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Putative Neuroprotective and Neurotoxic Kynurenine Pathway Metabolites Are Associated with Hippocampal and Amygdalar Volumes in Subjects with Major Depressive Disorder

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Inflammation-related changes in the concentrations of kynurenine pathway metabolites occur in depression secondary to medical conditions but are not firmly established in primary mood disorders. Reductions in hippocampal and amygdalar volume that putatively reflect dendritic atrophy are widely reported in major depressive disorder (MDD). Here we tested whether the relative serum concentrations of putatively neuroprotective (kynurenic acid (KA)) and neurotoxic (3-hydroxykynurenine (3HK) and quinolinic acid (QA)) kynurenine pathway metabolites were altered in primary MDD and whether these metabolites were associated with hippocampal and amygdalar volume. A total of 29 moderately to severely depressed *unmedicated* subjects who met DSM-IV criteria for MDD and 20 healthy controls (HCs) completed a structural MRI scan and provided blood sample for kynurenine metabolite analysis, performed using high-performance liquid chromatography with tandem mass spectrometry. Cytokine concentrations were measured with ELISA and gray matter volumes were measured with the automated segmentation software, FreeSurfer. An *a priori* defined variable of interest, the KA/QA ratio, a putative neuroprotective index, trended lower in the MDD *versus* the HC group and correlated negatively with anhedonia but positively with the total hippocampal and amygdala volume in the MDD subjects. The *post hoc* data reduction methods yielded three principal components. Component 1 (interleukin-1 receptor antagonist, QA, and kynurenine) was significantly elevated in MDD participants *versus* the HCs, whereas component 2 (KA, tryptophan, and kynurenine) was positively correlated with hippocampal and amygdala volume within the MDD group. Our results raise the possibility that an immune-related imbalance in the relative metabolism of KA and QA predisposes to depression-associated dendritic atrophy and anhedonia.
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INTRODUCTION

Major depressive disorder (MDD) has been associated with reductions in hippocampal and amygdalar volume that are thought to reflect dendritic atrophy occurring in the context of excitotoxicity, decreased neurogenesis, and impaired neurotrophic function (Koolschijn *et al*, 2009; Pereira *et al*, 2007; Petrik *et al*, 2012; Savitz and Drevets, 2009; Stockmeier *et al*, 2004). At least a subgroup of individuals with MDD also display putative signs of inflammation such as elevated circulating concentrations of C-reactive protein (CRP), interleukin-6 (IL-6), and interleukin-1β (IL-1β) (Dowlati *et al*, 2010; Howren *et al*, 2009). Inflammation can also lead to the activation of the tryptophan (TRP)-degrading enzyme indoleamine 2,3 dioxygenase (IDO), ultimately increasing the formation of kynurenine (KYN) metabolites, including kynurenic acid (KA), a putatively neuroprotective *antagonist* of *N*-methyl-D-aspartate (NMDA) receptors that also decreases glutamate levels via inhibition of α7 nicotinic receptors 3-hydroxykynurenine (3HK), a free radical generator, and quinolinic acid (QA), an NMDA receptor *agonist* that also exerts neurotoxic effects via lipid peroxidation, and disruption of the blood–brain barrier (Dantzer *et al*, 2011; Maes *et al*, 2011; Perkins and Stone, 1982; Schwarcz *et al*, 2012; Schwarcz *et al*, 1983) (Figure 1).

A causal association between neuroactive cytokine release and changes in brain structure and/or function has been reported in putative animal models of depression. IL-1 plays

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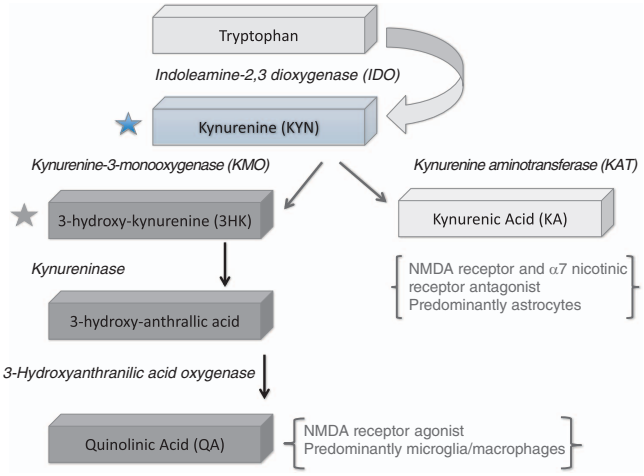

