondii* infection on despair-like behavior, we measured total time spent floating (immobility duration) in the FST at 10 and 30 dpi (Figure 2A). At 10 dpi, but not at 30 dpi, increased immobility duration was observed, indicating despair-like behavior during acute infection. To determine whether *T. gondii*-induced despair-like behavior is dependent on inflammatory responses, wild-type mice were treated with Mino (Figure 2B). Treatment of infected mice with Mino reduced immobility duration compared with untreated mice, indicating despair-like behavior is mediated by inflammatory responses. In the case of IFN-$\gamma^{-/-}$ mice, there was no significant difference in immobility duration between infected and non-infected mice; whereas, immobility duration of *T. gondii*-infected IFN-$\gamma^{-/-}$ mice was lower than that of infected wild-type mice (Figure 2C), indicating IFN-γ-dependent despair-like behavior. However, immobility duration of IFN-$\gamma^{-/-}$ mice was higher than that of uninfected wild-type mice (Figure 2C), suggesting higher sensitivity of IFN-$\gamma^{-/-}$ mice to despair-like behavior. To evaluate effects of IDO activity on despair-like behavior, wild-type mice were treated with 1-DL-MT (Figure 2D). Similar to results from Mino treatment, reduced immobility duration was observed in infected mice treated with 1-DL-MT compared with untreated infected animals. Taken together, despair-like behavior observed during acute infection may be induced by IDO activity via inflammatory responses.

Sickness behavior in IFN-$\gamma^{-/-}$ mice and wild-type mice treated with Mino and 1-MT

Acute *T. gondii* infection induces sickness behavior in terms of considerably elevated clinical score (prepared ethogram) and reduced locomotor activity (stride length in footprint test). Treatment of infected mice with Mino reduced clinical signs and improved stride length in the footprint test of *T. gondii*-infected mice (Figure 3A), suggesting clinical symptoms and locomotor activity may be mediated by inflammatory responses. Conversely, IFN-$\gamma^{-/-}$ mice exhibited clinical symptoms similar to wild-type animals including a marked reduction in locomotor activity, as indicated by shortened stride in the footprint test in infected compared with uninfected wild-type mice (Figure 3B), suggesting IFN-γ may not be directly linked to sickness behavior. Treatment with 1-DL-MT attenuated *T. gondii*-induced sickness symptoms, as indicated by a reduction in clinical score from 5 to 10 dpi; whereas, 1-DL-MT did not alleviate the reduced stride length caused by infection (Figure 3C), suggesting IDO inhibition may reduce clinical symptoms but not affect locomotor activity. Considered with observations of anhedonic- and despair-like behaviors, inflammatory responses during acute infection (including IDO activation) may induce both sickness and depressive-like behaviors. However, sickness and depressive-like behaviors may result from independent pathways, as in the case of IFN-$\gamma^{-/-}$ mice.

Expression of pro-inflammatory cytokines in IFN-$\gamma^{-/-}$ mice and wild-type mice treated with Mino and 1-DL-MT

T. gondii infection increased the expression of IL-1β, TNF-α and IFN-γ in the brain (Figure 4). The correlation analysis between levels of pro-inflammatory cytokine expression and depressive-like behavior indicated anhedonic- and despair-like behaviors, and levels of IFN-γ and IL-1β were strongly and moderately correlated,

respectively (Table S2). Further, inhibitory effects of Mino on the expression of IL-1β, TNF-α and IFN-γ mRNA was observed in brains at 10 dpi (Figure 4A). In addition, plasma levels of IL-1β and IFN-γ were reduced by Mino treatment in infected mice (Table 1). These results indicate that the changes in pro-inflammatory cytokine expression were associated with altered behaviors after *T. gondii* infection. IFN-$\gamma^{-/-}$ mice exhibited attenuation of IL-1β and TNF-α mRNA (Figure 4B); however, 1-DL-MT treatment did not reduce mRNA expression of IL-1β, TNF-α and IFN-γ in infected mice (Figure 4C). In addition, compared with untreated and *T. gondii*-infected mice, 1-DL-MT treatment did not alter production of IL-1β and IFN-γ after *T. gondii* infection (Table 1). Therefore, these results suggest an involvement of IDO activity in induction of anhedonic- and despair-like behaviors, as well as clinical symptoms during acute *T. gondii* infection.

Expression of IDO in IFN-$\gamma^{-/-}$ mice and wild-type mice treated with Mino and 1-DL-MT

Expression of IDO1 and its other form IDO2 have been described in both mice and humans (43). To determine whether IDO1 and IDO2 expression was correlated with altered-behavior exhibited during *T. gondii* infection, qRT-PCR of brains was performed (Figure 5). As shown in Table S2, expression levels of IDO1 and IDO2 in the brain correlated with immobility duration, but not with sucrose preference, suggesting expression of IDO1 and IDO2 play a role in the induction of despair-like behaviors. At 10 dpi, decreased expression of IDO1 and IDO2 was observed in Mino-treated wild-type mice compared with *T. gondii*-infected mice (Figure 5A). Decreased expression of IDO1 was also observed in IFN-$\gamma^{-/-}$ mice compared with

infected wild-type mice (Figure 5B). However, 1-DL-MT treatment did not affect IDO2 mRNA expression, but significantly increased IDO1 expression in *T. gondii*-infected mice at 10 dpi (Figure 5C). Thus, genetic deletion of IFN-γ and blockage of pro-inflammatory cytokines by Mino was associated with reduced IDO expression. However, 1-DL-MT treatment was not associated with reduced IDO expression in *T. gondii* infected mice.

Tryptophan metabolism in IFN-$\gamma^{-/-}$ mice and wild-type mice treated with Mino or 1-DL-MT

To determine the mechanism by which alterations in Trp degradation contribute to depressive-like behavior following *T. gondii*-infection, we measured Trp, Kyn and 5-hydroxytryptmine (5-HT) in brains, as well as plasma levels of Trp and Kyn (Figure 6). Calculated Kyn/Trp ratio in brains at 10 dpi was approximately 3.6-fold greater than at 30 dpi, whereas in plasma it was approximately 1.8-fold (Figure 6A). It was noted that *T. gondii* infection did not affect Trp turnover to 5-HT at 10 and at 30 dpi in wild-type mice, as measured by comparing the ratio of 5-HT/Trp (Figure 6A). At 10 dpi, Kyn/Trp ratios in plasma and brain exhibited a weak to moderate correlation with anhedonic-like behaviors and a moderately strong correlation with despair-like behaviors (Table S2). To further investigate the effects of inflammatory response and IFN-γ action on Trp metabolism, we measured Kyn/Typ and 5-HT/Trp in Mino-treated wild-type mice (Figure 6B) and IFN-$\gamma^{-/-}$ mice (Figure 6C). Reproducibly, *T. gondii* infection markedly increased the Kyn/Trp ratio in both plasma and the brain. These altered ratios of Kyn/Trp were significantly reduced in the plasma and brains of Mino-treated wild-type mice and in IFN-$\gamma^{-/-}$ mice; whereas, 5-HT/Trp was not affected

(Figure 6B, C). In parallel, treatment of *T. gondii*-infected wild-type mice with 1-DL-MT normalized the Kyn/Trp ratio in both plasma and the brain (Figure 6D). Next, we examined levels of 5-HT and dopamine in the brain (Figure 7). Lower levels of 5-HT and dopamine were observed in infected wild-type mice at both 10 and 30 dpi. Dopamine metabolism was upregulated in infected mice at 30 dpi as the result of lower DA levels and higher DA turnover compared with uninfected mice (Figure 7B). Thus, 5-HT and dopamine may not be directly involved in depressive-like behavior because the behavioral changes were not seen at 30 dpi. Together, these findings indicate *T. gondii* enhanced Trp catabolism towards Kyn, not 5-HT.

Effect of Mino and 1-DL-MT on parasite growth *in vivo* and *in vitro*

At 14 dpi, parasite number in brain DNA was measured by qPCR (Figure 8). We found that treatment of infected mice with Mino and 1-MT reduced brain parasite load (Fig. 8A). This effect was validated *in vitro* in *T. gondii*-infected microglia and astrocyte cell lines, MG6 and OS3, respectively. Pretreatment with Mino (5–40 nM) and 1-DL-MT (0.05–1 μM) had dose-dependent effects in restricting *T. gondii* replication in MG6 and OS3 (Figure 8B and C). No effect on cell viability at the selected dose ranges was observed (data not shown). These findings indicate that both 1-DL-MT and Mino exhibit significant anti-*Toxoplasma* activities *in vivo* and *in vitro*.

Discussion

Causes of depression do not stem from single source; rather, depression is likely to be caused by a combination of factors. Perpetuation of neurotropic parasite *T. gondii* may further complicate this syndrome. In the present study, we observed anhedonic- and despair-like behaviors during the acute stage of *T. gondii* infection. Therefore, immune enhancement may be a prerequisite to develop such a depressive phenotype. Here, we hypothesized that activation of host immunity mediates depressive-like behaviors in *T. gondii*-infected mice. Our results support the view that interactions of the immune system, IFN-γ, IDO and Trp metabolism play crucial roles in developing *T. gondii*–induced depression-like behaviors. In animal models, studies of Kyn pathway and Trp catabolic shunt, indicate these mechanisms are conserved not only between human and rodents but also in lower organisms such as yeast (44). Several lines of evidence illustrate depressive-like behavior is associated with enhancement of Trp catabolism caused by either inflammation or the release of pro-inflammatory cytokines (44, 45). Investigation of this pathway following infection with a chronic pathogen, such as *T. gondii*, will likely continue to advance our understanding of mechanisms by which neurotropic parasites induce some psychiatric disorders (46). Induction of depressive-like behaviors in *T. gondii*-infected mice and enhancement of Trp catabolism towards Kyn may be relevant to interpreting studies of psychobehavioral disorders in *T. gondii*-infected humans.

