Induction of depression-related behaviors by reactivation of chronic *Toxoplasma gondii* infection in mice

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ABSTRACT

Although *Toxoplasma gondii* (*T. gondii*) infection is relevant to many psychiatric disorders, the fundamental mechanisms of its neurobiological correlation with depression are poorly understood. Here, we show that reactivation of chronic infection by an immunosuppressive regimen caused induction of depressive-like behaviors without obvious sickness symptoms. However, the depression-related behaviors in *T. gondii*-infected mice, specifically, reduced sucrose preference and increased immobility in the forced-swim test were observed at the reactivation stage, but not in the chronic infection. Interestingly, reactivation of *T. gondii* was associated with production of interferon-gamma and activation of brain indoleamine 2, 3-dioxygenase, which converts tryptophan to kynurenine and makes it unavailable for serotonin synthesis. Furthermore, serotonin turnover to its major metabolite, 5-hydroxyindoleacetic acid, was also enhanced at the reactivation stage. Thus, enhanced tryptophan catabolic shunt and serotonin turnover may be implicated in development of depressive-like behaviors in mice with reactivated *T. gondii*.

KEYWORDS: *Toxoplasma gondii*, Reactivation, Depressive-like behaviors, Indoleamine 2, 3 dioxygenase, mouse.

1. Introduction

Toxoplasma gondii is the most successful neurotropic parasite, and chronically infects approximately one third of the world's population [1, 2]. To gain the advantage of completing its own life cycle, *T. gondii* can cause nervous system dysfunction, which is the resulting influence of host behavior [3]. *T. gondii* exists in two distinct forms: an immune-stimulating tachyzoite and an immune-encrypted bradyzoite. The brain is an immune-privileged site for lifelong existence of *T. gondii* bradyzoite cysts. During the tachyzoite stage, stimulation of the host immune response, e.g., cytokines including interleukin (IL-1, IL-6), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ), may indirectly alter brain neurotransmitter levels, with the changes not regionally selective [4]. Effects of IFN-γ have been linked to depressive symptoms via induction of indoleamine-2, 3-dioxygenase (IDO) activation and tryptophan (Trp) depletion, which results in decreased 5-hydroxytryptamine (5-HT, serotonin) production in the brain [5, 6]. Unavailability of Trp for 5-HT synthesis is due to stress-induced activation of the liver enzyme, tryptophan 2, 3-dioxygenase, and/or the pro-inflammatory cytokine-induced extrahepatic enzyme, IDO, with both enzymes being rate-limiting for Trp catabolism [5–8]. In addition to resulting low serotonergic neurotransmission, kynurenine (Kyn) is a neuroreactive metabolite that readily crosses the blood–brain barrier (BBB) and is linked to certain neurodegenerative and depressive disorders [9–11].

Conversion from a tachyzoite to a bradyzoite occurs because of parasitic cyst formation in certain parts of the brain, such as those involved in emotion/anxiety, and thereby directly influences these brain regions [12]. In contrast, during the bradyzoite stage, the parasite may enhance dopamine (DA) metabolism via genes that encode for

the enzyme tyrosine hydroxylase [13, 14]. The acute stage of infection is associated with increased DA turnover to homovanillic acid (HVA), while the chronic stage shows a 14% increase in DA levels in mouse brain [15]. *T. gondii* induces a proinflammatory response that leads to dysregulated dopaminergic and serotonergic neurotransmitter systems [4, 16, 17], which in turn may lead to psychiatric symptoms and precipitation of schizophrenia in vulnerable individuals [18].

Although *T. gondii* exposure is unlikely to reactivate in immune-competent individuals, new findings suggest that reactivation may be triggered by immune imbalance [18, 19]. Latent *T. gondii* infection has also been associated with a range of altered behavioral profiles [20]. Upon immunosuppression, *T. gondii* bradyzoites become reactivated and induce fatal toxoplasmic encephalitis [21, 22]. The presence of conflicting data on the inter-relationship between bipolar depressive disorder and *T. gondii* infection [23–26], highlights the need for studies examining the development of depressive-like behavior during *T. gondii* infection.

Depression is one of the most common affective disorders, and the world leading cause of chronic illness [27, 28]. As a mood disorder, depression is characterized by loss of interest, pleasure, cognitive function, sleep, appetite, and energy [29]. Retrospective studies have referred to *T. gondii* seroprevalence in psychiatric disorders such as schizophrenia, bipolar mood disorder [23], and self-directed violence [24, 25]. However, the causal link between *T. gondii* and bipolar disease is controversial, with some studies showing that *T. gondii* seroprevalence is not associated with major depressive disorder, generalized-anxiety disorder, or panic disorder [26].

In our previous study, transcriptome analysis of the brain of BALB/c mice infected with *T. gondii* showed that parasite number negatively correlated with expression of

genes predicted to be involved in neurological functions, yet positively correlated with those involved in the immune response [30]. In the present study, we examined the role of the reactivation of chronic *T. gondii* on behavioral changes in female BALB/c mice. We then determined the neurobiological correlate with the depressive-like behavior. Importantly, tryptophan catabolic shunt and serotonin turnover may contribute to the development of depressive-like behavior during reactivation stage of *T. gondii* infection.

2. **Methods**

2.1. Animals

Experiments were performed using wild-type female BALB/c mice (7 weeks old) obtained from Clea Japan, Inc. Animals were housed under specific pathogen-free conditions at the National Research Center for Protozoan Diseases (Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan). Mice were maintained for one week in stable conditions (12-h light dark cycle; light on from 7 a.m. to 7 p.m.) with food and water *ad libitum* before starting all behavioral experiments at 09:00 a.m. This study was performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of Ministry of Education, Culture, Sports, Science and Technology, Japan. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Obihiro University of Agriculture and Veterinary Medicine (Permit number 24-13, 24-15, 25-59, 25-61). All injections were performed under isoflurane anesthesia, and all efforts were made to minimize animal suffering.

