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Kynurenine pathway metabolism and neurobiology of treatment-resistant depression: Comparison of multiple ketamine infusions and electroconvulsive therapy

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Abstract

Current first-line antidepressants can take weeks or months to decrease depressive symptoms. Low dose ketamine, an N-methyl-D-aspartate (NMDA) receptor antagonist, shows potential for a more rapid antidepressant effect, with efficacy also evident in previously treatment-resistant populations. However, a greater understanding of the physiological mechanisms underlying such effects is required. We assessed the potential impact of ketamine infusion on neurobiological drivers of kynurenine pathway metabolism in major depression (HPA axis hyperactivity, inflammation) in patients with treatment-resistant depression compared to gender-matched healthy controls. Furthermore, we assessed these biomarkers before and after electroconvulsive therapy (ECT), which is currently the gold standard for management of treatment-resistant depression. As previously demonstrated, treatment with ketamine and ECT was associated with improved depressive symptoms in patients. At baseline, waking cortisol output was greater in the ECT cohort, kynurenine was greater in the ketamine cohort, and kynurenic acid was less in patients compared to healthy controls, although inflammatory markers (IL-6, IL-8, IL-10 or IFN- γ) were similar in patients and controls. Furthermore, in patients who responded to ECT, the cortisol awakening response was decreased following treatment. Despite a trend towards lesser kynurenine concentrations in those who responded to ketamine, ketamine was not associated with significant alterations in any of the biomarkers assessed.

Keywords: Depression; ketamine; cortisol; immune; cytokine; kynurenine

Introduction

Major depression is the leading cause of disability in the world (WHO, 2016). Molecular biological markers of depression and remission may aid diagnosis, prognosis and prediction of which treatment strategies will work best (see Gururajan et al., 2016a, for a review). Hypothalamic-pituitary-adrenal (HPA) axis hyperactivity features prominently in the neurobiology of depression (Pariante & Lightman, 2008). This dysregulation may be due to upregulation of the immune system in depression (e.g. Raison et al., 2006), creating a vicious cycle in major depression (Kim et al., 2016). Furthermore, treatment of major depression can enhance anti-inflammatory cytokine activity (e.g. Maes et al., 1997; O'Brien et al., 2004; Dowlati et al., 2010).

Altered HPA axis and inflammatory activity may in turn upregulate enzymes which will lead to increased metabolism of tryptophan along the kynurenine pathway; indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) (e.g. Ruddick et al., 2006; Badawy, 2017; Cervenka et al., 2017). The kynurenine pathway has been implicated in a number of neuropsychiatric as well as neurodegenerative diseases (see O'Farrell & Harkin, 2017, for a review), and may even play a key role in the etiology of psychiatric disorder (Schwarcz & Stone, 2017). In patients with major depression, heightened plasma kynurenine levels have been observed (Sublette et al., 2011), and there is also evidence of lowered peripheral concentrations of kynurenic acid, a neuroprotective metabolite of kynurenine (Myint et al., 2007). The interplay of HPA axis activity, immune system activity and tryptophan metabolism provides a unifying neurobiological framework for depression that might explain its key symptoms (see Clarke et al., 2017, Kennedy et al., 2017).

Serotonin has become a major target for treatment of major depression via first-line monoaminergic antidepressants such as selective serotonin reuptake inhibitors (e.g.

Ramachandrai et al., 2011; cf. Andrews et al., 2015, for an alternative account). However, these drugs can take a number of weeks to work, and a substantial proportion of patients do not respond (e.g. Trivedi et al., 2006). New pharmacological targets and therapies are thus urgently needed (O'Leary et al., 2015). Evidence suggests that ketamine, which affects the glutamergic system (see review by Naughton et al., 2014), leads to treatment response in treatment-resistant depression (Zarate et al., 2006). However, the side effect profile of ketamine, which still requires further research in clinical settings (Short et al., 2017), and which necessitates careful patient screening and monitoring (Sanacora et al., 2017), as well as the potential for abuse of the drug (Kalsi et al., 2011), mean that there is a need for safer precision therapies. A better understanding of ketamine's underlying mechanistic effects could allow for the development of such treatment options. Furthermore, the antidepressant effect of a single ketamine infusion may not persist beyond a week, although repeated infusions may maintain these effects for at least 2-3 weeks (Murrough et al., 2013; Singh et al., 2016), and so multiple infusions may be given in a clinical context to maintain effects. Consequently, a better understanding of the effects of multiple infusions is required.

The pro-inflammatory cytokine IL-6 was predictive of treatment response to ketamine in one study (Yang et al., 2014), though not in a more recent study (Park et al., 2017). There is also evidence that ketamine can increase cortisol output in healthy volunteers (from 30 minutes before and to three hours after ketamine infusion in the morning; Khalili-Mahani et al., 2015). However, there is a lack of research examining the impact of ketamine on the cortisol awakening response, a key variable in psychoneuroendocrinological research related to depression (Stalder et al., 2016). Similarly, there is a paucity of research examining treatment effects on kynurenine levels in patients with major depression, although there is preclinical evidence indicating that ketamine prevented the development of lipopolysaccharide-induced depressive like behaviour, which occurs via the activation of IDO

(O'Connor et al., 2009). Indeed, it has been proposed that the kynurenine pathway might represent a common target for ketamine with respect to the inflammatory and glutamate hypotheses of depression (Miller, 2013).

In the current research, we examined the neurobiology of major depression via cortisol awakening response, plasma cytokine levels (IL-6, IL-8, IL-10, and IFN- γ), and kynurenine pathway metabolites. We compared these measures in patients with treatment-resistant major depressive disorder to healthy controls. Furthermore, we examined whether these biological factors were altered by three infusions of ketamine, compared to electroconvulsive therapy, a highly established and well-validated intervention for treatment-resistant depression (UK ECT Review Group, 2003).

Material and methods

Ethical approval for this research was obtained from the Clinical Research Ethics Committee of the Cork Teaching Hospitals (EMC 3(nn) 08/11/11), which is nationally recognised by the Department of Health & Children, Ireland, and the Irish Medicine Board, Ireland (now the Health Products Regulatory Authority; IMB: EudraCT number: 2011-003654-40). The ECT component of this research was approved by the Research Ethics Committee of St. Patrick's Mental Health Services (Protocol No, 21/12).

Design

The impact of treatment-resistant depression (TRD) was examined through cross-sectional comparisons between patients with TRD and healthy controls. A repeated measures design was used to assess the impact of treatment (ketamine or ECT) on biomarkers of depression (cortisol awakening response, cytokines and tryptophan/kynurenine pathway metabolites), with biomarkers measured before and after treatment. Depressive symptoms were assessed using the 17-item Hamilton Depression Rating Scale (HDRS), and patients who showed a reduction in their HDRS scores of greater than 50% were classed as responders.

Participants

Patients had a current DSM-IV diagnosis of major depressive disorder at the time of the clinical trial. Seventeen patients were recruited for the ketamine arm of the trial, which was conducted at South Lee Community Mental Health Service in Cork, Ireland. Twenty patients with major depressive disorder, currently depressed who were being assessed for ECT were recruited at St Patrick's University Hospital (see Supplementary Table 1 for participant demographics). All patients had failed to respond to at least two adequate trials of antidepressant medication, as assessed with a modified version of the Antidepressant Treatment History Form (Prudic et al., 1990, 1996; Sackheim et al., 1990, 2001). Participants

were excluded in the case of any significant physical illness including acute or chronic infections, endocrine, immune or metabolic disorder such as autoimmune disorders, inflammatory bowel disease, acquired immunodeficiency syndrome, or if they were greater than 10% above ideal body weight. In the ketamine arm of the trial, five participants dropped out following the first infusion of ketamine and a further two dropped out after the second infusion. Two participants dropped out of the ECT arm of the trial prior to completion of post-ECT assessment.

Twenty control participants were recruited from Cork University Hospital staff. Control participants were screened for personal or family history (1st degree relative) of mental disorder and excluded if positive. All healthy controls were within 10% of ideal body weight.

Procedure

Participants received either twice-weekly brief-pulse bitemporal ECT (Semkovska et al., 2016) or sub-anaesthetic (0.5mg/kg) intravenous infusions of ketamine once a week for up to three weeks (see Allen et al., 2015, for detailed information).

Saliva samples were collected using Salivettes (Sarstedt, Germany); baseline samples were collected on the morning prior to study visits, and post-intervention samples were collected one week following each ketamine infusion or 4-7 days following the final ECT session. Saliva samples were taken upon waking, 30 minutes post waking and 150 minutes post waking. Participants were advised to take the first saliva sample as soon as they woke up, and not to eat or drink 15 minutes prior to the second or third sample, and not to brush their teeth until all samples had been collected. We did not require that participants wake at a specific time, but followed their normal routine as closely as possible. Upon receipt of the samples, the salivettes or test tubes were immediately centrifuged (3000 rpm for 15 minutes) and the saliva collected was transferred to sterile 1.5ml containers and frozen at -80 degrees Celsius.