In the present study, *T. gondii* infection induced depressive-like behavior in forms of anhedonic and despair-like behaviors in mice during the acute stage. Additionally, sickness behavior was also observed in mice following acute infection. As behavioral symptoms of sickness and depression are triggered by pro-inflammatory cytokines (17),

424 it may be difficult to determine whether *T. gondii* directly induced depression-like
425 behavior or generalized inflammatory responses induced by the infection triggered
426 behavioral changes. Although both sickness and depressive-like behaviors are induced
427 by the same pro-inflammatory cytokines, IDO induction has been proposed to lie at the
428 interface between chronic inflammatory disease and depression (17). Although *T.*
429 *gondii*-infected IFN-$\gamma^{-/-}$ mice showed similar clinical scores as infected wild-type mice,
430 infected IFN-$\gamma^{-/-}$ mice did not exhibit anhedonic-like behavior (reduced sucrose
431 preference) at 6–9 dpi, except for at 10 dpi, compared with uninfected IFN-$\gamma^{-/-}$ mice. At
432 10 dpi, both wild-type BALB/c and IFN-$\gamma^{-/-}$ mice showed a comparably sever clinical
433 symptom following the infection. These symptoms at 10 dpi were categorized as
434 sickness-related symptoms. Additionally, locomotor activity of IFN-$\gamma^{-/-}$ mice infected
435 with *T. gondii* reduced compared with that of the infected wild-type BALB/c mice. In
436 fact, IFN-$\gamma^{-/-}$ mice showed reduced intake of both water and sucrose (total fluid intake)
437 that ranges from 0 to 2 ml of each bottle during 24 h. Actually, these IFN-$\gamma^{-/-}$ mice
438 mostly succumbed after 10 days of infection. Thus, the severe reduction of locomotor
439 activity may results in reduced sucrose preference (or total fluid intake) in IFN-$\gamma^{-/-}$ mice
440 infected with *T. gondii* at 10 dpi. It should be noted that uninfected IFN-$\gamma^{-/-}$ mice
441 showed higher immobility duration at FST compared with uninfected wild-type mice.
442 Further, *T. gondii* infection did not increase the immobility duration in IFN-$\gamma^{-/-}$ mice at
443 10 dpi compared with uninfected IFN-$\gamma^{-/-}$ mice (Figure 2C). This result suggests
444 sickness and anhedonic-like behaviors may occur in *T. gondii*-infected mice by
445 independent mechanisms. However, at present, it is unclear why genetic deletion of
446 IFN-γ induced despair behavior (increased immobility duration in FST) in uninfected
447 IFN-$\gamma^{-/-}$ mice compared with uninfected wild-type mice. Perhaps, the relationship

between IFN-γ and Trp metabolism may be linked to the immunogenetics of major depression as reported in some correlation studies (11). Similarly deficiency of toll-like receptor 2 was found to be associated with induction of schizophrenia-like symptoms in mice (47). Collectively, these results suggest deficiency of an immunity-related gene, such as IFN-γ, was associated with despair- and anhedonic-like behaviors in uninfected mice.

IFN-γ is essential for host defense mechanisms and the survival of mice during *T. gondii* infection. IFN-γ deficient mice succumb to acute infection (48). Further, mice lacking IL-12 or T-cells that regulate or produce IFN-γ, respectively, do not survive the chronic stage of *T. gondii* infection (49-51). IFN-γ can suppress symptoms in *T. gondii* infection and, in fact, can inhibit *T. gondii* proliferation through various mechanisms including: (i) depletion of arginine and production of free radical nitric oxide via enzymatic activation (52) (ii) disruption of parasitophorous vacuoles via IFN-γ-inducible genes, such as immunity-related genes (IRGs) and guanylate-binding proteins (GBPs) in murine macrophages (34, 53, 54) or (iii) starvation of an essential amino acid, Trp, mediated through IDO activity, as shown in human fibroblasts (55), but direct effect of IDO on parasites in mice remains unclear. Thus, IFN-γ and IDO activation was associated with depletion of Trp, suggesting a correlation between host-defense mechanism correlated and display of anhedonic- and despair-like behaviors. Therefore, it is plausible anhedonic- and despair-like behaviors were dependent on IFN-γ or IDO activation, rather than simply linked to sickness behaviors.

Aside from the association of sickness symptoms with depressive-like behavior during the acute stage of *T. gondii* infection, our findings support a role for the Trp to Kyn shunt in depression pathophysiology induced by *T. gondii* infection. Anhedonic-

and despair-like behaviors during the acute stage were present with low 5-HT levels (associated with a high Kyn/Trp ratio in plasma and brain) and normal 5-HT turnover. However, such behaviors were not displayed at 30 dpi in the context of low brain 5-HT and DA levels. In support of these results, we found that Kyn/Trp ratios in the brain of infected mice at 60 dpi did not change from control uninfected mice, indicating lower IDO activity in the chronic stage (3). Thus, development of anhedonic- and despair-like behaviors may not be simply dependent on lower serotonergic and dopaminergic neurotransmission during the acute stage of *T. gondii* infection in mice. It has been reported that IDO induction, Trp degradation, and Kyn formation are completely absent in *T. gondii*-infected IFN-γ deficient mice (56, 57). Our data also confirmed the importance of IFN-γ for IDO activity induction and resultant depressive-like behaviors. Since IFN-γ-mediated IDO activation may induce such behaviors in mice following infection with *T. gondii* during the acute stage, *T. gondii* infection can induce changes in Trp metabolism via pro-inflammatory cytokines, especially IFN-γ.

However at present, it is unclear why the sustained decrease in DA and 5-HT levels were not reflected in the precipitation of anhedonic- and despair-like symptoms in *T. gondii*-infected mice during chronic infection. On other hand, low 5-HT and DA co-existed with anhedonic- and despair-like behaviors during acute infection. Thus, contribution of DA and 5-HT to depressive-like behaviors could not be concluded. Compared with uninfected mice, unchanged 5-HT/Trp ratio and serotonin turnover, and higher Kyn/Trp ratio indicated enhanced Trp catabolism towards Kyn in infected mice during acute infection. Together, these findings suggest enhanced Trp catabolism towards Kyn resulting from inflammatory response, IFN-γ action and IDO

activity-dependent mechanisms of *T. gondii* infection may be related to the appearance of anhedonic- and despair-like behaviors during acute infection.

In present study, Mino abrogated *T. gondii*-induced anhedonic- and despair-like behaviors; it also normalized Kyn/Trp ratios in the brain and plasma in *T. gondii*-infected mice. It is well known that pro-inflammatory cytokines are involved in depressive-like behaviors through generation of neuroreactive Trp metabolite, Kyn. Kyn metabolite, quinolinic acid, acts as an NMDA receptor agonist (58) and is implicated in the development of core symptoms of depression-like anhedonia and behavioral despair following peripheral administration of cytokine-inducing bacterial LPS (8). Our data showed that treatment with Mino inhibited IDO expression in the brain of *T. gondii*-infected mice, resulting in normalization of Kyn/Trp ratios in both plasma and the brain of infected mice. Mino, a prototype of anti-inflammatory drugs, was used to prevent ischemic neuronal death. In addition, Mino protects neurons from glutamate toxicity and blocks expression of pro-inflammatory cytokines in both peripheral and central organs (17–19). Mino is also a selective inhibitor of microglia activation, and it inhibits apoptosis by decreasing IL-1β, TNF-α and their converting enzymes caspase 1 and caspase 3 (59, 60). Additionally, Mino reduced permeability of the BBB by inhibiting IL-1β, TNF-α, matrix metalloproteinases (MMP)-2 and -9, and VCAM expression, as well as reducing transmigration of T-cells across the fibronectin matrix barrier (61, 62). Therefore, Mino may be a potential therapeutic for psychiatric diseases including early schizophrenia, multiple sclerosis, Huntington disease and Parkinson's disease (59, 60, 62–64). Activated microglia contribute to neuronal apoptosis; thus, inhibition of microglia activation by Mino may represent a novel therapeutic strategy for treating neuronal apoptosis in cases of toxoplasmic encephalitis (66). Mino also

519 prevented anhedonia and sickness behavior in mice challenged with LPS (67). Although
520 LPS induces sickness behaviors followed by depressive-like behaviors, this appeared
521 within a relatively short time-course compared with immune challenge with BCG
522 vaccine (9, 68) or *T. gondii* infection. In the present study, we treated *T. gondii*-infected
523 mice with Mino for 4 days at doses of 10 mg/kg, ip. Our treatment regimen decreased
524 clinical symptoms induced by the infection without inducing side effects. Furthermore,
525 Mino lowered brain parasite burden (Figure 5A), thus conferring the strongest
526 protective effect against *T. gondii* in murine microglia and astrocyte cells (Figure 4B
527 and C). *T. gondii* tachyzoites invade murine microglia, astrocytes, and neurons;
528 thereafter, the parasite forms cysts within these cells (69). Our data for parasite
529 growth indicated microglia have 17 times the ability of astrocytes to control *T. gondii*.
530 Within the brain, microglia are reported to be the major effector cells for prevention of
531 *T. gondii* tachyzoite proliferation (69). At the neuronal level, Mino salvaged glutamate
532 toxicity in cultured primary neurons (16). Thus, Mino may have the potential to control
533 acute toxoplasmosis while anti-*Toxoplasma* mechanism of Mino is still unknown.

534 1-DL-MT has previously been used as a reference drug for blocking IDO activity
535 in human and murine cells (20, 22, 70). In clinical trials, 1-DL-MT has been used as a
536 vaccine adjuvant and an add-on immunotherapeutic agent for cancer patients (71). As
537 IDO triggers Trp depletion in the cellular microenvironment, it can also inhibit
538 proliferation of T-cells and permit tumor cells to escape the immune system (72).
539 Therefore, IDO inhibition by 1-DL-MT reverses the immunosuppressive effects of IDO
540 (73). Interestingly, chronic treatment with 1-L-MT or 1-DL-MT results in controversial
541 antiparasitic activity, but subchronic treatment exerts some anti-*Toxoplasma* activity (23,
542 24). Furthermore, $IDO^{-/-}$ mice and mice with IDO inhibited by 1-DL-MT exhibit

543 reduced *T. gondii* mRNA expression in the lung (24). In our study, 1-DL-MT treatment
544 attenuated anhedonic- and despair-like behaviors in *T. gondii*-infected mice without an
545 obvious effect on pro-inflammatory cytokine expression, suggesting the effect of
546 1-DL-MT is independent of pro-inflammatory cytokine expression. Furthermore, IDO
547 activity may be involved in anhedonic- and despair-like behaviors, and such behaviors
548 are unlikely to be linked to cytokine production in *T. gondii*-infected mice. Our results
549 indicate that 1-DL-MT, like Mino, significantly reduced the parasite load *in vivo* and *in*
550 *vitro* (Figure 8). It would be of interest in future studies to examine mechanisms
551 underlying the ability of Mino and 1-DL-MT to control growth of *T. gondii*.

552 In summary, the present study suggests depressive-like behaviors are likely mediated
553 by host defense mechanisms against *T. gondii* infection. As a host immune response
554 against acute *T. gondii* infection, IFN-γ produced by T-cells or natural killer cells
555 stimulates IDO activity. Metabolites of IDO activity, Trp to Kyn, resulted in
556 depressive-like behaviors. Treatment with Mino and 1-DL-MT ameliorated *T.*
557 *gondii*-induced anhedonic- and despair-like behaviors, suggesting the potential of this
558 drug to treat core depression symptoms. As these drugs are highly lipid soluble and
559 capable of penetrating the BBB (17), they may be of potential interest for future clinical
560 studies. Since these drugs inhibit *Toxoplasma* growth, use for *T. gondii* infection may
561 be beneficial. Moreover, their effect was associated with symptomatic relief of
562 anhedonia and despair, as well as improvement of clinical symptoms or locomotor
563 activity. Our results demonstrate that immune enhancement in response to infection of
564 immunocompetent mice with *T. gondii* resulted in IFN-γ production, IDO activation,
565 and inflammation associated with sickness, anhedonic- and despair-like behaviors. In
566 contrast, after inhibition of IFN-γ production, sickness symptoms were displayed

without the other behaviors. Inactivation of IDO improved clinical symptoms and depressive-like behaviors. To conclude, host defense mechanisms against *T. gondii* infection may be involved in anhedonic- and despair-like behaviors in mice.

Funding information

This research was supported by the Japan Society for the Promotion of Science's Funding Program for Next-Generation World-Leading Researchers (NEXT Program), initiated by the Council for Science and Technology Policy (2011/LS003). This work was also supported by a Grant-in-Aid for Challenging Exploratory Research from MEXT KAKENHI (15K15118). Motamed Mahmoud was supported by postdoctoral fellowship from the Egyptian Ministry of Higher Education and Scientific Research.