2.2. Parasite and infection.

T. gondii (PLK strain, Type II) tachyzoites were maintained in cultured African green monkey kidney epithelial (Vero) cells and purified as previously described [31]. Each mouse was intraperitoneally infected with 10^3 tachyzoites suspended in 0.2 mL sterile phosphate-buffered saline (PBS). At the end of experiments, mice were decapitated without anesthesia, blood was collected in heparinized tubes to obtain plasma, and organs were harvested in liquid nitrogen and stored at −80 °C until further analysis.

2.3. *Reactivation of T. gondii by dexamethasone treatment.*

The procedure used in this study has been described to reactivate toxoplasmosis in chronically infected mice [32, 33]. All *T. gondii*-infected or PBS-injected mice were randomly assigned to two groups: water or dexamethasone (DXM: dexamethasone 21-phosphate disodium salt, Sigma). DXM was continuously dissolved at a concentration of 10 mg/L in the drinking water and provided to the mice for 15 days. Bottles were changed with new ones every other day.

2.4. *Clinical score*

We customized ethograms according to the appearance of clinical symptoms during the first weeks of *T. gondii* infection within home and naïve cages. Most of these signs were reported in infection studies with protozoan parasites [34–36]. Scores varied from 0 (no signs) to 10 (all signs). We recorded the signs from the first day of DXM treatment included hunching, piloerection, warmth-seeking behavior (hiding in corner of the cage beneath bedding), ptosis, sunken eyes, ataxia, reluctant movement, deficient evacuation and touch reflexes, and finally lying on belly.

2.5. Assessment of locomotor activity

Animal activity was measured in a naïve cage identical to the home cage (16 × 26 × 13 cm^3), but devoid of bedding as described previously [37]. The number of line crossings and rearings over 12 virtual identical quadrants were quantified during a period of 180 s. To avoid the accommodation effect, each mouse was exposed to the test once. Line crossings and rearings were used to assess alterations in general exploratory and locomotor activity following infection or vehicle control. Test cages were kept in a testing room within the same housing room.

2.6. *Sucrose preference test.*

Mice were first habituated with two bottles of water for 1 week before infection, followed by one bottle with 1% sucrose solution and the other with tap water. The bottle position was switched every night according to the reward test protocol [38]. The preference to sucrose was measured on the same animal, and all bottles were simultaneously weighed each morning in all groups to calculate the percentage of consumed sucrose solution of the total amount of fluid consumed. The following formula was adopted: [sucrose intake/(water intake + sucrose intake)] × 100. An additional 2 bottles were inserted in an empty cage to subtract the amount of fluid loss. When performing sucrose preference for experimental groups subjected to DXM treatment, the mice were also allowed to drink from two bottles with and without the drug.

2.7. *Forced swim test (FST).*

FST procedures were performed according to previously described methods [38–40]. Mice were individually placed in water, in the FST cylinder (12 cm in diameter, filled with 25 cm water depth, Coulbourn Instruments). The water temperature was adjusted within a thermoneutral range (31 ± 1 °C) for rodents [40]. Immobility was defined as remaining motionless, except for necessary movements to maintain floating. The first 2 test minutes were allowed for accommodation. The duration of immobility within a 6-min session was recorded as an immobility score. The latency to begin immobility was scored as the time between introduction of the mouse and the first moment of floating. 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.

2.8. *Quantitative reverse-transcription-PCR*

Total RNA was extracted using TRI Reagent (Sigma) from the left brain hemispheres. Reverse transcription was performed using Superscript III™ Reverse Transcriptase (Invitrogen), according to the manufacturer's instruction. Amplification was performed by 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 (Applied Biosystems), and the calculated cycle threshold (Ct) values were exported to Microsoft Excel for analysis. Expression levels of each gene relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were calculated using the $2^{\Delta Ct}$ method (User Bulletin no. 2; Perkin-Elmer). The optimal reference gene was selected based on the Cotton EST database (http://www.leonxie.com). Specific primers for each gene were designed using PRIMER EXPRESS software (Perkin-Elmer). A list of primer sequences is shown in Table S1.

2.9. *High-performance liquid chromatography*

Major monoamines, L-Trp and their metabolites, were measured in supernatants obtained from the right brain hemispheres, using 5-μm octyldecyl silane columns (Eicompak SC-5ODS) and an electrochemical detector, according to the monoamine analysis application manual (Eicom). Wet samples (100 mg) were homogenized in 0.5

mL 0.2 M perchloric acid (including 100 μM EDTA 2Na and 1 μg/mL isoproterenol as an internal standard). 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.).

2.10. *IDO activity assay*

To detect IDO activity, Kyn was measured in brain homogenates (per mg tissue) according to Däubener et al. [41] with previously described modifications [31]. In brief, equal amounts (250 μL) of homogenate and 2xIDO buffer (100 mM PBS, 40 mM ascorbate, 20 μM methylene blue, and 0.8 mM L-Tryptophan, pH 6.5) were mixed, and 10 min after adding the color developing solution Ehrlich's reagent (0.8% p-dimethylaminobenzaldehyde in acetic acid), color was detected at 490 nm.

2.11. *ELISA*

Plasma L-Trp was measured by fluorescence (excitation: 485 nm, emission: 665 nm) using the Bridge-It L-Trp Fluorescence Assay kit (Mediomics, LLC). Plasma L-Kyn was assayed by competitive ELISA with a mouse L-kyn monoclonal antibody (Abcam). ELISA readings at 490 nm were plotted against L-Kyn (Sigma) standard curves.

