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Relationship between neurotoxic kynurenine metabolites and reductions in right medial prefrontal cortical thickness in major depressive disorder

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Abstract

Reductions in gray matter volume of the medial prefrontal cortex (mPFC), especially the rostral and subgenual anterior cingulate cortex (rACC, sgACC) are a widely reported finding in major depressive disorder (MDD). Inflammatory mediators, which are elevated in a subgroup of patients with MDD, activate the kynurenine metabolic pathway and increase production of neuroactive metabolites such as kynurenic acid (KynA), 3-hydroxykynurenine (3HK) and quinolinic acid

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(QA) which influence neuroplasticity. It is not known whether the alterations in brain structure and function observed in major depressive disorders are due to the direct effect of inflammatory mediators or the effects of neurotoxic kynurenine metabolites. Here, using partial posterior predictive distribution mediation analysis, we tested whether the serum concentrations of kynurenine pathway metabolites mediated reductions in cortical thickness in mPFC regions in MDD. Further, we tested whether any association between C-reactive protein (CRP) and cortical thickness would be mediated by kynurenine pathway metabolites. Seventy-three unmedicated subjects who met DSM-IV-TR criteria for MDD and 91 healthy controls (HC) completed MRI scanning using a pulse sequence optimized for tissue contrast resolution. Automated cortical parcellation was performed using the PALS-B12 Brodmann area atlas as implemented in FreeSurfer in order to compare the cortical thickness and cortical area of six PFC regions: Brodmann areas (BA) 9, 10, 11, 24, 25, and 32. Serum concentrations of kynurenine pathway metabolites were determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection, while high-sensitivity CRP concentration was measured immunoturbidimetrically. Compared with HCs, the MDD group showed a reduction in cortical thickness of the right BA24 ($p < 0.01$) and BA32 ($p < 0.05$) regions and MDD patients with a greater number of depressive episodes displayed thinner cortex in BA32 ($p < 0.05$). Consistent with our previous findings in an overlapping sample, the KynA/3HK ratio and the log KynA/QA were reduced in the MDD group relative to the HC group (p 's < 0.05) and symptoms of anhedonia were negatively correlated with log KynA/QA in the MDD group ($p < 0.05$). Both KynA/3HK and log KynA/QA at least partially mediated the relationship between diagnosis and cortical thickness of right BA32 (p 's < 0.05). CRP was inversely associated with BA32 thickness ($p < 0.01$) and KynA/3HK partially mediated the relationship between CRP and the thickness of right BA32 ($p < 0.05$). The results raise the possibility that the relative imbalance between KynA and neurotoxic kynurenine metabolites may partially explain the reductions in mPFC thickness observed in MDD, and further that these changes are more strongly linked to the putative effects of neuroactive kynurenine metabolites than those of inflammatory mediators.

Keywords

Major depressive disorder; magnetic resonance imaging; medial prefrontal cortex; quinolinic acid; inflammation; kynurenine

INTRODUCTION

Major depressive disorder (MDD) consistently has been associated with a reduction in gray matter (GM) volume in several brain regions. In the case of the cortex, the evidence for the loss of GM is most persuasive in the case of the medial prefrontal cortex (mPFC), especially the rostral and/or subgenual anterior cingulate cortex (rACC, sgACC) – for systematic reviews and meta-analyses see (Arnone et al., 2012; Kempton et al., 2011; Koolschijn et al., 2009; Price and Drevets, 2010; Savitz and Drevets, 2009). Similarly, in a recent meta-analysis of voxel-based morphometry (VBM) data, the areas of GM volume reduction in MDD patients were reported to be confined to the rACC (BA24 and BA32), bilaterally, and the dorsomedial frontal cortex, bilaterally (BA 9, BA 8, BA 32) (Bora et al., 2012).

Unlike manual tracing or VBM, the automated neuroimaging analysis software, FreeSurfer, allows for the measurement of two separate components of cortical volume, cortical thickness and surface area. Comparatively fewer cortical thickness studies have been performed in MDD but nonetheless, reductions in thickness of the prefrontal cortex, including the rACC, the orbitofrontal cortex (OFC), middle frontal gyrus, superior frontal gyrus, and dorsolateral prefrontal cortex (DLPFC) comprising Brodmann areas 9, 10, 11, 24, 25, 32, and 47 appear to be prominent (Lim et al., 2012; Tu et al., 2012; Wagner et al., 2012). Consistent with the neuroimaging data, *postmortem* studies have found reduced neuronal and glial cell densities, neuronal size, and/or cortical thickness in the dorsolateral prefrontal cortex (DLPFC), orbitofrontal cortex (OFC), rACC, and sgACC of patients with mood disorders (Cotter et al., 2002; Ongur et al., 1998; Rajkowska et al., 2005).

The origins of these changes appear complex and heterogeneous, and are likely to involve both neurodevelopmental and neurodegenerative components (Savitz and Drevets, 2009; Savitz et al., 2014b). One factor that may theoretically contribute to neuropathophysiological abnormalities in a subset of depressed patients is inflammation. However, despite evidence for elevations in peripheral biomarkers of inflammation such as C-reactive protein (CRP), interleukin 6 (IL-6), and interleukin 1 beta (IL-1 β) in mood and psychotic disorders (Dowlati et al., 2010; Howren et al., 2009; Potvin et al., 2008), few studies have examined the association between inflammation and brain structure in the context of psychiatric disorders. We previously reported an inverse association between the mRNA expression of CD160, a gene which plays a key role in natural killer cell-mediated IFN- γ production (Tu et al., 2015), and thickness of the left sgACC in 29 patients with mood disorders (Savitz et al., 2013). In a recent longitudinal study of subjects at high risk of psychosis, a composite measure of inflammation at baseline (the serum concentrations of several pro-inflammatory cytokines) was associated with faster thinning of the right prefrontal cortex (right superior frontal, middle frontal, and medial OFC) over the 12 month follow-up period, especially among individuals who subsequently developed a psychotic disorder (Cannon et al., 2015).