Conflicts of interest

The authors declare that there are no conflicts of interest to disclose.

References

1. **Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT.** 2012. "*Toxoplasma gondii* infection and self-directed violence in mothers". Arch Gen Psychiatry **69**: 1123–1130.
2. **Zhang Y, Träskman-Bendz L, Janelidze S, Langenberg P, Saleh A.** 2012. Constantine N, Okusaga O, Bay-Richter C, Brundin L, Postolache TT. *Toxoplasma gondii* immunoglobulin G antibodies and nonfatal suicidal self-directed violence. J Clin Psychiatry **73**: 1069–1076.
3. **Mahmoud ME, Ihara F, Fereig RM, Nishimura M, Nishikawa Y.** 2016. Induction of depression-related behaviors by reactivation of chronic *Toxoplasma gondii* infection in mice. Behav Brain Res **298**: 125–133.
4. **Suzuki Y, Orellana MA, Schreiber R, Remington JS.** 1988..Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. Science **240**: 516–518.
5. **Dantzer R, Kelley KW.** 2007. Twenty years of research on cytokine-induced sickness. Behavior. Brain Behav Immun **21**:153–160.
6. **Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, Gulati M, Thornton AJ, Schultz RL, Valentine AD, Meyers CA, Howell CD.** 2002. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. Mol Psychiatry **7**: 942–947.
7. **Raison CL, Miller AH.** 2013. The evolutionary significance of depression in Pathogen Host Defense (PATHOS-D). Mol Psychiatry **18**: 15–37.
8. **O'Connor JC, André C, Wang Y, Lawson MA, Szegedi SS, Lestage J, Castanon N, Kelley KW, Dantzer R.** 2009. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2, 3-dioxygenase activation in

mice. Mol Psychiatry **14**: 511–522.

9. **Moreau M, André C, O'Connor JC, Dumich SA, Woods JA, Kelley KW, Dantzer R, Lestage J, Castanon N.** 2008. Inoculation of Bacillus Calmette-Guerin to mice induces an acute episode of sickness behavior followed by chronic depressive-like behavior. Brain Behav Immun **22**:1087–1095.
10. **Lestage J, Verrier D, Palin K, Dantzer R.** 2002. The enzyme indoleamine 2, 3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. Brain Behav Immun **16**: 596–601.
11. **Myint AM, Bondy B, Baghai TC, Eser D, Nothdurfter C, Schüle C, Zill P, Müller N, Rupprecht R, Schwarz MJ.** 2013. Tryptophan metabolism and immunogenetics in major depression: a role for interferon-γ gene. Brain Behav Immun **31**:128–133.
12. **Wichers MC, Koek GH, Robaeys G, Verkerk R, Scharpé S, Maes M**. 2005. IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. Mol Psychiatry **10**: 538–544.
13. **Russo S, Kema IP, Fokkema MR, Boon JC, Willemse PH, de Vries EG, den Boer JA, Korf J.** 2003. Tryptophan as a link between psychopathology and somatic states. Psychosom Med **65**: 665–671.
14. **Fukui S, Schwarcz R, Rapoport SI, Takada Y, Smith QR.** 1991. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. J Neurochem **56**: 2007–2017.
15. **Yrjänheikki J, Keinänen R, Pellikka M, Hökfelt T, Koistinaho J.** 1998. Tetracyclines inhibit microglial activation and are neuroprotective in global brain

ischemia. Proc Natl Acad Sci USA **95**: 15769–15774.

16. **Yrjänheikki J, Tikka T, Keinänen R, Goldsteins G, Chan PH, Koistinaho JA.** 1999. Tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci USA **96**: 13496–13500.
17. **Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW.** 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci **9**: 46–56.
18. **Rifkin BR, Vernillo A, Golub LM, Ramamurthy N.** 1994. Modulation of bone resorption by tetracyclines. Ann N Y Acad Sci **732**:165–180.
19. **Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, Patel IR, Abramson SB**. 1996. A novel mechanism of action of tetracyclines: Effects on nitric oxide synthases. Proc Natl Acad Sci USA **93**: 14014–14019.
20. **Munn DH, Sharma MD, Lee JR, Jhaver KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CLJr, Mellor AL.** 2002. Potential regulatory function of human dendritic cells expressing indoleamine 2, 3-dioxygenase. Science **297**: 1867–1870.
21. **Sakurai K, Zou JP, Tschetter JR, Ward JM, Shearer GM.** 2002. Effect of indoleamine 2, 3-dioxygenase on induction of experimental autoimmune encephalomyelitis. J Neuroimmunol **129**: 186–196.
22. **Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, Choi BK, Vinay DS, Kwon BS.** 2004. 4-1BB-mediated immunotherapy of rheumatoid arthritis. Nat Med **10**: 1088–1094.
23. **Divanovic S, Sawtell NM, Trompette A, Warning JI, Dias A, Cooper AM, Yap**

GS, Arditi M, Shimada K, Duhadaway JB, Prendergast GC, Basaraba RJ, Mellor AL, Munn DH, Aliberti J, Karp CL. 2012. Opposing biological function of tryptophan degrading enzyme during intracellular infection. J Infect Dis **205**: 152–161.

24. **Murakami Y, Hoshi M, Hara A, Takemura M, Arioka Y, Yamamoto Y, Matsunami H, Funato T, Seithima M, Saito K.** 2012. Inhibition of increased indoleamine 2, 3 dioxygenase activity attenuates *Toxoplasma gondii* replication in the lung during acute infection. Cytokine **59**: 245–251.
25. **Xie W, Cai L, Yu Y, Gao L, Xiao L, He Q, Ren Z, Liu Z.** 2014. Activation of brain indoleamine 2, 3-dioxygenase contributes to epilepsy-associated depressive-like behavior in rats with chronic temporal lobe epilepsy. J Neuroinflammation **11**: 1–41.
26. **Guillemin GJ, Williams KR, Smith DG, Smythe GA, CroitoruLamoury J, Brew BJ.** 2003. Quinolinic acid in the pathogenesis of Alzheimer's disease. Adv Exp Med Biol **527**: 167–176.
27. **Sanni LA, Thomas SR, Tattam BN, Moore DE, Chaudhri G, Stocker R, Hunt NH.** 1998. Dramatic changes in oxidative tryptophan metabolism along the kynurenine pathway in experimental cerebral and noncerebral malaria. Am J Pathol **152**: 611–619.
28. **McLeod R, Skamene E, Brown CR, Eisenhauer PB, Mack DG.** 1989. Genetic regulation of early survival and cyst number after peroral *Toxoplasma gondii* infection of A 3 B/B 3 A recombinant inbred and B10 congenic mice. J. Immunol. 143:3031. 16.
29. **Suzuki Y, Joh K, Orellana MA, Conley FK, Remington JS.** 1991. A gene(s)

within the H-2D region determines the development of toxoplasmic encephalitis in mice. Immunology **74**:732–739.

30. **Brown CR, Hunter CA, Estes RG, Beckmann E, Forman J, David C, Remington JS, McLeod R.** 1995. Definitive identification of a gene that confers resistance against *Toxoplasma* cyst burden and encephalitis. Immunology **85**: 419–428.
31. **Suzuki Y, Yang Q, Remington JS.** 1995. Genetic resistance against acute toxoplasmosis depends on the strain of *T. gondii*. J Parasitol **81**:1032–1034.
32. **Suzuki Y, Kang H, Parmley S, Lim S, Park D.** 2000. Induction of tumor necrosis factor-alpha and inducible nitric oxide synthase fails to prevent toxoplasmic encephalitis in the absence of interferon-gamma in genetically resistant BALB/c mice. Microbes Infect **2**: 455–462.
33. **Tanaka S, Nishimura M, Ihara F, Yamagishi J, Suzuki Y, Nishikawa Y.** 2013. Transcriptome Analysis of Mouse Brain Infected with *Toxoplasma gondii*. Infect Immun **81**: 3609–3619.
34. **Mahmoud ME, Ui F, Salman D, Nishimura M, Nishikawa Y.** 2015. Mechanisms of interferon-beta-induced inhibition of *Toxoplasma gondii* growth in murine macrophages and embryonic fibroblasts: role of immunity-related GTPaseM1. Cell Microbiol **17**: 1069–1083.
35. **Takenouchi T, Ogihara K, Sato M, Kitani H.** 2005. Inhibitory effects of U73122 and U73343 on Ca2+ influx and pore formation induced by the activation of P2X7 nucleotide receptors in mouse microglial cell line. Biochim Biophys Acta **1726**:177–186.

36. **Nakamichi K, Saiki M, Kitani H, Kuboyama Y, Morimoto K, Takayama-Ito M, Kurane I.** 2006. Suppressive effect of simvastatin on interferon-beta-induced expression of CC chemokine ligand 5 in microglia. Neurosci Lett. **407**: 205–210.

37. **Ohtani K, Suzumura A, Sawada M, Marunouchi T, Nakashima I, Takahashi A.** 1992. Establishment of mouse oligodendrocyte/type-2 astrocyte lineage cell line by transfection with origin-defective simian virus 40 DNA. Cell Struct Funct **17**: 325–333.

38. **Strekalova T, Spanagel R, Bartsch D, Henn FA, Gass P.** 2004. Stress-induced anhedonia in mice is associated with deficits in forced swimming and exploration. Neuropsychopharmacology **29**: 2007–2017.

39. **Lucki I.** 1997. The forced swimming test as a model for core and component behavioral effects of antidepressant drugs. Behav Pharmacol **8**: 523–532.

40. **Carter RJ, Lione LA, Humby T, Mangiarini L, Mahal A, Bates GP, Dunnett SB, Morton AJ.** 1999. Characterization of progressive motordeficits in mice transgenic for the human Huntington's disease mutation. J Neurosci **19**: 3248–3257.

41. **Contini C, Seraceni S, Cultrera R, Incorvaia C, Sebastiani A, and Picot S.** (2005) Evaluation of a Realtime PCR-based assay using the light-cycler system for detection of *Toxoplasma gondii* bradyzoite genes in blood specimens from patients with toxoplasmic retinochoroiditis. Int J Parasitol35: 275–283.

42. **Däubener W, Wanagat N, Pilz K, Seghrouchni S, Fischer, HG, Hadding U.** 1994. **A** new, simple, bioassay for human IFN-gamma. J Immunol Methods **168**: 39–47.