Figure 1 Main branches of the kynurenine pathway. Each box represents a metabolite resulting from the oxidation of tryptophan. Putative neurotoxic metabolites are colored red whereas KA, which is putatively neuroprotective, is colored green. The black italicized text shows the enzymes that catalyze each step in the metabolic pathway. The blue and red stars indicate that the metabolite is able to cross the blood–brain barrier. The effects on NMDA receptor activity listed for some metabolites have been established *in vitro*, but the extent to which the metabolite concentrations achieve sufficiently high levels *in vivo* to impact the function of this receptor system remains unclear. NMDA, *N*-methyl-D-aspartate glutamate receptor. A full text version of this figure is available at *Neuropsychopharmacology* Journal online.

a key role in the development of depression-like behavior and in the dendritic atrophy, synaptic loss, suppressed neurogenesis, and decreased brain-derived neurotrophic factor (BDNF) concentrations that are apparent in the hippocampi of rodents subjected to various inflammatory challenges (Barrientos *et al*, 2009; Goshen *et al*, 2008; Koo and Duman, 2008; Williamson and Bilbo, 2013; Yirmiya and Goshen, 2011). The effect of IL-1 may be partly mediated by the production of neurotoxic kynurenine metabolites. IL-1 increases the expression of IDO, kynurenine 3-monooxygenase (KMO), and kynureninase—that is, the enzymes that produce potentially neurotoxic kynurenine metabolites (Zunszain *et al*, 2012). Moreover, IL-1-driven synthesis of 3HK decreases human hippocampal cell neurogenesis *in vitro* (Zunszain *et al*, 2012). Consistent with these data, IDO transcripts were upregulated in the hippocampi of rats displaying comorbid pain and depression in response to inflammation in the paw, and these comorbid symptoms were IDO dependent (Kim *et al*, 2012).

Other cytokines also play an important role in activating IDO. Dendritic cells express the highest level of IDO activity in response to interferon-γ (IFN-γ) (Fallarino *et al*, 2002), thus aiding in the inhibition of the local replication of pathogens. Similarly, IDO is upregulated by the tumor necrosis factor-α (TNF-α) secretion associated with viral infections such as the Epstein–Barr virus (Liu *et al*, 2014). Kynurenine pathway metabolites are also regulators of T-cell tolerance and maintenance, an effect that occurs in the context of transforming growth factor-β (TGF-β) signaling (Orabona *et al*, 2012; Puccetti and Grohmann, 2007).

Nevertheless, it is not yet known whether the relationship between inflammation and hippocampal physiology extends to patients with MDD *in vivo*. Reductions in the gray matter (GM) volume of limbic structures in depressed humans appear to reflect dendritic atrophy, a hypothesis confirmed in subjects with MDD post-mortem (Stockmeier *et al*, 2004) and more recently in a dual *ex vivo* MRI and histopathological study of rats exposed to antipsychotic medications (Vernon *et al*, 2013).

In our study, we therefore hypothesized that reductions in the hippocampal and the amygdalar volumes measured with T1-weighted MRI could be secondary to inflammation and activation of the kynurenine metabolite pathway. If this is the case, these volumes should be associated with increased concentrations of potentially neurotoxic kynurenine metabolites and/or decreased concentrations of potentially neuroprotective kynurenine metabolites. Because the effects of 'neurotoxic' metabolites on GM volumes may depend on the corresponding concentration of 'neuroprotective' metabolites, we maximized sensitivity by focusing *a priori* on the ratios of 'neuroprotective' to 'neurotoxic metabolites'—KA *versus* 3HK (KA/3HK) as well as KA *versus* QA (KA/QA)—considered 'neuroprotective indices' (Johansson *et al*, 2013; Kocki *et al*, 2012). In addition, in order to reduce the probability of type I error while maintaining a hypothesis-free approach, we conducted a principal components analysis (PCA) and used the extracted principal components in a *post hoc* analysis. Here we show that a higher neuroprotective index (KA/QA) and a principal component that was highly associated with KA correlated positively with hippocampal and amygdalar volumes in unmedicated moderately to severely depressed subjects with MDD.

MATERIALS AND METHODS

Subjects

Subjects provided written informed consent after receiving a full explanation of the study procedures and risks, as approved by the local IRB.

All depressed subjects ($n = 29$) and healthy controls ($n = 20$) were interviewed with the Structured Clinical Interview for the DSM-IV-TR (First *et al*, 1996). In addition, unstructured psychiatric interviews with board-certified psychiatrists were obtained on all depressed participants.

