2016

Reciprocal Moderation by *Toxoplasma gondii* Seropositivity and Blood Phenylalanine – Tyrosine Ratio of Their Associations with Trait Aggression

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Reciprocal moderation by *Toxoplasma gondii* seropositivity and blood phenylalanine – tyrosine ratio of their associations with trait aggression

DOI 10.1515/pterid-2016-0006
Received May 27, 2016; accepted August 11, 2016; previously published online October 21, 2016

Abstract: We previously reported that trait aggression, proposed as an endophenotype for suicidal behavior, is positively associated with *Toxoplasma gondii* (*T. gondii*) seropositivity in females, but not in males. Additionally, older males seropositive for *T. gondii* had lower scores on measures of trait aggression, including self-aggression. Trait aggression may be influenced by dopaminergic signaling, which is known to be moderated by gender and age, and potentially enhanced in *T. gondii* positives through the intrinsic production of dopamine by the microorganism. Therefore, we investigated associations between trait aggression and interactions between *T. gondii* enzyme-linked immunoabsorbant assay (ELISA) IgG titer-determined seropositivity and high-performance liquid chromatography- (HPLC-) measured blood levels of dopamine precursors phenylalanine (Phe), tyrosine (Tyr), and their ratio in a sample of 1000 psychiatrically healthy participants. Aggressive traits were assessed using the questionnaire for measuring factors of aggression (FAF), the German version of the Buss-Durkee hostility questionnaire. We found that 1) the decrease in trait aggression scores in *T. gondii*-positive older males was only present in individuals with a low Phe:Tyr ratio, and 2) that there was a positive correlation between Phe:Tyr ratio and total aggression and selected subscales of aggression in *T. gondii*-positive males, but not in *T. gondii*-negative males. These findings point toward a gender-specific reciprocal moderation by Phe:Tyr ratio and *T. gondii* seropositivity of their associations with aggression scores, and lead to experimental interventions geared to manipulating levels of dopamine precursors in selected *T. gondii* positive individuals with increased propensity for aggression.

Keywords: aggressive personality traits; phenylalanine; seropositivity; *Toxoplasma gondii*; tyrosine.

Introduction

We previously reported [1] that trait aggression, proposed as an endophenotype for suicidal behavior, is positively associated with *Toxoplasma gondii* seropositivity in females, but

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not in males. Converging lines of evidence have catalyzed an interest in the link between inflammation and mental illness [2, 3]. While elevated proinflammatory cytokines and glial cell abnormalities represent trait markers of major mental illnesses like major depressive disorder [4, 5], bipolar disorder [5, 6] and schizophrenia [5, 7], there also appears to be a state-mediated effect of suicidal behavior [8–10], acuity of mood episode or psychosis [6, 11, 12] and treatment [11–14] on cytokine levels, microglial activation and other indicators of central inflammation. Trait aggression [15–18] and impulsivity [19, 20], behaviors often present in these illnesses, are also associated with elevations of inflammatory biomarkers as suggested by human [21–25] and animal studies [26–29]. Higher rates of latent infection with neurotropic pathogens like *Toxoplasma gondii* (*T. gondii*) and Herpesvidae family viruses [30–34], may contribute to the low-grade immune activation observed in these conditions. Latent toxoplasmosis, during which only bradyzoites (tissue cysts) are present, previously considered harmless, is linked to major psychiatric illnesses [30, 31, 33] as well as suicidal behavior [35–41].

We recently reported, in psychiatrically healthy individuals, that trait aggression, proposed as an endophenotype for suicide [42–44], was associated with *T. gondii* seropositivity [1] in females but not in males. We also noted that males over the age of 60 who were seropositive for *T. gondii* had significantly lower scores on measures of trait aggression including self-aggression. This is consistent with previous reports that the differences in personality measures associated with *T. gondii* seropositivity are sex-specific [45, 46]. Sex-specific differences in personality dimensions in relationship to *T. gondii* have been described in the past in subjects in whom mental illness was not ruled out in a systematic manner [47–50].

The associations between trait measures like aggression and *T. gondii* seropositivity may be mediated by dopaminergic neurotransmission. Previous studies have demonstrated an increase in dopamine synthesis and release, as well as elevated homovanillic acid (HVA), a metabolite of dopamine, in *T. gondii*-infected neurons in animals [51–54]. Additionally, anti-dopaminergic agents prevent behavioral alterations induced by *T. gondii* in animals [55, 56]. Of potential high relevance, two genes encoding tyrosine hydroxylase, the rate-limiting enzyme of dopamine synthesis, were identified in the genome of *T. gondii* [57]. *Toxoplasma gondii* and its influence on dopamine signaling is also suggested by a decrease in the psychobiological construct of novelty-seeking, one of the temperaments in the Temperament and Character Inventory (TCI) related to dopamine [58], in association with *T. gondii* seropositivity in humans [45, 59, 60].

Dopaminergic neurotransmission has also been implicated in the neurobiology of aggression [61–64]. Genetic polymorphisms involved in altered dopamine receptor and transporter activities as well as enzymes involved in the metabolism of dopamine have been identified in association with aggression in humans and animals [65, 66]. The direction of the relationship between dopamine and aggression is far from unequivocal. Some studies have reported a positive relationship between dopaminergic neurotransmission and aggression [67, 68], while others point to a dopaminergic deficit in the relationship with aggression, in particular impulsive aggression [64, 65, 69, 70]. These conflicting findings may be due in part to both context dependency of the relationship between dopamine and aggression, and genetic influences, such as functional allelic variants of monoamine oxidase A, which metabolizes dopamine [71].