43. **Ball HJ, Yuasa HJ, Austin CJ, Weiser S, Hunt NH.** 2009. Indoleamine 2,

724 3-dioxygenase-2; a new enzyme in the kynurenine pathway. Int J Biochem Cell
725 Biol **41**: 467–471.
726 44. **Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ.** 2012. Kynurenines in the
727 mammalian brain: when physiology meets pathology. Nat Rev Neurosci **13**: 465–
728 477.
729 45. **Parrott JM, O'Connor JC.** 2015. Kynurenine 3-monooxygenase: an influential
730 mediator of neuropathology. Front Psychiatry **6**:116.
731 46. **Noakes R.** 2015. The Aryl Hydrocarbon Receptor: A Review of Its Role in the
732 Physiology and Pathology of the Integument and Its Relationship to the Tryptophan
733 Metabolism. Int J Tryptophan Res **8**: 7–18.
734 47. **Park SJ, Lee JY, Kim SJ, Choi SY, Yune TY, Ryu JH.** 2015. Toll-like
735 receptor-2 deficiency induces schizophrenia-like behaviors in mice. Sci Rep
736 5:8502.
737 48. **Suzuki Y, Orellana MA, Schreiber RD, Remington JS.** 1988. Interferon-gamma:
738 the major mediator of resistance against *Toxoplasma gondii*. Science **240**: 516–518.
739 49. **Khan IA, Matsuura T, Kasper LH.** 1994. Interleukin-12 enhances murine
740 survival against acute toxoplasmosis. Infect Immun **62**:1639–1642.
741 50. **Hunter CA, Subauste CS, Van Cleave VH, Remington JS.** 1994. Production of
742 gamma interferon by natural killer cells from *Toxoplasma gondii*-infected SCID
743 mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha.
744 Infect Immun **62**: 2818–2824.
745 51. **Adams LB, Hibbs JB Jr, Taintor RR, Krahenbuhl JL.** 1990. Microbiostatic
746 effect of murine activated macrophages for *Toxoplasma gondii*: Role for synthesis
747 of inorganic nitrogen oxides from L-arginine. J Immunol **144**: 2725–2729.

52. **Collazo C M, Yap, GS, Sempowski GD, Lusby KC, Tessarollo L, Vande Woude GF, Sher A, Taylor GA.** 2001. Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. J Exp Med **194**: 181–188.

53. **Butcher BA, Greene R I, Henry SC, Annecharico KL, Weinberg JB, Denkers EY, Sher A, Taylor GA**. 2005. p47 GTPases regulate *Toxoplasma gondii* survival in activated macrophages. Infect Immun **73**: 3278–3286.

54. **Pfefferkorn ER.** 1984. Interferon-γ blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. Proc Natl Acad Sci USA **81**: 908–912.

55. **Chao CC, Anderson WR, Hu S, Martella A, Gekker G, Peterson PK**.1993. Acti vated microglia inhibit *Toxoplasma gondii* via a nitric oxide—mechanism. Clin Immunol Immunopathol **67**:178–183.

56. **Fujigaki S, Saito K, Takemura M, Maekawa N, Yamada Y, Wada H, Seishima M.** 2002. L-Tryptophan-l-Kynurenine Pathway Metabolism Accelerated by *Toxoplasma gondii* Infection Is Abolished in Gamma Interferon-Gene-Deficient Mice: Cross-Regulation between Inducible Nitric Oxide Synthase and Indoleamine-2, 3-Dioxygenase. Infect Immun **70**: 779–786.

57. **Silva NM, Rodrigues CV, Santoro MM, Reis LF, Alvarez-Leite JI, Gazzinelli RT.** 2002. Expression of indoleamine 2, 3-dioxygenase, tryptophan degradation, and kynurenine formation during in vivo infection with *Toxoplasma gondii*: induction by endogenous gamma interferon and requirement of interferon regulatory factor 1. Infect Immun **70**: 859–868.

58. **Walker AK, Budac DP, Bisulco S, Lee AW, Smith RA, Beenders B, Kelley**

KW, Dantzer R. 2013. NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J Mice. Neuropsychopharmacology **38**: 1609–1616.

59. **Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu A.S., Hartley, DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, Friedlander RM.** 2002. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. Nature **417**: 74–78.

60. **Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, Bian J, Guo L, Farrell, LA. Hersch SM, Hobbs W, Vonsattel JP, Cha JH, Friedlander RM.** 2000. "Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease". Nat Med **6**: 797–801.

61. **Giuliani F, Hader W, Yong VW.** 2005. "Minocycline attenuates T cell and microglia activity to impair cytokine production in T cell-microglia interaction". J Leukoc Biol **78**: 135–143.

62. **Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW.** 2002. Targeting leukocyte MMPs and transmigration: Minocycline as a potential therapy for multiple sclerosis. Brain **125**: 1297–1308.

63. **Du Y, Ma Z, Lin S, Dodel RC, Gao F, Bales KR, Triarhou LC, Chernet E, Perry KW, Nelson DL, Luecke S, Phebus LA, Bymaster FP, Paul SM.** 2001. Minocycline prevents nigrostriatal dopaminergic neurodegeneration in the MPTP model of Parkinson's disease. Proc Natl Acad Sci USA. **98**: 14669–14674.

64. **Zabad RK, Metz LM, Todoruk TR, Zhang Y, Mitchell JR, Yeung M, Patry DG, Bell RB, Yong VW.** 2007. "The clinical response to minocycline in multiple

796 sclerosis is accompanied by beneficial immune changes: a pilot study". Mult Scler
797 **13**: 517–526.
798 65. **Levkovitz Y, Mendlovich S, Riwkes S, Braw Y, Levkovitch-Verbin H, Gal G,**
799 **Fennig S, Treves I, Kron S.** 2010. "A Double-Blind, Randomized Study of
800 Minocycline for the Treatment of Negative and Cognitive Symptoms in
801 Early-Phase Schizophrenia". J Clin Psychiatry **71**: 138–149.
802 66. **Zhang YH, Chen H, Chen Y, Wang L, Cai YH, Li M, Wen HQ, Du J, An R,**
803 **Luo QL, Wang XL, Lun ZR, Xu YH, Shen JL.** 2014. Activated microglia
804 contribute to neuronal apoptosis in Toxoplasmic encephalitis. Parasit Vectors **13**:
805 372–379.
806 67. **Carruthers VB, Suzuki Y.** 2007. Effects of *Toxoplasma gondii* infection on
807 the brain. Schizophr Bull **33**:745–51.
808 68. **Henry CJ, Huang Y, Wynne A, Hanke M, Himler J, Bailey MT, Sheridan JF,**
809 **Godbout JP.** 2008. Minocycline attenuates lipopolysaccharide (LPS)-induced
810 neuroinflammation, sickness behavior, and anhedonia. J Neuroinflammation **5**: 15.
811 69. **Frenois F, Moreau M, O'Connor J, Lawson M, Micon C, Lestage J, Kelley**
812 **KW, Dantzer R, Castanon N.** 2007. Lipopolysaccharide induces delayed
813 FosB/DeltaFosB immunostaining within the mouse extended amygdala,
814 hippocampus and hypothalamus, that parallel the expression of depressive-like
815 behavior. Psychoneuroendocrinology 32:516–531.
816 70. **Ueno A, Cho S, Cheng L, Wang J, Hou S, Nakano H, Nakano H, Santamaria**
817 **P, Yang Y.** 2007. Transient upregulation of indoleamine 2, 3-dioxygenase in
818 dendritic cells by human chorionic gonadotropin downregulates autoimmune

diabetes. Diabetes **56**: 1686–1693.

71. **Uyttenhove C, Pilotte L, Théate I, Stroobant V, Colau D, Parmentier N, Boon T, Van den Eynde BJ.** 2003. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2, 3-dioxygenase. Nat Med **9**: 1269–1274.

72. **Muller AJ, DuHadaway JB, Donover PS, Sutanto-Ward E, Prendergast GC.** 2005. Inhibition of indoleamine 2, 3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. Nat Med **11**: 312–319.

73. **Kwidzinski E, Bunse J, Aktas O, Richter D, Mutlu L, Zipp F, Nitsch R, Bechmann I.** 2005. Indoleamine 2, 3 dioxygenase is expressed in the CNS and down-regulates autoimmune inflammation. FASEB J **19**: 1347–1349.

Figure legends

Figure 1. Analysis of anhedonic behavior by sucrose consumption following *T. gondii* infection. (A) Sucrose consumption of wild-type BALB/c mice after injection with PBS or infection with *T. gondii*. Data are summarized from two independent experiments and presented as mean ± SD (PBS, N = 4 + 6; *T. gondii*, N = 2 + 6, two mice died due to the infection during trial 1, $F_{(30, 496)} = 4.15$, $P < 0.0001$). * indicates significant difference between PBS-injected and *T. gondii*-infected groups by two-way ANOVA plus Bonferroni *post hoc* analysis. **(B)** Sucrose consumption of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* under treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 day-post infection (dpi). Data are representative from two independent experiments with similar results and presented as mean ± SD (N = 6, $F_{(42, 300)} = 3.27$, $P < 0.0001$). * indicates significant difference between PBS/PBS and *T. gondii*/PBS groups and # indicates significant difference between *T. gondii*/PBS and *T. gondii*/Mino groups by two-way ANOVA plus Bonferroni *post hoc* analysis. **(C)** Sucrose consumption of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after injection with PBS or infection with *T. gondii*. Data are representative from four independent experiments with similar results, presented as mean ± SD (N = 5, $F_{(30, 176)} = 3.77$, $P < 0.0001$). * indicates significant difference between PBS/IFN-$\gamma^{+/+}$ and *T. gondii*/IFN-$\gamma^{+/+}$ groups and # indicates significant difference between *T. gondii*/IFN-$\gamma^{+/+}$ and *T. gondii*/ IFN-$\gamma^{-/-}$ groups by two-way ANOVA plus Bonferroni *post hoc* analysis. **(D)** Sucrose consumption of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are representative from two independent experiments with similar

867 results, presented as mean ± SD (N = 6, $F_{(42, 300)}$ = 4.24, P < 0.0001). * indicates
868 significant difference between PBS/Vehicle and *T. gondii*/Vehicle groups and #
869 indicates significant difference between *T. gondii*/Vehicle and *T. gondii*/1-MT groups
870 by two-way ANOVA plus Bonferroni *post hoc* analysis.
871
872 **Figure 2. Analysis of despair behavior by immobility duration following *T. gondii***
873 **infection.** Total time spent floating (immobility duration) in the forced swim test (FST)
874 was measured as a putative indicator of despair behavior. **(A)** Immobility duration of
875 wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 and
876 30 day-post infection (dpi). Data are summarized from two independent experiments,
877 presented as mean ± SD (PBS, N = 4 + 6; *T. gondii*, N = 4 + 6, $F_{(1, 36)}$ = 4.24, P =
878 0.0002). **(B)** Immobility duration of wild-type BALB/c mice after injection with PBS or
879 infection with *T. gondii* at 10 dpi under treatment with minocycline (Mino, 10 mg/kg,
880 ip) or injection with PBS from 4 to 7 dpi. Data are representative from two independent
881 experiments with similar results, presented as mean ± SD (N = 5–6, one mouse died due
882 to infection in *T. gondii*-infected and PBS-injected group, $F_{(1, 19)}$ = 10.54, P = 0.0042).
883 **(C)** Immobility duration of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after
884 injection with PBS or infection with *T. gondii* at 10 dpi. Data are summarized from
885 three independent experiments and presented as mean ± SD (N = 3+5+5, $F_{(1, 48)}$ = 46.50,
886 P < 0.0001). **(D)** Immobility duration of wild-type BALB/c mice after injection with
887 PBS or infection with *T. gondii* at 10 dpi under treatment with 1-methyl-DL-tryptophan
888 (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are
889 representative from two independent experiments with similar results, presented as
890 mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and

Vehicle-injected groups, $F_{(1, 19)} = 35.81$, $P < 0.0001$). Different letters above bars in the graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis.