The depressed subjects met DSM-IV-TR criteria for primary MDD in a current major depressive episode and had a Hamilton Depression Rating Scale (HDRS, 24-item) score in the moderately to severely depressed range (Table 1). The MDD patients did not receive any psychotropic medications for at least 3 weeks (8 for fluoxetine) before the blood draw and MRI scanning (mean length of time off medication: 42.3 ± 42.3 months; 9 participants were treatment naive). Exclusion criteria were as follows: serious suicidal ideation or behavior; bipolar disorder; medical conditions or concomitant medications likely to influence CNS or immunological function including cardiovascular, respiratory, endocrine, and neurological diseases; a history of drug or alcohol abuse within 6 months or a history of drug or alcohol dependence within 1 year (DSM-IV-TR criteria), and general MRI exclusion criteria such as magnetic implants or claustrophobia. Details are provided in the Supplementary Data File, including Supplementary Tables S1 and S2.

The healthy control (HC) individuals met the same exclusion criteria except that they had no personal or

Table 1 Demographic and Clinical Information for the MDD and the HC Groups

	MDD	HC
N	29	20
Sex (% F)	83	52
Age	36.4 ± 10.0	35.0 ± 10.9
BMI	27.3 ± 4.4	29.2 ± 5.1
HAM-D (24-item)	27.1 ± 6.6	0.48 ± 0.67
SHAPS	31.4 ± 5.9	18.6 ± 5.9
Intracranial volume (ml)	1310 ± 237	1419 ± 198
Total hippocampus (ml)	7.75 ± 0.52	8.22 ± 0.84
Total amygdala (ml)	2.86 ± 0.26	3.14 ± 0.64
TRP (μM)	50.9 ± 8.7	52.5 ± 8.8
CRP (ng/ml)	1012 ± 1428	668 ± 484
IL1-RA (pg/ml)	788 ± 717	569 ± 401
BDNF (pg/ml)	317 ± 1013	463 ± 998
KYN (nM)	1.99 ± 0.54	1.98 ± 0.52
KA (nM)	40.5 ± 8.7	46.4 ± 12.9
3HK (nM)	41.5 ± 13.4	37.8 ± 12.8
QA (nM)	436 ± 214	382 ± 130
Component 1	0.22 ± 1.17	− 0.26 ± 0.75
Component 2	− 0.19 ± 0.84	0.17 ± 1.18
Component 3	− 0.11 ± 1.12	0.09 ± 0.92

Abbreviations: BMI, body mass index; HAM-D, Hamilton Depression Rating Scale; HC, healthy control; KYN, kynurenine; 3HK, 3-hydroxykynurenine; MDD, major depressive disorder; KA, kynurenic acid; QA, quinolinic acid; SHAPS, Snaith–Hamilton Pleasure Scale; TRP, tryptophan.
Each of the principal components has a mean of 0 and SD of 1. SHAPS data were not available for four subjects.

family (first-degree relatives) history of psychiatric illness assessed using the Structured Clinical Interview for the DSM-IV-TR and the Family Interview for Genetic Studies (FIGS) (Maxwell, 1992).

MRI

The scans were acquired on a 3 Tesla GE Discovery MR750 MRI scanner (GE Medical Systems) with a receive-only 32 elements surface coil brain array (Nova Medical) using a magnetization-prepared, rapid gradient echo (MP-RAGE) pulse sequence with sensitivity encoding (SENSE) optimized for tissue contrast resolution: (TR = 5 ms, TE = 2.01 ms, FOV 240 × 192 mm; voxel size = 0.94 × 0.94 × 0.90 mm; prep = 725 ms, delay = 1400 ms, flip = 8° SENSE acceleration R = 2). The automated segmentation program, FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), was used to obtain unbiased GM volumes of the hippocampus, amygdala, and whole brain. The T1-weighted anatomical images were processed using the default analysis settings of FreeSurfer. Visual inspection of the FreeSurfer-based segmentations was performed to confirm accurate segmentation of the hippocampus and amygdala. The accuracy of FreeSurfer-derived volumetric measures estimates have been validated by histological analysis (Rosas *et al*, 2002) and compare favorably with manual measurements (Kuperberg *et al*, 2003). Representative examples of the hippocampal and amygdala masks from a single subject are shown in Supplementary Figures S1 and S2.