Nevertheless, most research to date has examined the relationship between cortical thickness and markers of inflammation in healthy individuals within the context of aging. In healthy middle-aged adults, elevations of CRP and IL-6 were inversely associated with total cortical surface area but not cortical thickness (Marsland et al., 2015). In another study, adults between the ages of 40 and 60 years showed an inverse association between serum levels of interleukin 2 (IL-2) and thickness of the inferior frontal gyrus (Kaur et al., 2014). Similarly, in middle-aged, neurologically healthy males, CRP and intercellular adhesion molecule (ICAM-1) were associated with cortical thinning of the dorsolateral prefrontal cortex (Krishnadas et al., 2013) while transforming growth factor β (TGF- β), a cytokine with anti-inflammatory properties, was positively correlated with thickness of the rACC in young adults (Piras et al., 2012).

These changes in structural volume are potentially consistent with several papers that have demonstrated inflammation-induced alterations in the BOLD signal or glucose metabolism in the medial PFC (Capuron et al., 2005; Eisenberger et al., 2009; Hannestad et al., 2012; Haroon et al., 2014; Harrison et al., 2009). In addition, using magnetic resonance

spectroscopy, (Haroon et al., 2015) showed that IFN- α treatment was associated with a significant increase in the glutamate to creatine ratio (Glu/Cr) in the dorsal ACC and left basal ganglia in older individuals.

Cytokines and other molecules released during inflammation such as nitric oxide and free radicals may exert direct cytotoxic effects and cause neuronal damage (Block and Hong, 2005). For instance, in patients with multiple sclerosis, IL-1 β concentrations in the cerebrospinal fluid (CSF) correlated inversely with both cortical lesion load and global cortical thickness (Seppi et al., 2014). However, inflammatory mediators may also affect brain structure and/or neuronal function indirectly by activating the tryptophan degrading enzyme, indoleamine 2,3 dioxygenase (*IDO*), increasing the formation of kynurenine, which in turn is metabolized into neuroactive kynurenine-pathway metabolites, including kynurenic acid (KynA), 3-hydroxykynurenine (3HK), and quinolinic acid (QA) (Dantzer et al., 2011) (Figure S1). While activation of *IDO* may also reduce serotonin concentrations, the behavioral and neural effects of *IDO* are believed to be primarily dependent on the formation of neuroactive kynurenine metabolites rather than the reduction in serotonin (Dantzer et al., 2011; O'Connor et al., 2009).

Kynurenine is metabolized along two main branches to form either KynA, a putative NMDA receptor antagonist, or alternatively, 3HK, a free-radical generator, 3-hydroxyanthrallic acid (3-HAA), and QA, a putative NMDA receptor agonist. Partly as a result of the competing effects of KynA and QA at the NMDA receptor, both the preclinical literature and human studies of known inflammatory and/or neurodegenerative disorders have led to the hypothesis that microglial-derived 3HK and QA are neurotoxic while astrocyte-derived KynA is neuroprotective (Amaral et al., 2013; Myint and Kim, 2003; Stone et al., 2012). We and others have previously hypothesized that the effects of activation of the kynurenine metabolic pathway on limbic structures are mediated by glutamate-induced neuroplasticity and/or excitotoxicity – in part by virtue of the competing actions of KynA and QA on the NMDA receptor (Miller, 2013; Savitz et al., 2014a; Savitz et al., 2015a; Steiner et al., 2012; Walker et al., 2013). While this model may be overly simplistic, our previous results showing reductions in the ratio of KynA to 3HK and/or KynA to QA in depressed patients with MDD and/or bipolar disorder (BD) along with positive correlations between these indices and hippocampal or amygdalar volume in the MDD and BD groups (Savitz et al., 2014a; Savitz et al., 2015a), are consistent with this model.

Here we test whether these results can be extended to another neuroanatomical region that has been widely implicated in mood disorders and has been shown to undergo stress-related dendritic remodeling in preclinical studies, the medial PFC (mPFC).

METHODS

Participants

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

All MDD (n=73) and healthy control (HC, n=91) participants were interviewed with the Structured Clinical Interview for the DSM-IV-TR. In addition, unstructured diagnostic interviews with a psychiatrist were obtained on all MDD participants. We previously published results from 51 of the 73 MDD subjects and 58 of the 91 HCs making up the current sample. These previous publications reported associations between kynurenine metabolites and GM volumes of the hippocampus and amygdala in depressed subjects with MDD (Savitz et al., 2015a) and kynurenine pathway abnormalities in remitted subjects with MDD (Savitz et al., 2015b).

The majority of the MDD subjects had Montgomery-Asberg Depression Rating Scale scores (MADRS) in the moderately-to-severely depressed range (table 1). Anhedonia was assessed with the Snaith-Hamilton Pleasure Scale (SHAPS; higher scores being indicative of greater anhedonia). The unmedicated MDD subjects had not received any psychotropic medication for at least 4 weeks (8 for fluoxetine) prior to the blood-draw. Exclusion criteria were as follows: contra-indications to MRI scanning; serious suicidal ideation or behavior; medical conditions or concomitant medications likely to influence CNS or immunological function including cardiovascular, respiratory, endocrine, neurological, and known autoimmune diseases, and a history of drug or alcohol abuse within 6 months or a history of drug or alcohol dependence within 1 year.