Six millilitre samples of whole blood (fasting) were collected between 8 a.m. and 11 a.m. on the morning of ketamine infusion for determination of baseline IL-6, IL-8, IL-10, IFN- γ concentrations. Similar samples (non-fasting) were taken two hours after ketamine infusion. A fasting sample was taken 24 hours later and one week later in the ketamine cohort. This was repeated two hours and one week post ketamine infusion over the second and third infusions for participants who received these infusions. In the ECT cohort, whole blood (fasting) was similarly collected between 8 a.m. and 11 a.m. on the morning of ketamine infusion for determination of baseline cytokine concentrations. Fasting samples were taken 4-7 days following the final ECT session. Samples were centrifuged immediately and frozen at -80 degrees Centigrade. See Supplementary Figure 1 for timeline of sampling for the ketamine and ECT cohort.

Biochemical analysis

Cortisol concentrations were measured in saliva using Enzo® enzyme immunoassay according to manufacturer's instructions. Assay detection limit was 0.16 nmol/L. Inter and intra-assay % C.Vs were 11.24% and 8.2% respectively.

Measurement of IL-6, IL-8, IL-10, and IFN- γ concentrations were conducted in duplicate using MesoScale Discovery custom assays according to manufacturer's instructions. IL-8: Lower limit of detection = 0.04 pg/ml. Intra-assay CV = 3.6% and inter-assay CV = 7.1%. IL-6: Lower limit of detection = 0.06 pg/ml. Intra-assay CV = 4.5% and inter-assay CV = 7.3%. IL-10: Lower limit of detection = 0.03 pg/ml. Intra-assay CV = 3.7% and inter-assay CV = 10.1%. IFN- γ : Lower limit of detection = 0.2 pg/ml. Intra-assay CV = 5% and inter-assay CV = 9.2%.

Total tryptophan and kynurenine pathway metabolites were determined as previously described (Clarke et al., 2009). Briefly, plasma samples were spiked with internal standard

(3-Nitro-l-tyrosine) prior to being deproteinised by the addition of 20 μ l of 4 M perchloric acid to 200 μ l of sample. Samples were centrifuged at 21,000g on a Hettich Mikro 22R centrifuge (AGB, Dublin, Ireland) for 20 minutes at 4 °C and 100 μ l of supernatant transferred to a HPLC vial for analysis on the HPLC system (UV and FLD detection). All samples were injected onto a reversed phase Luna 3 μ m C18 (2) 150 \times 2 mm column (Phenomenex), which was protected by KrudKatcher disposable pre-column filters (Phenomenex) and SecurityGuard cartridges (Phenomenex). The mobile phase consisted of 50 mM acetic acid and 100 mM zinc acetate with 3 % (v/v) acetonitrile and was filtered through Millipore 0.45- μ m HV Durapore membrane filters (AGB) and vacuum degassed prior to use. Compounds were eluted isocratically over a 30-min runtime at a flow rate of 0.3 ml/min after a 20- μ l injection. The column was maintained at a temperature of 30 °C, and samples/standards were kept at 8 °C in the cooled autoinjector prior to injection. The fluorescent detector was set at an excitation wavelength of 254 nm and an emission wavelength of 404 nm. The UV detector was set to 330 nm. l-Tryptophan and kynurenine were identified by their characteristic retention times as determined by standard injections which were run at regular intervals during the sample analysis. Analyte: Internal standard peak height ratios were measured and compared with standard injections, and results were expressed as nanogram per millilitre of plasma.

Statistical analysis

Statistics were calculated using SPSS-18. A p-value of 0.05 was selected as the threshold of statistical significance. Graph Pad Prism version 5.0 for windows (Graph Pad Software, San Diego, CA, USA, (www.graphpad.com)) was used for graph design. Where normality was violated, square root or natural log transformations were used as necessary. Area under the curve with respect to ground (AUC_g) and increase (AUC_i) were calculated according to guidelines of Pruessner et al (2003). Differences at baseline between patients in the ketamine

and ECT cohorts and healthy controls were calculated using ANOVA (with LSD post-hoc test). The effect of ketamine or ECT was assessed using either repeated measures ANOVA (ketamine) or t-tests (ECT). Furthermore, to assess the relationship between clinical symptoms and the biological variables examined, we computed bivariate (Pearson) correlations between the change in symptom severity (as measured with the HDRS) and the corresponding changes in the biological variables at each timepoint.

Results

Participant characteristics

The characteristics of the participants group are outlined in Supplementary Table 1. The three groups were well matched on gender, although the ECT group were somewhat older than the ketamine group and the healthy controls, who were similar to each other in age.

Treatment-resistant depression and assessed biomarkers

There was no significant difference between patients in the ketamine and ECT cohorts and controls in terms of IL-6, IL-8, IL-10 or IFN- γ concentrations. However, salivary cortisol output differed between groups (AUCg: $F(2, 48) = 4.74, p = .013$), and was greater at baseline in the ECT cohort compared to either the ketamine cohort ($p = .006$) or healthy controls ($p = .018$); the ketamine cohort and healthy controls did not differ from each other in cortisol levels.

Plasma kynurenine concentrations differed between groups, $F(2, 53) = 3.93, p = .026$; it was significantly greater in the ketamine cohort compared to both healthy controls ($p = .045$) and the ECT cohort ($p = .009$), who did not differ from each other. There was a marginally significant difference between groups in terms of plasma tryptophan concentrations, $F(2, 54) = 2.91, p = .063$; the ketamine cohort had greater tryptophan levels than the ECT cohort ($p = .02$), while the healthy control group did not differ from either cohort. The groups were similar in terms of kynurenine:tryptophan ratio. Kynurenic acid also differed between groups, $F(2, 28) = 4.93, p = .015$, and was lower in both the ketamine ($p = .006$) and ECT cohort ($p = .013$) compared to the healthy controls, although the two cohorts of TRD patients did not differ from each other. Similarly, the kynurenic acid:kynurenine also differed between

groups, $F(2, 28) = 13.41$, $p < .001$, and was lower in both the ketamine ($p < .001$) and ECT cohort ($p = .036$) compared to the healthy controls.

INSERT FIGURE 1 ABOUT HERE

Impact of treatment with ketamine and ECT

A majority of patients indicated a clinical response (a reduction in HDRS of 50% or more) to ketamine at all post-baseline timepoints except for one week following the first infusion. Of 17 participants receiving the first ketamine infusion, 13 showed a clinical response at 2 hours and 24 hours post-infusion, and 7 at one week post-infusion. Of 12 participants receiving the second infusion, 12 showed a response at 2 hours post-infusion and 8 at one week. Of 10 participants receiving the third infusion, 9 showed a response at 2 hours and 6 at one week. Of 18 participants who completed the HDRS following ECT, 9 showed a clinical response.

For the ketamine cohort as a whole, treatment did not affect kynurenine pathway metabolism. However, there was a trend towards a decrease in kynurenine concentration in ketamine responders at two hours after the first infusion, $t(12) = 2.01$, $p = .067$, and a trend for a reduced kynurenine/tryptophan ratio at 24 hours following the first infusion, $t(11) = 2.15$, $p = .054$. ECT did not significantly normalise kynurenine pathway metabolism, for the ECT cohort as a whole or for responders to ECT intervention. Reduction in depressive severity following ketamine/ECT was correlated with increased kynurenic acid at 2 hours following the second infusion ($r = -.1$, $p = .013$), and at one week after the third infusion ($r = -.1$, $p = .005$), but there was no consistent pattern of correlation between level of treatment response and changes in the kynurenine pathway.

INSERT FIGURES 2 AND 3 ABOUT HERE

Ketamine treatment was not associated with a change in the cortisol awakening response. Ketamine responders did not have significantly altered cortisol awakening response post-treatment. ECT treatment did not affect cortisol in the ECT cohort overall, nor was there was an impact of ECT treatment on overall salivary cortisol profile. However, the area under the curve with respect to increase was significantly lesser following ECT in patients who responded to ECT, $t(5) = 2.68$, $p = .044$; this is likely due to a highly significant attenuation in the cortisol increase between waking and 30 minutes post-waking, $t(5) = 4.41$, $p = .007$. Change in depressive severity following ketamine/ECT was not correlated with any change in cortisol awakening response.

INSERT FIGURES 4 AND 5 ABOUT HERE

There was no significant change in the immune markers following ketamine treatment or ECT treatment for these cohorts as a whole. ECT responders did not show significantly altered cytokine levels post-ECT; nor did ketamine responders have any significant alterations in any of the cytokines examined. Reduction in depressive severity following ketamine/ECT was correlated with higher IL-6 at 24 hours following the first infusion ($r = -0.66$, $p = .007$), and higher IL-8 at two hours after the third infusion ($r = -0.64$, $p = .048$), but

there was no consistent pattern of correlation between level of treatment response and changes in immune markers.