Kynurenine Pathway Metabolites

A blood sample was obtained from each subject within 3 days of completing the MRI scan. Subjects fasted overnight and the blood sample was drawn between 0800 and 1100 h. Serum samples were collected with BD Vacutainer serum tubes, processed according to the standard BD Vacutainer protocol, and stored at − 80 °C.

Concentrations of TRP, KYN, KA, 3HK, and QA were measured blind to diagnosis by Brains Online, LLC (www.brainsonline.org/home). The metabolite concentrations were determined by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection using their standard protocols. The lower limit of quantification and the coefficients of variation for the analytes are provided in Supplementary Table S4.

ELISAs

Commercially available colorimetric sandwich ELISA kits were used to quantify plasma levels of BDNF (EMD Millipore, Billerica, MA), IL-6, CRP, and IL-1RA (eBioscience, San Diego, CA) blind to diagnosis. Plasma samples were stored at − 80 °C until use and thawed on ice the day of the assays. To remove any precipitate, samples were centrifuged for 15 min at 3000 r.p.m. Each sample was run in duplicate according to the manufacturer's instructions using the provided reagents. Two control plasma samples were run on each plate to determine interassay variation and were used to normalize the data across plates. The respective mean interassay coefficient of variation and lower detection limit for each kit are as follows: BDNF 6%, 7.8 pg/ml; IL-6 7%, 0.03 pg/ml; CRP 14%, 3 pg/ml; and IL-1RA 2%, 30 pg/ml

Statistical Analysis

We took two approaches to statistical analysis, an *a priori* approach and an agnostic approach. Regarding the *a priori* analysis, analysis of variance was used to test for differences in KA/3HK and KA/QA between the HC and MDD groups after controlling for age, sex, and body mass index (BMI). Second, linear regression was used to test for associations between KA/3HK and KA/QA and hippocampal and amygdala volume within the MDD group, controlling for intracranial volume. Analysis of variance was used to test for differences between the MDD and HC groups in hippocampal and amygdala volumes using sex, age, BMI, and intracranial volumes as covariates.

Regarding the agnostic approach, we conducted the PCA on the kynurenine pathway metabolites and other immune markers, followed by linear regression analyses using the extracted principal components as independent variables.

The PCA was performed using Statistical Package for the Social Sciences (SPSS) (V17) (oblimin rotation). The Kaiser–Meyer–Olkin measure was used to verify sampling adequacy for the analysis (KMO = 0.54). Bartlett's test of

sphericity $\chi^2 = 63$, 1, $p < 0.001$, indicated that the correlations between kynurenine metabolites and cytokines were sufficiently large for PCA. Three components had eigenvalues over Kaiser's criterion of 1, and in combination explained 68% of the variance. Table 2 shows the factor loadings after rotation. The items that cluster on the same components suggest that component 1 (IL-1RA, QA, and KYN) represents inflammation-related activation of the QA branch of the kynurenine pathway. Component 2 (KA, TRP, and KYN) appears to represent the activity of the KA branch of the kynurenine pathway, whereas component 3 (3HK, BDNF, and TRP) is more difficult to interpret but conceivably suggests an interaction between BDNF and 3HK.

Analysis of variance with sex, age, and BMI as covariates was used to test for mean differences between the MDD and HC groups in the three principal components derived from the PCA.

In order to test for associations between the principal components and GM volumes of the hippocampus and amygdala, we conducted linear regression analyses with components 1, 2, and 3 as independent variables, intracranial volume as a covariate, and hippocampal volume or amygdala volume as the dependent variables.

RESULTS

After excluding subjects who failed quality control checks of the metabolite analyses or the FreeSurfer segmentations or who had missing data points, 29 MDD subjects and 20 HCs had both valid MRI and metabolite data available for analysis. Correlations between the kynurenine metabolite measurements and the cytokine measurements are provided in Supplementary Table S3.

The MDD group had significantly smaller total hippocampal volumes and total amygdalar volumes than the HCs, although these differences were no longer statistically significant after regressing out sex, age, BMI, and intracranial volumes.

Regarding the *a priori* analyses, the ratios of KA/3HK and KA/QA, putative neuroprotective indices (Johansson *et al*, 2013; Kocki *et al*, 2012), were lower in the MDD group relative to the HCs (KA/3HK: $t = 2.42$, $p = 0.019$; KA/QA: $t = 2.04$, $p = 0.047$). However, after regressing out sex, age, and BMI, these differences no longer remained statistically significant (Figure 2).