Figure 3. Clinical score and locomotor activity of mice following *T. gondii* infection. **(A)** Clinical score (left) and locomotor activity in terms of stride length in footprint test (right) of wild-type BALB/c mice after infection with *T. gondii* and treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 day-post infection (dpi). Clinical scores are summarized from two independent experiments and presented as mean ± SD ($N = 6 + 6$, $F_{(10, 242)} = 52.97$, $P < 0.0001$). Locomotor activity data are presented as mean ± SD (N = 5, $F_{(1, 16)} = 47.15$, $P < 0.0001$) **(B)** Clinical score (left) and locomotor activity (right) of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after infection with *T. gondii*. Data are summarized from two independent experiments and presented as mean ± SD (clinical score, N = 6 + 6, $F_{(10, 242)} = 3.05$, $P = 0.0012$; ㄷㄷ locomotor activity, $n = 5 + 5$, $F_{(1, 36)} = 81.69$, $P < 0.0001$). **(C)** Clinical score (left) and locomotor activity (right) of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Clinical score data are summarized from two independent experiments and presented as mean ± SD (N = 6 + 6, $F_{(10, 242)} = 41.79$, $P < 0.0001$). Data of locomotor activity are presented as mean ± SD (N = 5, $F_{(1, 16)} = 7.877$, $P = 0.0127$). * indicates significant differences of clinical score (left) between two groups by two-way ANOVA plus Bonferroni *post hoc* analysis. Different letters above bars in the graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis (right).

Figure 4. Expression of cytokines in brains of mice following *T. gondii* infection. **(A)** Expression of IL-1β, TNF-α and IFN-γ in brain of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 day-post infection (dpi) under treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 dpi. Data are representative from two independent experiments with similar results and presented as mean ± SD (N = 5–6, one mouse died due to the infection in *T. gondii*-infected and PBS-injected group, IL-1β; $F_{(1, 19)} = 13.66$, $P = 0.0015$, TNF-α; $F_{(1, 19)} = 8.235$, $P = 0.0098$, IFN-γ; $F_{(1, 19)} = 6.325$, $P = 0.0211$). **(B)** Expression of IL-1β and TNF-α in brain of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after injection with PBS or infection with *T. gondii* at 10 dpi. Data are summarized from two independent experiments and presented as mean ± SD (N = 5 + 5, IL-1β; $F_{(1, 36)} = 35.98$, $P < 0.0001$, TNF-α; $F_{(1, 36)} = 50.97$, $P < 0.0001$). **(C)** Expression of IL-1β, TNF-α and IFN-γ in brain of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 dpi under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are representative from two independent experiments with similar results and presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and vehicle-injected groups, IL-1β; $F_{(1, 19)} = 0.937$, $P = 0.3452$, TNF-α; $F_{(1, 19)} = 0.1289$, $P = 0.7235$, IFN-γ; $F_{(1, 19)} = 0.1108$, $P = 0.7429$). Different letters above bars in the graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis.

Figure 5. Expression of indoleamine 2, 3-dioxygenase (IDO) in brain of mice following *T. gondii* infection. (A) Expression of IDO1 and IDO2 in brain of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 day-post infection (dpi) under treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 dpi. Data are representative from two independent experiments with similar results and presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and PBS-injected groups, IDO1; $F_{(1, 19)} = 6.480$, $P = 0.0197$, IDO2; $F_{(1, 19)} = 26.09$, $P < 0.0001$). **(B)** Expression of IDO1 and IDO2 in brain of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after injection with PBS or infection with *T. gondii* at 10 dpi. Data are summarized from two independent experiments and presented as mean ± SD (N = 5 + 5, IDO1; $F_{(1, 36)} = 13.06$, $P = 0.0009$, IDO2; $F_{(1, 36)} = 3.313$, $P = 0.077$). **(C)** Expression of IDO1 and IDO2 in brain of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 dpi under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are representative from two independent experiments with similar results and presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and vehicle-injected groups, IDO1; $F_{(1, 19)} = 7.313$, $P = 0.0141$, IDO2; $F_{(1, 19)} = 0.158$, $P = 0.6954$). Different letters above bars in the graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis.

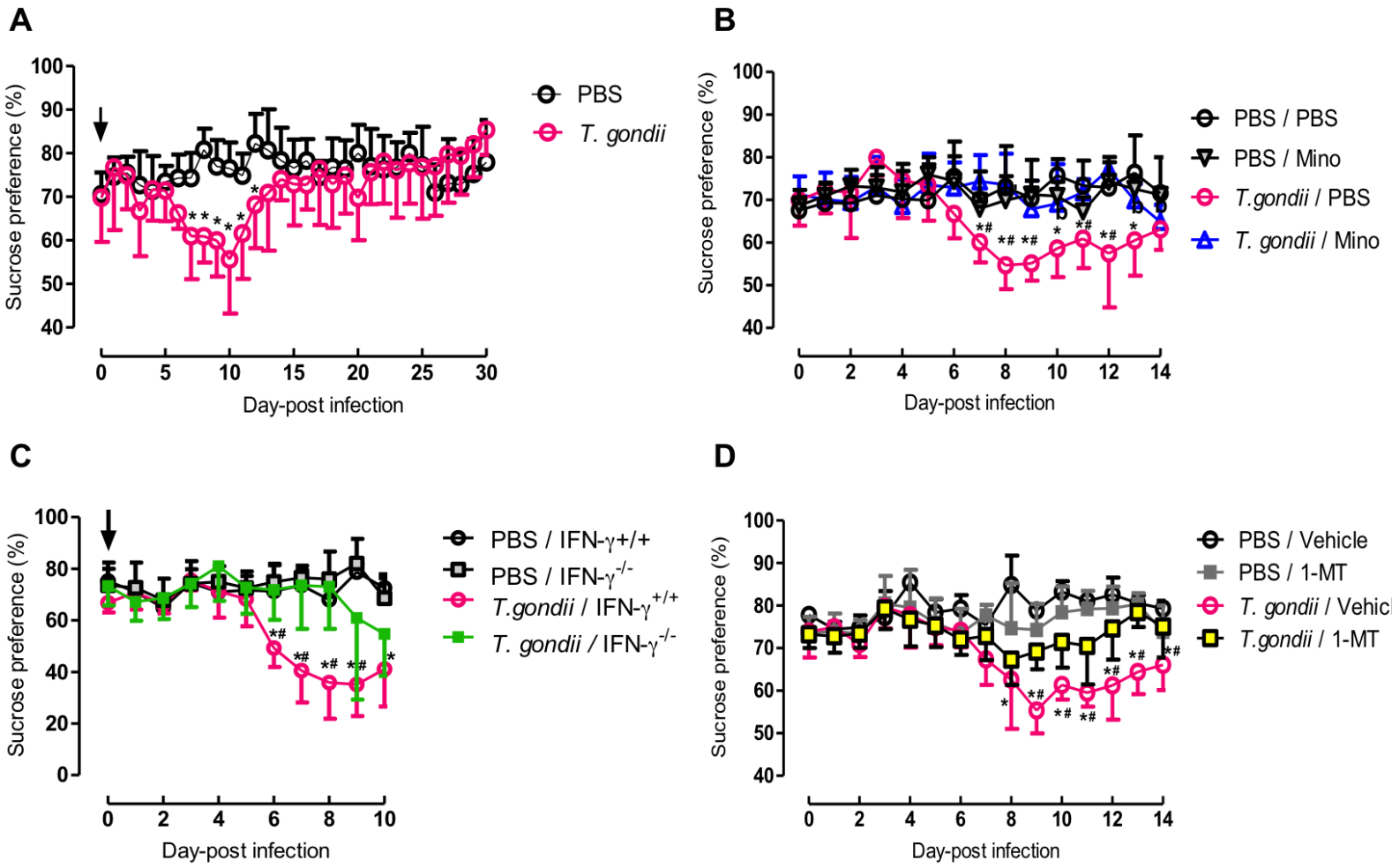
Figure 6. Trp turnover to Kyn or 5-HT in mice following *T. gondii* infection. Trp turnover to Kyn (Kyn/Trp ratio) in plasma and brain, and Trp turnover to brain serotonin (5-HT/Trp ratio) were determined. **(A)** Trp turnover in wild-type BALB/c

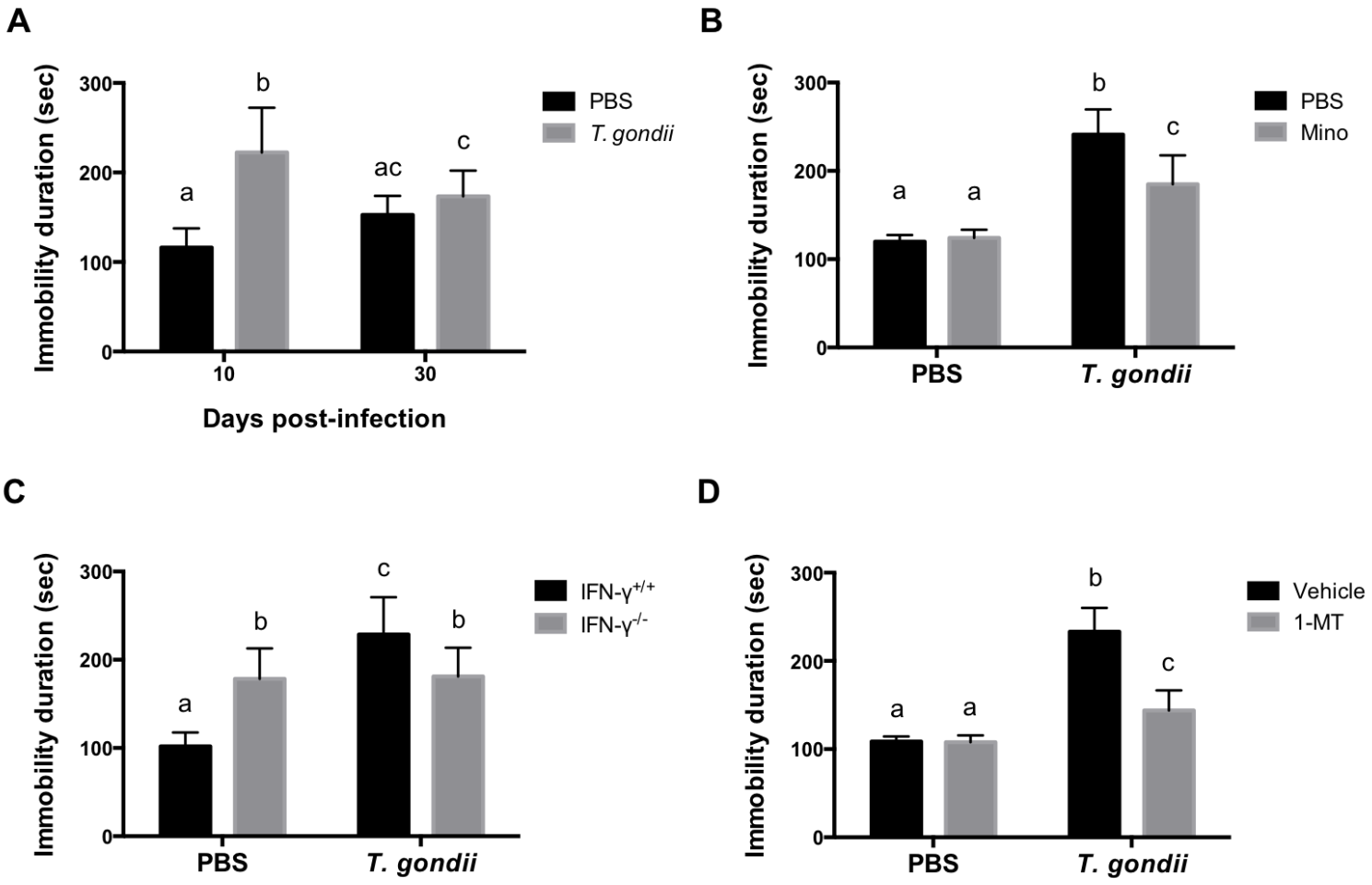
mice after injection with PBS or infection with *T. gondii* at 10 and 30 day-post infection (dpi). Plasma Kyn/Trp ratios are presented as mean ± SD (N = 5, $F_{(1, 16)} = 8.039$, $P = 0.0119$). Data of brain Kyn/Trp and brain 5-HT/Trp are summarized from two independent experiments and presented as mean ± SD (N = 4–5 + 5, Brain Kyn/Trp; $F_{(1, 32)} = 274.6$, $P < 0.0001$, Trp Turnover; $F_{(1, 36)} = 0.01294$, $P = 0.9101$). **(B)** Trp turnover of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 dpi under treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 dpi. Data are presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and PBS-injected groups, Plasma Kyn/Trp; $F_{(1, 19)} = 15.91$, $P = 0.0008$, Brain Kyn/Trp; $F_{(1, 19)} = 12.44$, $P = 0.0023$, Trp Turnover; $F_{(1, 19)} = 0.2663$, $P = 0.6118$). **(C)** Trp turnover of wild-type BALB/c mice (IFN-$\gamma^{+/+}$) and IFN-$\gamma^{-/-}$ mice after injection with PBS or infection with *T. gondii* at 10 dpi. Data are summarized from two independent experiments and presented as mean ± SD (N = 4 + 4, plasma Kyn/Trp; $F_{(1, 28)} = 21.44$, $P < 0.001$, N = 5 + 5, brain Kyn/Trp; $F_{(1, 36)} = 6.016$, $P = 0.0192$, N = 5 + 5, brain 5-HT/Trp; $F_{(1, 36)} = 3.19$, $P = 0.0825$). **(D)** Trp turnover of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 dpi under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and Vehicle-injected groups, Plasma Kyn/Trp; $F_{(1, 16)} = 7.838$, $P = 0.0129$, Brain Kyn/Trp; $F_{(1, 19)} = 11.51$, $P = 0.0031$, Trp Turnover; $F_{(1, 19)} = 4.43$, $P = 0.0489$). Different letters above bars in graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis.