INSERT FIGURE 6 AND 7 ABOUT HERE

Discussion

To the best of our knowledge, this is the first research in patients to examine whether the antidepressant effects of ketamine are associated with changes in kynurenine pathway metabolism. The cohort of patients receiving ketamine had greater plasma kynurenine concentrations at baseline compared to healthy controls, consistent with previous research (e.g. Sublette et al., 2011). There was also a trend towards lesser kynurenine concentrations two hours after a first ketamine infusion in those patients who responded to ketamine at this time, although this was not statistically significant; nor was it sustained following later infusions. Concentrations of the neuroprotective metabolite kynurenic acid were also lower in both cohorts of patients with treatment-resistant depression (consistent with Schwieler et al., 2016), although this was not affected by treatment with either ECT or ketamine, despite previous evidence suggesting broad changes in the kynurenine pathway during ECT (Guloksuz et al., 2015). Other studies have not shown differences between depressed patients and controls in tryptophan, kynurenine, or tryptophan: kynurenine ratio (Sorgdrager et al., 2017). As ketamine nonetheless reduced depressive severity, the current results suggest that resolution of kynurenine pathway activation may not be necessary for symptomatic improvement. However, it will be important in future studies to conduct a more comprehensive assessment of kynurenine pathway metabolites that will allow more definitive conclusions regarding their role in the pathophysiology and treatment of depression. This includes an assessment of the neurotoxic arm of the kynurenine pathway, where the use of liquid chromatography or gas chromatography/mass spectrometry may be of particular utility. It will also be of interest to determine whether different ketamine protocols have different effects on the tryptophan-metabolic pathway, and to what extent this is mediated by the immune system or the HPA axis.

Both cohort of patients, as well as the healthy volunteers, displayed an increase in cortisol following waking. However, it is notable that only the group that received ECT had either of the posited biological conditions leading to increased kynurenine pathway metabolism (increased cortisol), but it was the group that received ketamine (who did *not* differ from healthy controls in inflammatory markers or cortisol) that had increased kynurenine. Furthermore, both groups of TRD patients had a modest reduction in the kynurenine acid:kynurenine ratio, despite their different inflammatory/cortisol profiles. Patients were not randomised to the ketamine or ECT arm of the study, and it was not possible to match participants on all relevant characteristics; it is possible that the differences in baseline levels of cortisol may have influenced treatment responses. Nonetheless, our results suggest that the neurobiological abnormality in kynurenine pathway metabolism can be an ongoing effect of depression, despite resolution of the biology thought to drive it, and furthermore that the inflammatory and HPA axis abnormality can resolve without symptomatic improvement. The observed lack of altered cytokine levels is inconsistent with previous evidence of heightened pro-inflammatory activity in depressed patients (e.g. Maes et al., 1997; Dowlati et al., 2010). The current results may be due to the fact that, due to ethical and recruitment considerations, the patients assessed in this study were not treatment-naïve. Antidepressant treatments may have already normalised inflammation levels in patients (Köhler et al., 2017) and HPA axis activity in the ketamine cohort (Schüle, 2017) when the patients were assessed for the first time in the current study. In future research, it would also be of interest to assess the more short-term effects of ECT, as a review has suggested that there may be differential short- and long-term effects of ECT on immune activity, with a single session leading to immune activation, whereas repetitive ECT leads to downregulation (Guloksuz et al., 2014).

Patients in the ECT cohort had heightened cortisol at awakening, and this was attenuated following ECT treatment in those who responded to ECT. However, there was a lack of HPA

axis alteration in the ketamine cohort of TRD patients compared to healthy controls. Careful adherence to sample timing is important when assessing waking cortisol (see consensus guidelines, Stalder et al., 2016). As the ECT cohort were inpatients at the time of salivary sampling, sampling could be monitored more closely; it is thus possible that differences in the process of sampling may account for some of the observed differences in cortisol output in this cohort. Despite the rapid improvement in depressive symptoms in a majority of TRD patients following ketamine treatment, symptom improvement following ketamine treatment was not associated with changes in waking cortisol or cytokines. Again, we cannot rule out that ketamine infusion in treatment-naïve patients may have shown changes in these biomarkers, which may have been normalised due to antidepressant treatment.

Of course, other biomarkers of depression may interact with the effects observed in the current work; consistent with other research (e.g. Haile et al., 2014), our group has previously demonstrated that treatment-resistant depression is associated with lower peripheral concentrations of the neurotrophin brain-derived neurotrophic factor (BDNF), and BDNF levels were heightened following a single infusion of ketamine in patients who responded to ketamine treatment (Allen et al., 2015). Patients with treatment-resistant depression also had altered levels of microRNAs associated with the PI3k-Akt-mTOR signalling pathway (microRNAs are small nucleotide sequences that can regulate gene expression at the transcriptomic level; see review by O'Connor et al., 2012), although these microRNAs were not altered by treatment (Gururajan et al., 2016b). Future work could establish if differences in pre-treatment kynurenine levels could help in optimising treatments for patients with depression. There is evidence that acute tryptophan depletion can reduce kynurenine as well as tryptophan levels (Kennedy et al., 2015), but is also associated with a transient relapse in depressive symptoms in people with remitted depression (Booij et al., 2005). The relationship between tryptophan metabolism and depressive symptomatology is therefore complex, and

the impact of tryptophan metabolites (including serotonin) may differ depending on the timeframe over which it is studied (e.g. Andrews et al., 2015).

It should be acknowledged that major depression is a heterogeneous disorder, and attempts to reconceptualise psychiatric disorders such as depression in neurobiological terms may allow for better prediction of treatment response to pharmacological intervention (e.g. Insel, 2014; Kelly et al., 2017). Recent research in patients with major depression has also identified biotypes in central nervous system neurophysiology which were predictive of responsiveness to treatment with transcranial magnetic stimulation (Drysdale et al., 2017). There are encouraging developments in preclinical evidence of ketamine metabolites that may be associated with an improved adverse effect profile, although they may act through a mechanism other than NMDA receptor inhibition (Zanos et al., 2016); substantial future research will be required into their efficacy in humans. Such research, in combination with findings on peripheral markers of depression such as plasma concentrations of kynurenine or BDNF, may help to better predict treatment to novel treatments such as ketamine, although in identifying key biomarkers, we should bear in mind that more extensive work has been conducted on more clinically established, slower-acting antidepressants.

Furthermore, we cannot ignore the possibility that the antidepressant effects of ketamine observed in this cohort may be related to factors outside the mechanistically oriented biomarkers observed. There is some evidence that the dissociative side effects of ketamine may in fact mediate its antidepressant effects (Luckenbaugh et al., 2014). Consequently, in addressing questions concerning the mechanisms of ketamine, psychological and subjective properties of the drug should not be ignored. Non-pharmacological approaches such as mindfulness meditation may be able to tap into similar psychological mechanisms, for example by creating a psychological “distance from the self”. Such approaches, which have demonstrated positive effect under conditions of chronic stress (e.g. Allen et al., 2017) as

well as in anxiety and depression (e.g. Hofmann et al., 2010) may complement novel pharmacological therapies such as ketamine (Pradhan et al., 2015).

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The authors declare no conflict of interest.

References

- Allen, A.P., Curran, E.A., Duggan, Á., Cryan, J.F., Chorcóráin, A.N., Dinan, T.G., Molloy, D.W., Kearney, P.M., Clarke, G. (2017). A systematic review of the psychobiological burden of informal caregiving for patients with dementia: Focus on cognitive and biological markers of chronic stress. *Neurosci. Biobehav. Rev.* 73, 123-164.
- Allen, A.P., Naughton, M., Dowling, J., Walsh, A., Ismail, F., Shorten, G., Scott, L., McLoughlin, D.M., Cryan, J.F., Dinan, T.G., Clarke, G. (2015). Serum BDNF as a peripheral biomarker of treatment-resistant depression and the rapid antidepressant response: a comparison of ketamine and ECT. *J. Affect. Disord.* 186, 306-311.
- Andrews, P.W., Bharwani, A., Lee, K.R., Fox, M., Thomson, J.A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neurosci. Biobehav. Rev.* 51, 164-188.
- Badawy, A.A. (2017). Tryptophan availability for kynurenine pathway metabolism across the life span: control mechanisms and focus on aging, exercise, diet and nutritional supplements. *Neuropharmacology* 112, 248-263.
- Booij, L., Haffmans, P.J., Spinhoven, P., McNally, R.J. (2005). Acute tryptophan depletion as a model of depressive relapse. *Br. J. Psychiatry* 187(2), 148-154.
- Cervenka, I., Agudelo, L.Z., Ruas, J.L. (2017). Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. *Science*, 357(6349), eaaf9794.
- Clarke, G., Fitzgerald, P., Cryan, J.F., Cassidy, E.M., Quigley, E.M., Dinan, T.G. (2009a). Tryptophan degradation in irritable bowel syndrome: evidence of indoleamine 2,3-dioxygenase activation in a male cohort. *BMC Gastroenterology* 9(1), 6.
- Clarke, G., Stone, T.W., Schwarcz, R. (2017). The kynurenine pathway in health and disease. *Neuropharmacology* 112(B), 235-412.
- Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctôt, K.L. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67(5), 446-457.