Table 2 Results of the Principal Component Analysis (Pattern Matrix) in the MDD and HC Groups

	Component 1	Component 2	Component 3
IL-1RA	0.892		
QA	0.839		
KYN	0.496	0.416	
KA		0.901	
TRP		0.696	0.403
3HK			−0.745
BDNF			0.668

Component 1 explained 32% of the variance, component 2 explained 19% of the variance, and component 3 explained 17% of the variance. The cumulative percentage of variance explained was 68%.
For abbreviations, see Table 1.

Within the MDD group the KA/QA ratio was positively correlated with larger hippocampal (β-weight = 0.42, $t = 2.5$, $p = 0.022$) and amygdala volumes (β-weight = 0.37, t = 2.2, $p = 0.041$; Figure 3) but the KA/3HK ratio did not predict either hippocampal or amygdala volumes ($p > 0.1$). Neither KA/QA nor KA/3HK was significantly associated with GM volumes in the HC group. Regarding the clinical ratings, KA/QA was significantly correlated (negatively) with the SHAPS score ($r = -0.41$, $p = 0.039$) but not the HAM-D score ($r = -0.25$, $p = 0.183$). Higher scores on the SHAPS are indicative of greater anhedonia. KA/3HK was not significantly correlated with the HAM-D or the SHAPS.

After controlling for sex, age, and BMI, the MDD group showed higher levels of component 1 relative to the HCs ($F = 4.5$, $p = 0.039$; Figure 2). There was no significant group difference in components 2 or 3.

In the combined MDD and HC sample, component 2 was significantly associated with total hippocampal volume (β-weight = 0.30, $t = 2.3$, $p = 0.028$) and trended significant for total amygdala volume (β-weight = 0.25, $t = 1.8$, $p = 0.073$) after controlling for intracranial volume. The association between component 2 and GM volume was present in the MDD group alone (Figure 3), hippocampus (β-weight = 0.43, $t = 2.4$, $p = 0.024$) and amygdala (β-weight = 0.38, $t = 2.2$, $p = 0.038$), but was not significant in the HC group alone. There were no significant associations between components 1 and 3 and GM volume in either the MDD or HC groups. There were also no significant associations between the principal components and the clinical rating scales.

DISCUSSION

The main finding of this study is that within the MDD group, both the KA/QA ratio and principal component 2, which largely reflects KA, were associated with larger hippocampal and amygdalar volumes in clinically depressed, unmedicated patients.

The positive association between KA/QA and principal component 2 with hippocampal and amygdala volumes is arguably consistent with a heuristic model that construes dendritic atrophy and/or decreased hippocampal neurogenesis as a consequence of an inflammation-driven decrease in the ratio of KA to QA metabolism. Whereas QA is a neurotoxin that in elevated concentrations can act as an agonist of the NMDA receptor, and 3HK is a potent free radical donor that promotes oxidative stress, and KA acts as an antagonist of both the glycine co-agonist site of the NMDA receptor and the $\alpha 7$ nicotinic receptor situated on glutamatergic neuronal terminals in the hippocampus and other brain regions, reducing glutamate release (Hilmas *et al*, 2001; Kessler *et al*, 1989; Perkins and Stone, 1982; Potter *et al*, 2010; Schwarcz *et al*, 1983).

Preclinical, *in vitro*, and post-mortem data demonstrate that elevated concentrations of 'neurotoxic' kynurenine metabolites are associated with neuronal damage and/or suppression of neurogenesis—particularly in the hippocampus. For instance, a rat model of pneumococcal meningitis showed that acute infection resulted in the