Conversion of phenylalanine, an essential amino acid, to tyrosine via the activity of phenylalanine hydroxylase (PAH), is the first step towards the synthesis of dopamine. Tyrosine is further converted into dopamine via a two-step enzymatic reaction involving tyrosine hydroxylase (rate-limiting enzyme in the biosynthesis of catecholamines) and aromatic L-type amino acid decarboxylase [72, 73]. Type 1 T-helper cell immunity (Th1), which acts against intracellular pathogens like *T. gondii* [74], is linked to dysfunction of PAH, and subsequently, an elevated Phe:Tyr ratio [75, 76]. This is thought to be mediated by oxidative stress and reactive oxygen species- (ROS-) induced depletion of (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4), an essential cofactor for PAH [77, 78]. The ratio between phenylalanine and tyrosine (Phe:Tyr) provides an estimate of PAH activity [78] and is elevated in proinflammatory conditions like cancer [79], trauma, sepsis [80], human immunodeficiency virus (HIV) infection [81] as well as depression [76, 82] and schizophrenia [83–85]. Additionally, children with phenylketonuria, a congenital decrease in activity of PAH leading to elevated phenylalanine levels, exhibit higher levels of aggression [86], with a positive correlation between aggression ratings and phenylalanine levels [87].

In this add-on project, we sought to investigate interactions among Phe, Tyr, and their ratio, *T. gondii* seropositivity, and aggression measures in a sample of psychiatrically healthy participants.

Materials and methods

Sample

This is an add-on study to a previous protocol on the genetics of mental illness. As a component of the "parent" protocol, 1000 healthy adults were recruited from the Munich area in Germany. All the healthy

controls provided written informed consent to take part in the parent study after the study procedures were explained in detail. The local Ethics Committee of Ludwig Maximilians University, Munich, Germany, approved the study, and the University of Maryland Institutional Review Board (IRB) granted an exempt status considering that the data were deidentified and recontacting participants in the future was rendered impossible. Axis I or II diagnoses were ruled out by the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision (DSM-IV TR) [88]. History of suicide attempts was also ruled out. Mental illness and suicidal behavior were ruled out in first degree-relatives as well. Blood samples were drawn without observing any fasting protocols or dietary restrictions from a forearm vein into ethylenediaminetetraacetic acid (EDTA)-containing tubes. Plasma obtained after centrifuging the sample for 10 min at 4°C was aliquoted into Eppendorf tubes and frozen immediately at −80°C and stored at this temperature until analysis of plasma Phe and Tyr.

Measurement of plasma phenylalanine and tyrosine

Using high performance liquid chromatography (HPLC), plasma phenylalanine and tyrosine levels were measured using 3-nitro-L-tyrosine as an internal standard as described elsewhere [84, 89].

Toxoplasma gondii serological analysis

Solid phase enzyme-linked immunosorbent assay (ELISA) was used to measure immunoglobulin G (IgG) to *T. gondii* [35] (*T. gondii* IgG ELISA, IBL Laboratories, Hamburg, Germany). After exposing diluted serum to immobilized *T. gondii* antigens on the wells of microtiter plates, specific antibodies binding to the antigen-coated wells were detected using a human IgG-specific secondary enzyme-conjugated antibody. Serointensity was determined by quantitative measurement of antibodies after substrate reaction, using optical density ratios between the blood sample and a standard containing 10 international units of anti-*T. gondii* antibody. A titer ≥0.8 was used to define seropositivity. All *T. gondii* antibody measurements were performed at the Stanley Laboratory of Developmental Neurovirology, Baltimore, MD and the technician was blind to the study hypothesis.

Measures of aggression

Questionnaire for measuring factors of aggression (FAF-Fragebogen zur Erfassung von Aggressivitätsfaktoren), a German version of the Buss-Durkee hostility questionnaire [90, 91], was used to assess trait-aggression in all participants. FAF is a self-administered inventory consisting of 76 items, 66 of which explore five components of aggressive behavior including FAF-self-aggression (11 items), FAF-total aggression (35 items) and its three component subscales: FAF-spontaneous aggression (19 items); FAF-reactive aggression (13 items); FAF-irritability (13 items). FAF demonstrates good internal consistency and test-retest reliability with Cronbach's α-values range from 0.61 to 0.79 [90].

Statistical analysis

Analysis of covariance (ANCOVA) was used to determine differences in FAF-total aggression, its subscales and FAF-self aggression between *T. gondii* seropositive and seronegative individuals. Then subjects were stratified further based on age and sex to detect differences in aggression scores between seropositive and seronegative individuals. Phe:Tyr ratio was converted into a categorical variable by assigning all individuals with a ratio in the upper quartile as high and all individuals with a ratio in the lower three quartiles as low, a quartile-to-binary threshold we applied to monoamine precursors in our previous work [92].

Further analysis by stratification based on high vs. low ratio was performed.

Adjusted linear regression models, accounting for body mass index (BMI), age, education level and sex were then used to determine if Phe or Tyr levels or the Phe:Tyr ratio were related to specific subscales of aggression. All models were stratified by sex due to previous evidence of sex-dependent shifts in personality traits by *T. gondii* status.

Results

Sample characteristics

Approximately half the sample (n = 476, 50.1%) of patients were positive for *T. gondii* based on the criteria of seropositivity of IgG > 0.8 with higher seroprevalence among older subjects (60%) vs. younger subjects (34%) based on a median age split (Table 1). This seroprevalence pattern is similar to previously published estimates of seroprevalence of *T. gondii* from Germany [34, 93]. Mean age of the sample was 53.6 (±15.8) with *T. gondii*-positive participants being significantly older (59.1 ± 12.5) when compared to *T. gondii*-negative patients (48.1 ± 16.8). Close to half the sample was university educated (45.8%) with *T. gondii*-seropositive patients having lower educational attainment ($p < 0.004$). Mean Phe:Tyr ratio was significantly lower in *T. gondii*-positive individuals when compared to *T. gondii*-negative individuals. BMI was higher in *T. gondii*-positive vs. *T. gondii*-negative individuals.