Plasma cytokine (IL-1β and IFN-γ) levels were measured using an ELISA kit (BD Biosciences Pharmingen), according to the manufacturer's recommendations. On each immunoplate, a standard cytokine curve run was included to calculate the amount of

cytokine produced.

2.12. Statistical analysis

GraphPad Prism 5 software (GraphPad Software Inc.) was used. Data were presented as mean ± SD. Statistical analyses were performed using Student's *t*-test, one-way analysis of variance (ANOVA), or two-way ANOVA repeated measures for group comparisons. The levels of statistical significance were presented as asterisks or different letters and defined in each figure legend together with the name of the statistical test. A P value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Reduced sucrose consumption after reactivation of T. gondii.

Reduced sucrose preference is a putative indicator of anhedonic behavior. To confirm the anhedonic behavior at the reactivation stage, we induced reactivation of chronic infection by oral DXM treatment for 15 days to determine. Successful tachyzoite-bradyzoite stage interconversion was confirmed by gene expression of tachyzoite and bradyzoite markers for each stage (Fig. 1A). DXM treatment induced the expression of tachyzoite marker, indicating the reactivation of *T. gondii*. Prior to reactivation, *T. gondii*-infected mice did not exhibit a difference in sucrose consumption compared with PBS-injected animals at 42 dpi. However, a highly significant reduction in sucrose consumption was observed in *T. gondii*-infected and DXM-treated mice compared with other groups (at 60 dpi) ($F_{(3, 23)} = 24.89, p < 0.0001$, Fig. 2A). This result indicated that anhedonic behavior appears during the reactivation stages of *T. gondii* infection.

3.2. Increased immobility in the FST during reactivation stages of infection

To test the effect of *T. gondii* infection on despair-like behavior, we measured the immobility duration and onset latency before and after *T. gondii* reactivation (Fig. 2B). Increased duration and reduced onset of immobility indicate despair behavior, and we found that DXM treatment in infected mice resulted in significantly increased immobility duration ($F_{(3, 41)} = 15.46, p < 0.0001$, Fig. 2B right) and reduced immobility latency ($F_{(3, 41)} = 21.34, p < 0.0001$, Fig. 2C left) at 60 dpi compared with the other experimental groups. However, at 42 dpi, before DXM treatment, there were no significant differences between *T. gondii*-infected and PBS-injected mice in immobility

duration and immobility latency (Fig. 2B, C, left). Ten days after treatment with DXM, infected mice showed some clinical symptoms, such as piloerection and warmth-seeking behavior, but no differences in locomotor activity were observed between groups at 60 dpi (Fig. 1B). Thus, our findings indicate that depressive-like behaviors, in terms of anhedonic and despair-like behaviors, is observed during the reactivation stage of *T. gondii* infection but not during chronic infection.

3.4. Enhanced expression of brain IDO and IFN-γ after reactivation of T. gondii

Next, we determined if expression of inflammatory markers is associated with reduced sucrose preference and increased immobility in the FST. In the brain, enhanced expression of IL-6, IFN-γ and IDO1 was induced in *T. gondii*-infected and DXM-treated mouse brain at 60 dpi, compared with other groups (Fig. 3A). Consistently, plasma levels of IFN-γ in *T. gondii*-infected and DXM-treated groups were approximately 10-fold greater than infected and untreated animals at 60 dpi (Fig. 3B). Together, upregulation of IDO1, IL-6, and IFN-γ in the brain may be associated with reduced sucrose preference and increased immobility in the FST during the reactivation stage of infection.

3.5. Enhanced Trp turnover to Kyn and serotonin turnover following reactivation of T. gondii

Subsequently, we investigated the neurobiological correlates of the observed reduced sucrose consumption and increased immobility in the FST. We examined Trp catabolites collected at the end of behavioral experiments. We found that the Kyn/Trp

ratio in the brain of infected and DXM-treated mice was significantly greater than in the other groups ($F_{(3,41)} = 11.75$, $p < 0.0001$, Fig. 4A). However, the plasma Kyn/Trp ratio was not different in the infected and DXM-treated groups compared with the infected and untreated group, although it remained considerably higher than in the uninfected group ($F_{(3,41)} = 5.50$, $p < 0.003$). In contrast, *T. gondii* infection did not affect Trp turnover to serotonin (5-hydroxytryptamine; 5-HT) at 60 dpi (Fig. 4A).

To determine if the observed behavioral changes are associated with 5-HT, DA, and norepinephrine levels, we examined production of these neurotransmitters in the brain. Although decreased levels of these neurotransmitters were observed in the infected mice at 60 dpi, treatment of the infected mice with DXM did not change neurotransmitter levels between infected and untreated mice (Fig. 4B). Furthermore, we investigated the effect of *T. gondii* infection on 5-HT, DA, and norepinephrine turnover (Fig. 4C). DA turnover was increased in infected mice with/without DXM at 60 dpi, while DXM treatment of infected mice increased 5-HT turnover. No difference of norepinephrine turnover was observed among the groups. These results indicate that DA and norepinephrine levels are not associated with the observed behavioral changes, but enhancement of serotonin turnover may contribute on this behavior.

4. Discussion

Our study suggests an apparent relationship between reduced sucrose preference (a putative indicator of anhedonic-like behavior, [38] and increased FST immobility (despair-like behavior) [42], during the reactivation stages of *T. gondii* infection. Depressive-like behavior was determined by taking together the results from both tests (i.e., sucrose preference test and FST [38, 42]). During reactivated *T. gondii* the depressive-like behavior was evident without apparent sickness symptoms. Although *T. gondii*-infected mice showed mild clinical symptoms upon DXM treatment, their locomotor activity was normal compared with uninfected mice. Together, these finding validated the reactivated *T. gondii* by DXM treatment, but not chronic infection of *T. gondii*, induced depressive-like behavior in the BALB/c mouse with minimum impact on motor activity.