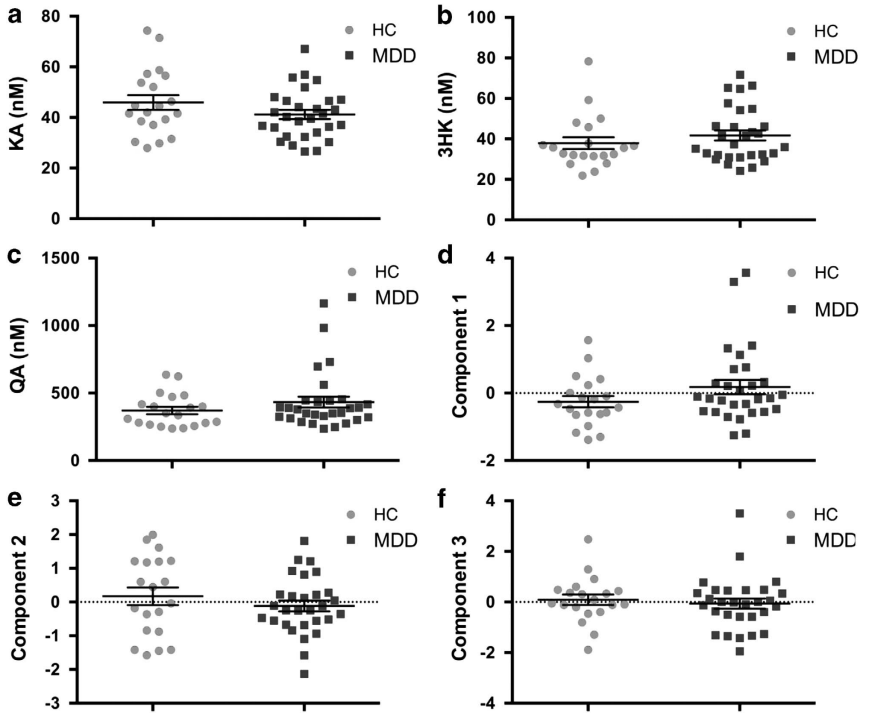

Figure 2 Scatterplots showing the distributions of kynurenine pathway metabolites and the principal components in MDD subjects and HCs. (a) Kynurenic acid. (b) 3-hydroxykynurenine. (c) Quinolinic acid. (d) Principal component 1 (IL-1RA, QA, and KYN). After controlling for sex, age, and BMI, the MDD group showed higher levels of component 1 relative to the healthy controls ($F = 4.5$, $p = 0.039$). (e) Principal component 2 (KA, TRP, KYN). (f) Principal component 3 (3HK, BDNF, and TRP). The MDD subjects are represented by blue squares and the HC subjects by pink circles. The black lines show the mean concentration and the error bars show the SEM. A full text version of this figure is available at *Neuropsychopharmacology* Journal online.

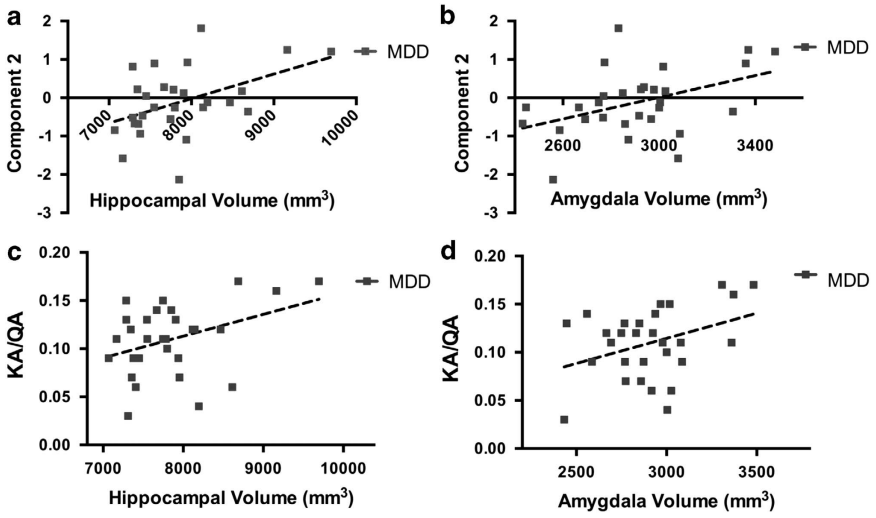

Figure 3 (a) Scatterplot showing the correlation between total hippocampal GM volume (X axis) and principal component 2 in subjects with MDD (β-weight = 0.43, $t = 2.4$, $p = 0.024$). Hippocampal volumes are provided in cubic millimeters. The linear trendline is displayed in black. (b) Scatterplot showing the correlation between total amygdala GM volume (X axis) and principal component 2 in subjects with MDD (β-weight = 0.38, $t = 2.2$, $p = 0.038$). Amygdala volumes are provided in cubic millimeters. (c) Scatterplot showing the correlation between total hippocampal GM volume (X axis) and the serum concentration of the ratio of kynurenic acid (KA) to quinolinic acid (QA) (Y axis) in subjects with MDD (β-weight = 0.42, $t = 2.5$, $p = 0.022$). (d) Scatterplot showing the relationship between total amygdalar GM volume (X axis) and the ratio of serum KA to QA (Y axis) in subjects with MDD (β-weight = 0.37, $t = 2.2$, $p = 0.041$).