Toxoplasma gondii seropositivity and trait aggression

There were no differences in aggression scores between seropositive and seronegative individuals. As previously reported [1], upon stratification by sex, females who were positive for *T. gondii* had higher FAF-reactive aggression scores when compared to *T. gondii*-negative females after adjustment for age, BMI and education [$F_{(1480)} = 4.1$, $p = 0.04$] but did not differ on any other scales of aggression including total aggression.

In males, *T. gondii*-seropositive individuals had decreased scores on FAF-total aggression [$F_{(1457)} = 6.98$, $p = 0.009$], FAF-spontaneous aggression [$F_{(1457)} = 5.0$,

Table 1: Characteristics of study sample (n = 950) by *T. gondii* status.

	T. gondii IgG+[a] (n = 476)	*T. gondii* IgG– (n = 474)	Total (n = 950)	p-Value[b]
Age, mean ± SD	59.1 ± 12.5	48.1 ± 16.8	53.6 ± 15.8	p < 0.001
Sex, n (%)				
Male	243 (51.0)	220 (46.4)	463 (48.7)	p = 0.153
Female	233 (48.9)	254 (53.6)	487 (51.3)	
Education, n (%)				
Primary	145 (30.5)	86 (18.1)	230 (24.3)	p < 0.001
Secondary	147 (30.9)	137 (28.9)	284 (29.9)	
University	184 (38.6)	251 (53.0)	435 (45.8)	
BMI[c], mean ± SD	25.3 ± 4.0	24.3 ± 3.7	24.8 ± 3.9	p < 0.001
Phe:Tyr[d], mean ± SD	0.734 ± 0.31	0.821 ± 0.39	0.803 ± 0.57	p < 0.001

IgG, immunoglobulin G serum antibody status, tested by ELISA. [a]Optical density ratio between the blood sample and a standard containing 10 international units of anti-*Toxoplasma gondii* IgG ≥ 0.8. [b]Statistical tests using t-test for continuous and χ^2-test for categorical variables. [c]BMI, body mass index (kg/m^2). [d]Phe:Tyr, ratio between plasma phenylalanine and tyrosine levels.

p = 0.03], FAF-irritability $F_{(1457)} = 5.0$, p = 0.03] and FAF-self-aggression [$F_{(1456)} = 5.2$, p = 0.03] when compared to *T. gondii*-negative males after adjusting for age, BMI and education. On further stratification by age, this strong effect was observed only in older males (≥ 60 years old) but not in younger males [$F_{(1274)} = 10.5$, p = 0.001]. Post hoc analysis showed that the average difference between seropositive and seronegative older males in FAF-total aggression was 2.5 points (CI = 1.1–4.0, p = 0.001). Similar observations of lower aggression scores in seropositive males over the age of 60 were observed with FAF-spontaneous aggression [$F_{(1274)} = 6.2$, p = 0.01]. FAF-reactive aggression [$F_{(1274)} = 6.4$, p = 0.01], FAF-irritability $F_{(1274)} = 9.2$, p = 0.003), and FAF-self-aggression [$F_{(1273)} = 9.0$, p = 0.003].

High vs. low Phe:Tyr ratio

Upon further stratification by high vs. low Phe:Tyr ratio, seropositive males over the age of 60 continued to demonstrate decreased FAF-total aggression scores [$F_{(1198)} = 8.7$, p = 0.004] and FAF-self-aggression [$F_{(1197)} = 6.1$, p = 0.02] if the Phe:Tyr ratio was low, but no differences were observed if the Phe:Tyr ratio was high (p-value was 0.24 and 0.90 respectively). This effect was also present for the subscale scores of FAF-reactive aggression [$F_{(1198)} = 7.4$, p = 0.007] and FAF-irritability [$F_{(1198)} = 6.6$, p = 0.01].

Associations between Phe, Tyr, Phe:Tyr ratio and trait aggression score

There were no relationships detected between Phe, Tyr or Phe:Tyr ratio and FAF-total aggression, self-aggression or the subscale scores (reactive aggression, spontaneous aggression and irritability) initially, after adjusting for age, sex, BMI and education (Table 2). After stratifying by *T. gondii* status and sex, there was a positive correlation between FAF-total aggression/FAF-spontaneous aggression and Phe:Tyr ratio in *T. gondii*-seropositive males but not in seronegative males.

Discussion

Our main findings were that; a) the decrease in trait aggression scores in *T. gondii*-positive older males and aggression was only present in individuals with low Phe:Tyr ratio, with no significant association observed in subjects with a high Phe:Tyr ratio, and b) that there was a positive correlation between Phe:Tyr ratio and Total aggression and selected subscales of aggression in *T. gondii*-positive males but not in seronegative males. To our knowledge, this is the first study reporting the moderating role by the Phe:Tyr ratio of the association between *T. gondii*-seropositivity and trait aggression.

Our findings point to Phe:Tyr ratio moderating the association between *T. gondii*-seropositivity and aggression in older males, with a lowering of aggression in *T. gondii*-positives only present in those who have a lower Phe:Tyr.

A higher Phe:Tyr ratio may lead to impedance in dopaminergic neurotransmission, which may negate the potentially dopamine-mediated protective link between *T. gondii* and aggression in older males. Supporting this hypothesis, decreased dopamine synthesis capacity in the midbrain was linked with aggression in humans using positron-emission tomography (PET) [64]. A negative relationship between cerebrospinal fluid HVA levels, a

Table 2: Associations of FAF total score and selected subscale scores with Phe:Tyr rank in *T. gondii* positive vs. *T. gondii* negative individuals stratified by sex after adjustment for age, BMI and education.