The HCs met the same exclusion criteria except that they had no personal history of psychiatric illness and no family history of a mood disorder (first-degree relatives) assessed using the Structured Clinical Interview for the DSM-IV-TR and the Family Interview for Genetic Studies.

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=5msec, TE=2.01 msec, FOV 240×192 mm; voxel size=0.94 × 0.94 × 0.90 mm; prep=725 msec, delay=1400 msec, flip=8° SENSE acceleration R=2). Cortical reconstruction and volume segmentation was performed using the FreeSurfer image analysis suite version 5.3. Technical details of these procedures are described elsewhere (Fischl et al., 2002; Fischl et al., 1999). Automated cortical parcellation was performed using the PALS-B12 Brodmann area Atlas as implemented in FreeSurfer (Fischl et al., 2004; Van Essen, 2005). The cortical surface area of each Brodmann area was calculated as the sum of the areas of all vertices within that region and cortical thickness was measured as the average distance between the gray-white matter boundary and the pial surface within each Brodmann Area (Fischl and Dale, 2000). The result from each step was inspected for every subject as part of a quality assurance protocol, and corrections were made when necessary.

CRP and Kynurenine Pathway Metabolites

A blood sample was obtained from each subject within three days of completing the MRI scan. Subjects fasted overnight and the blood sample was drawn by venipuncture between

8am and 11am. Serum samples were collected with BD Vacutainer serum tubes, processed according to the standard BD Vacutainer protocol, and stored at -80°C .

Concentrations of tryptophan (TRP), kynurenine, kynurenic acid (KynA), 3-hydroxykynurenine (3HK), and quinolinic acid (QA) were measured blind to diagnosis by Brains Online, LLC (www.brainsonline.org/home). The serum metabolite concentrations were determined by high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection using their standard protocols. The lowest level of quantification and intra-assay percentage of coefficient of variation for each of the kynurenine pathway metabolites was as follows: TRP: 10 μM , 5.7%; kynurenine: 0.75 μM , 5.8%; KynA: 12.5 nM, 5.4%; 3HK: 10 nM, 4.5%, and QA: 50 nM and 3.7%. High-sensitivity C-reactive protein (hs-CRP) was measured in a clinical laboratory using the immunoturbidimetric assay. The analytical measurement range for the assay is 0.2 mg/L to 480.0 mg/L.

Statistical Analysis

Analyses were performed with Statistical Package for the Social Sciences (V.17). Deviations from normality were tested using the Shapiro-Wilk test and non-normally distributed variables were log normalized. All statistical t-tests were 2-tailed. Group differences in demographic data were assessed using independent sample t-tests for continuous data and a chi-squared test for sex.

Despite the *a priori* evidence implicating volumetric changes of the mPFC in depression, given that we measured both the cortical area and the cortical thickness of 6 different Brodmann areas in both the left and the right hemispheres (i.e. 24 measurements in total) we ran four separate MANOVAs in order to reduce type I error: thickness of the left hemisphere, thickness of the right hemisphere, area of the left hemisphere and area of the right hemisphere. Univariate analyses of the individual Brodmann areas were only performed when the multivariate statistic for the relevant ANOVA was significant. We used two different statistical models: (a) with no covariates (i.e. MANOVA) and (b) with the addition of potentially confounding variables that correlated significantly with the dependent variable/s (i.e. MANCOVA). Where the thickness or area of the regions of interest (ROIs) differed between the groups, exploratory Pearson's correlation coefficients and independent sample t-tests were used to evaluate the effect of depression severity and illness chronicity on cortical thickness/area.

Differences in the kynurenine metabolites and CRP concentrations between the groups were assessed using MANOVA or MANCOVA in order to evaluate the continuity of these data with our previous results. Since we had previously shown KynA/3HK and/or KynA/QA to differ between healthy and depressed subjects in an overlapping sample, we considered a 2-tailed, uncorrected p-value of 0.05 to be statistically significant for these comparisons. For the remaining kynurenine metabolites and CRP, we used the Benjamini-Hochberg FDR correction for multiple comparisons. Because the kynurenine metabolites were analyzed in 3 different batches and since significant correlations were found between analysis batch and concentrations of several of the kynurenine metabolites, we also regressed out the effect of batch in the MANCOVA analysis.

The association between the concentration of CRP and the concentrations of the kynurenine metabolites was tested using Pearson's correlation with the Benjamini-Hochberg FDR correction for multiple comparisons. Similarly, the associations between CRP, kynurenine metabolites, and anhedonic and depressive symptoms were tested using Pearson's correlation with the Benjamini-Hochberg FDR correction for multiple comparisons. However, since we had previously reported a significant correlation between KynA/QA and the severity of anhedonic symptoms, this specific statistic was not adjusted for multiple comparisons.

In order to test whether any cortical differences between groups were mediated by differences in concentrations of kynurenine pathway metabolites, we performed mediation analyses using the partial posterior predictive distribution method (which has greater power than Sobel's statistic and compares favorably with boot-strapping methods (Biesanz et al., 2010)). Additional mediation analyses were performed in order to evaluate whether the relationship between CRP and cortical thickness was mediated by KynA/3HK and/or KynA/QA.