accumulation of 3HK in the hippocampus and that the concentration of 3HK was positively correlated ($r = 0.5$) with the extent of apoptosis in this region (Bellac *et al*, 2006). Another study demonstrated that neurons exposed to extracellular glutamate show a reduction in dendritic growth that is prevented by administration of KA

(Monnerie *et al*, 2003). IL-1 was shown not only to decrease hippocampal neurogenesis in human hippocampal progenitor cells but also to upregulate the expression of the enzymes, *IDO*, *KMO*, and *kynureninase* and, crucially, treatment with a KMO inhibitor reversed the effects of IL-1 on hippocampal neurogenesis (Zunszain *et al*, 2012). The reduction in GM volume and the decrease in neuronal density that has been found in some depressed patients post-mortem may be related to higher brain concentrations of microglia-derived QA, as was recently reported in subjects with MDD (Steiner *et al*, 2008, 2011).

Conversely, psychiatric medication may increase the synthesis of the potentially neuroprotective compound, KA, and/or decrease the synthesis of 3HK. (Kocki *et al* (2012) reported that 24–48 h of exposure to selective serotonin reuptake inhibitors or tricyclic antidepressant medications stimulated the *de novo* synthesis of KA and decreased 3HK production in astroglial cultures, thus resulting in an increase in the KA to 3HK ratio. Patients with schizophrenia had lower plasma concentrations of KA and higher plasma concentrations of 3HK than HCs, an effect that was ameliorated by 6 weeks of treatment with antipsychotic treatment such that a significant increase in the KA/3HK ratio was observed after treatment (Myint *et al*, 2011). Interestingly, atypical antipsychotic medications have been reported to attenuate the typical pattern of hippocampal volume loss observed over time in schizophrenic patients (Koolschijn *et al*, 2010). In addition, administration of valproic acid, which we previously demonstrated to be associated with larger amygdala volumes in patients with bipolar disorder (BD) (Savitz *et al*, 2010), reportedly increased the levels of KA in the rat brain (Maciejak *et al*, 2013). In mice, peripheral administration of low-dose ketamine after injection with lipopolysaccharide (LPS) abrogated the LPS-induced depressive behavior, an effect that was due to ketamine's antagonistic effect at the NMDA receptor rather than suppression of cytokine release *per se* (Walker *et al*, 2013). Interestingly, burn victims with post-traumatic stress disorder (PTSD) who were treated with ketamine were found to have larger hippocampal volumes than nonketamine-treated patients (Winter and Irle, 2004).

Nevertheless, the above model that characterizes KA as 'neuroprotective' and 3HK and QA as 'neurotoxic' is heuristic and should be interpreted with caution as there are contradictory reports in the literature. For instance, the QA and the KA enzymatic pathways were reported to be equally activated in patients receiving IFN-α treatment, with QA and KA contributing approximately equally to symptom changes (Raison *et al*, 2010). Consistent with these data, a recent study reported an upregulation of KA in the saliva samples of HCs and schizophrenic subjects after a psychological stress challenge (Chiappelli *et al*, 2014). Conceivably, the relative effects of KA *versus* 3HK and QA are dependent on the type of the disorder under study (eg, schizophrenia *versus* primary MDD), the nature of the inflammatory stimulus, and as yet uncharacterized molecular actors. Reports indicative of elevations of KA in schizophrenia or BD with psychosis (Olsson *et al*, 2012; Wonodi *et al*, 2011) may be particularly relevant as elevations in KA concentrations may potentiate dopaminergic neurotransmission potentially explaining the putative link with psychosis (Olsson *et al*, 2009).

Second, relative to HCs, unmedicated, depressed subjects with primary MDD had higher levels of principal component 1 that is largely reflective of IL-1RA and QA. Although elevations in pro-inflammatory cytokines and kynurenine metabolites such as QA have been widely reported in patients with depression secondary to a general medical condition (Capuron *et al*, 2003; Raison *et al*, 2010), the kynurenine pathway has been understudied in primary mood disorders. The increase in principal component 1 is partially consistent with prior reports in the MDD (Gabbay *et al*, 2010; Myint *et al*, 2007) and BD (Johansson *et al*, 2013) literature that are indicative of an increased production of 'neurotoxic' *versus* 'neuroprotective' metabolites in depressed patients.