FAF scores	*T. gondii* IgG+[c]			*T. gondii* IgG–			Total		
	B	SE	p-Value	*B*	SE	p-Value	*B*	SE	p-Value
Total aggression									
Male	2.51	1.12	**0.026**	0.55	1.23	0.6	1.23	0.83	0.1
Female	−1.5	1.17	0.2	−1.33	0.79	0.09	−1.32	0.68	0.06
Total	0.02	0.81	0.9	−0.07	0.73	0.9	−0.019	0.54	0.9
Self-aggression									
Male	0.77	0.39	**0.050**	−0.31	0.36	0.4	0.03	0.26	0.9
Female	0.31	0.36	0.4	−0.22	0.29	0.4	0.07	0.22	0.7
Total	0.46	0.26	0.09	−0.22	0.23	0.3	0.06	0.17	0.7
Spontaneous aggression									
Male	1.38	0.47	**0.004**	0.46	0.55	0.4	0.77	0.36	**0.04**
Female	−0.83	0.51	0.1	−0.29	0.28	0.3	−0.50	0.28	0.08
Total	0.024	0.35	0.95	0.20	0.30	0.5	0.14	0.23	0.5
Reactive aggression									
Male	1.00	0.5	**0.046**	0.20	0.41	0.6	0.5	0.31	0.1
Female	0.28	0.42	0.5	−0.24	0.3	0.4	0.02	0.25	0.9
Total	0.54	0.32	0.09	0.07	0.25	0.8	0.26	0.2	0.2
Irritability									
Male	0.12	0.48	0.8	−0.11	0.44	0.8	−0.03	0.32	0.9
Female	−0.98	0.48	**0.042**	−0.79	0.43	0.07	−0.85	0.32	**0.008**
Total	−0.54	0.34	0.1	−0.34	0.30	0.3	−0.42	0.22	0.06

FAF, Fragebogen zur Erfassung von Aggressivitätsfaktoren (German adaptation of the Buss-Durkee hostility inventory); Phe:Tyr, ratio between plasma phenylalanine and tyrosine; IgG, immunoglobulin G serum antibody status, tested by ELISA. [a]Individual scale score differences are model-based estimates from linear regression model adjusted for age, education and BMI and stratified by sex/*T. gondii* status. [b]Total FAF score and selected FAF component score (self-aggression, spontaneous aggression, reactive aggression and irritability). [c]Optical density ratio between the blood sample and a standard containing 10 international units of anti-*Toxoplasma gondii* IgG ≥ 0.8. Bold face values denote statistically significant associations.

major metabolite of dopamine, and impulsive aggression also points to decreased dopaminergic function [94] in aggressive behaviors. Lower functioning dopamine receptor profiles were reported to predict aggressive responses in an analysis of dopamine receptor polymorphisms and their relationships to aggression [65]. *Toxoplasma gondii*-infected dopaminergic neurons show an increase in intracellular dopamine in rodents [52]. In rodent-models, an activation of dopamine-responsive sexual pathways, has been described in *T. gondii*-infected rodents upon exposure to cat urine [95]. Moreover, *T. gondii* genome contains two genes that code for tyrosine hydroxylase enzyme, one of which is exclusively activated in the bradyzoite or tissue cyst stage, which localizes to brain and muscle tissue in latent toxoplasmosis [57].

Alternatively, Phe:Tyr ratio, a proxy for PAH activity, and thereby proinflammatory states [75], may also reflect the moderation of aggression by inflammation in *T. gondii*-positive individuals. Patients with intermittent explosive disorder (IED), a psychiatric condition, with recurrent impulsive aggression, have been reported to manifest elevated blood markers of inflammation like C-reactive protein (CRP) and interleukin (IL)-6 [23, 24], and very recently, increased rates of *T. gondii* seropositivity [96]. Moreover, in these patients with "rage" traits, and documented episodes of aggression, the aggression scores were higher in *T. gondii*-positive individuals. Our findings of a positive correlation between aggression and Phe:Tyr in *T. gondii*-positive males may lend credence to the possibility of low grade inflammation, triggered and perpetuated by the parasitic infection, leads to aggression either directly, or, in part, through a higher Phe:Tyr ratio.

Sex-specific differences in personality profile with regards to *T. gondii* have been described in the past and our results suggest that the correlations between Phe:Tyr ratio and aggression are specific to male subjects positive for *T. gondii*. We have previously reported age- and sex-dependent differences in trait aggression and impulsivity in *T. gondii*-positive individuals, with higher reactive aggression in *T. gondii* positive females and higher impulsivity in *T. gondii*-positive young males [1]. Despite the possibility of correlations among personality factors that overlap and co-vary by sex, animal studies point to the parasite inducing sex-specific changes in neurotransmitter

activity [97], mating behavior [49, 98, 99] and production of sex hormones [100].

One of the limitations of the study is that we did not obtain fasting levels of Phe and Tyr as there is evidence for heterogeneity in plasma levels of amino acids based on timing of meals and dietary preferences [101]. Moreover, the cross-sectional nature of the study precludes estimating causal relationships between dopamine precursors and aggression in *T. gondii*-positive individuals. The effect of stress and the influence of stress-related hormones were not analyzed, thus not statistically accounting for these potentially relevant confounders. We did not correct the level of significance for multiple comparisons, and thus our results and interpretation are prone to Type I error. Yet, we position our study as, primarily, hypothesis generating rather than testing, leading to future hypothesis testing study designs. Direct markers of immune response like cytokines and acute phase reactants were not measured due to the insufficient volume of plasma samples, considering that this was an add-on study. Additionally, we only used self-report questionnaires instead of observed aggressive events, or collateral informants. For all the above reasons, the results of this study must be considered preliminary and, thus, requiring replication.

