# The Kynurenine Pathway and Bipolar Disorder: Intersection of the Monoaminergic and Glutamatergic Systems and Immune Response

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## Abstract

Dysfunction in a wide array of systems—including the immune, monoaminergic, and glutamatergic systems—is implicated in the pathophysiology of depression. One potential intersection point for these three systems is the kynurenine (KYN) pathway. This study explored the impact of the prototypic glutamatergic modulator ketamine on the endogenous KYN pathway in individuals with bipolar depression (BD), as well as the relationship between response to ketamine and depression-related behavioral and peripheral inflammatory markers. Thirty-nine participants with treatment-resistant BD (23F, ages 18–65) received a single ketamine infusion (0.5mg/kg) over 40 minutes. KYN pathway analytes—including plasma concentrations of indoleamine-2,3-dioxygenase (IDO), KYN, kynurenic acid (KynA), and quinolinic acid (QA)—were assessed at baseline (pre-infusion), 230 minutes, Day 1, and Day 3 post-ketamine. General linear models with restricted maximum likelihood estimation and robust sandwich variance estimators were implemented. A repeated effect of time was used to model the covariance of

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Conflict of Interest

Dr. Zarate is listed as a co-inventor on a patent for the use of ketamine in major depression and suicidal ideation. Drs. Zarate and Moaddel are listed as co-inventors on a patent for the use of (2*R*,6*R*)-hydroxynorketamine, (*S*)-dehydronorketamine, and other stereoisomeric dehydro and hydroxylated metabolites of (*R*,*S*)-ketamine metabolites in the treatment of depression and neuropathic pain; and as co-inventors on a patent application for the use of (2*R*,6*R*)-hydroxynorketamine and (2*S*,6*S*)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation, and post-traumatic stress disorders. They have assigned their patent rights to the U.S. government but will share a percentage of any royalties that may be received by the government. All other authors have no conflict of interest to disclose, financial or otherwise.

the residuals with an unstructured matrix. After controlling for age, sex, and body-mass index (BMI), post-ketamine IDO levels were significantly lower than baseline at all three time points. Conversely, ketamine treatment significantly increased KYN and KynA levels at Days 1 and 3 versus baseline. No change in QA levels was observed post-ketamine. A lower post-ketamine ratio of QA/KYN was observed at Day 1. In addition, baseline levels of pro-inflammatory cytokines and behavioral measures predicted KYN pathway changes post-ketamine. The results suggest that, in addition to having rapid and sustained antidepressant effects in BD participants, ketamine also impacts key components of the KYN pathway.

## INTRODUCTION

The etiology of bipolar depression (BD) remains unknown, in part due to both the heterogeneous nature of the disease and its complex underlying neuropathology (1). In addition, the lack of reliable biomarkers substantially complicates treatment of the disorder, worsens prognosis, and increases treatment-refractoriness. Recent studies have implicated both altered glutamatergic neurotransmission and excessive immune activation in the neurobiology of BD as well as treatment-resistance (2, 3). In particular, evidence from preclinical and clinical studies suggests that abnormal activation of the kynurenine (KYN) pathway may underlie BD (3, 4). Neuroactive byproducts of the KYN pathway are involved in the interface between inflammatory/immune response (5, 6) and serotoninergic neurotransmission via catabolism of tryptophan to kynurenine (7), ultimately altering downstream synaptic glutamatergic neurotransmission (8). The KYN pathway also facilitates inter-organ communication between the brain and the immune system by affecting neural afferents and circulating immune mediators that activate brain endothelial and innate immune cells (microglia) (9, 10). This communication is primarily achieved via two rate-limiting enzymes: tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) (11, 12) (see Figure 1), both of which are implicated in the catabolism of tryptophan into KYN. Of the two enzymes, TDO is mostly intra-hepatic and regulated by glucocorticoid induction (13), whereas IDO is extra-hepatic, highly expressed in the brain, and tightly upregulated in response to proinflammatory mediators, glucocorticoids, and psychosocial stress (14).

In the brain, KYN can be differentially processed by either astrocytes or microglia to produce distinct neuroactive compounds. During homeostasis, kynurenine amino transferase converts KYN to kynurenic acid (KynA), a metabolite of the astrocytic process (15), which clears accumulated KYN in the central nervous system (CNS). KynA binds at the glycine co-agonist site of the NR2B N-methyl-D-aspartate (NMDA) receptor and also has antagonist properties at the α7-nicotinic acetylcholine receptor; both of these receptors are neuroprotective when activated physiologically (16, 17) and are closely linked to synaptic plasticity and cognitive processes (18), partly via their anti-inflammatory properties (19) and ability to clear glutamate spillover in the brain (2, 17).

Conversely, the microglial processing of KYN results in the formation of quinolinic acid (QA), a byproduct that exerts powerful excitotoxic effects and promotes neuronal apoptosis (3, 19, 20) (Figure 1). Evidence suggests that during depressive episodes, proinflammatory

components of the KYN system in the brain are either directly or indirectly activated, altering the neuroprotective/neurotoxic balance of the KYN pathway and leading to the overproduction of neurotoxic microglial byproducts (such as QA) that potentiate NMDA activation (3, 5, 21–23). This increased activation of inflammatory circuitry within the brain, in turn, contributes to the pathological activation of the glutamatergic system (5, 24), which leads to decreased neurotrophic support, synaptic dysregulation, oxidative stress, excitotoxicity, and loss of glial tissue in multiple sites in the CNS (17, 25).