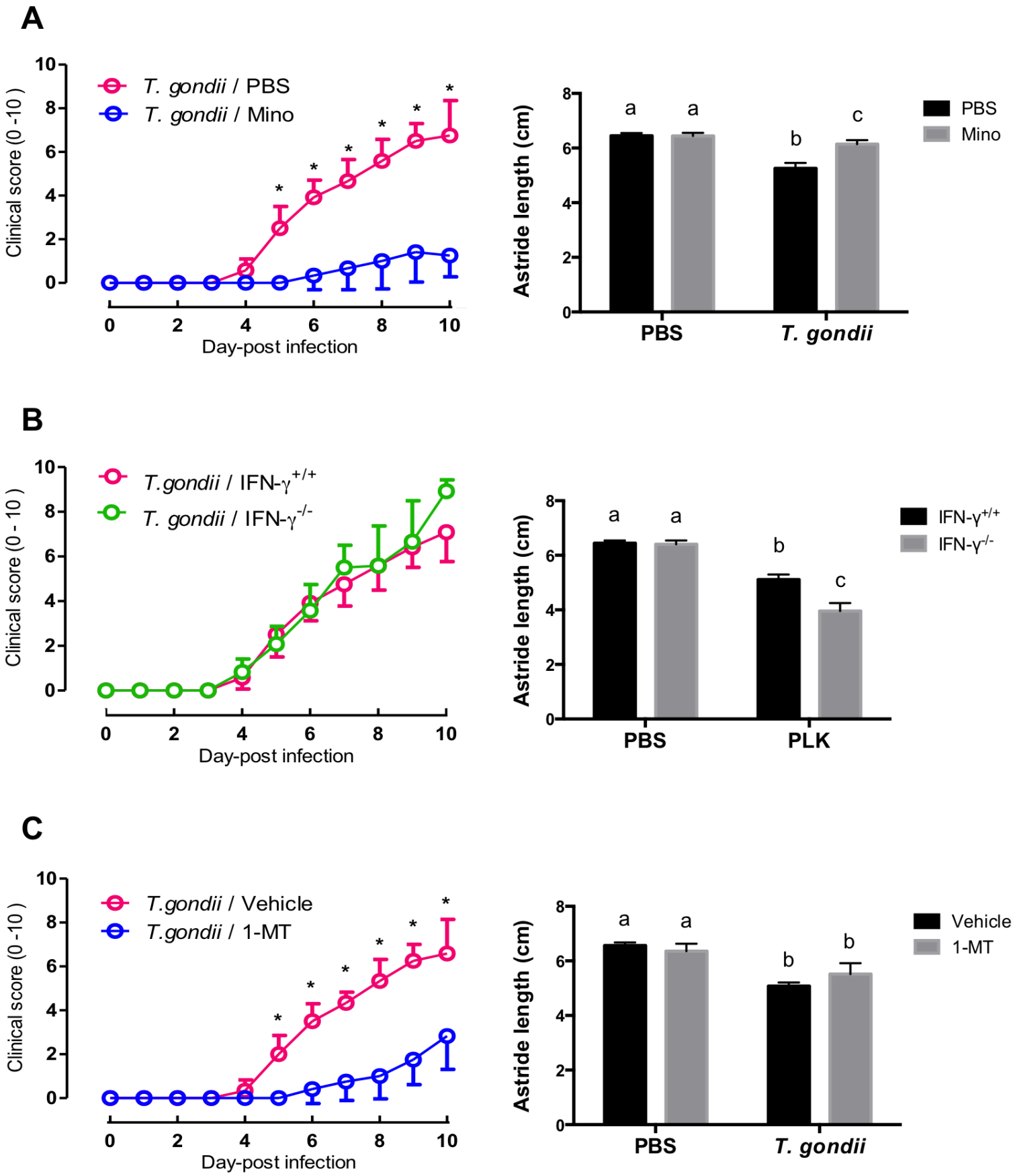
Figure 7. Levels of serotonin and dopamine in brain of mice following *T. gondii* infection. Serotonin (5-HT) and its turnover to 5-hydroxyindoleacetic acid (5-HIAA/5-HT ratio), as well as dopamine (DA) and its turnover to homovanillic acid (HVA/DA ratio) were measured in brains of mice. **(A)** 5-HT and its turnover of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 and 30 day-post infection (dpi). Data are summarized from two independent experiments and presented as mean ± SD (N = 5 + 5, 5-HT: $F_{(1,\ 36)}$ = 2.538, P = 0.1199, 5-HIAA/5-HT ratio; $F_{(1,\ 36)}$ = 0.1521, P = 0.6989). **(B)** DA and its turnover of wild-type BALB/c mice after injection with PBS or infection with *T. gondii* at 10 and 30 dpi. Data are summarized from two independent experiments and presented as mean ± SD (N = 5 + 5, DA: $F_{(1,\ 36)}$ = 5.27, P = 0.0276, 5-HIAA/5-HT ratio; $F_{(1,\ 36)}$ = 0.1521, P = 0.6989). Different letters above bars in graphs indicate statistically significant differences among the groups by two-way ANOVA plus Tukey–Kramer *post hoc* analysis.

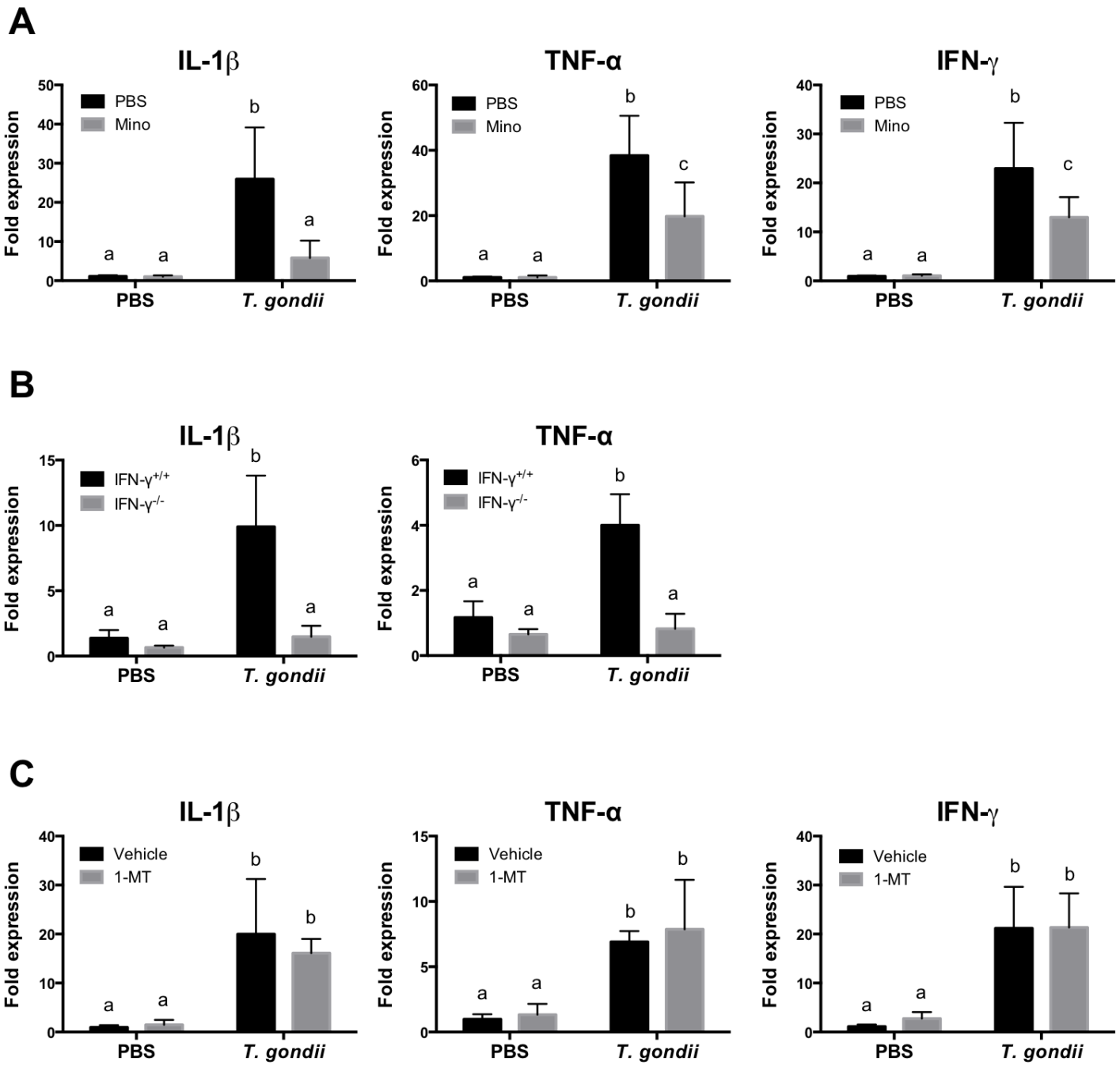
Figure 8. Effects of 1-methyl-DL-tryptophan (1-MT) and minocycline (Mino) on *T. gondii* growth *in vivo* and *in vitro*. (A) Parasite number in the brain of wild-type BALB/c mice after infection with *T. gondii* at 14 day-post infection (dpi) under treatment with minocycline (Mino, 10 mg/kg, ip) or injection with PBS from 4 to 7 dpi and under treatment with 1-methyl-DL-tryptophan (1-MT, 50 mg/kg, subcutaneously) or injection with vehicle from 4 to 7 dpi. Data are presented as mean ± SD (N = 5–6, one mouse died due to infection in *T. gondii*-infected and PBS-injected groups and in *T. gondii*-infected and Vehicle-injected groups). * indicates significant differences between groups by student's *t*-test. **(B, C)** Effects of IFN-γ (250 U/ml), Mino (5–40