Despite these limitations, our study had several strengths; we studied a large sample of normal individuals without any overt psychopathology, family history of mental illness suicidal behavior or substance use [1]. The majority of studies exploring the relationship between personality and *T. gondii* involved subjects in whom mental illnesses was not ruled out using a structured and validated tool [45, 47, 49, 59, 60]. This previous approach runs the risk of spurious associations between *T. gondii* seropositivity and personality changes, as they may be confounded by unmeasured mental illness. This study supports the possibility that aggressive traits are directly linked to *T. gondii* seropositivity, and not as a mere byproduct of the established link between mental illness, and implicitly, its treatment, and *T. gondii* infection [30, 33]. These findings, as was the case in our previous work [1], persisted after post-hoc controlling for cytomegalovirus (CMV) and herpes simplex virus 1 (HSV1) titers and were independent of CMV/HSV1 seropositivity (for methodology see our previous article Cook et al. (2015) [1].

In conclusion, if replicated and confirmed by further longitudinal and, ideally, experimental designs, our findings may pave the way for individualized interventions geared towards optimizing blood levels of dopamine precursors in *T. gondii*-positive individuals with aggressive personality traits, and in particular those with overt history of aggression and suicidal behavior.

Acknowledgments: This work was supported by the Distinguished Investigator Award from the American Foundation for Suicide Prevention (Postolache, PI, Rujescu Co-I, DIG 1-162-12), with additional funding from the P30 DK072488 NIDDK (NORC pilot/developmental grant Postolache) from the National Institutes of Health, Bethesda, MD, and the Joint Institute for Food Safety and Applied Nutrition/UMD, through the cooperative agreement FDU.001418 (Postolache, PI). Further support was received from the MVM-CoRE, Denver CO (Postolache, Brenner, Lowry), Rocky Mountain MIRECC for Suicide Prevention, Denver, CO (Postolache, Brenner, Lowry), VISN5 MIRECC, Baltimore, MD (Postolache) and the DC Department of Behavioral Health (Mathai). The findings and conclusions in this study are those of the authors and do not necessarily represent the view of the NIH, FDA, VA or the United States Government.

Conflict of interest statement: All authors have declared no conflicts of interest. All authors contributed to the manuscript and approved its final version.

References

1. Cook TB, Brenner LA, Cloninger CR, Langenberg P, Igbide A, Giegling I, et al. "Latent" infection with Toxoplasma gondii: association with trait aggression and impulsivity in healthy adults. J Psychiatr Res 2015;60:87–94.
2. Najjar S, Pearlman DM, Alper K, Najjar A, Devinsky O. Neuroinflammation and psychiatric illness. J Neuroinflammation 2013;10:43.
3. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology meets neuropsychopharmacology: translational implications of the impact of inflammation on behavior. Neuropsychopharmacology 2012;37:137–62.
4. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry 2009;65:732–41.
5. Cotter DR, Pariante CM, Everall IP. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. Brain Res Bull 2001;55:585–95.
6. Brietzke E, Stabellini R, Grassi-Oliveira R, Lafer B. Cytokines in bipolar disorder: recent findings, deleterious effects but promise for future therapeutics. CNS Spectr 2011;16:157–68.
7. Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. Biol Psychiatry 2008;63:801–8.
8. Bay-Richter C, Linderholm KR, Lim CK, Samuelsson M, Traskman-Bendz L, Guillemin GJ, et al. A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. Brain Behav Immun 2015;43:110–7.
9. Brundin L, Erhardt S, Bryleva EY, Achtyes ED, Postolache TT. The role of inflammation in suicidal behaviour. Acta Psychiatr Scand 2015;132:192–203.