- Drysdale, A.T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., Fetcho, R.N., Zebley, B., Oathes, D.J., Etkin, A., Schatzberg, A.F. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nat. Med.* 23, 29-38.
- Gold, P.W., Chrousos, G.P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol. Psychiatry* 7(3), 254-275.
- Guloksuz, S., Arts, B., Walter, S., Drukker, M., Rodriguez, L., Myint, A.M., Schwarz, M.J., Ponds, R., van Os, J., Kenis, G., Rutten, B.P. (2015). The impact of electroconvulsive therapy on the tryptophan–kynurenine metabolic pathway. *Brain Behav. Immun.* 48, 48-52.
- Guloksuz, S., Rutten, B.P., Arts, B., van Os, J., & Kenis, G. (2014). The immune system and electroconvulsive therapy for depression. *J ECT* 30(2), 132-137.
- Gururajan, A., Clarke, G., Dinan, T.G., Cryan, J.F. (2016a). Molecular biomarkers of depression. *Neurosci. Biobehav. Rev.* 64, 101-133.
- Gururajan, A., Naughton, M.E., Scott, K.A., O'Connor, R.M., Moloney, G., Clarke, G., Dowling, J., Walsh, A., Ismail, F., Shorten, G., Scott, L., McLoughlin, D.M., Cryan, J.F., Dinan, T.G., (2016b). MicroRNAs as biomarkers for major depression: a role for let-7b and let-7c. *Transl. Psychiatry* 6(8), e862.
- Haile, C.N., Murrough, J.W., Iosifescu, D.V., Chang, L.C., Al Jurdi, R.K., Foulkes, A., Iqbal, S., Mahoney III, J.J., De La Garza, R., Charney, D.S., Newton, T.F. (2014). Plasma brain derived neurotrophic factor (BDNF) and response to ketamine in treatment-resistant depression. *Int. J. Neuropsychopharmacology* 17(2), 331-336.
- Hofmann, S.G., Sawyer, A.T., Witt, A.A., Oh, D. (2010). The effect of mindfulness-based therapy on anxiety and depression: A meta-analytic review. *J. Consult. Clin. Psychol.* 78(2), 169-183.
- Insel, T.R. (2014). The NIMH research domain criteria (RDoC) project: precision medicine for psychiatry. *Am. J. Psychiatry* 171(4), 395-397.
- Kalsi, S.S., Wood, D.M., Dargan, P.I. (2011). The epidemiology and patterns of acute and chronic toxicity associated with recreational ketamine use. *Emerg. Health Threats J.* 4(1), 7107.
- Kelly, J.R., Clarke, G., Cryan, J.F., Dinan, T.G. (2017). Dimensional thinking in psychiatry in the era of the research domain criteria (RDoC). *Ir. J. Psychol. Med.* doi: 10.1017/ipm.2017.7.

- Kennedy, P.J., Allen, A.P., O'Neill, A., Quigley, E.M., Cryan, J.F., Dinan, T.G., Clarke, G. (2015). Acute tryptophan depletion reduces kynurenine levels: implications for treatment of impaired visuospatial memory performance in irritable bowel syndrome. *Psychopharmacology* 232(8), 1357-1371.
- Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G. (2017). Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology* 112, 399-412.
- Khalili-Mahani, N., Martini, C.H., Olofsen, E., Dahan, A., Niesters, M. (2015). Effect of subanaesthetic ketamine on plasma and saliva cortisol secretion. *Br. J. Anaesth.* 115(1), 68-75.
- Kim, Y.K., Na, K.S., Myint, A.M., Leonard, B.E. (2016). The role of pro-inflammatory cytokines in neuroinflammation, neurogenesis and the neuroendocrine system in major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 64, 277-284.
- Luckenbaugh, D.A., Niciu, M.J., Ionescu, D.F., Nolan, N.M., Richards, E.M., Brutsche, N.E., Guevara, S., Zarate, C.A. (2014). Do the dissociative side effects of ketamine mediate its antidepressant effects? *J. of Affect. Disord.* 159, 56-61.
- Maes, M., Bosmans, E., De Jongh, R., Kenis, G., Vandoelaeghe, E., Neels, H. (1997). Increased serum IL-6 and IL-1 receptor antagonist concentrations in major depression and treatment resistant depression. *Cytokine* 9(11), 853-858.
- Miller, A.H. (2013). Conceptual confluence: the kynurenine pathway as a common target for ketamine and the convergence of the inflammation and glutamate hypotheses of depression. *Neuropsychopharmacology* 38(9), 1607-1608.
- Murrough, J.W., Perez, A.M., Pillemer, S., Stern, J., Parides, M.K., van der Rot, M., Collins, K.A., Mathew, S.J., Charney, D.S., Iosifescu, D.V. (2013). Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol. Psychiatry* 74(4), 250-256.
- Myint, A.M., Kim, Y.K., Verkerk, R., Scharpé, S., Steinbusch, H., Leonard, B. (2007). Kynurenine pathway in major depression: evidence of impaired neuroprotection. *J. Affect. Disord.* 98(1), 143-151.
- Naughton, M., Clarke, G., Olivia, O.F., Cryan, J.F., Dinan, T.G. (2014). A review of ketamine in affective disorders: current evidence of clinical efficacy, limitations of use and pre-clinical evidence on proposed mechanisms of action. *J. Affect. Disord.* 156, 24-35.

O'Brien, S.M., Scott, L.V., Dinan, T.G. (2004). Cytokines: abnormalities in major depression and implications for pharmacological treatment. *Hum. Psychopharmacol.* 19(6), 397-403.

O'Connor, R.M., Dinan, T.G., Cryan, J.F. (2012). Little things on which happiness depends: microRNAs as novel therapeutic targets for the treatment of anxiety and depression. *Mol. Psychiatry* 17(4), 359-376.

O'Connor, J.C., Lawson, M.A., Andre, C., Moreau, M., Lestage, J., Castanon, N., Kelley, K.W., Dantzer, R. (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2, 3-dioxygenase activation in mice. *Mol. Psychiatry* 14(5), 511-522.

O'Farrell, K., Harkin, A. (2017). Stress-related regulation of the kynurenine pathway: Relevance to neuropsychiatric and degenerative disorders. *Neuropharmacology* 112, 307-323.

O'Leary, O.F., Dinan, T.G., Cryan, J.F. (2015). Faster, better, stronger: Towards new antidepressant therapeutic strategies. *Eur. J. Pharmacol.* 753, 32-50.

Pariante, C.M., Lightman, S.L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci.* 31(9), 464-468.

Park, M., Newman, L.E., Gold, P.W., Luckenbaugh, D.A., Yuan, P., Machado-Vieira, R., Zarate, C.A. (2017). Change in cytokine levels is not associated with rapid antidepressant response to ketamine in treatment-resistant depression. *J. Psychiatr. Res.* 84, 113-118.

Pradhan, B., Parikh, T., Makani, R., Sahoo, M. (2015). Ketamine, transcranial magnetic stimulation, and depression specific yoga and mindfulness based cognitive therapy in management of treatment resistant depression: review and some data on efficacy. *Depress. Res. Treat.* 2015, 842817.

Prudic, J., Haskett, R.F., Mulsant, B., Malone, K.M., Pettinati, H.M., Stephens, S., ... & Sackeim, H.A. (1996). Resistance to antidepressant medications and short-term clinical response to ECT. *Am. J. Psychiatry* 153(8), 985-992.

Prudic, J., Sackeim, H. A., & Devanand, D. P. (1990). Medication resistance and clinical response to electroconvulsive therapy. *Psychiatry Res.* 31(3), 287-296.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28(7), 916-931.

- Raison, C.L., Capuron, L., & Miller, A.H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* 27(1), 24-31.
- Ramachandrai, C.T., Subramanyam, N., Bar, K.J., Baker, G., Yeragani, V.K. (2011). Antidepressants: From MAOIs to SSRIs and more. *Indian J. Psychiatry* 53(2), 180-182.
- Ruddick, J.P., Evans, A.K., Nutt, D.J., Lightman, S.L., Rook, G.A., Lowry, C.A. (2006). Tryptophan metabolism in the central nervous system: medical implications. *Expert Rev. Mol. Med.* 8(20), 1-27.
- Sackeim, H.A., Haskett, R.F., Mulsant, B.H., Thase, M.E., Mann, J.J., Pettinati, H.M., ... & Prudic, J. (2001). Continuation pharmacotherapy in the prevention of relapse following electroconvulsive therapy: a randomized controlled trial. *JAMA*, 285(10), 1299-1307.
- Sackeim, H. A., Prudic, J., Devanand, D. P., Decina, P., Kerr, B., & Malitz, S. (1990). The impact of medication resistance and continuation pharmacotherapy on relapse following response to electroconvulsive therapy in major depression. *J. Clin. Psychopharmacol.* 10(2), 96-104.
- Sanacora, G., Frye, M.A., McDonald, W., Mathew, S.J., Turner, M.S., Schatzberg, A.F., Summergrad, P., Nemeroff, C.B. (2017). A consensus statement on the use of ketamine in the treatment of mood disorders. *JAMA Psychiatry* 74(4), 399-405.
- Schwarcz, R., Stone, T.W. (2017). The kynurenine pathway and the brain: challenges, controversies and promises. *Neuropharmacology* 112, 237-247.
- Schwieler, L., Samuelsson, M., Frye, M.A., Bhat, M., Schuppe-Koistinen, I., Jungholm, O., Johansson, A.G., Landén, M., Sellgren, C.M., Erhardt, S. (2016). Electroconvulsive therapy suppresses the neurotoxic branch of the kynurenine pathway in treatment-resistant depressed patients. *J. Neuroinflammation* 13(1), 51.
- Semkovska M, Landau S, Dunne R, Kolshus E, Kavanagh A, Jelovac A, Noone M, Carton M, Lambe S, McHugh C, McLoughlin DM (2016) Bitemporal versus high-dose unilateral twice-weekly electroconvulsive therapy for depression (EFFECT-Dep): a pragmatic, randomised, non-inferiority trial. *Am. J. Psychiatry* 173(4), 408-17.
- Short, B., Fong, J., Galvez, V., Shelker, W., Loo, C.K. (2017). Side-effects associated with ketamine use in depression: a systematic review. *Lancet Psychiatry* doi: 10.1016/S2215-0366(17)30272-9.