RESULTS

Cortical Thickness and Cortical Area Differences between Groups

Multivariate analyses with no covariates showed no overall group differences in left hemisphere area (Wilks's Lambda: 0.94, $F_{1,157}=1.61$, $p=0.148$), right hemisphere area (Wilks's Lambda: 0.94, $F_{6,157}=1.67$, $p=0.131$), and left hemisphere thickness (Wilks's Lambda: 0.99, $F_{6,157}=0.22$, $p=0.887$). Although a number of individual regions of interest showed significant univariate test statistics (table S1), we did not follow-up on these individual regions in order to minimize Type I error. There was however, a significant difference between cortical thickness of the right hemisphere between groups (Wilks's Lambda: 0.91, $F_{6,157}=2.57$, $p=0.021$). Univariate tests showed that MDD subjects had thinner cortices compared with HCs in BA24 ($F_{1,162}=7.8$, $p=0.006$) and BA32 ($F_{1,162}=3.9$, $p=0.024$, table 1, figure 1). There were no significant correlations between the severity of depressive symptoms (MADRS score) and thickness of right BA32 or BA24 using Pearson's test although there was a non-significant trend for an association between depression severity and right BA32 thickness with Spearman's test ($n=68$, $r_s=-0.24$, $p=0.051$). In an exploratory analysis we tested whether the number of previous depressive episodes was associated with cortical thickness of right BA24 and right BA32 in the MDD group. Given the skewness of the data and potential inaccuracies in the recall of episodes, we performed a median split of the MDD group based on number of prior depressive episodes. Note that accurate remote historical data were not available for 29 individuals and thus the analysis was performed on 44 MDD participants. The "low" and "high" episode groups did not differ significantly from each other in sex, age, BMI, MADRS scores, and SHAPS scores. Compared with the "low" episode MDD group ($n=21$, mean episodes = 2.1 ± 0.89), the "high" episode group ($n=23$, mean episodes = 12.2 ± 2.8) had thinner cortex in BA24 (2.65mm versus 2.72mm) and BA32 (2.49mm versus 2.61mm) although only the difference in thickness of BA32 was significant ($t_{42}=2.6$, $p=0.014$).

Since sex and age (but not BMI or batch) were significantly correlated with cortical area, bilaterally, and cortical thickness of several ROIs in the left mPFC, we performed an additional MANCOVA analysis with sex and age as covariates. In the case of right hemisphere thickness, age, sex, and BMI were significantly correlated with several ROIs, and thus BMI was used as an additional covariate.

Multivariate analyses with age and sex as covariates showed no overall group differences in left hemisphere area (Wilks's Lambda: 0.96, $F_{6,155}=1.02$, $p=0.414$), right hemisphere area (Wilks's Lambda: 0.97, $F_{6,155}=0.69$, $p=0.662$), and left hemisphere thickness (Wilks's Lambda: 0.97, $F_{6,155}=0.69$, $p=0.657$). Similarly, there was no significant group difference in overall thickness of the right mPFC after controlling for sex, age, and BMI (Wilks's Lambda: 0.93, $F_{6,155}=1.97$, $p=0.073$).

KP and CRP Differences between Groups

With the exception of the KynA/3HK ratio, all KP metabolites as well as CRP were non-normally distributed and therefore log normalized as appropriate. In the combined MDD and HC samples, log CRP was significantly correlated with log TRP ($n=97$, $r=-0.28$, $p=0.006$), log 3HK ($n=97$, $r=0.27$, $p=0.007$), log Kyn/TRP ($n=97$, $r=0.34$, $p=0.001$), KynA/3HK ($n=92$, $r=-0.36$, $p=0.001$), and log KynA/QA ($n=92$, $r=-0.26$, $p=0.012$). All of these correlations remained significant after FDR correction.

Symptoms of anhedonia measured with the SHAPS were correlated with log KynA/QA in the MDD group ($n=51$, $r=-0.33$, $p=0.019$), an association that was driven by log QA ($r=0.23$, $p=0.083$) rather than log KynA ($r=-0.08$, $p=0.562$). Further, log Kyn/Trp ($n=60$, $r=0.31$, $p=0.017$) and log 3HK ($n=60$, $r=0.29$, $p=0.025$) were positively correlated with the severity of depressive symptoms measured with the MADRS although these correlations were no longer significant after FDR correction. Log TRP additionally showed a nominal correlation with depressive symptoms prior to FDR correction ($n=60$, $r=-0.25$, $p=0.055$).

There was no significant difference in log CRP between groups (without covariates: $F_{1,109}=1.3$, $p=0.252$; with sex and BMI: $F_{1,107}=2.9$, $p=0.093$). The individual kynurenine pathway metabolites and the log Kyn/TRP ratio did not differ between groups (without covariates: Wilks's Lambda: 0.95, $F_{6,123}=1.24$, $p=0.297$; with sex, batch, and BMI: Wilks's Lambda: 0.98, $F_{6,121}=0.47$, $p=0.798$). Consistent with our previous findings in an overlapping sample, KynA/3HK ($F_{1,128}=4.1$, $p=0.045$) and Log KynA/QA ($F_{1,128}=4.3$, $p=0.039$) were reduced in the MDD group relative to the HC group. Nevertheless, after controlling for sex which was significantly correlated with KynA/3HK and log KynA/QA, these differences were no longer statistically significant (KynA/3HK: $F_{1,127}=1.1$, $p=0.289$; Log KynA/QA: $F_{1,127}=2.0$, $p=0.157$). In the new sample of participants the mean KynA/3HK and KynA/QA ratios in the 22 MDD versus 33 HC participants was in the same direction as reported previously: 1.303 ± 0.427 versus 1.342 ± 0.438 and 0.102 ± 0.037 versus 0.107 ± 0.038 , respectively.