The KYN pathway is best known for regulating the interaction between the immune and stress pathways (see Figure 1) (26). Psychosocial stress stimulates inflammatory mediators in the periphery and brain via multiple mechanisms; for example, peripheral immune cells, including T-cells, can gain access to the brain (27), resulting in increased IDO production. This, in turn, triggers the activation of resident microglial cells that secrete the proinflammatory cytokine tumor necrosis factor alpha (TNF-α), with neuronal injury as the net result (see Figure 1). Postmortem human data support this result, with studies showing that proinflammatory cytokines (such as interleukin-6 (IL-6) and TNF-α) and other byproducts of microglial activation (mainly QA) were significantly upregulated in the frontal cortex of individuals with BD (28). Positive correlations have also been found between QA and specific proinflammatory immunologic variables, such as IL-6, in the CSF of individuals with previous suicide attempts (29). In contrast, KynA levels in the CSF were found to be inversely correlated with the severity of depressive symptoms (30), as well as significantly downregulated in suicidal individuals (29). Another study found that the QA/KynA ratio was significantly elevated in the CSF of suicide attempters compared to healthy participants (31), suggesting that net positive QA levels in the brain might result in detrimental structural and functional deficits, likely due to enhanced activation of microglial cells and a corresponding upsurge in NMDA receptor agonism (32, 33).

Previous studies found that a single infusion of ketamine leads to rapid (within hours), robust, and sustained antidepressant effects in individuals with BD (34, 35). Preclinical evidence also suggests that, within this time frame, ketamine reverses deficits in neuroplasticity and neurogenesis (36, 37). Ketamine has also been found to reduce levels of key proinflammatory mediators (38) as well as bone biomarkers and adipokines (39, 40) within hours to days following acute treatment. In addition, ketamine abolished lipopolysaccharide (LPS)-induced depressive-like behaviors in preclinical mouse models (41). Previous work from our laboratory also found that ketamine's antidepressant effects may depend on its ability to affect KYN pathway metabolites (42).

This study explores the complex interactions between ketamine and relevant behavioral and biological immune/inflammatory markers that affect the KYN pathway in depression. The study had four main goals: 1) to explore the impact of a single dose of ketamine on specific proinflammatory KYN pathway metabolites in participants with treatment-resistant BD; 2) to assess whether baseline values of specific KYN pathway analytes predicted change in depressive symptom ratings post-ketamine; 3) to investigate whether baseline levels of inflammatory markers predicted change in KYN pathway analytes post-ketamine infusion; and 4) to determine whether inflammatory markers were longitudinally associated with KYN pathway analytes post-ketamine infusion.

# MATERIALS AND METHODS

## Study Design and Participants

The current data were drawn from the ketamine condition of a randomized, placebo (saline)-controlled, crossover study designed to assess the antidepressant efficacy of adjunctive ketamine administered intravenously at 0.5 mg/kg over 40 minutes in participants with BD; all participants were receiving adjunctive treatment with a mood stabilizer (either lithium or valproate). Details regarding study design have been previously published (34, 35). Clinical and demographic data are presented in Table 1, and additional information about the participant sample can be found in the Supplementary Materials.

Psychiatric rating scales included the Beck Depression Inventory (BDI) (43), the Hamilton Depression Rating Scale (HAM-D) (44), the Montgomery-Asberg Depression Rating Scale (MADRS) (45), and the Snaith-Hamilton Pleasure Scale (SHAPS) (46). For the purposes of this analysis, empirically-derived unidimensional scores comprising items from all four scales were used (47). The three largest subscales—*Depressed Mood*, *Negative Cognition*, and *Anhedonia*—were selected for use, though results for all subscales are available upon request.

## ELISA and multiplex immunoassay

Plasma samples were collected 60 minutes prior to ketamine infusion (baseline) and at 230 minutes, Day 1, and Day 3 post-ketamine infusion. Levels of human KYN, KynA, QA, and IDO were measured using specific ELISA kits. Circulating levels of several inflammatory cytokines (TNF-$\alpha$, soluble tumor necrosis factor receptor 1 (sTNFR1), interferon gamma (IFN-$\gamma$), IL-2, IL-5, IL-6, IL-8, and IL-10) were measured in plasma using a high-sensitivity multiplex Luminex immunoassay. For additional details, see the Supplementary Materials.

## Statistical Analysis

General linear models with restricted maximum likelihood estimation and robust sandwich variance estimators were implemented using PROC MIXED in SAS/STAT Version 9.3. The syntax for all models is provided in the Supplementary Materials. A repeated effect of time was used to model the covariance of the residuals with an unstructured matrix. Mood stabilizer (lithium or valproic acid), age, and body mass index (BMI) were entered as covariates in all analyses. The first research question—which sought to assess changes to the KYN pathway in response to ketamine—was investigated via pre-specified contrasts between baseline and follow-up points (230 minutes, Day 1, Day 3). The second research question—whether baseline KYN pathway values predicted change in depressive symptom ratings post-ketamine—was assessed by estimating the simple slopes for KYN pathway member-by-time interactions. The third research question—whether baseline levels of inflammatory markers predicted KYN pathway changes post-ketamine—was similarly assessed by estimating the simple slope for each baseline-to-follow-up contrast (i.e., simple slopes for the inflammatory marker-by-time interactions in predicting KYN pathway changes post-ketamine). Finally, the fourth research question—whether inflammatory markers were longitudinally associated with KYN pathway analytes post-ketamine infusion—was evaluated by determining whether the inflammatory markers changed over time (a

mixed model predicting inflammatory marker from time and covariates); if so, whether that change correlated with changes in the KYN pathway was then assessed (Spearman correlations). Prior to the analyses, plasma data (for the KYN pathway and for inflammatory cytokines) were natural-log transformed after adding 1 to all values. Ratios (e.g., KYN/KynA) are the ratios of the transformed variables.