Singh, J.B., Fedgchin, M., Daly, E.J., De Boer, P., Cooper, K., Lim, P., Pinter, C., Murrough, J.W., Sanacora, G., Shelton, R.C., Kurian, B. (2016). A double-blind, randomized, placebo-controlled, dose-frequency study of intravenous ketamine in patients with treatment-resistant depression. *Am. J. Psychiatry* 173(8), 816-826.

Sorgdrager, F.J.H., Doornbos, B., Penninx, B.W.J.H., de Jonge, P., Kema, I.P. (2017). The association between the hypothalamic pituitary adrenal axis and tryptophan metabolism in persons with recurrent major depressive disorder and healthy controls. *J. Affect. Disord.* 222, 32-39.

Stalder, T., Kirschbaum, C., Kudielka, B.M., Adam, E.K., Pruessner, J.C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D.H., Miller, R. (2016). Assessment of the cortisol awakening response: expert consensus guidelines. *Psychoneuroendocrinology* 63, 414-432.

Sublette, M.E., Galfalvy, H.C., Fuchs, D., Lapidus, M., Grunebaum, M.F., Oquendo, M.A., Mann, J.J., Postolache, T.T. (2011). Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. *Brain Behav. Immun.* 25(6), 1272-1278.

Trivedi, M.H., Rush, A.J., Wisniewski, S.R., Nierenberg, A.A., Warden, D., Ritz, L., Norquist, G., Howland, R.H., Lebowitz, B., McGrath, P.J., Shores-Wilson, K. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR* D: implications for clinical practice. *Am. J. Psychiatry* 163(1), 28-40.

U.K. ECT Review Group (2003). Efficacy and safety of electroconvulsive therapy in depressive disorders: a systematic review and meta-analysis. *Lancet* 361(9360), 799-808.

World Health Organisation (2016). Depression. Fact Sheet. Retrieved 26/01/2017 from <http://www.who.int/mediacentre/factsheets/fs369/en/>

Yang, J.J., Wang, N., Yang, C., Shi, J.Y., Yu, H.Y., Hashimoto, K. (2015). Serum interleukin-6 is a predictive biomarker for ketamine's antidepressant effect in treatment-resistant patients with major depression. *Biol. Psychiatry* 77(3), e19-e20.

Zanos, P., Moaddel, R., Morris, P.J., Georgiou, P., Fischell, J., Elmer, G.I., Alkondon, M., Yuan, P., Pribut, H.J., Singh, N.S., Dossou, K.S. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature* 533, 481-486.

Zarate, C.A., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen Psychiatry* 63(8), 856-864.

Figure Captions

Figure 1: Baseline levels in healthy controls, ketamine cohort and ECT cohort of (A). plasma IL-6, (B). IL-8, (C). IL-10, (D). IFN- γ , (E). cortisol awakening response, (F). cortisol area under the curve with respect to ground (G). cortisol area under the curve with respect to increase, (H). plasma kynurenine, (I). plasma tryptophan, (J). kynurenine:tryptophan ratio, (K). plasma kynurenine acid, (L). kynurenic acid:kynurenine.

Figure 2: Ketamine treatment and kynurenine pathway metabolism: (A). plasma kynurenine (B). plasma tryptophan (C). kynurenine:tryptophan ratio (D). kynurenic acid (E). kynurenic acid: kynurenine ratio for all participants completing three infusion (A-E). (F). Plasma kynurenine at baseline and at 2 hours following first infusion in those who responded at this timepoint (G). Kynurenine:tryptophan ratio at baseline and at 24 hours following first infusion in those who responded at this timepoint.

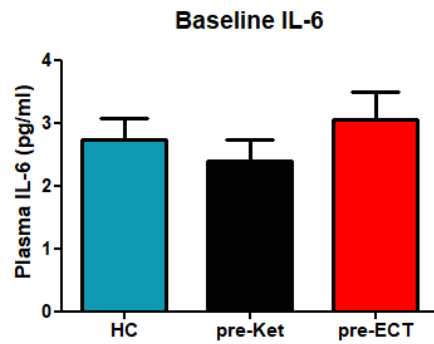
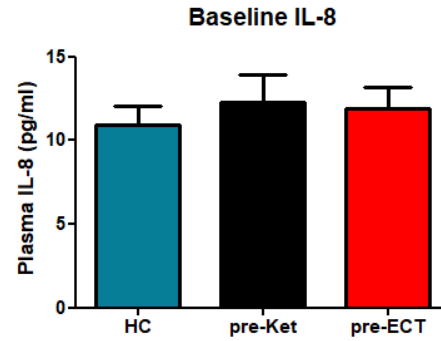
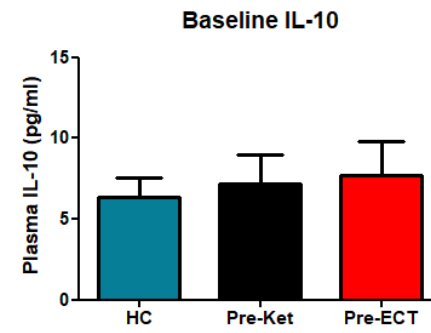
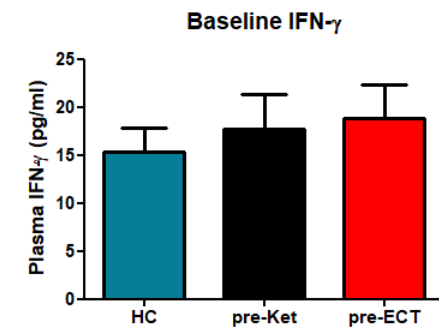
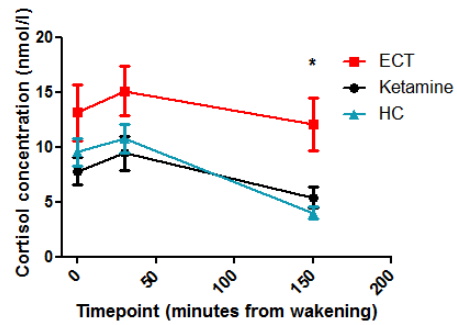
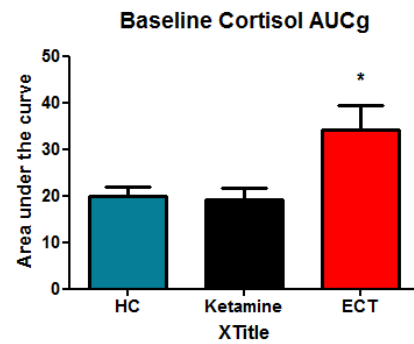
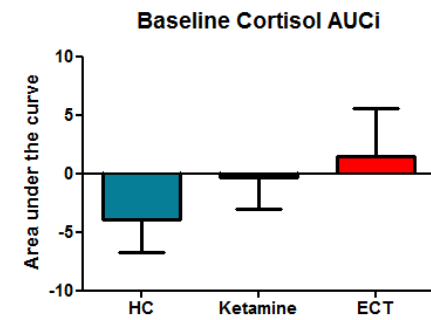
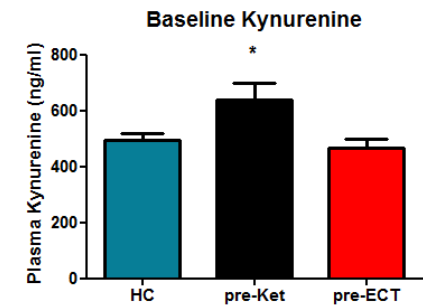
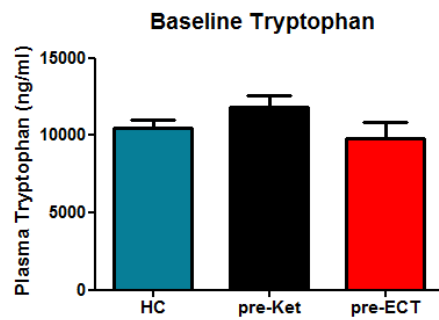
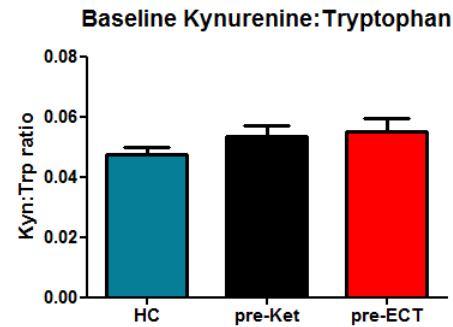
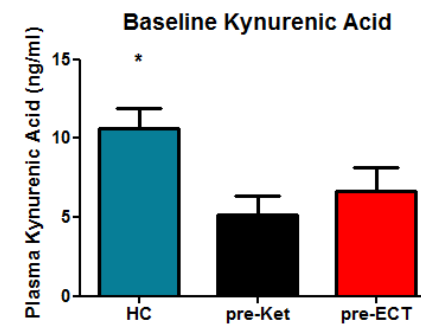
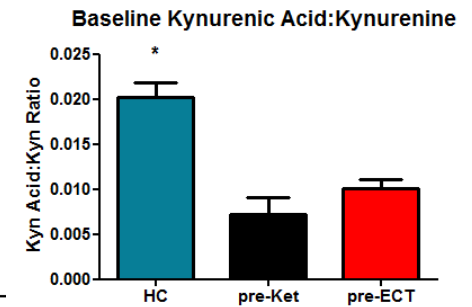
Figure 3: ECT treatment and kynurenine pathway metabolism: (A). plasma kynurenine (B). plasma tryptophan (C). kynurenine:tryptophan ratio (D). kynurenic acid (E). kynurenic acid: kynurenine ratio.