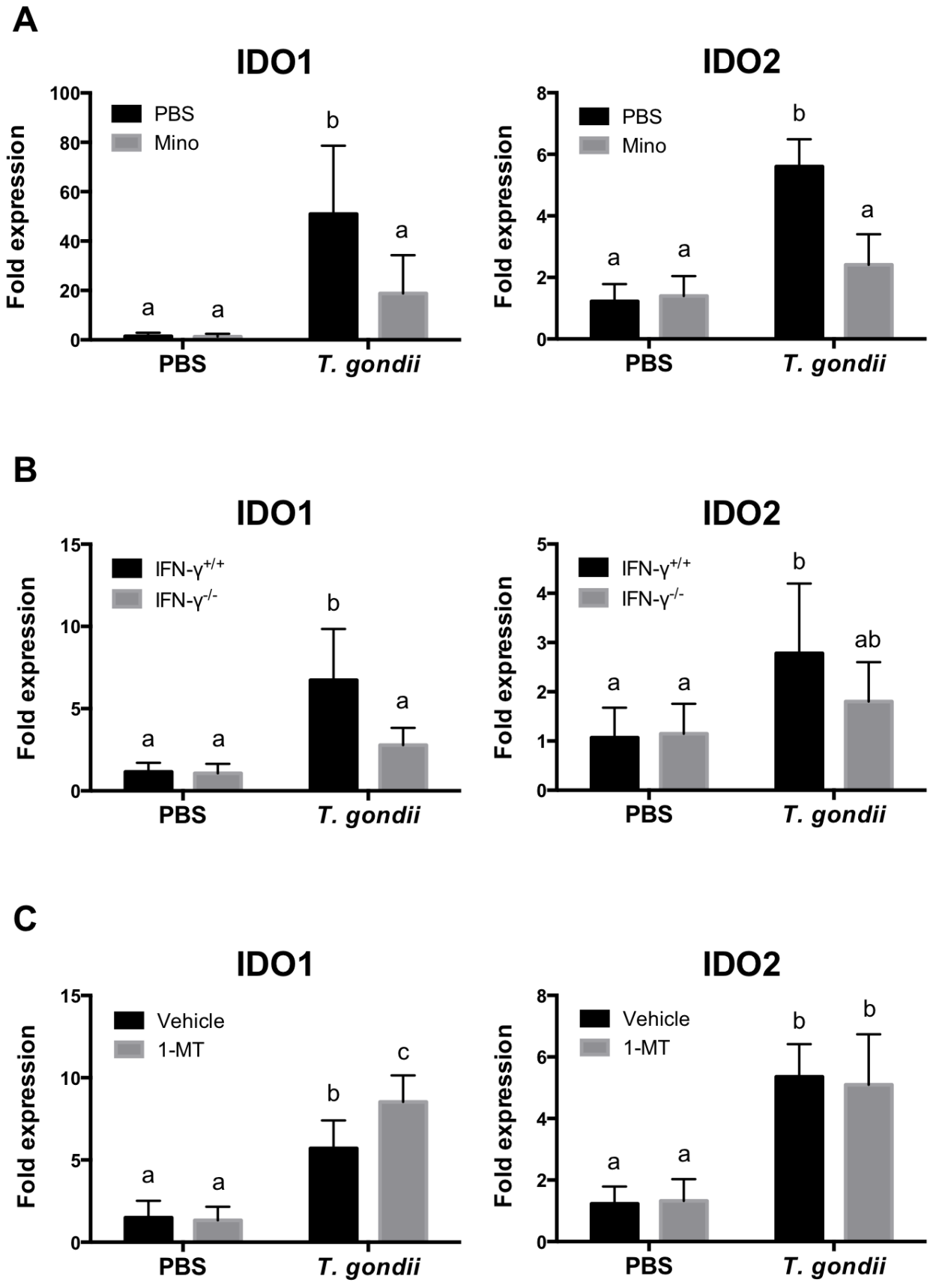
1010 nM) and 1-MT (50–1,000 nM) on *T. gondii* growth in murine microglia MG6 **(B)** and
1011 murine astrocyte OS3 **(C)**. Data are presented as mean ± SD (N = 3).

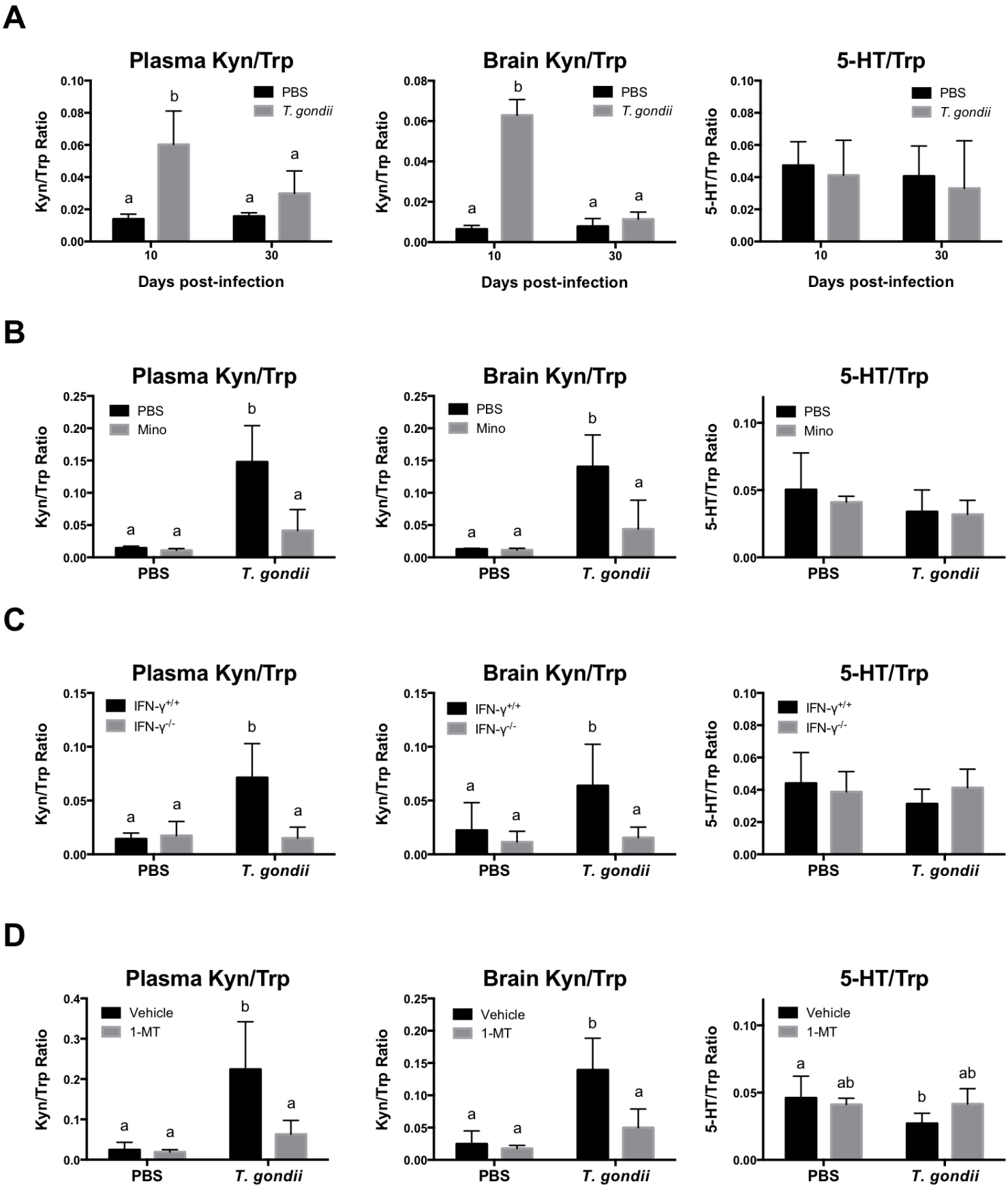

A
Sucrose preference (%)
Day-post infection
PBS
T. gondii
B
Sucrose preference (%)
Day-post infection
PBS / PBS
PBS / Mino
T.gondii / PBS
T. gondii / Mino
C
Sucrose preference (%)
Day-post infection
PBS / IFN-γ+/+
PBS / IFN-γ-/-
T.gondii / IFN-γ+/+
T. gondii / IFN-γ-/-
D
Sucrose preference (%)
Day-post infection
PBS / Vehicle
PBS / 1-MT
T. gondii / Vehicle
T.gondii / 1-MT


A
Immobility duration (sec)
300
200
100
0
a
b
ac
c
10
30
Days post-infection
PBS
T. gondii
B
Immobility duration (sec)
300
200
100
0
a
a
b
c
PBS
T. gondii
PBS
Mino
C
Immobility duration (sec)
300
200
100
0
a
b
c
b
PBS
T. gondii
IFN-γ+/+
IFN-γ-/-
D
Immobility duration (sec)
300
200
100
0
a
a
b
c
PBS
T. gondii
Vehicle
1-MT

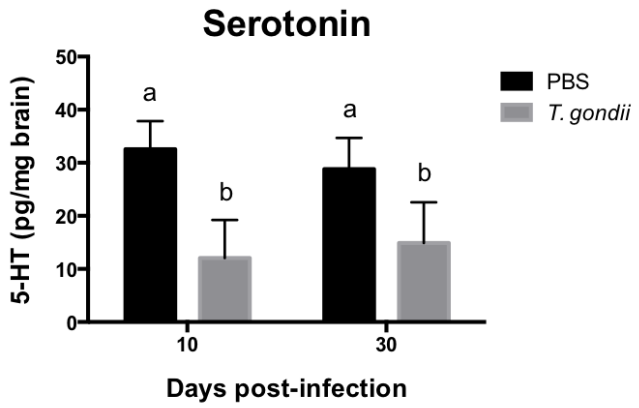

A
T. gondii / PBS
T. gondii / Mino
Clinical score (0 -10)
Day-post infection
Astride length (cm)
PBS
Mino
PBS
T. gondii
B
T.gondii / IFN-γ+/+
T. gondii / IFN-γ-/-
Clinical score (0 - 10)
Day-post infection
Astride length (cm)
IFN-γ+/+
IFN-γ-/-
PBS
PLK
C
T.gondii / Vehicle
T.gondii / 1-MT
Clinical score (0 -10)
Day-post infection
Astride length (cm)
Vehicle
1-MT
PBS
T. gondii