A third finding of interest was the inverse association between KA/QA and the degree of anhedonia in subjects with MDD. The link between inflammation-induced sickness behavior and anhedonia is well characterized (Dantzer *et al*, 2008; Maes *et al*, 2012), and a previous study hypothesized that it is primarily MDD patients with melancholic features who show alterations in immune function (Gabbay *et al*, 2010). As CRP and pro-inflammatory cytokines were not significantly correlated with the SHAPS score, our finding additionally raises the possibility that the relative metabolism of KA to QA might influence the development of anhedonic symptoms over and above the severity of inflammation *per se*.

Another finding of interest was the composition of principal component 3 (inverse relationship between BDNF and 3HK) given that inflammation-induced synthesis of 3HK decreases human hippocampal cell neurogenesis *in vitro* (Zunszain *et al*, 2012) and that BDNF is a key regulator of hippocampal neurogenesis and neuroplasticity (Gray *et al*, 2013; Vithlani *et al*, 2013). Future studies of the potential interactions between BDNF and 3HK may provide greater insight.

The neuroanatomical specificity of our findings warrants follow-up. Regression analyses showed no association between KA/QA or component 2 and either whole-brain GM volume or intracranial volume (data not shown), thus raising the possibility that any putative effect of cytokines or kynurenine metabolites on brain structure could be limited to brain regions that are known to display reductions in GM volume in MDD. Future functional imaging may conceivably clarify any differential impact of inflammation on hippocampal-centric and amygdalar-centric circuits.

The main limitation of our study is that we examined kynurenine metabolites in serum rather than in brain, and thus it is theoretically possible that our data are not reflective of central kynurenine metabolite concentrations. KYN and 3HK are, however, able to cross the blood–brain barrier into the brain parenchyma *via* the neutral amino acid transporter (Fukui *et al*, 1991), where KYN and 3HK may be metabolized into QA by microglial cells and KYN metabolized into KA by astrocytes (Schwarcz *et al*, 2012). This finding raises the possibility that peripheral measures of kynurenine pathway metabolites may be at least partially reflective of metabolite concentrations within the brain parenchyma. In support of this hypothesis, Raison *et al* (2010) reported that INFα-treated hepatitis C patients showed both plasma and CSF increases in KYN and QA

that correlated with depressive symptoms. Furthermore, peripheral administration of KYN has been shown to induce behavioral analogs of depression in rodents (O'Connor *et al*, 2009), and Laugeray *et al* (2011) reported that mice subjected to chronic mild stress showed a peripheral increase in kynurenine pathway activity that was inversely correlated with KA concentration in the amygdala.

Second, this was a cross-sectional study that was designed to investigate the relationship between kynurenine pathway metabolites and pro-inflammatory cytokines and GM volume of two regions widely implicated in MDD: the hippocampus and amygdala. The data reported here are correlative in nature and, unlike some of the animal studies cited above, do not provide information concerning cause and effect relationships.

Third, because we were only able to measure circulating levels of kynurenine pathway metabolites, it remains unclear whether the hippocampal and amygdalar concentrations of KA, 3HK, and QA in the brains of the volunteers were sufficient to affect NMDA or $\alpha 7$ nicotinic receptor function.

In sum, our results raise the possibility that dysregulation of the innate immune system contributes to the neuromorphometric abnormalities observed in some patients with MDD. The potential existence of a positive association between the peripheral KA/QA ratio and principal component 2 and hippocampal and amygdalar GM volume is of both theoretical and clinical interest. Firstly, it raises the possibility that at least with respect to the kynurenine pathway, peripheral concentrations of metabolites may be reflective of depression-related changes in brain structure. Second, it has been hypothesized by other researchers that immune dysregulation predisposes to mood disorders via its effect on glutamatergic neurotransmission. In other words, abnormal NMDA receptor signaling may be the unifying mechanism underlying the glutamate and inflammation hypotheses of depression (Miller, 2013; Steiner *et al*, 2012; Walker *et al*, 2013). Although we cannot demonstrate that KA and QA are at high enough physiological concentrations in the hippocampus and amygdala to affect glutamate release, the correlations between the neuroprotective ratios and GM volumes reported here suggest that the potential effects of KA and QA on glutamatergic neurotransmission are worthy of future study. Third, our results support the hypothesis that pharmacological agents such as *KMO* inhibitors (the *KMO* enzyme metabolizes KYN into 3HK) that shunt the catabolism of KYN away from 3HK and toward KA may exert neuroprotective and/or antidepressant effects and therefore may constitute novel targets for drug development (Haroon *et al*, 2012; Stone and Darlington, 2002).

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