Figure 4: Ketamine treatment and waking cortisol-in the ketamine cohort as a whole: (A). salivary cortisol at each timepoint, (B). area under the curve with respect to ground, (C). area under the curve with respect to increase.

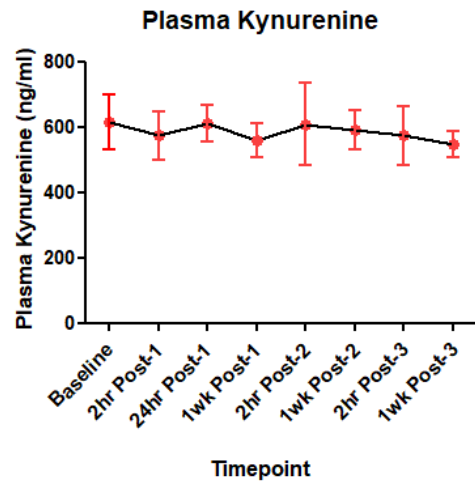
Figure 5: ECT treatment and waking cortisol: in the ECT cohort as a whole: (A). salivary cortisol, (B). cortisol area under the curve with respect to ground, (C). cortisol area under the curve with respect to increase. (D). area under the curve with respect to ground in ECT responders.

Figure 6: Ketamine treatment and cytokines for participants completing all three infusions: (A). plasma IL-6, (B). IL-8, (C). IL-10, (D). IFN- γ .

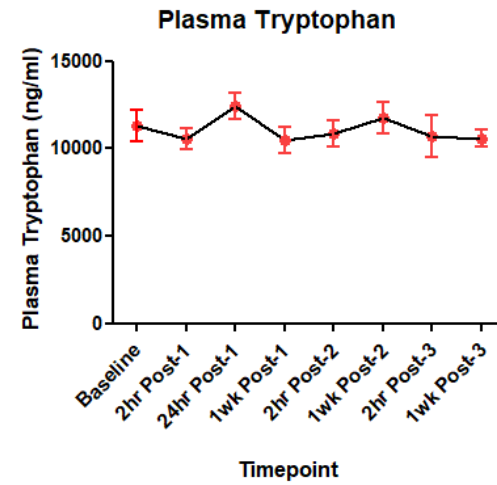
Figure 7: ECT treatment and cytokines: in the ECT cohort as a whole: (A). plasma IL-6, (B). IL-8, (C). IL-10, (D). IFN- γ .

A.**B.****C.****D.****E.****Baseline Cortisol Awakening Response****F.****G.****H.****I.****J.****K.****L.**

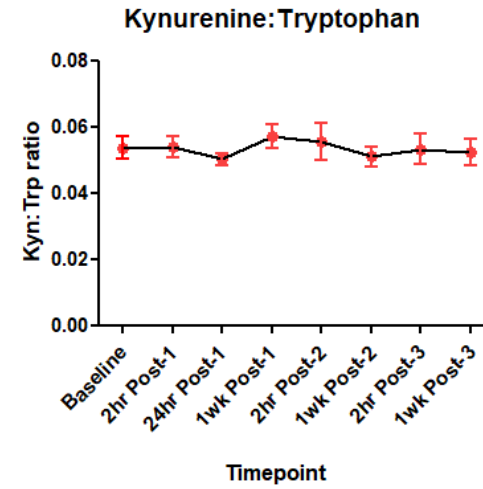
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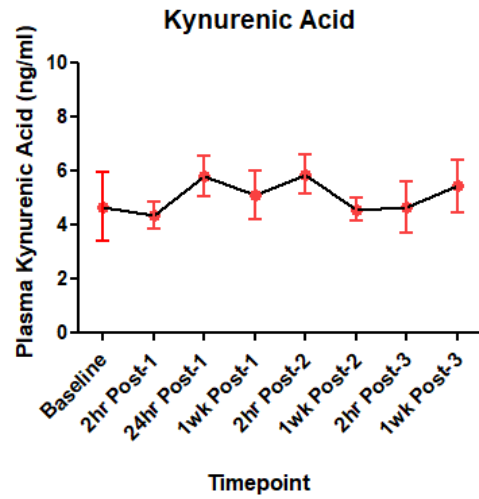
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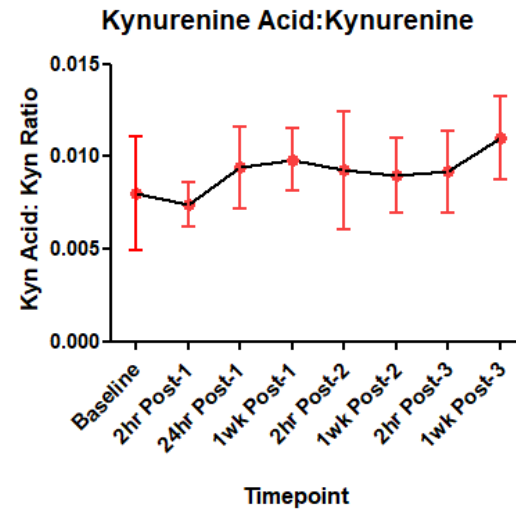
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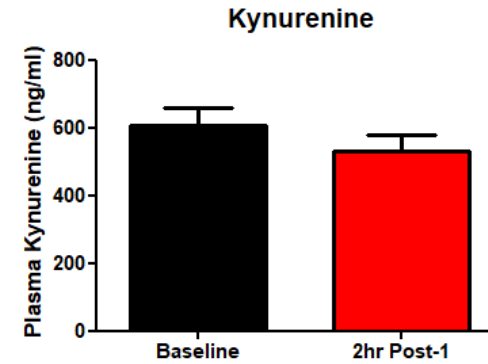
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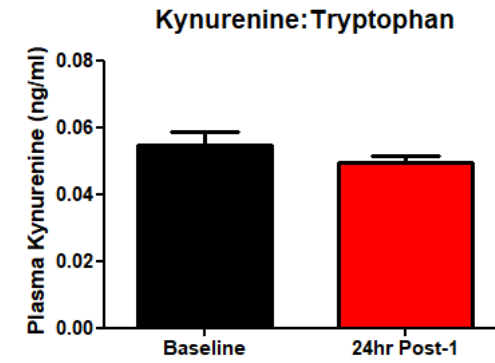
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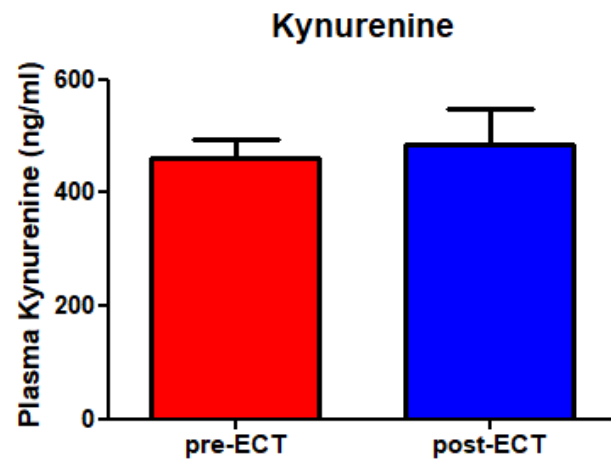
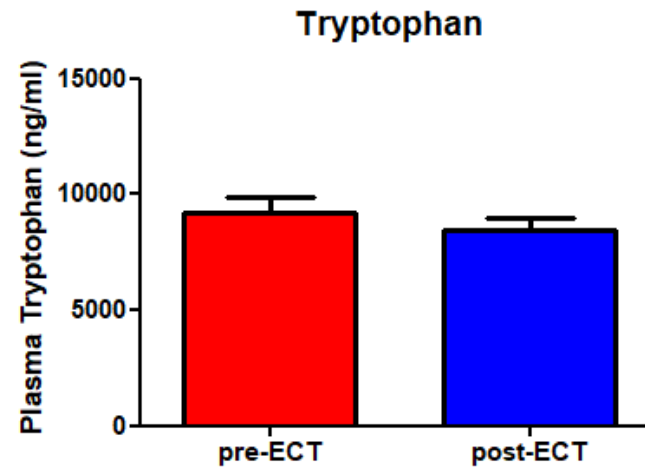
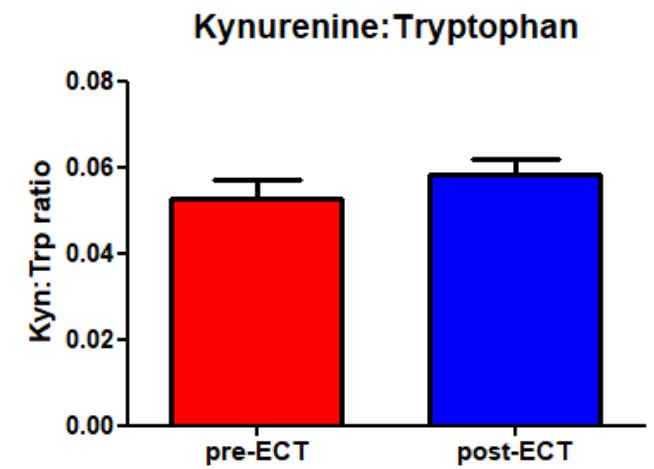
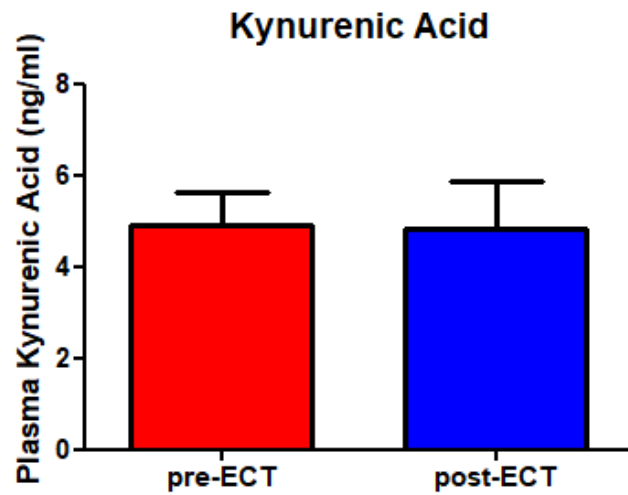
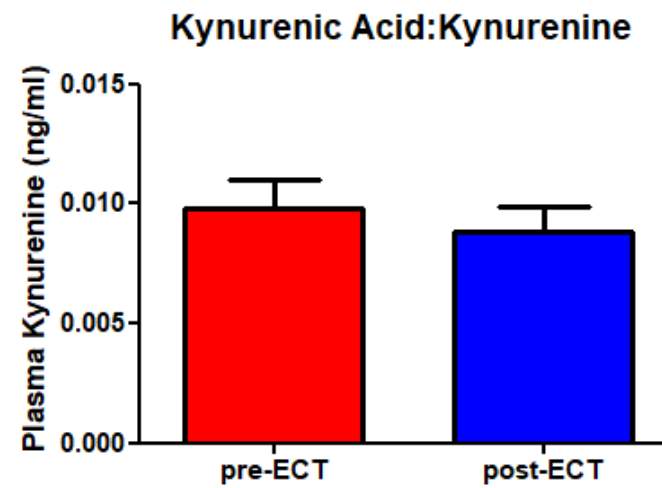