10. Janelidze S, Mattei D, Westrin A, Traskman-Bendz L, Brundin L. Cytokine levels in the blood may distinguish suicide attempters from depressed patients. Brain Behav Immun 2011;25:335–9.
11. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011;70:663–71.
12. McNally L, Bhagwagar Z, Hannestad J. Inflammation, glutamate, and glia in depression: a literature review. CNS Spectr 2008;13:501–10.
13. de Witte L, Tomasik J, Schwarz E, Guest PC, Rahmoune H, Kahn RS, et al. Cytokine alterations in first-episode schizophrenia patients before and after antipsychotic treatment. Schizophr Res 2014;154:23–9.
14. Kubera M, Kenis G, Bosmans E, Zieba A, Dudek D, Nowak G, et al. Plasma levels of interleukin-6, interleukin-10, and interleukin-1 receptor antagonist in depression: comparison between the acute state and after remission. Pol J Pharmacol 2000;52:237–41.
15. Ballester J, Goldstein T, Goldstein B, Obreja M, Axelson D, Monk K, et al. Is bipolar disorder specifically associated with aggression? Bipolar Disord 2012;14:283–90.
16. Soyka M. Neurobiology of aggression and violence in schizophrenia. Schizophr Bull 2011;37:913–20.
17. Volavka J, Citrome L. Pathways to aggression in schizophrenia affect results of treatment. Schizophr Bull 2011;37:921–9.
18. Oquendo MA, Galfalvy H, Russo S, Ellis SP, Grunebaum MF, Burke A, et al. Prospective study of clinical predictors of suicidal acts after a major depressive episode in patients with major depressive disorder or bipolar disorder. Am J Psychiatry 2004;161:1433–41.
19. Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC. Psychiatric aspects of impulsivity. Am J Psychiatry 2001;158:1783–93.
20. Swann AC, Lijffijt M, Lane SD, Steinberg JL, Moeller FG. Increased trait-like impulsivity and course of illness in bipolar disorder. Bipolar Disord 2009;11:280–8.
21. Suarez EC. Joint effect of hostility and severity of depressive symptoms on plasma interleukin-6 concentration. Psychosom Med 2003;65:523–7.
22. Suarez EC, Lewis JG, Krishnan RR, Young KH. Enhanced expression of cytokines and chemokines by blood monocytes to in vitro lipopolysaccharide stimulation are associated with hostility and severity of depressive symptoms in healthy women. Psychoneuroendocrinology 2004;29:1119–28.
23. Coccaro EF. Association of C-reactive protein elevation with trait aggression and hostility in personality disordered subjects: a pilot study. J Psychiatr Res 2006;40:460–5.
24. Coccaro EF, Lee R, Coussons-Read M. Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans. JAMA Psychiatry 2014;71:158–65.
25. Graham JE, Robles TF, Kiecolt-Glaser JK, Malarkey WB, Bissell MG, Glaser R. Hostility and pain are related to inflammation in older adults. Brain Behav Immun 2006;20:389–400.
26. Bhatt S, Bhatt R, Zalcman SS, Siegel A. Role of IL-1 beta and 5-HT2 receptors in midbrain periaqueductal gray (PAG) in potentiating defensive rage behavior in cat. Brain Behav Immun 2008;22:224–33.
27. Patel A, Siegel A, Zalcman SS. Lack of aggression and anxiolytic-like behavior in TNF receptor (TNF-R1 and TNF-R2) deficient mice. Brain Behav Immun 2010;24:1276–80.
28. Pesce M, Speranza L, Franceschelli S, Ialenti V, Patruno A, Febo MA, et al. Biological role of interleukin-1beta in defensive-aggressive behaviour. J Biol Regul Homeost Agents 2011;25:323–9.
29. Zalcman SS, Siegel A. The neurobiology of aggression and rage: role of cytokines. Brain Behav Immun 2006;20:507–14.
30. Pearce BD, Kruszon-Moran D, Jones JL. The relationship between Toxoplasma gondii infection and mood disorders in the third National Health and Nutrition Survey. Biol Psychiatry 2012;72:290–5.
31. Tedla Y, Shibre T, Ali O, Tadele G, Woldeamanuel Y, Asrat D, et al. Serum antibodies to Toxoplasma gondii and Herpesvidae family viruses in individuals with schizophrenia and bipolar disorder: a case-control study. Ethiop Med J 2011;49:211–20.
32. Prasad KM, Watson AM, Dickerson FB, Yolken RH, Nimgaonkar VL. Exposure to herpes simplex virus type 1 and cognitive impairments in individuals with schizophrenia. Schizophr Bull 2012;38:1137–48.
33. Torrey EF, Bartko JJ, Yolken RH. Toxoplasma gondii and other risk factors for schizophrenia: an update. Schizophr Bull 2012;38:642–7.
34. Hinze-Selch D, Daubener W, Erdag S, Wilms S. The diagnosis of a personality disorder increases the likelihood for seropositivity to Toxoplasma gondii in psychiatric patients. Folia Parasitol (Praha) 2010;57:129–35.
35. Arling TA, Yolken RH, Lapidus M, Langenberg P, Dickerson FB, Zimmerman SA, et al. Toxoplasma gondii antibody titers and history of suicide attempts in patients with recurrent mood disorders. J Nerv Ment Dis 2009;197:905–8.
36. Pedersen MG, Mortensen PB, Norgaard-Pedersen B, Postolache TT. Toxoplasma gondii infection and self-directed violence in mothers. Arch Gen Psychiatry 2012;69:1123–30.
37. Ling VJ, Lester D, Mortensen PB, Langenberg PW, Postolache TT. Toxoplasma gondii seropositivity and suicide rates in women. J Nerv Ment Dis 2011;199:440–4.
38. Okusaga O, Langenberg P, Sleemi A, Vaswani D, Giegling I, Hartmann AM, et al. Toxoplasma gondii antibody titers and history of suicide attempts in patients with schizophrenia. Schizophr Res 2011;133:150–5.
39. Postolache TT, Cook TB. Is latent infection with Toxoplasma gondii a risk factor for suicidal behavior? Expert Rev Anti Infect Ther 2013;11:339–42.
40. Yagmur F, Yazar S, Temel HO, Cavusoglu M. May Toxoplasma gondii increase suicide attempt-preliminary results in Turkish subjects? Forensic Sci Int 2010;199:15–7.
41. Zhang Y, Traskman-Bendz L, Janelidze S, Langenberg P, Saleh A, Constantine N, et al. Toxoplasma gondii immunoglobulin G antibodies and nonfatal suicidal self-directed violence. J Clin Psychiatry 2012;73:1069–76.
42. Mann JJ, Arango VA, Avenevoli S, Brent DA, Champagne FA, Clayton P, et al. Candidate endophenotypes for genetic studies of suicidal behavior. Biol Psychiatry 2009;65:556–63.
43. Turecki G. Dissecting the suicide phenotype: the role of impulsive-aggressive behaviours. J Psychiatry Neurosci 2005;30:398–408.
44. Kovacsics CE, Gottesman, II, Gould TD. Lithium's antisuicidal efficacy: elucidation of neurobiological targets using endophenotype strategies. Annu Rev Pharmacol Toxicol 2009;49:175–98.