It should be noted that with regard to the third research question, high intercorrelations were observed among the pro- and anti-inflammatory circulating markers (i.e., TNF-α, sTNFR1, IFN-γ, IL-2, IL-5, IL-6, IL-8, and IL-10), and a principal components analysis was therefore implemented. Two components with eigenvalues greater than 1.0 explained 55% of the variance in the inflammatory markers (see Supplementary Table S1); the first component included mainly pro-inflammatory markers and represented TNF-α, sTNFR1, IFN-γ, IL-5, IL-6, and IL-8. The second component, which included mainly anti-inflammatory markers, represented IL-2, IL-8 (reverse loading), and IL-10. These pro- and anti-inflammatory component scores were used in the analysis rather than the values of the individual circulating inflammatory cytokines.

In addition, because this was a secondary analysis of participants from a clinical trial, an *a priori* power analysis was not performed. Given the exploratory nature of this study, we also did not adjust alpha or p-values for multiplicity. All parameter estimates with standard errors, as well as exact p-values, are shown in Supplementary Tables S1–S8.

## RESULTS

### Changes in KYN pathway metabolites post-ketamine infusion

After controlling for type of mood stabilizer, age, and BMI, post-ketamine levels of IDO were significantly lower than baseline at all time points (Table 2, Figure 2). Both KYN and KynA levels were significantly higher at Days 1 and 3, as was their ratio (KYN/KynA). No changes in QA levels were observed post-ketamine administration, though the ratio of QA to KYN was significantly lower from baseline to Day 1. No post-ketamine difference was observed for KynA/QA ratio, a measure of NMDA agonist/antagonist balance. Model results appear in Table 2 and Supplementary Table S2.

### Members of the KYN pathway as predictors of change in depressive symptoms post-ketamine infusion

Baseline concentrations of IDO, KYN, QA, and the KynA/QA ratio were evaluated as predictors of *Depressed Mood*, *Negative Cognition*, and *Anhedonia* subscale scores. Higher baseline IDO levels ($t(33)=-3.84$, $p=.0005$) and lower baseline QA levels ($t(33)=2.35$, $p=.02$) were associated with less severe baseline *Depressed Mood* scores, and lower QA/KYN ratio was associated with less severe baseline *Negative Cognition* scores ($t(33)=2.58$, $p=.01$) (see Supplementary Table S3 for slopes).

Baseline IDO levels were unrelated to antidepressant response to ketamine on any subscale, and lower baseline KYN levels were nominally related to improved *Anhedonia* scores at Day 3 ($t(33)=2.09$, $p=.04$) (Figure 3B, Supplementary Table S4). Higher baseline QA levels predicted improvement in *Depressed Mood* scores at 230 minutes ($t(33)=-2.34$, $p=.03$)

and at Day 3 (t(33)=−3.21, p=.003) and improved *Negative Cognition* scores at Day 3 (t(33)=−2.79, p=.009) (Supplementary Table S4). A lower baseline ratio of KynA/QA (a measure of NMDA antagonist/agonist balance) predicted improvement in *Depressed Mood* score at 230 minutes (t(33)=2.53, p=.02) and in *Anhedonia* score at Day 3 (t(33)=2.29, p=.02) (Supplementary Table S4).

### Baseline inflammatory markers as predictors of change in the KYN pathway post-ketamine infusion

Ten participants who were missing inflammatory marker concentrations were excluded from this analysis. The baseline values of the two inflammatory cytokine principal component scores (the pro-inflammatory cytokine component score, representing TNF-α, sTNFR1, IFN-γ, IL-5, IL-6, and IL-8, and the anti-inflammatory cytokine component score, representing IL-2, reverse loading IL-8, and IL-10) were entered as predictors of IDO, KYN, and KynA levels, as well as KYN/KynA and QA/KYN ratios. Baseline pro-inflammatory component score was not related to baseline KYN pathway values (Supplementary Table S5). Nominal trends were observed for higher baseline pro-inflammatory cytokine component levels to predict larger increases in KYN levels and in the KYN/KynA ratio. These were strongest at the 230-minute time point (t(24)=2.46, p=0.02 and t(24)=2.42, p=0.02, respectively) (Figure 3A, Supplementary Table S6), when neither KYN levels nor the KYN/KynA ratio had yet changed significantly from baseline.