We also show a central effect for proinflammatory cytokine-mediated IDO activity on depressive-like behavior. Several studies have investigated the involvement of proinflammatory cytokines in depressive disorders and comorbid conditions [5, 6, 39, 43, 44]. In this study, depressive-like behavior during the reactivation stage was associated with an enhanced Kyn/Trp ratio in the brain, but not plasma, together with enhanced brain IDO expression, may induce anhedonic and despair-like behaviors. These behaviors were also associated with elevated IL-6 and IFN-γ expression in the brain during the reactivation stages. These findings are consistent with studies examining different inflammatory conditions [5, 6, 39, 43, 44], and with depressive-like behavior associated with an elevated Kyn/Trp ratio. Induction of depression symptoms may be explained by enhanced IDO activity via proinflammatory cytokines, thereby increasing the Trp to Kyn shunt within the brain and causing reduced bioavailability of

Trp.

An alternative mechanistic explanation is that the Kyn generated may induce core symptoms of depression *per se*, or following further generation of downstream neuroreactive metabolites, such as kynurenic acid and quinolinic acid, which could impact glutamatergic neurotransmission in addition to their roles as N-methyl D-aspartate receptor agonists or antagonists [7, 10, 11, 46]. To identify the molecules triggering core depression symptoms during reactivated *T. gondii*, experimental studies are required to examine downstream signaling of Trp metabolic enzymes (e.g., IDO, kynurenine 3-hydroxylase, and kynurenine aminotransferase).

In contrast, decreased serotonergic neurotransmission may not be involved in the depressive-like behavior in the present study because we did not observe behavioral changes in mice during the chronic stage when 5-HT levels were low. DXM selectively induces monoamine oxidase mRNA expression and its activity in *in vitro* cultured astrocytes [47]. Therefore, the enhanced serotonin turnover in *T. gondii*/DXM group may be due to the effect of the DXM treatment plus *T. gondii* infection on serotonin level. *T. gondii* infection resulted in significant DA reduction and increased DA turnover at 60 dpi. However, norepinephrine level and norepinephrine turnover did not change in the reactivation stage. Therefore, our results suggest enhanced monoamine oxidase or catechol-O-methyl transferase activity after *T. gondii* infection. Another possible explanation of reduced DA levels could be due to *T. gondii*-associated loss of DA terminals in certain brain regions [48, 49]. Thus, the low levels of DA and increased DA turnover induced by *T. gondii* infection were neither reactivation stage-specific nor associated with depressive-like symptoms.

The depressive-like behavior observed during *T. gondii* reactivation appears to be

non-specific in parts to *T. gondii* infection. Hence, the initiation mechanisms of depressive-like behavior may be conserved in mammals, beginning with common involvement of pro-inflammatory cytokine release, consequent IDO activation, and enhancement of the Kyn pathway. This conserved pathway is supported by several lines of evidence suggesting that IDO activation and subsequent enhancement of the cerebral Kyn pathway play a central role in developing depressive disorders [50]. First, a number of pro-inflammatory cytokines are known to stimulate IDO (e.g., IFN-γ and TNF-α) and precipitate depressive symptoms in mice injected with peripheral immune-stimulatory and inflammatory agents [5, 6, 43]. Reciprocally, Kyn pathway catabolites are involved in generation of the inflammatory reaction induced by pathogens such as *Trypanosoma*, malaria parasites, or experimental endotoxemia [51–53]. Second, following bacterial infection in mice, chronic IDO up-regulation by IFN-γ or TNF-α induces long-lasting depressive-like behavior [44, 50]. These effects are largely attenuated when the increased IDO activity is diminished [44]. Third, cytokine-induced depression is associated with viral infection by neurotropic influenza A virus in mice [54], and in HIV-1-infected monkeys [55] and individuals [56]. Fourth, and the most persuasive argument for a conserved mechanism within the Kyn pathway, comes from work in the lower model organism, yeast, via identification of kynurenine 3-monooxygenase, an enzyme in the Kyn pathway of Trp degradation [57]. Therefore, fundamental mechanisms for developing depressive-like behavior during infection appear similar. Altogether, chronic neuroinflammation, as a conserved etiological factor, plays a crucial role in the pathogenesis of depressive-disorders [58–60].

Some studies have suggested that *T. gondii* as a neurotropic parasite does not produce encephalitis during the bradyzoite cyst stage in BALB/c mice [61–63].

However, we previously reported pathological and neurological changes in mice chronically infected with *T. gondii* [30]. In fact, at present it is difficult to determine if the effects of *T. gondii* are due to mild encephalitis or a direct effect of the parasite on the brain. The difference in depressive-like behavior induced by reactivated *T. gondii* is due to an increased Kyn/Trp ratio in the brain, but not the periphery. This suggests that locally produced Kyn, rather than blood-derived, is one of the initiating factors in precipitating depressive symptoms. Although lowered 5-HT levels are not specific to the reactivated *T. gondii* stage, enhanced 5-HT turnover (as indicated by an increased brain 5-HIAA/5-HT ratio) is not observed in other studies performed on acute or chronic IDO up-regulation by inflammatory agents [43, 44]. Moreover, although depressive-like behavior in chronic *Trypanosoma cruzi* infection is paralleled by increased IDO mRNA expression in brain without encephalitis [64], inflammation is still suggested to be a major contributor to the pathogenesis of depression. Endotoxin administration can directly precipitate depression symptoms [65, 66]. Furthermore, patients suffering from chronic inflammatory diseases develop symptoms of depression when compared with healthy adults [67]. Thus, although latent toxoplasmosis is asymptomatic in healthy adults, *T. gondii* reactivation by immune suppressive conditions may be a factor in inducing depression.