Given the significant effect of sex on KynA/3HK and log KynA/QA we performed an exploratory analysis to test whether the significant group differences in KynA/3HK and log KynA/QA were only present in one gender. ANOVA with diagnosis, sex, and diagnosis x

sex as the independent variables showed that compared to the HC group, the MDD group had significant or nominally significant reductions in log KynA/QA ($F_{1,126}=4.5$, $p=0.035$) and KynA/3HK ($F_{1,126}=3.7$, $p=0.056$), respectively. Females had significantly lower log KynA/QA ($F_{1,126}=9.2$, $p<0.001$) and KynA/3HK ($F_{1,126}=17.1$, $p<0.001$) than males. There was also a trend towards a diagnosis x sex interaction for log KynA/QA ($F_{1,126}=3.0$, $p=0.087$) or KynA/3HK ($F_{1,126}=3.7$, $p=0.056$) indicating that the group differences in KynA/3HK and KynA/QA were driven by males (figure S2).

Mediation Analyses

Firstly, we tested whether the reduction in KynA/3HK and log KynA/QA in MDD mediated the difference between MDD and HC groups in BA24 and BA32 thickness. The mediation analyses for both KynA/3HK ($p=0.034$) and log KynA/QA ($p=0.036$) were significant for BA32 but not BA24 (KynA/3HK: $p=0.082$; log KynA/QA: $p=0.556$). See figure 3. Although log CRP did not differ between the diagnostic groups (see above), it was significantly associated with BA32 thickness in the combined MDD and HC samples ($n=110$, β -weight = -0.27 , $p=0.004$). Similarly, KynA/3HK ($n=129$, β -weight = 0.27 , $p=0.001$) and log KynA/QA ($n=129$, β -weight = 0.23 , $p=0.009$) were significantly associated with BA32 thickness. Thus in order to assess the relative influence of CRP versus KynA/3HK and KynA/QA on BA32 thickness, we performed two additional mediation analyses which showed that KynA/3HK ($p=0.013$) but not log KynA/QA ($p=0.190$) at least partially mediated the association between log CRP and BA32 thickness across all subjects (figure 4).

DISCUSSION

Summary of the Principal Findings

Relative to HCs, the MDD group showed a reduction in cortical thickness of BA24 and BA32 in the right hemisphere that was associated with a greater number of depressive episodes. However, after controlling for age, sex, and BMI, the multivariate statistic for right hemisphere thickness was no longer statistically significant at $p<0.05$ although there was a non-significant effect ($p<0.10$).

Secondly, KynA/3HK and log KynA/QA were reduced in the MDD group relative to the HC group but these results were no longer significant after controlling for sex. An exploratory analysis showed that the diagnostic differences in KynA/3HK and log KynA/QA were driven by differences in males. In addition, the severity of anhedonic symptoms was inversely correlated with log KynA/QA in the MDD group.

Thirdly, the thinner right BA32 thickness in the MDD group was found to be at least partially mediated by the reduction in KynA/3HK and log KynA/QA in the MDD versus HC group. Further, the inverse association between log CRP and right BA32 thickness in the combined MDD and HC sample was at least partially mediated by KynA/3HK.

Cortical Thickness and Cortical Area Differences between Groups

The significant decrease in cortical thickness of BA24 and BA32 in MDD patients is partially consistent with the results of (Spati et al., 2015) who reported only one region of

decreased cortical thickness in MDD - the right dorsal mid-anterior ACC. Similarly, reductions in thickness of the right rACC (Oertel-Knochel et al., 2015), the right dorsomedial PFC, including BA32 (Elvsashagen et al., 2013), and the left rACC (Foland-Ross et al., 2011; Lyoo et al., 2006) have been reported in patients with bipolar disorder.

The finding that group differences in BA24 and BA32 were limited to thickness rather than area may be related to the fact that cortical area and cortical thickness are genetically and ontogenetically independent (Panizzon et al., 2009). Cortical neurons are organized into columns that run perpendicular to the cortical surface. Surface area is determined by the number of columns while thickness is determined by the number of cells and the amount of neuropil within each column (Rakic, 1988; Stockmeier et al., 2004). (Storsve et al., 2014) found that not only are temporal changes in cortical surface area and cortical thickness often independent of each other, they may be negatively correlated in the context of healthy aging. Surface area and thickness thus are reflective of independent neurobiological processes and should be evaluated as separate metrics of development, aging, or disease (Storsve et al., 2014). While most of the variation in individual cortical volume is due to variation in surface area (Im et al., 2008), the primary basis of alterations in cortical volume over time in healthy aging is considered to be change in cortical thickness (Storsve et al., 2014).