45. Flegr J, Kodym P, Tolarova V. Correlation of duration of latent Toxoplasma gondii infection with personality changes in women. Biol Psychol 2000;53:57–68.
46. Flegr J, Zitkova S, Kodym P, Frynta D. Induction of changes in human behaviour by the parasitic protozoan Toxoplasma gondii. Parasitology 1996;113 (Pt 1):49–54.
47. Flegr J. Effects of toxoplasma on human behavior. Schizophr Bull 2007;33:757–60.
48. Flegr J, Lindova J, Kodym P. Sex-dependent toxoplasmosis-associated differences in testosterone concentration in humans. Parasitology 2008;135:427–31.
49. Lindova J, Novotna M, Havlicek J, Jozifkova E, Skallova A, Kolbekova P, et al. Gender differences in behavioural changes induced by latent toxoplasmosis. Int J Parasitol 2006;36:14 85–92.
50. Lindova J, Kubena AA, Sturcova H, Krivohlava R, Novotna M, Rubesova A, et al. Pattern of money allocation in experimental games supports the stress hypothesis of gender differences in Toxoplasma gondii-induced behavioural changes. Folia Parasitol (Praha) 2010;57:136–42.
51. Stibbs HH. Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. Ann Trop Med Parasitol 1985;79:153–7.
52. Prandovszky E, Gaskell E, Martin H, Dubey JP, Webster JP, McConkey GA. The neurotropic parasite Toxoplasma gondii increases dopamine metabolism. PLoS One 2011;6:e23866.
53. Xiao J, Li Y, Prandovszky E, Karuppagounder SS, Talbot CC, Jr., Dawson VL, et al. MicroRNA-132 dysregulation in Toxoplasma gondii infection has implications for dopamine signaling pathway. Neuroscience 2014;268:128–38.
54. Martin HL, Alsaady I, Howell G, Prandovszky E, Peers C, Robinson P, et al. Effect of parasitic infection on dopamine biosynthesis in dopaminergic cells. Neuroscience 2015;306:50–62.
55. Webster JP, Lamberton PH, Donnelly CA, Torrey EF. Parasites as causative agents of human affective disorders? The impact of anti-psychotic, mood-stabilizer and anti-parasite medication on Toxoplasma gondii's ability to alter host behaviour. Proc Biol Sci 2006;273:1023–30.
56. Skallova A, Kodym P, Frynta D, Flegr J. The role of dopamine in Toxoplasma-induced behavioural alterations in mice: an ethological and ethopharmacological study. Parasitology 2006;133(Pt 5):525–35.
57. Gaskell EA, Smith JE, Pinney JW, Westhead DR, McConkey GA. A unique dual activity amino acid hydroxylase in Toxoplasma gondii. PLoS One 2009;4:e4801.
58. Cloninger CR, Svrakic DM, Przybeck TR. A psychobiological model of temperament and character. Arch Gen Psychiatry 1993;50:975–90.
59. Flegr J, Preiss M, Klose J, Havlicek J, Vitakova M, Kodym P. Decreased level of psychobiological factor novelty seeking and lower intelligence in men latently infected with the protozoan parasite Toxoplasma gondii Dopamine, a missing link between schizophrenia and toxoplasmosis? Biol Psychol 2003;63:253–68.
60. Novotna M, Hanusova J, Klose J, Preiss M, Havlicek J, Roubalova K, et al. Probable neuroimmunological link between Toxoplasma and cytomegalovirus infections and personality changes in the human host. BMC Infect Dis 2005;5:54.
61. Guo G, Roettger ME, Shih JC. Contributions of the DAT1 and DRD2 genes to serious and violent delinquency among adolescents and young adults. Hum Genet 2007;121:125–36.
62. Yu Q, Teixeira CM, Mahadevia D, Huang Y, Balsam D, Mann JJ, et al. Dopamine and serotonin signaling during two sensitive developmental periods differentially impact adult aggressive and affective behaviors in mice. Mol Psychiatry 2014;19:688–98.
63. Soderstrom H, Blennow K, Manhem A, Forsman A. CSF studies in violent offenders. I. 5-HIAA as a negative and HVA as a positive predictor of psychopathy. J Neural Transm (Vienna) 2001;108:869–78.
64. Schluter T, Winz O, Henkel K, Prinz S, Rademacher L, Schmaljohann J, et al. The impact of dopamine on aggression: an [18F]-FDOPA PET Study in healthy males. J Neurosci 2013;33:16889–96.
65. Chester DS, DeWall CN, Derefinko KJ, Estus S, Lynam DR, Peters JR, et al. Looking for reward in all the wrong places: dopamine receptor gene polymorphisms indirectly affect aggression through sensation-seeking. Soc Neurosci 2016;11:487–94.
66. Zhang-James Y, Faraone SV. Genetic architecture for human aggression: a study of gene-phenotype relationship in OMIM. Am J Med Genet B Neuropsychiatr Genet 2016;171:641–9.
67. Ferrari PF, van Erp AM, Tornatzky W, Miczek KA. Accumbal dopamine and serotonin in anticipation of the next aggressive episode in rats. Eur J Neurosci 2003;17:371–8.
68. Couppis MH, Kennedy CH. The rewarding effect of aggression is reduced by nucleus accumbens dopamine receptor antagonism in mice. Psychopharmacology (Berl) 2008;197:449–56.
69. Kayser AS, Allen DC, Navarro-Cebrian A, Mitchell JM, Fields HL. Dopamine, corticostriatal connectivity, and intertemporal choice. J Neurosci 2012;32:9402–9.
70. Oswald LM, Wong DF, Zhou Y, Kumar A, Brasic J, Alexander M, et al. Impulsivity and chronic stress are associated with amphetamine-induced striatal dopamine release. Neuroimage 2007;36:153–66.
71. Schluter T, Winz O, Henkel K, Eggermann T, Mohammadkhani-Shali S, Dietrich C, et al. MAOA-VNTR polymorphism modulates context-dependent dopamine release and aggressive behavior in males. Neuroimage 2016;125:378–85.
72. van Spronsen FJ, Hoeksma M, Reijngoud DJ. Brain dysfunction in phenylketonuria: is phenylalanine toxicity the only possible cause? J Inherit Metab Dis 2009;32:46–51.
73. Daubner SC, Le T, Wang S. Tyrosine hydroxylase and regulation of dopamine synthesis. Arch Biochem Biophys 2011;508:1–12.
74. Spellberg B, Edwards JE, Jr. Type 1/Type 2 immunity in infectious diseases. Clin Infect Dis 2001;32:76–102.
75. Neurauter G, Schrocksnadel K, Scholl-Burgi S, Sperner-Unterweger B, Schubert C, Ledochowski M, et al. Chronic immune stimulation correlates with reduced phenylalanine turnover. Curr Drug Metab 2008;9:622–7.
76. Capuron L, Schroecksnadel S, Feart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: role in neuropsychiatric symptoms. Biol Psychiatry 2011;70:175–82.
77. Werner ER, Blau N, Thony B. Tetrahydrobiopterin: biochemistry and pathophysiology. Biochem J 2011;438:397–414.
78. Anderson DN, Wilkinson AM, Abou-Saleh MT, Blair JA. Recovery from depression after electroconvulsive therapy is accompanied by evidence of increased tetrahydrobiopterin-dependent hydroxylation. Acta Psychiatr Scand 1994;90:10–3.
79. Neurauter G, Grahmann AV, Klieber M, Zeimet A, Ledochowski M, Sperner-Unterweger B, et al. Serum phenylalanine