In addition, higher baseline anti-inflammatory cytokine component scores were associated with higher baseline KYN levels (t(24)=2.94, p=.007) and higher baseline KYN/KynA ratio (t(24)=3.47, p=.002), but not with KynA levels or the QA/KynA ratio (Supplementary Table S5). Baseline anti-inflammatory component values predicted change in IDO levels at 230 minutes post-ketamine, such that higher anti-inflammatory levels were associated with less of a decrease in IDO levels (t(24)=2.74, p=0.01) (Figure 3A, Supplementary Table S6). Higher baseline anti-inflammatory levels were also associated with less of an increase in KYN levels (t(24)=−2.85, p=0.009) and in the KYN/KynA ratio (t(24)=−2.40, p=0.02) at Day 1 (Figure 3A, Supplementary Table S6).

The pro-inflammatory component did not change over time (Supplementary Table S7), but the anti-inflammatory component was increased at Day 3 (t(26)=2.87, p=.008). Change in the anti-inflammatory component was moderately and positively correlated with change in KynA levels at Day 3 (r=0.39, p=0.45) (see Supplementary Table S8).

## DISCUSSION

This study is the first to explore the impact of ketamine on KYN pathway metabolites in individuals with treatment-resistant BD. A number of salient findings emerged. First, ketamine significantly lowered IDO levels at 230 minutes, Day 1, and Day 3 post-ketamine infusion. Surprisingly, participants who had higher baseline levels of anti-inflammatory cytokines experienced less of an immediate (230 minutes post-infusion) decrease in IDO levels. Second, and in contrast, plasma levels of KYN and KynA were significantly increased on Days 1 and 3 relative to baseline. Third, the relatively neuroprotective index ratio KYN/KynA was upregulated in response to ketamine at Day 1. Interestingly, increased

baseline levels of anti-inflammatory cytokines were associated with less change at Day 1 for KYN and the KYN/KynA ratio, but not for KynA itself. Finally, a significant lower QA/KYN index ratio was observed, but only at Day 1 post-ketamine infusion, which coincides with ketamine's peak antidepressant effects (34); given that this ratio is broadly accepted as an index of high inflammation and excitotoxicity, any decrease reflects a desirable outcome.

IDO is an intracellular enzyme mainly produced by immune cells and the brain and appears to be critical to some forms of immunologically-mediated depression (11, 21, 23, 29, 48). Preclinical studies also found that IDO is induced in both brain and periphery after a systemic inflammatory challenge (14, 48, 49). During depressive episodes, stress and immune activation enhance the conversion of tryptophan to KYN, mostly via IDO induction. In addition, numerous studies have shown that proinflammatory cytokines such as IFN-γ, IFN-α, IL-6, and TNF-α robustly induce IDO production via immune cells (50–52), which subsequently shifts tryptophan metabolism away from the liver (53). Furthermore, IDO levels are also affected by oxidative stress and LPS injection (5). During LPS-induced brain inflammation, most KYN and QA production is mediated via IDO induction (54). In humans, vulnerability to cytokine-induced depression was found to be enhanced by a polymorphism in the *IDO* gene, providing a possible explanation for the clinical heterogeneity surrounding the occurrence of depressive symptoms following immune system activation (55). In this context, genetic manipulation or pharmacological inhibition of IDO was found to abolish the LPS-induced depressive phenotype in mouse models independently of cytokine induction, suggesting that IDO itself is sufficient to trigger depressive-like behaviors in mice (56). Studies assessing indirect IDO activity (measured via the KYN/tryptophan ratio) found that increased plasma IDO levels mediated the link between inflammation and depressive symptomatology (57). The present data support the evidence that IDO is critical for shunting KYN towards the microglial pathway, but also suggest that ketamine's robust ability to significantly lower IDO levels at 230 minutes, Day 1, and Day 3 may protect against this eventuality. Interestingly, we found that higher baseline IDO levels were associated with less severe depressive symptoms at baseline but were unrelated to degree of antidepressant response to ketamine.

In addition, our finding that KYN and KynA levels were significantly upregulated in individuals with BD compared to baseline in response to a single ketamine infusion at both Days 1 and 3 (Figure 2) broadly supports a number of recent results linking the KYN pathway and depression. For instance, a large, recent meta-analysis demonstrated that, compared to healthy volunteers, individuals with depression had lower overall plasma levels of KYN and KynA and higher levels of QA (58). Other studies found lower levels of KYN metabolites in depressed individuals (22) as well as in adolescents with melancholic depression (59). The severity of depressive symptoms in suicidal individuals has also been linked to increased plasma levels of QA and decreased plasma levels of KynA (20, 31). Another study found lower KYN levels and a higher QA/KYN ratio in participants with major depressive disorder (MDD) compared to healthy controls; notably, in that study, higher baseline KYN levels predicted remission in response to adjunctive treatment with celecoxib, suggesting that these metabolites were associated with better anti-inflammatory outcomes (60).

In this context, the present study found that higher baseline levels of KynA were associated with greater improvement in *Depressed Mood* score at 230 minutes and Day 3 and with *Negative Cognition* score at Day 3. In contrast, lower baseline KYN levels were linked to improved *Anhedonia* score at Day 3. Interestingly, higher baseline QA levels were associated with greater improvement in *Depressed Mood* score post-ketamine infusion at 230 minutes and Day 3, and with greater improvement in *Negative Cognition* score at Day 3.