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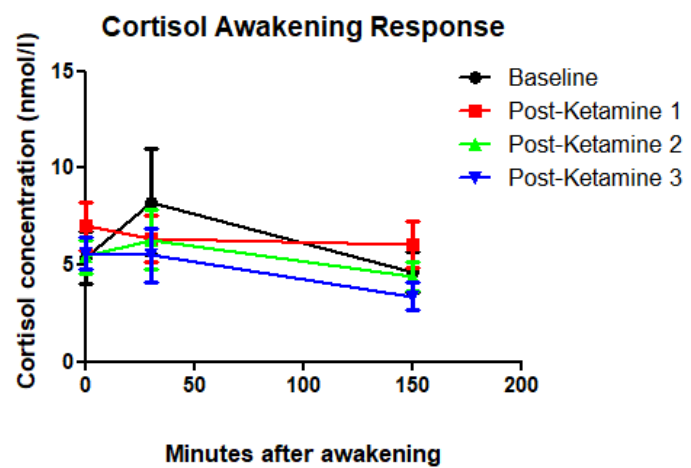


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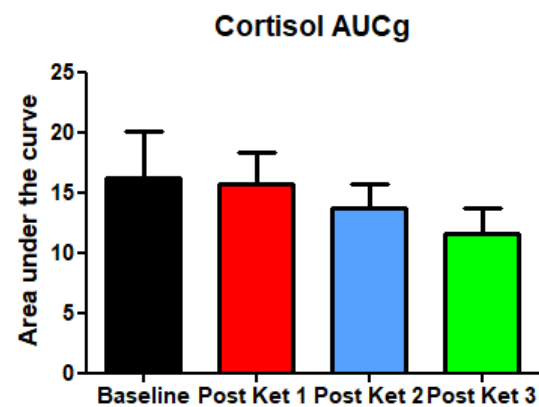


A.**B.****C.****D.****E.**

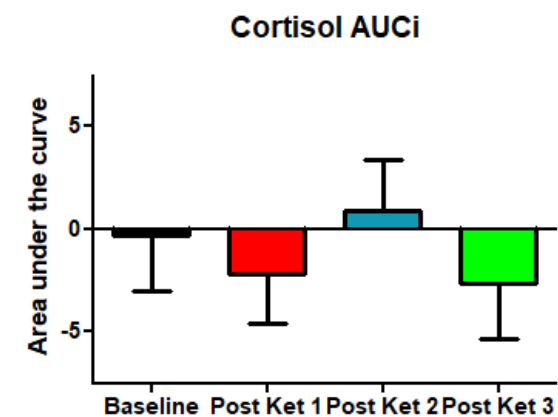
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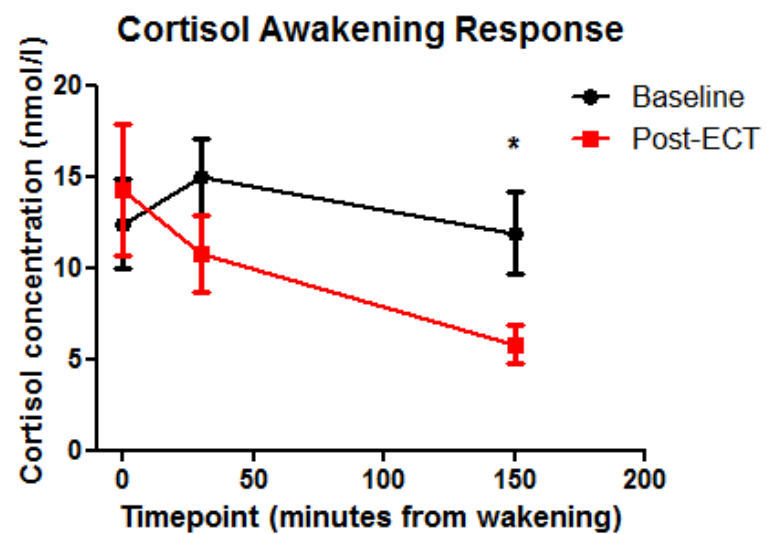
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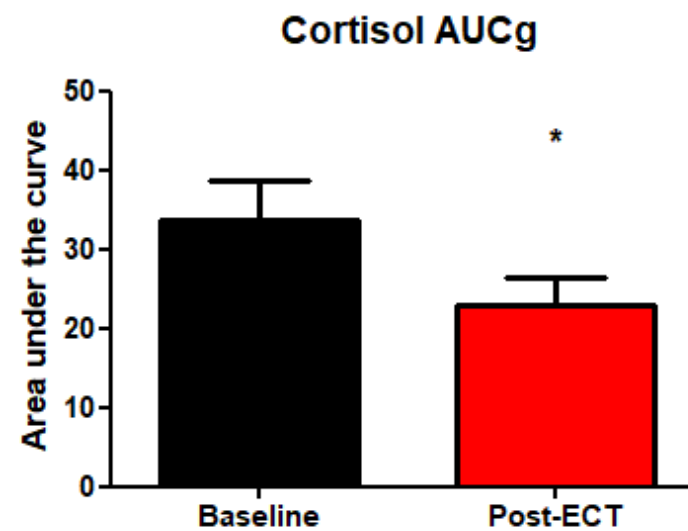
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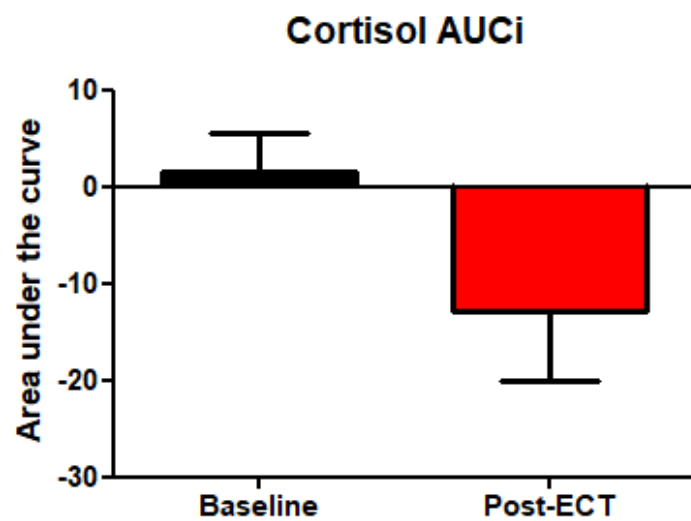
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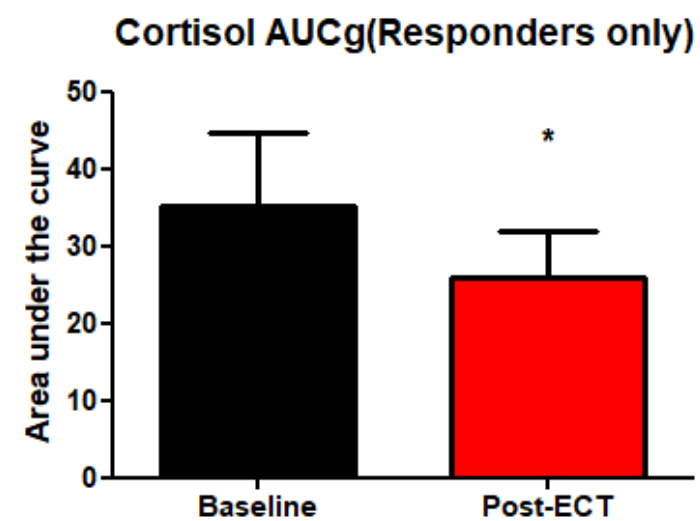
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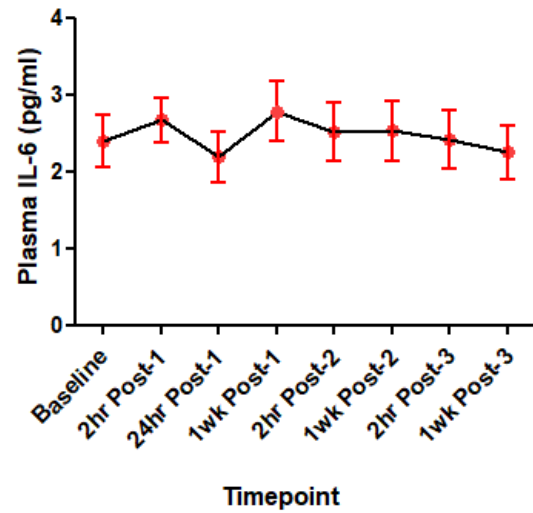