The reason for the right lateralized nature of the reduction in cortical thickness in MDD is unclear but also may be related to differences between cortical thickness and cortical area. Volumetric studies have generally found more pronounced reductions in volume of the left ACC, particularly the left sgACC (Drevets et al., 2008). The number of cortical columns has been reported to remain relatively constant after birth with most of the increase in cortical volume due to the generation of glial cells, the expansion of the vasculature and connective tissue, the myelination of axons, and the elaboration of axonal and dendritic processes (Keil et al., 2010; Rimol et al., 2012). In other words, cortical thickness may be a sensitive measure of dendritic remodeling occurring at all stages in life while cortical area might be more reflective of early developmental processes. In a recent twin study, (Bootsman et al., 2015) found that environmental factors accounted for a significant percentage of variance in the cortical thickness and thus the liability to develop BD. Conceivably, the left lateralized reductions in volume may be genetically-driven, and therefore more strongly correlated with cortical area than cortical thickness *per se*. In this regard, it is interesting that reductions in the left sgACC are more pronounced in depressed individuals with a family-history of mood disorders (Drevets et al., 2008). In the current sample, the MDD group indeed showed a highly significant reduction in the surface area (but not the thickness) of the left BA24 ($p=0.008$, see Table S1) although the region examined herein extended well beyond the subgenual portion of the BA24. Similarly, the MDD group showed a significant reduction in the surface area (but not the thickness) of the left ($p=0.031$) as well as the right BA25 ($p=0.026$) in the posterior portion of the subgenual PFC (, Table S1). However, we did not pursue these findings because the multivariate statistics for left and right hemisphere area were not statistically significant.

The nominal association between depression severity and BA32 thickness is partially consistent with a previous study that reported that depressive symptoms were inversely correlated with the thickness of the rACC in a group of patients with late onset depression

(Lim et al., 2012). Similarly, (Phillips et al., 2015) reported that a greater decrease in MADRS scores in the interval between the baseline assessment and a one year follow-up was associated with an increase in thickness of the right supragenual/caudal ACC. Further, our finding of thinner cortex of BA32 in the “high” episode MDD group is consistent with the literature. Specifically, an inverse association between number of depressive episodes or length of illness and cortical thickness of the mPFC, including the rACC and sgACC has been reported in MDD and BD (Foland-Ross et al., 2011; Treadway et al., 2015). Additionally, a meta-analysis of VBM studies concluded that longer illness duration in MDD patients is associated with greater GM volume decrease in the rACC and dorsomedial frontal cortex (Bora et al., 2012).

KP and CRP Differences between Groups

The reduction in Kyn/3HK and log Kyn/QA in the MDD sample is partially consistent with our previous findings (Savitz et al., 2015a; Savitz et al., 2015b) although the differences did not remain significant after regressing out the effects of sex and batch. The significant effect of sex on KynA/3HK and log KynA/QA may be an important to consider in future studies especially since males and females have been shown to differ immunologically in other contexts (Eisenberger et al., 2009; Furman et al., 2014; Moieni et al., 2015). In the new subset of MDD and HC participants, the diagnostic group difference in the mean KynA/3HK and log KynA/QA values was nominally in the same direction as those of the previously studied subjects although these mean differences did not reach significance, possibly because of the smaller sample size.

The reductions in Kyn/3HK and log Kyn/QA in the MDD group relative to the HCs are consistent with the results of (Bay-Richter et al., 2015) who recently reported persistent decreases in KynA and increases in QA in the cerebrospinal fluid of predominantly depressed subjects up to 2 years after a suicide attempt. Furthermore, our results are partially consistent with two studies that found reductions in peripheral KynA concentrations in groups of depressed patients compared with healthy controls (Maes et al., 2011; Myint et al., 2007).

The significant negative correlation between KynA/QA and the severity of anhedonic symptoms in the MDD group is consistent with the well characterized link between inflammation-induced sickness behavior and anhedonia (Dantzer et al., 2008; Maes et al., 2012; Yirmiya et al., 2000). Similarly, a previous study hypothesized that it is primarily MDD patients with melancholic features that show alterations in immune function (Gabbay et al., 2010).

The absence of significant differences in CRP between the MDD and HC groups is also consistent with our previous findings, and may be an artifact of the similar BMI scores in the MDD and HC groups and/or our fairly strict inclusion criteria (see above). A perusal of Table 1 shows that both the MDD and HC subjects had relatively high BMIs with means of 28 and 27, respectively and in the combined MDD and HC sample, CRP was significantly correlated with BMI ($r=0.27$, $p=0.002$).

Mediating effect of KynA/3HK and log KynA/QA on thickness differences in BA24 and BA32

We found that KynA/3HK and log KynA/QA mediated the difference between diagnostic groups in cortical thickness raising the possibility that depression-induced activation of *IDO* and/or kynurenine mono-oxygenase (*KMO*) may lead to the production of neurotoxic metabolites such as QA that lead to pathological changes in the mPFC. QA is a neurotoxin that is produced in the brain by microglia and infiltrating macrophages. QA activates NMDA receptors and additionally exerts neurotoxic effects via lipid peroxidation, and disruption of the blood-brain barrier (Schwarcz et al., 2012; Stone et al., 2012). Further, QA has the ability to initiate an inflammatory response or augment existing inflammation by enhancing the production of pro-inflammatory proteins (Stone et al., 2013). Elevated concentrations of QA have been reported in both the serum and the CSF of patients with neurodegenerative and inflammatory disorders such as Alzheimer's disease and systemic lupus erythematosus patients with neuropsychiatric symptoms (Ting et al., 2009; Vogelgesang et al., 1996).

The potential mediating effect of KynA/QA on BA32 thickness is particularly intriguing in light of a *postmortem* immunohistochemistry study showing that relative to controls, a mixed sample of MDD and BD subjects had increased QA-positive cell densities in the anterior mid-cingulate cortex and sgACC, suggesting microglial cell activation (Steiner et al., 2011). Consistent with these data, a recent positron emission tomography study found evidence for microglial activation in the PFC and ACC of patients with MDD as indexed by an increase in the distribution of volume of the ligand for the translocator protein ligand, TP50 (Setiawan et al., 2015). While we cannot measure central QA concentrations, a significant correlation has been found between QA levels in the serum and cerebrospinal fluid (L. Brundin, personal communication).