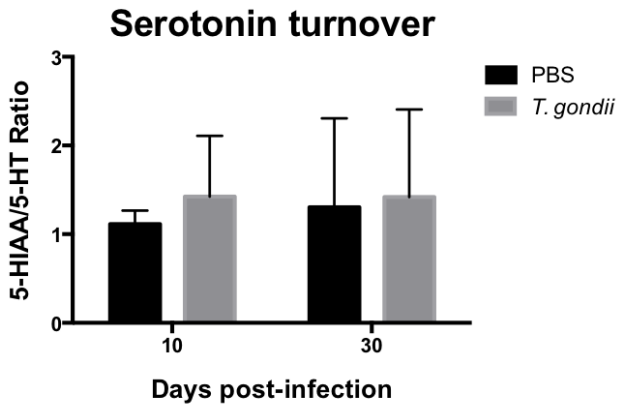

A
IL-1β
TNF-α
IFN-γ
Fold expression
PBS
Mino
a
a
b
a
a
b
c
a
a
b
c
PBS
T. gondii
B
IL-1β
TNF-α
Fold expression
IFN-γ+/+
IFN-γ-/-
a
a
b
a
a
a
b
a
PBS
T. gondii
C
IL-1β
TNF-α
IFN-γ
Fold expression
Vehicle
1-MT
a
a
b
b
a
a
b
b
a
a
b
b
PBS
T. gondii


A
IDO1
Fold expression
PBS
Mino
a
a
b
a
PBS
T. gondii
IDO2
Fold expression
PBS
Mino
a
a
b
a
PBS
T. gondii
B
IDO1
Fold expression
IFN-γ+/+
IFN-γ-/-
a
a
b
a
PBS
T. gondii
IDO2
Fold expression
IFN-γ+/+
IFN-γ-/-
a
a
b
ab
PBS
T. gondii
C
IDO1
Fold expression
Vehicle
1-MT
a
a
b
c
PBS
T. gondii
IDO2
Fold expression
Vehicle
1-MT
a
a
b
b
PBS
T. gondii

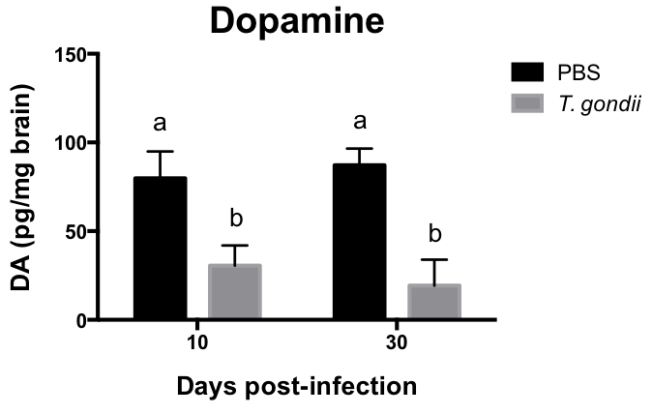

A
Plasma Kyn/Trp
Brain Kyn/Trp
5-HT/Trp
Kyn/Trp Ratio
5-HT/Trp Ratio
PBS
T. gondii
Days post-infection
B
Mino
C
IFN-γ+/+
IFN-γ-/-
D
Vehicle
1-MT

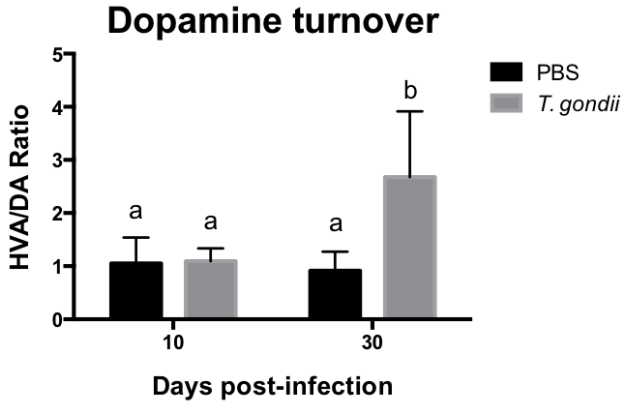
A


Serotonin
5-HT (pg/mg brain)
50
40
30
20
10
0
a
b
a
b
10
30
Days post-infection
PBS
T. gondii


Serotonin turnover
5-HIAA/5-HT Ratio
3
2
1
0
10
30
Days post-infection
PBS
T. gondii

B


Dopamine
DA (pg/mg brain)
150
100
50
0
a
b
a
b
10
30
Days post-infection
PBS
T. gondii


Dopamine turnover
HVA/DA Ratio
5
4
3
2
1
0
a
a
a
b
10
30
Days post-infection
PBS
T. gondii

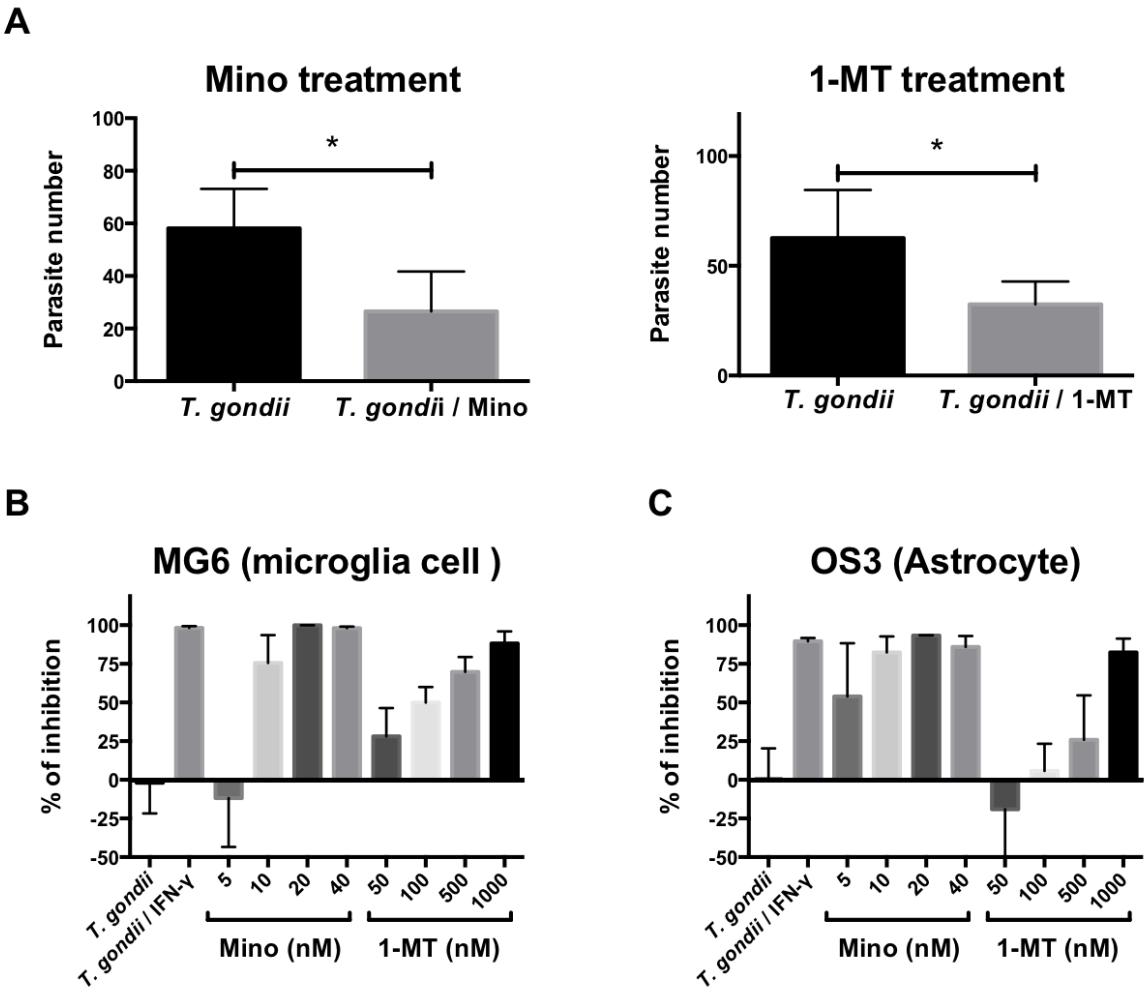

A
Mino treatment
Parasite number
100
80
60
40
20
0
*
T. gondii
T. gondii / Mino
1-MT treatment
Parasite number
100
50
0
*
T. gondii
T. gondii / 1-MT
B
MG6 (microglia cell)
% of inhibition
100
75
50
25
0
-25
-50
T. gondii
T. gondii / IFN-γ
5
10
20
40
50
100
500
1000
Mino (nM)
1-MT (nM)
C
OS3 (Astrocyte)
% of inhibition
100
75
50
25
0
-25
-50
T. gondii
T. gondii / IFN-γ
5
10
20
40
50
100
500
1000
Mino (nM)
1-MT (nM)

Table 1. Effect of minocycline (Mino) and 1-methyl DL-tryptophan (1-MT) treatment on plasma levels of inflammatory cytokines IFN-γ and IL-1β in mice infected with *T. gondii* at 10 day-post infection

A) Mino

Proteins (pg/ml)	**PBS/ PBS**	**PBS/ Mino**	***T. gondii*/PBS**	***T. gondii*/Mino**
IL-1β	nd	47.2 ± 4.6 [a]	645.6 ± 34.6 [b]	24.1 ± 4.4 [a]
IFN-γ	nd	82.5 ± 28.4 [a]	410.89 ± 87.7 [b]	136.6 ± 7.6 [c]

B) 1-MT

Proteins (pg/ml)	**PBS/ Vehicle**	**PBS/1-MT**	***T. gondii*/Vehicle**	***T. gondii*/1-MT**
IL-1β	nd	nd	676.0 ± 32.5	619.5 ± 26.7
IFN-γ	nd	26.5 ± 5.3 [a]	459.6 ± 43.2 [b]	433.3 ± 40.0 [b]

Abbreviations: IFN; interferon, IL; interleukin, nd; not detected.

Data represent average of values for means ± SD (N = 5–6, one mouse died due to infection in *T. gondii* / PBS group (A) (IL-1β; $F_{(3, 22)} = 231.7$, $P=0.0001$, IFN-γ; $F_{(3, 22)} = 329.4$, $P=0.0001$) and *T. gondii* / Vehicle group (B) (IL-1β; $F_{(3, 22)} = 156.7$, $P=0.0001$, IFN-γ; $F_{(3, 22)} = 326.0$, $P=0.0001$). Different letters indicate statistically significant differences among the groups by one-way ANOVA plus Tukey–Kramer *post hoc* analysis.