With regard to QA in particular, selective serotonin reuptake inhibitor (SSRI) treatment was found to reduce QA levels in the rodent brain (61); in contrast, this study found that ketamine had no direct impact on QA levels in humans, though—as noted above—it did significantly affect other KYN byproduct metabolites, including IDO, KYN, and KynA. Interestingly, previous postmortem studies found elevated QA levels in the brains of severely depressed or suicidal individuals (33), suggesting an excess of QA's precursor, KYN, which would thus be available for conversion to QA. There are two potential reasons why, under pathological conditions, the QA pathway appears to predominate over the neuroprotective KynA pathway (62). First, while microglia produce QA, astrocytes produce KynA; studies have shown that, in response to increased microglial activity, MDD participants typically have significant astrocytic dysfunction in critical areas such as the subgenual prefrontal cortex (63, 64). Second, QA binds with 10-fold greater affinity to NR2B NMDA glutamate receptor subunits, which are preferentially localized in the prefrontal cortex, amygdala, hippocampus, and ventral striatum (65). These regions thus bear a greater excitotoxic burden, which often reflects activation of the KYN pathway in the brain. KynA has no such preferential localization, though it should be noted that KynA inhibition can lead to inflammation and excitotoxicity. Previous studies found that a higher plasma KynA/QA ratio—a putative neuroprotective marker—was positively correlated with hippocampal and amygdalar volumes (66).

KYN readily crosses the blood-brain barrier. Overall, 40% of KYN in the CNS is produced in the brain, and 60% is supplied by TDO, which is present only in the liver (67). For this reason, it is almost certain that the increased plasma levels of KYN and KynA observed in the present study after ketamine administration derive from the liver. Interestingly, peripheral KYN possesses pronounced anti-inflammatory properties; it is known to block T-cell proliferation, induce T-cell death, and suppress the activity of natural killer cells and antigen-presenting cells (e.g., dendritic cells, monocytes, and macrophages) (29, 68). Thus, ketamine-induced increases in hepatic KYN production would be expected to counter the substantial peripheral proinflammatory state associated with depression. However, increased hepatic KYN production would also present a greater KYN load to the brain. While this might ordinarily increase production of toxic metabolites, the ketamine-induced suppression of IDO could protect against this potential eventuality.

Given the chronicity and long-lasting changes underlying BD, considerable evidence suggests that epigenetic modifications are involved in the pathophysiology of both depression (69) and glutamatergic dysregulation (70). Epigenetic mechanisms also help regulate kynurenine 3-monooxygenase (KMO), a critical enzyme in the KYN pathway that influences bioactive KYN pathway metabolites (71). Recent genetic, epigenetic, and pharmacological studies are targeting KMO as a way of impacting the bioactive byproducts

of the KYN pathway, including KynA (72, 73). Interestingly, preclinical studies found that ketamine's rapid-acting antidepressant actions are in part mediated epigenetically (74, 75). In this context, it is highly likely that ketamine's effects on the KYN pathway may be mediated by epigenetic mechanisms.

One key strength of this study is that our participants were well-characterized and hospitalized for several weeks before and after ketamine administration. The study was also associated with several limitations, including the absence of a control group. In addition, 10 participants were missing inflammatory cytokine data. Because CSF measures were lacking, another potential limitation is that only peripheral measures of KYN pathway mediators were assessed. Finally, the lack of ethnic diversity in the cohort may limit the generalizability of the results.

Despite these limitations, this study is the first to demonstrate that ketamine, in addition to exerting rapid and sustained antidepressant effects, also impacts key components of the KYN pathway. These rate-limiting enzymes and analytes are highly impacted by inflammation and stress, underscoring ketamine's acute anti-inflammatory effects.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