Despite no conclusive link between *T. gondii* infection and a history of major depressive disorder, there is a significant relationship with bipolar disorder [23]. A better understanding of the fundamental mechanisms of depressive behavior induced by *T. gondii* infection will have wide-ranging implications, spanning preclinical and clinical disciplines. However, challenges remain in extrapolating the data to clinical depression. Our study shows that reactivation of *T. gondii* induces core symptoms of

depression in mice, possibly via enhancement of the Trp to Kyn pathway as a result of IDO activity within the brain. Thus, stage interconversion may reflect a relapsing nature that may be of concern in recurrent or episodic psychiatric disorders. In conclusion, our study provides considerable insight into modulating psychiatric disorders induced by *T. gondii* infection during the reactivation stage.

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Author contributions

M.E.M. and Y.N. designed research. M.E.M., F.I, R.M.F. and M.N. performed experiments. M.E.M. and Y.N. discussed results and drafted the manuscript. All authors have primary responsibility for final content. All authors read and approved the manuscript.

Competing financial interests

The authors declare that they have no competing financial interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at: http://www.journals.elsevier.com/behavioural-brain-research/

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Figure captions

Fig. 1: Reactivation of chronic *T. gondii*. Dexamethasone (DXM) was continuously administered to *T. gondii*-infected and uninfected mice from 43 to 58 days post-infection (dpi). (A) Relative expression of *SAG1* (tachyzoite marker gene) and *BAG1* (bradyzoite marker gene) was measured by reverse transcription and real time-PCR. In *T. gondii*-infected mice, *SAG1* and *BAG1* gene expression levels were compared at 10 and 30 dpi. DXM or PBS was continuously administered to *T. gondii*-infected mice from 43 to 58 dpi. *SAG1* and *BAG1* gene expression levels were measured at 60 dpi (n = 6–10). (B) Clinical scores were measured in *T. gondii*-infected and uninfected mice after DXM treatment for 15 days (n = 10–12). Exploratory locomotor activity was measured at 60 dpi (n = 9–12). Data represent mean ± SD. * indicates significant differences between *T. gondii*/ DXM and *T. gondii*/ water group (two-way ANOVA plus Bonferroni *post-hoc* analysis).

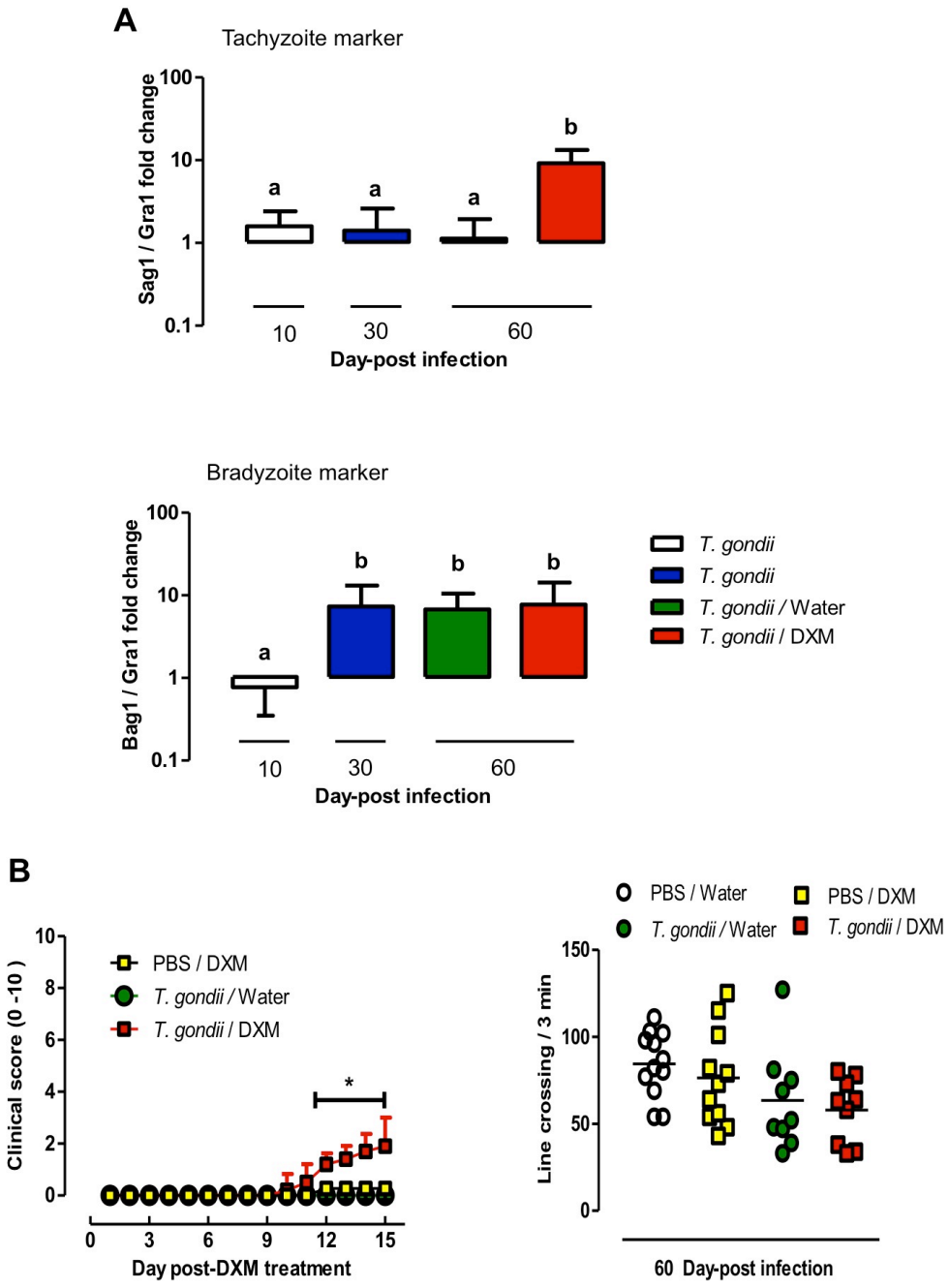
A
Tachyzoite marker
Sag1 / Gra1 fold change
100
10
1
0.1
a
a
a
b
10
30
60
Day-post infection
Bradyzoite marker
Bag1 / Gra1 fold change
100
10
1
0.1
a
b
b
b
10
30
60
Day-post infection
T. gondii
T. gondii
T. gondii / Water
T. gondii / DXM
B
Clinical score (0 -10)
10
8
6
4
2
0
PBS / DXM
T. gondii / Water
T. gondii / DXM
*
0
3
6
9
12
15
Day post-DXM treatment
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM
Line crossing / 3 min
150
100
50
0
60 Day-post infection