Mediating effect of KynA/3HK on the association between CRP and cortical thickness

While both pro-inflammatory cytokines and neuroactive kynurenine metabolites may affect brain structure and function, the neural effects of a general increase in inflammatory factors are challenging to disentangle from the effects of kynurenine metabolites because *IDO* is upregulated by inflammation. While mediation analysis does not allow us to draw causal conclusions, our data suggest that the ratio of KynA to neurotoxic metabolites may play a more direct role in neuroplasticity/excitotoxicity than more general markers of inflammation such as CRP. While preliminary, our results extend previous studies that have reported associations between inflammatory markers and cortical thickness in healthy populations (Kaur et al., 2014; Krishnadas et al., 2013; Marsland et al., 2015; Piras et al., 2012). Moreover, our results also are partially consistent with those of (O'Connor et al., 2009) who showed that LPS-induced depressive behavior in mice could be abrogated using the specific *IDO* blocker, 1-MT, without affecting the concentrations of pro-inflammatory cytokines. We could not use circulating levels of cytokines in this analysis because with the exception of IL-6 they are usually below the threshold of detection and their usefulness for assessing systemic inflammation is very limited.

Limitations

Given that we compared the MDD and the HC groups on the cortical thickness and cortical area of 12 ROIs (considering left and right sides independently), the possibility for type I errors due to multiple comparisons exists. Nevertheless, this possibility is partially mitigated by the *a priori* selection of regions of interest and the directional hypotheses concerning the association between kynurenine metabolites and cortical thickness. Although the multivariate statistic for right hemisphere thickness was not statistically significant after controlling for age, sex, and BMI ($p < 0.10$), age and BMI did not differ across diagnostic groups and further, because BMI is significantly correlated with inflammation, and inflammation in turn, may contribute to reductions in cortical thickness, the use of BMI as a covariate may remove a substantial portion of the variance associated with diagnosis, and may thus constitute a case of “over-control”.

We measured peripheral concentrations of CRP and the kynurenine pathway metabolites and it is unclear if these concentrations accurately reflect central concentrations. Nevertheless, 3HK and kynurenine can cross the blood brain barrier, and INF α -treated hepatitis C patients showed *both plasma and CSF* increases in KYN and QA that correlated with depressive symptoms (Raison et al., 2010). Consistent with our finding of decreased KynA/QA in the serum of MDD patients, chronic decreases in KynA and elevations in QA have been reported in the cerebrospinal fluid of suicide attempters (Bay-Richter et al., 2015) while (Steiner et al., 2011) reported increased QA-positive cell densities in the anterior mid-cingulate cortex and sgACC. Further, preclinical work demonstrates the relevance of peripheral kynurenine metabolites for brain function. In mouse models of Alzheimer’s Disease (AD) and Huntington’s Disease (HD) *peripheral* administration of a drug that directly inhibited *KMO*, elevated brain levels of KynA and decreased glutamate release and excitotoxicity in the brain parenchyma *despite the fact that neither the prodrug nor its metabolite were capable of crossing the blood brain barrier* (Zwilling et al., 2011). This finding is consistent with data indicating that increased production of KynA from kynurenine in the periphery through exercise training decreases the amount of kynurenine that can enter the brain and be transformed into QA in the context of inflammation (Agudelo et al., 2014). Another recent preclinical study also demonstrates the importance of the peripheral immune system function in the induction of depressive behavior (Hodes et al., 2014). Stress susceptible mice were found to produce higher levels of interleukin-6 (IL-6) compared with resilient mice. The authors subsequently generated bone marrow chimeras transplanted with hematopoietic progenitor cells from the stress susceptible mice or IL-6 knockout mice. The stress susceptible chimeras exhibited increased depression-like behavior, whereas the knockouts were resistant to the stress, *this despite the fact that the resident microglia population was left intact* in the chimeras (Hodes et al., 2014). Thus although CSF measurements of kynurenine pathway metabolites would have been advantageous in our study, there is enough evidence to accept the fact that peripheral concentrations of neuroactive kynurenine pathway metabolites are relevant to brain structure in the context of depression.

Our study may have benefited from the availability of cytokine measurements as it would have allowed us to make a direct comparison with other studies that reported negative

associations between several different pro-inflammatory cytokines and cortical thickness or area in healthy controls and subjects at high-risk of psychosis. Similarly, we may have had greater statistical power had we limited the neuromorphometric analyses to the subregions where greater evidence for volume loss exists in volumetric MRI and postmortem studies (e.g., subgenual portion of BA24, as opposed to the entire extent of BA24).

Finally we note that this is a cross-sectional study that was designed to investigate the relationship between kynurenine-pathway metabolites and CRP and cortical abnormalities of the mPFC. The data reported here, including the mediation analyses, are correlative in nature and cannot provide information concerning cause and effect relationships.

In sum, our results suggest that inflammation-induced changes in the ratio of KynA to QA-pathway metabolites may be one mechanism through which inflammatory processes affect cortical thickness in MDD and thus conceivably may partially explain the MDD-associated reductions in cortical thickness in the mPFC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Thickness of right BA24 and right BA32 was reduced in major depressive disorder
- Serum KynA/3HK and KynA/QA was reduced in MDD
- CRP was inversely associated with BA32 thickness
- KynA/3HK partially mediated the relationship between CRP and thickness of BA32

Figure 1.

A. Medial view of the right hemisphere illustrating the PALS-B12 Brodmann area atlas as implemented in FreeSurfer. BA24 is colored dark blue (pink dashed arrows) and the adjacent BA32, a slightly lighter shade of blue (purple dashed arrow).