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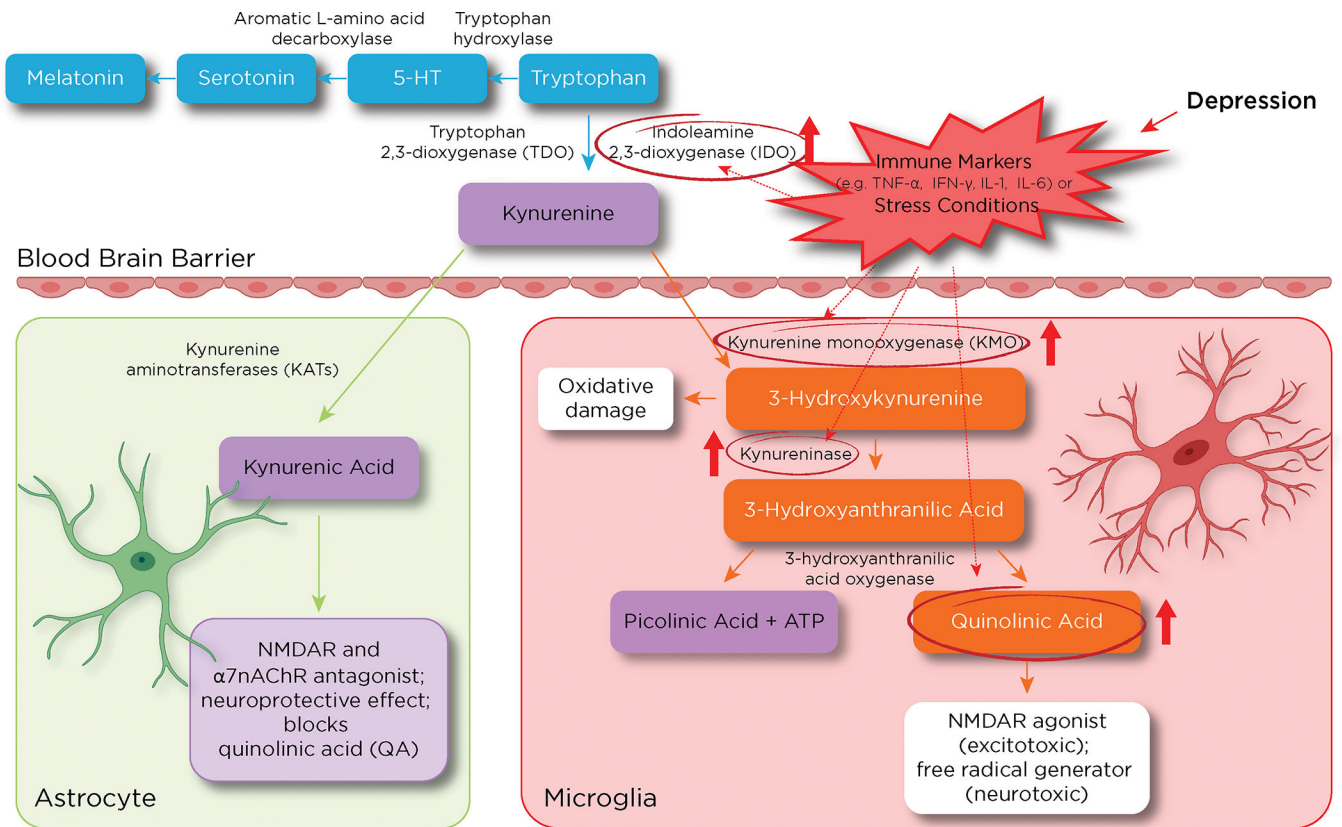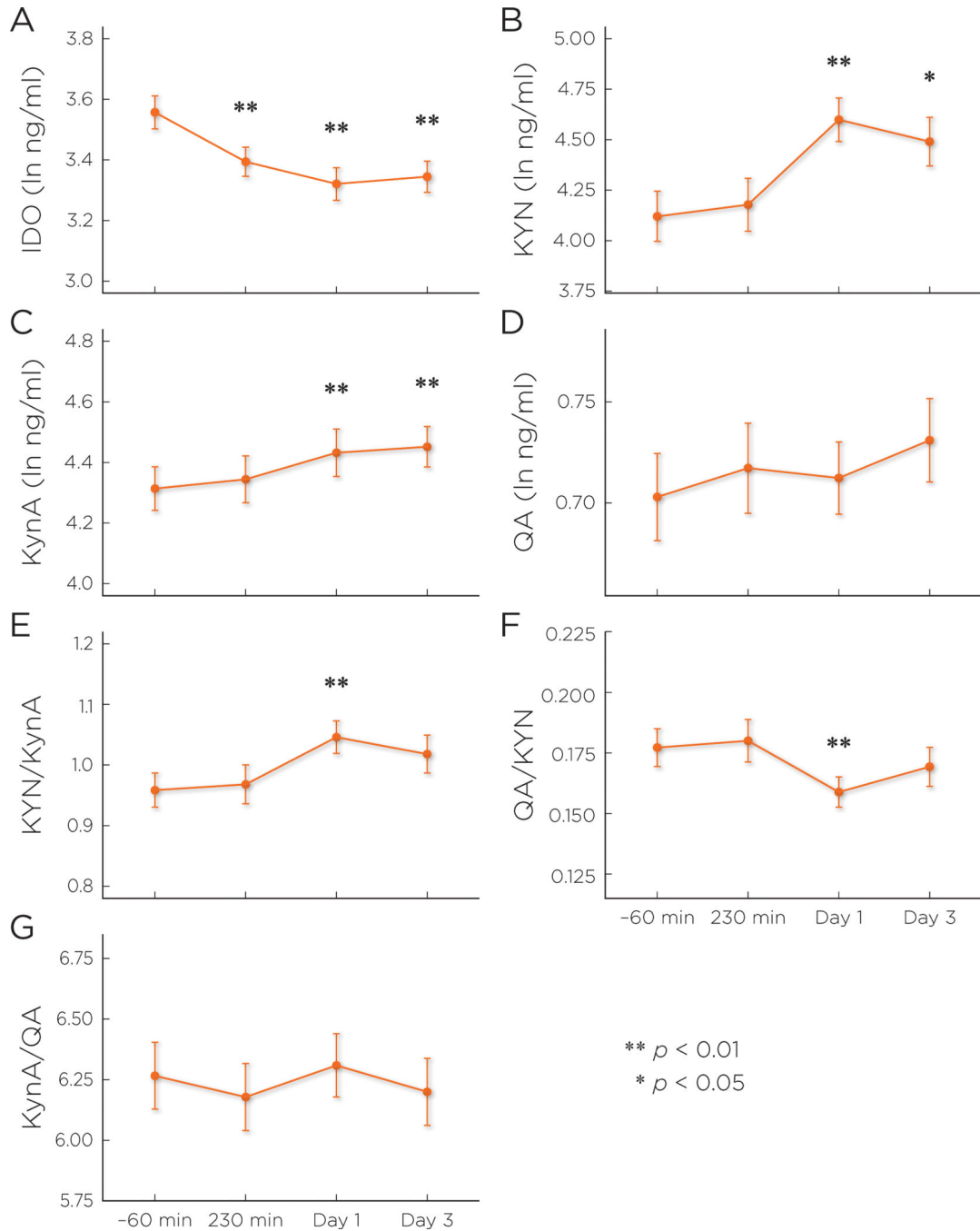
**Figure 1.**
The impact of depression on the kynurenine (KYN) pathway in brain and periphery. The figure depicts KYN metabolites and their related effects on neuronal cells (inside boxes) and the enzymes that metabolize them (arrows). The impact of inflammation or stress-related conditions on key rate-limiting enzymes such as indoleamine 2,3-dioxygenase (IDO) shifts KYN metabolism towards microglial byproducts such as 3-hydroxykynurenine (3-HK) and quinolinic acid (QA). This metabolic change is associated with elevated oxidative stress (3-HK and QA) and glutamate excitotoxicity (QA) that could contribute to depressive symptoms (right panel). Conversely, during homeostasis, substantial amounts of KYN are converted to kynurenic acid (KynA), a process mediated by kynurenine aminotransferase II (KAT II) in astrocytes (left panel). At physiologic levels, KynA is an N-methyl-D-aspartate receptor (NMDAR) antagonist and contributes to the clearance of glutamate spillover in the brain.