Figure 1

Fig. 2. Depressive-like behaviors following reactivation of *T. gondii*. In *T. gondii*-infected and PBS-injected wild-type mice, dexamethasone (DXM) was continuously administered from 43 to 58 days post-infection (dpi). (A) Sucrose preference was measured in two groups before DXM treatment at 42 dpi (n = 12) and in four groups after DXM treatment at 60 dpi (n = 6). Immobility (B) and latency (C) in the FST were examined before DXM treatment in *T. gondii*-infected and PBS-injected groups at 42 dpi (n = 22–24), and after DXM treatment in four groups at 60 dpi. Data represent mean ± SD. Different letters above the bars indicate significant differences (student's t-test (42 dpi), one-way ANOVA plus Tukey–Krammer *post-hoc* analysis (60 dpi).

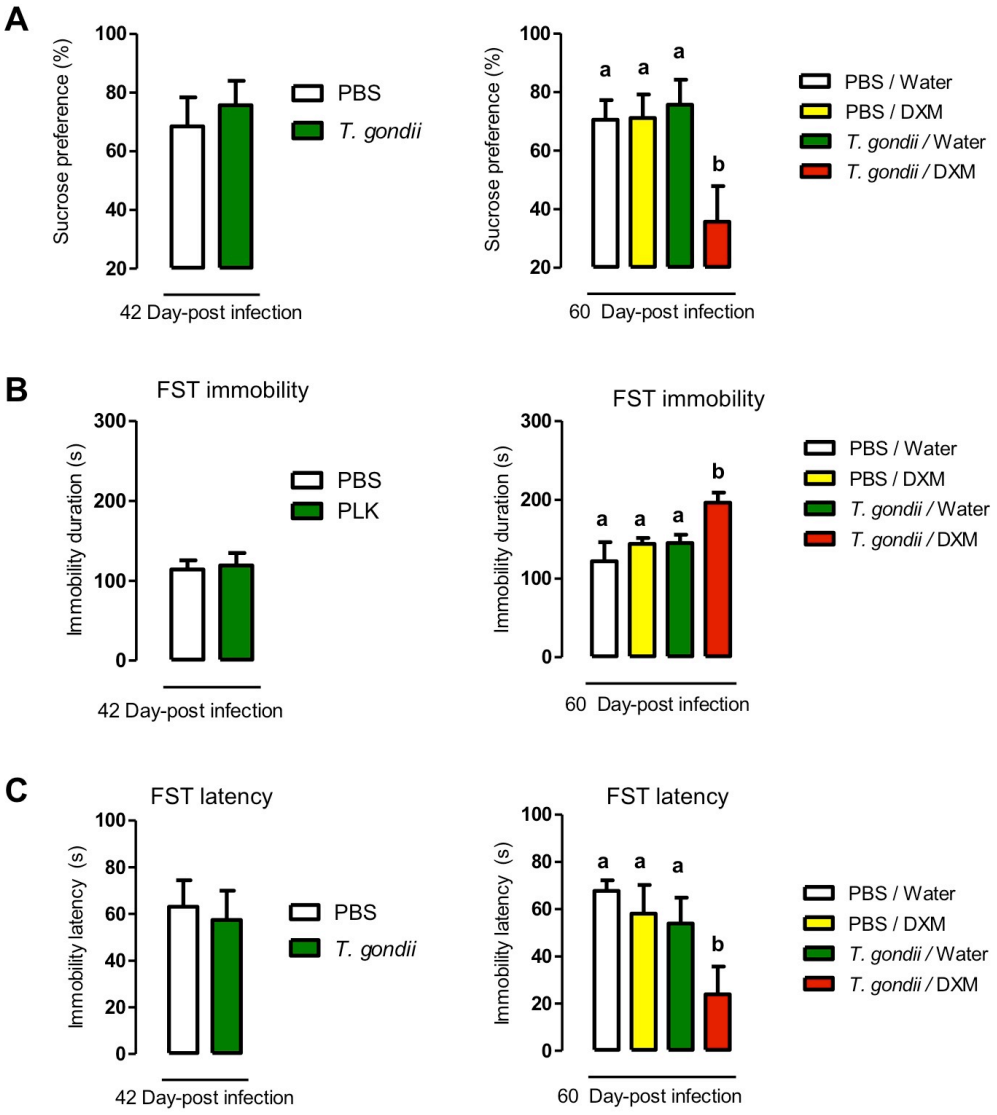
A
Sucrose preference (%)
100
80
60
40
20
PBS
T. gondii
42 Day-post infection
a
a
a
b
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM
60 Day-post infection
B
FST immobility
Immobility duration (s)
300
200
100
0
PBS
PLK
42 Day-post infection
FST immobility
60 Day-post infection
C
FST latency
Immobility latency (s)
PBS
T. gondii
42 Day-post infection
FST latency
60 Day-post infection