B. Original Brodmann area map showing the equivalent regions shown in the PALS-B12 atlas. BA24 is colored brown while BA32 is white.

C. Scatterplot illustrating a statistically significant difference between the thickness of the right BA32 between the healthy controls (green circles) and MDD participants (blue squares): $F_{1,162}=3.9$, $p=0.024$. The error bars shown in black represent the standard error of the mean.

D. Scatterplot illustrating a statistically significant difference between the thickness of the right BA24 between the healthy controls (green circles) and MDD participants (blue squares): $F_{1,162}=7.8$, $p=0.006$. The error bars shown in black represent the standard error of the mean.

Figure 2.

Scatterplots showing significant group differences in (A) KynA/3HK ($F_{1,128}=4.1$, $p=0.045$) and (B) Log KynA/QA ($F_{1,128}=4.3$, $p=0.039$). The error bars shown in black represent the standard error of the mean. Correlations between (C) anhedonia symptoms (SHAPS) and log KynA/QA ($r=-0.33$, $p=0.019$) and (D) depressive symptoms (MADRS) and log Kyn/Trp ($r=-0.31$, $p=0.017$, $q>0.05$).

Figure 3. Schematic representations of the potential mediating effects of (A) KynA/3HK ($p=0.034$) and (B) log KynA/QA ($p=0.036$) on the MDD-associated reduction in thickness of right BA32. The pathway from Diagnosis to KynA/3HK or log KynA/QA (a) and then from KynA/3HK or log KynA/QA to BA32 (b) represents the indirect effect of Diagnosis on right BA32 thickness (quantified as the product of paths a and b). The pathway from Diagnosis to BA32 represents the direct effect of Diagnosis on BA32 (c), while c' shows the strength of the direct effect after KynA/3HK or log KynA/QA is taken into account. Model coefficients are reported in unstandardized form.

Figure 4. Schematic representations of the potential mediating effects of (A) KynA/3HK ($p=0.013$) and (B) log KynA/QA ($p=0.190$) on the relationship between CRP and thickness of right BA32. The pathway from log CRP to KynA/3HK or log KynA/QA (a) and then from KynA/3HK or log KynA/QA to BA32 (b) represents the indirect effect of CRP on right BA32 thickness (quantified as the product of paths a and b). The pathway from CRP to BA32 represents the direct effect of CRP on BA32 (c), while c' shows the strength of the direct effect after KynA/3HK or log KynA/QA is taken into account. Model coefficients are reported in unstandardized form.

Table 1

Means and standard deviations of demographic data, clinical ratings, cortical area/thickness of the regions of interest, and kynurenine metabolite concentrations in the MDD and HC groups.

	MDD	Healthy	Univariate Statistic
N	73	91	-
Age	34.2±9.3	31.6±9.3	t=1.7, p=0.085
Sex (% F)	78	60	X ² =5.8, p=0.012
Ethnicity (%Caucasian)	82	83	NS
BMI	28.0±5.5	27.0±4.9	t=1.2, p=0.567
MADRS	27.6±8.8	1.0±2.1	t=24.5, p<0.001
SHAPS	30.6±7.1	19.0±4.9	t=11.3, p<0.001
Right BA9 Thickness	2.54±0.15	2.55±0.16	F=0.1, p=0.759
Right BA10 Thickness	2.36±0.16	2.36±0.15	F=0.1, p=0.780
Right BA11 Thickness	2.54±0.16	2.56±0.15	F=0.5, p=0.483
Right BA24 Thickness	2.64±0.15	2.70±0.14	F=7.8, p=0.006 *
Right BA25 Thickness	2.65±0.31	2.71±0.21	F=2.4, p=0.127
Right BA32 Thickness	2.51±0.19	2.58±0.19	F=5.2, p=0.024 *
hs-CRP	2.50±3.68	3.17±5.07	t=0.8, p=0.424
TRP	54.4±11.4	60.4±17.4	t=2.5, p=0.016
Kyn	1.90±0.49	1.95±0.48	t=0.6, p=0.578
KynA	39.1±11.1	42.1±15.5	t=1.3, p=0.205
3HK	36.1±14.7	34.4±15.0	t=0.7, p=0.495
QA	388.2±164.7	354.3±113.9	t=1.4, p=0.167
Kyn/TRP	0.036±0.013	0.033±0.008	t=1.5, p=0.126
KynA/3HK	1.161±0.377	1.304±0.418	t=2.0, p=0.022
KynA/QA	0.109±0.036	0.124±0.044	t=2.2, p=0.013

* Wilk's Lamda: F_{6,157}=2.2, p=0.021.

Note: CRP data were available for 39 individuals with MDD and 71 healthy controls (HC). Kynurenine pathway metabolite concentrations were available for 65 individuals with MDD and 74 HCs.

Abbreviations: MDD=major depressive disorder, BMI=body mass index, MADRS = Montgomery-Asberg Depression Rating Scale, SHAPS=Snaith Hamilton Pleasure Scale, BA=Brodmann's Area, hs-CRP=high-sensitivity c-reactive protein, TRP=tryptophan, Kyn=kynurenine, KynA=kynurenic acid, 3HK=3-hydroxykynurenine, QA=quinolinic acid, Kyn/TRP=ratio of kynurenine to tryptophan, KynA/3HK= ratio of kynurenic acid to 3- hydroxykynurenine, KynA/QA=ratio of kynurenic acid to quinolinic acid.