Abbreviations: TNF-α: tumor necrosis factor alpha; IFN-gamma: interferon gamma; IL-6: interleukin-6; α7nAChR: alpha-7-nicotinic acetylcholine receptor.

**Figure 2.**
Results of mixed models: post-ketamine change in the kynurenine (KYN) pathway. Least square mean estimated scores (with standard error) are plotted by time point. Significance refers to change from baseline (−60 minutes). IDO: indoleamine 2,3-dioxygenase; KYN: kynurenine; KynA: kynurenic acid; QA: quinolinic acid. Results of the full analysis can be found in Supplementary Table S2.
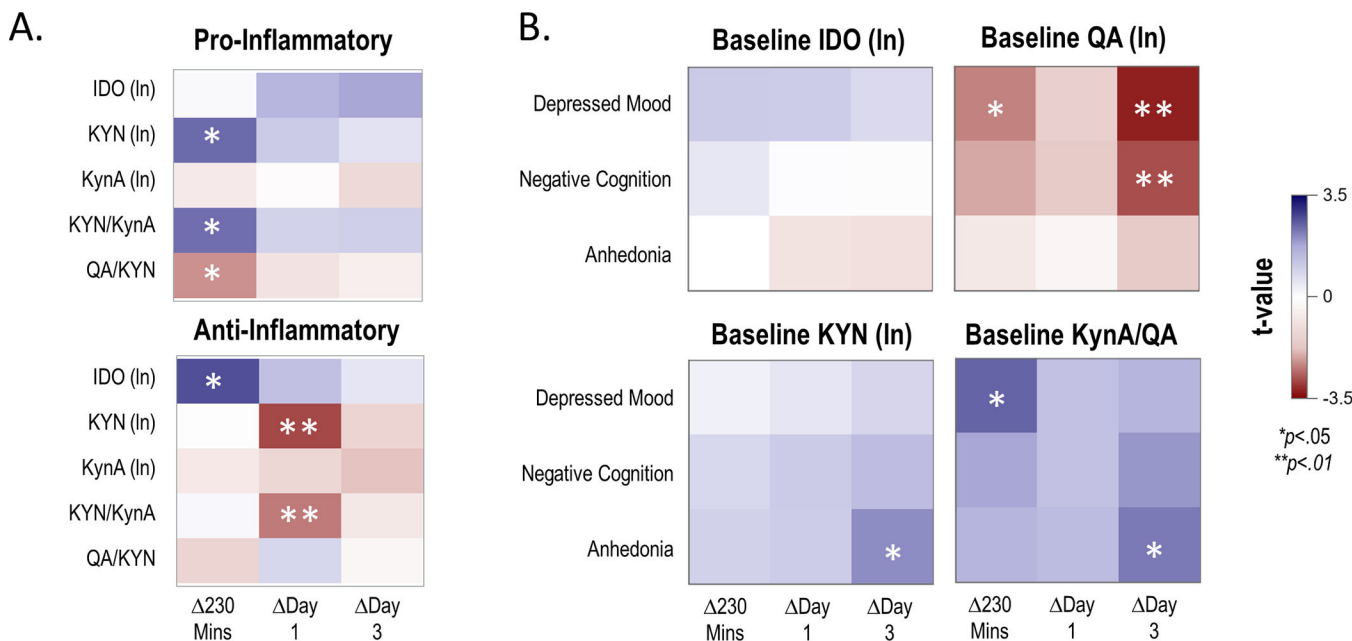
**Figure 3.**

Baseline inflammatory cytokine component scores as moderators of change in the kynurenine (KYN) pathway (Panel A), and baseline KYN levels as moderators of change in depressive symptom ratings (Panel B).