Figure 2

Fig. 3. Expression of proinflammatory cytokines and indoleamine dioxygenase (IDO) and serum cytokines following reactivation of *T. gondii*. (A) In *T. gondii*-infected or PBS-injected wildtype mice, dexamethasone (DXM) was continuously administered from 43 to 58 days post-infection (dpi). mRNA expression of IL-1β, IL-6, IFN-γ, IDO1, and IDO2 was measured in the brain of four groups after DXM treatment at 60 dpi by real time RT-PCR (n = 10–12). (B) Plasma levels of IL-1β and IFN-γ were measured at 60 dpi after reactivation of *T. gondii* (n = 10-12 mice/group). Data represent mean ± SD. Different letters above the bars indicate significant differences (one-way ANOVA plus Tukey–Kramer *post-hoc* analysis).

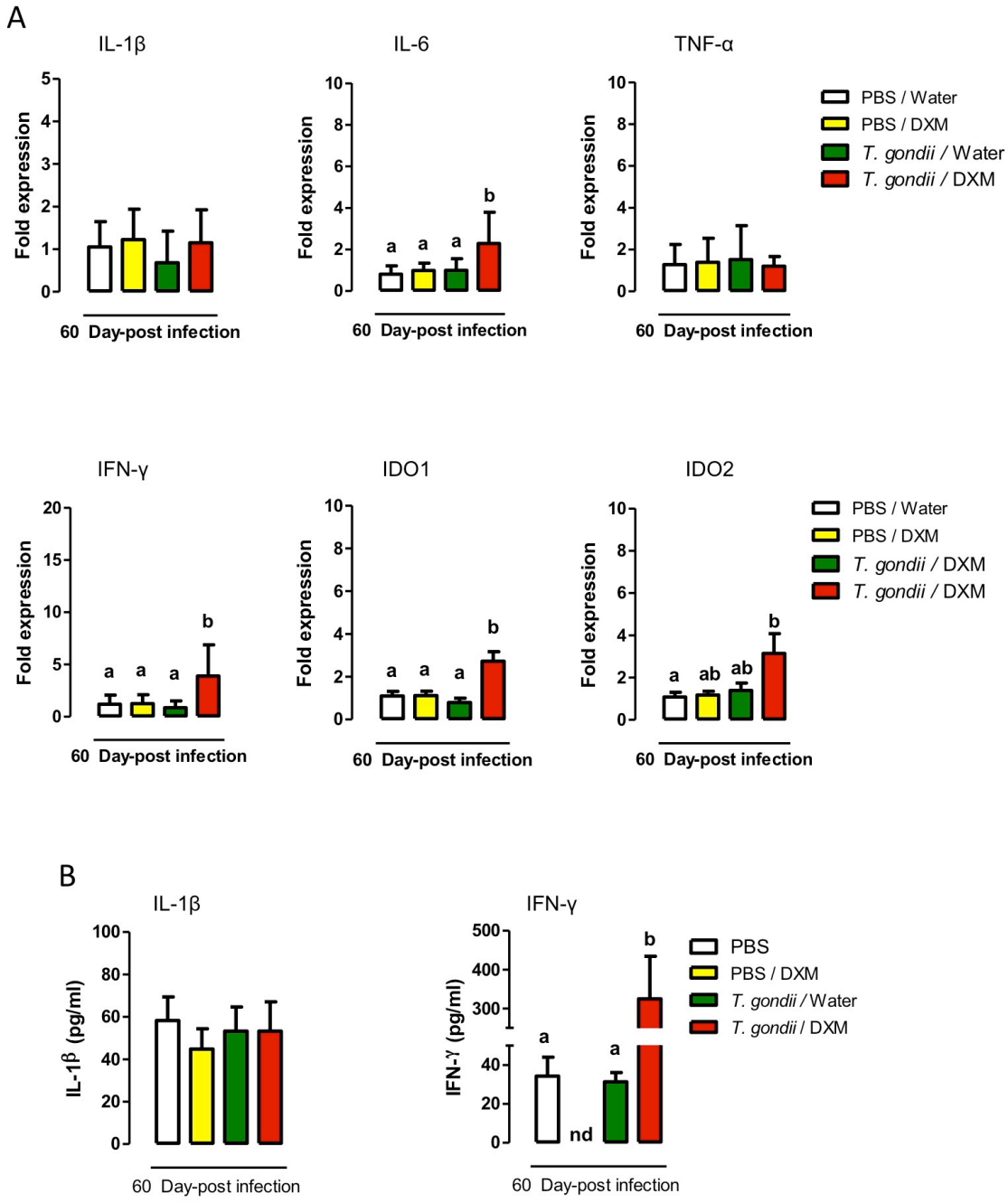
A
IL-1β
IL-6
TNF-α
IFN-γ
IDO1
IDO2
Fold expression
60 Day-post infection
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM
a
ab
b
B
IL-1β (pg/ml)
IFN-γ (pg/ml)
nd
PBS

Figure 3

Fig. 4. Levels of Kyn, 5-HT, DA and norepinephrine following *T. gondii* infection. In *T. gondii*-infected or PBS-injected mice, dexamethasone (DXM) was continuously administered from 43 to 58 days post-infection (dpi). (A) The Kyn/Trp ratio in plasma and brain, and Trp turnover (5-HT/Typ ratio) in brain, were examined after DXM treatment in four groups at 60 dpi (n = 10–12). (B) 5-HT, DA, and NE in the brain were measured after DXM treatment in four groups at 60 dpi (n = 10–12). (C) The ratios of 5-HIAA/5-HT, HVA/DA, and NM/NE in the brain were measured after DXM treatment in four groups at 60 dpi (n = 10–12). Data represent mean ± SD. Different letters above the bars indicate significant differences (one-way ANOVA plus Tukey–Kramer *post-hoc* analysis).