concentrations in patients with ovarian carcinoma correlate with concentrations of immune activation markers and of isoprostane-8. Cancer Lett 2008;272:141–7.
80. Ploder M, Neurauter G, Spittler A, Schroecksnadel K, Roth E, Fuchs D. Serum phenylalanine in patients post trauma and with sepsis correlate to neopterin concentrations. Amino Acids 2008;35:303–7.
81. Zangerle R, Kurz K, Neurauter G, Kitchen M, Sarcletti M, Fuchs D. Increased blood phenylalanine to tyrosine ratio in HIV-1 infection and correction following effective antiretroviral therapy. Brain Behav Immun 2010;24:403–8.
82. Hoekstra R, van den Broek WW, Fekkes D, Bruijn JA, Mulder PG, Pepplinkhuizen L. Effect of electroconvulsive therapy on biopterin and large neutral amino acids in severe, medication-resistant depression. Psychiatry Res 2001;103:115–23.
83. Rao ML, Gross G, Strebel B, Braunig P, Huber G, Klosterkotter J. Serum amino acids, central monoamines, and hormones in drug-naive, drug-free, and neuroleptic-treated schizophrenic patients and healthy subjects. Psychiatry Res 1990;34:243–57.
84. Okusaga O, Muravitskaja O, Fuchs D, Ashraf A, Hinman S, Giegling I, et al. Elevated levels of plasma phenylalanine in schizophrenia: a guanosine triphosphate cyclohydrolase-1 metabolic pathway abnormality? PLoS One 2014;9:e85945.
85. Wei J, Xu H, Ramchand CN, Hemmings GP. Low concentrations of serum tyrosine in neuroleptic-free schizophrenics with an early onset. Schizophr Res 1995;14:257–60.
86. Targum SD, Lang W. Neurobehavioral problems associated with phenylketonuria. Psychiatry (Edgmont) 2010;7:29–32.
87. Wu W, Sheng D, Shao J, Zhao Z. Mental and motor development and psychosocial adjustment of Chinese children with phenylketonuria. J Paediatr Child Health 2011;47:441–7.
88. First MB, Spitzer RL, Gibbon M, Williams JBW. Structured clinical interview for DSM-IV-TR axis I Disorders, research version, Patient Edition. (SCID-I/P). New York: Biometrics Research, New York State Psychiatric Institute, 2002.
89. Neurauter G, Scholl-Burgi S, Haara A, Geisler S, Mayersbach P, Schennach H, et al. Simultaneous measurement of phenylalanine and tyrosine by high performance liquid chromatography (HPLC) with fluorescence detection. Clin Biochem 2013;46:1848–51.
90. Buss AH, Durkee A. An inventory for assessing different kinds of hostility. J Consult Psychol 1957;21:343–9.
91. Hampel R, Selg H. FAF-Fragebogen zur Erfassung von Aggressivitätsfaktoren. Göttingen: Hogrefe, 1975.
92. Okusaga O, Duncan E, Langenberg P, Brundin L, Fuchs D, Groer MW, et al. Combined Toxoplasma gondii seropositivity and high blood kynurenine – Linked with nonfatal suicidal self-directed violence in patients with schizophrenia. J Psychiatr Res 2016;72:74–81.
93. Pappas G, Roussos N, Falagas ME. Toxoplasmosis snapshots: global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis. Int J Parasitol 2009;39:1385–94.
94. Coccaro EF, Lee R. Cerebrospinal fluid 5-hydroxyindolacetic acid and homovanillic acid: reciprocal relationships with impulsive aggression in human subjects. J Neural Transm (Vienna) 2010;117:241–8.
95. House PK, Vyas A, Sapolsky R. Predator cat odors activate sexual arousal pathways in brains of Toxoplasma gondii infected rats. PLoS One 2011;6:e23277.
96. Coccaro EF, Lee R, Groer MW, Can A, Coussons-Read M, Postolache TT. Toxoplasma gondii infection: relationship with aggression in psychiatric subjects. J Clin Psychiatry 2016;77:334–41.
97. Gatkowska J, Wieczorek M, Dziadek B, Dzitko K, Dlugonska H. Sex-dependent neurotransmitter level changes in brains of Toxoplasma gondii infected mice. Exp Parasitol 2013;133:1–7.
98. Dass SA, Vasudevan A, Dutta D, Soh LJ, Sapolsky RM, Vyas A. Protozoan parasite Toxoplasma gondii manipulates mate choice in rats by enhancing attractiveness of males. PLoS One 2011;6:e27229.
99. Vyas A. Parasite-augmented mate choice and reduction in innate fear in rats infected by Toxoplasma gondii. J Exp Biol 2013;216(Pt 1):120–6.
100. Lim A, Kumar V, Hari Dass SA, Vyas A. Toxoplasma gondii infection enhances testicular steroidogenesis in rats. Mol Ecol 2013;22:102–10.
101. Wurtman RJ, Rose CM, Chou C, Larin FF. Daily rhythms in the concentrations of various amino acids in human plasma. N Engl J Med 1968;279:171–5.