Panel A: Results of a mixed model with component scores of pro-inflammatory (top) or anti-inflammatory (bottom) cytokines entered as moderators of change in KYN pathway analytes (Y-axis) at three time points post-ketamine infusion (X-axis). T-values (all df=24) for the simple slope of the moderator for change at each time point are plotted; positive values indicate that higher cytokine component scores were associated with increases in respective KYN pathway analytes, and negative values indicate that higher cytokine component scores were associated with decreases in respective KYN pathway analytes.

Panel B: Results of a mixed model with baseline KYN pathway member entered as a moderator of change in depressive symptom ratings (Y-axis) post-ketamine infusion. T-values (all df=33) for the simple slope of the moderator for change at each time point are plotted; positive values indicate that higher baseline KYN pathway concentrations were associated with less improvement in depressive symptoms, and negative values indicate that higher baseline KYN pathway concentrations were associated with more improvement in depressive symptoms. In both models, age, body mass index (BMI), and type of mood stabilizer were included as covariates. Results of the full analysis can be found in Supplementary Table S5 and Supplementary Table S6.

IDO: indoleamine 2,3-dioxygenase; KYN: kynurenine; KynA: kynurenic acid; QA: quinolinic acid.

**TABLE 1.**

Participant Demographics

|  | N (%) | Mean ± SD |
|---|---|---|
| **Age** | 39 (100) | 45.92 ± 10.52 |
| **Sex** | | |
| Male | 16 (41) | |
| Female | 23 (59) | |
| **Race** | | |
| Caucasian | 32 (82) | |
| African-American | 4 (10) | |
| Other | 2 (5) | |
| Not reported | 1 (3) | |
| **BMI** | | 29.84 ± 5.83 |
| **Age of illness onset** | | 17.51 ± 6.88 |
| **MADRS Total** | | 33.00 ± 4.39 |
| **Substance Use Disorder** | 18 (46%) | |
| **Alcohol Dependence** | 14 (36%) | |
| **Generalized Anxiety Disorder** | 6 (15%) | |
| **Mood stabilizer** | | |
| Lithium | 26 (67) | |
| Valproic Acid | 13 (33) | |

**Note.** BMI data were missing for one participant; age of onset was missing for two participants; substance/alcohol abuse data were missing for one participant; and comorbid psychiatric diagnosis data were missing for five participants. BMI: body mass index; MADRS: Montgomery-Asberg Depression Rating Scale.

**TABLE 2.**

Results of mixed models predicting post-ketamine change from baseline in the KYN pathway

| Dependent Variable | | 230 minutes | Day 1 | Day 3 |
|---|---|---|---|---|
| IDO (ln) | Change from baseline (95% CI) | −0.16 (−0.27 – −0.06) | −0.24 (−0.35 – −0.12) | −0.2 (−0.31 – −0.1) |
| | Statistical Test | t(34) = −3.05, **p = .004** | t(34) = −4.12, **p = .0002** | t(34) = −3.91, **p = .0004** |
| KYN (ln) | Change from baseline (95% CI) | 0.07 (−0.11 – 0.26) | 0.5 (0.23 – 0.76) | 0.39 (0.08 – 0.7) |
| | Statistical Test | t(34) = 0.77, p = .45 | t(34) = 3.69, **p = .0008** | t(34) = 2.44, **p = .02** |
| KynA (ln) | Change from baseline (95% CI) | 0.03 (−0.04 – 0.1) | 0.12 (0.04 – 0.2) | 0.15 (0.06 – 0.23) |
| | Statistical Test | t(34) = 0.82, p = .42 | t(34) = 2.85, **p = .007** | t(34) = 3.36, **p = .002** |
| QA (ln) | Change from baseline (95% CI) | 0.01 (−0.01 – 0.04) | 0.01 (−0.01 – 0.04) | 0.03 (−0.02 – 0.08) |
| | Statistical Test | t(34) = 1.28, p = .21 | t(34) = 0.94, p = .35 | t(34) = 1.26, p = .22 |
| KYN/KynA | Change from baseline (95% CI) | 0.01 (−0.03 – 0.06) | 0.09 (0.03 – 0.15) | 0.07 (−0.01 – 0.14) |
| | Statistical Test | t(34) = 0.59, p = .56 | t(34) = 2.91, **p = .006** | t(34) = 1.76, p = .087 |
| QA/KYN | Change from baseline (95% CI) | 0 (−0.01 – 0.01) | −0.02 (−0.03 – −0.01) | −0.01 (−0.02 – 0.01) |
| | Statistical Test | t(34) = 0.24, p = .81 | t(34) = −3.31, **p = .002** | t(34) = −0.91, p = .37 |
| KynA/QA | Change from baseline (95% CI) | −0.09 (−0.32 – 0.13) | 0.03 (−0.18 – 0.25) | −0.12 (−0.42 – 0.19) |
| | Statistical Test | t(34) = −0.82, p = .42 | t(34) = 0.29, p = .78 | t(34) = −0.75, p = .46 |

IDO: indoleamine 2,3-dioxygenase; KYN: kynurenine; KynA: kynurenic acid; QA: quinolinic acid; ln: natural logarithm

**Note:** Results of a mixed model with empirical sandwich variance estimator and repeated effect of time. Type 3 tests are shown in Supplementary Table S2. Table shows specific contrast between estimated values at baseline and follow-up (illustrated in Figure 2). Age, body mass index (BMI), and type of mood stabilizer were included as covariates in all analyses.