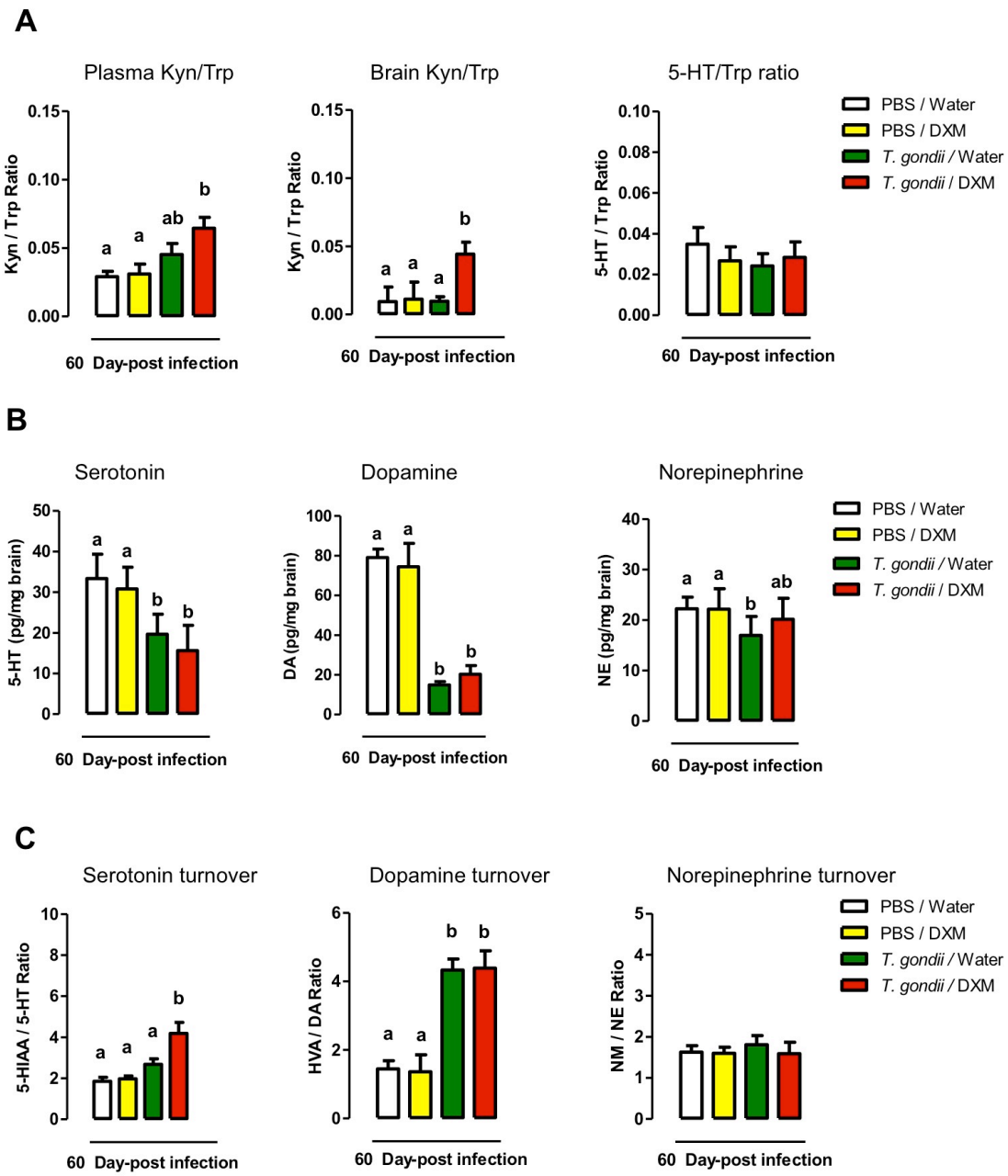
A
Plasma Kyn/Trp
Kyn / Trp Ratio
0.15
0.10
0.05
0.00
a
a
ab
b
60 Day-post infection
Brain Kyn/Trp
Kyn / Trp Ratio
0.15
0.10
0.05
0.00
a
a
a
b
60 Day-post infection
5-HT/Trp ratio
5-HT / Trp Ratio
0.10
0.08
0.06
0.04
0.02
0.00
60 Day-post infection
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM
B
Serotonin
5-HT (pg/mg brain)
50
40
30
20
10
0
a
a
b
b
60 Day-post infection
Dopamine
DA (pg/mg brain)
100
80
60
40
20
0
a
a
b
b
60 Day-post infection
Norepinephrine
NE (pg/mg brain)
40
30
20
10
0
a
a
b
ab
60 Day-post infection
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM
C
Serotonin turnover
5-HIAA / 5-HT Ratio
10
8
6
4
2
0
a
a
a
b
60 Day-post infection
Dopamine turnover
HVA / DA Ratio
6
4
2
0
a
a
b
b
60 Day-post infection
Norepinephrine turnover
NM / NE Ratio
5
4
3
2
1
0
60 Day-post infection
PBS / Water
PBS / DXM
T. gondii / Water
T. gondii / DXM

Figure 4