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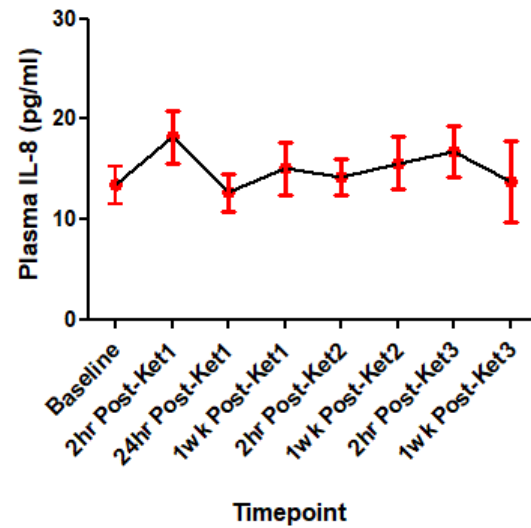
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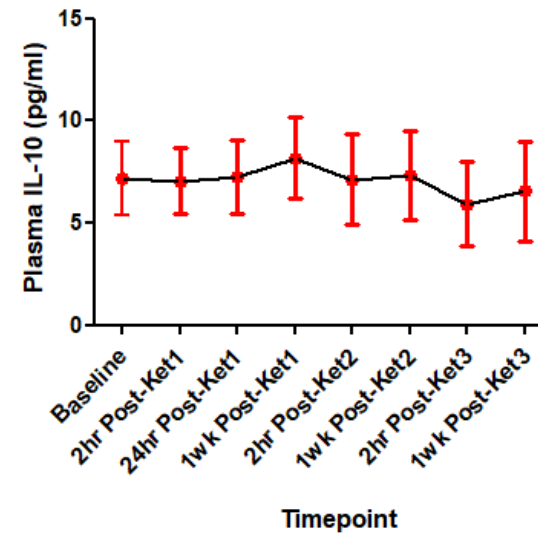
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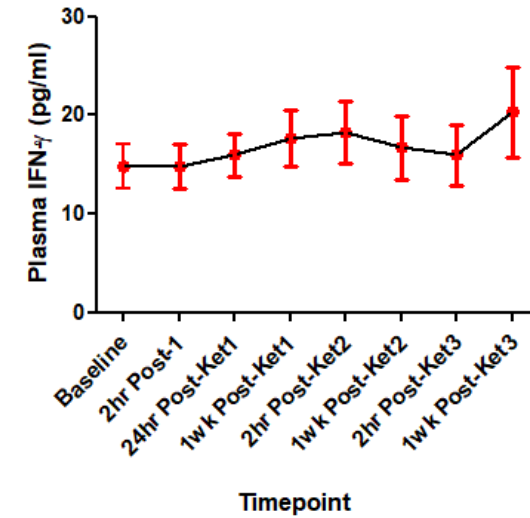


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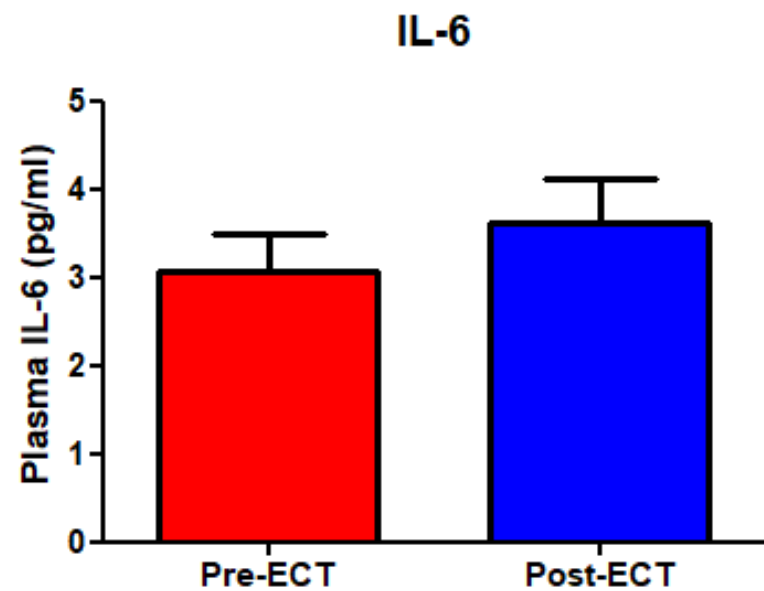
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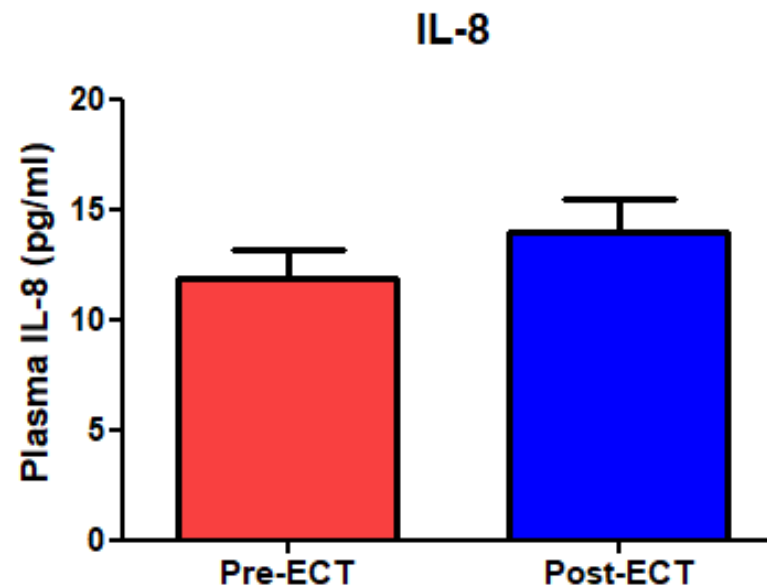
D.

IFN- γ 

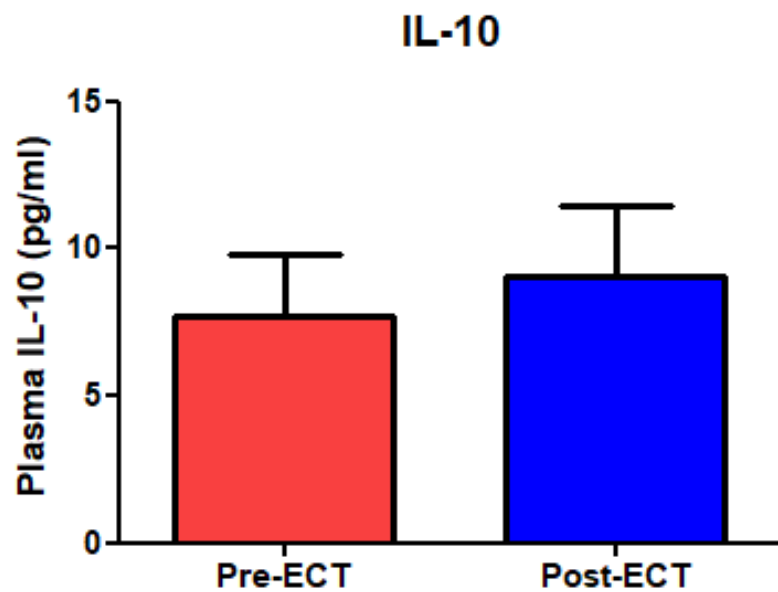
A.



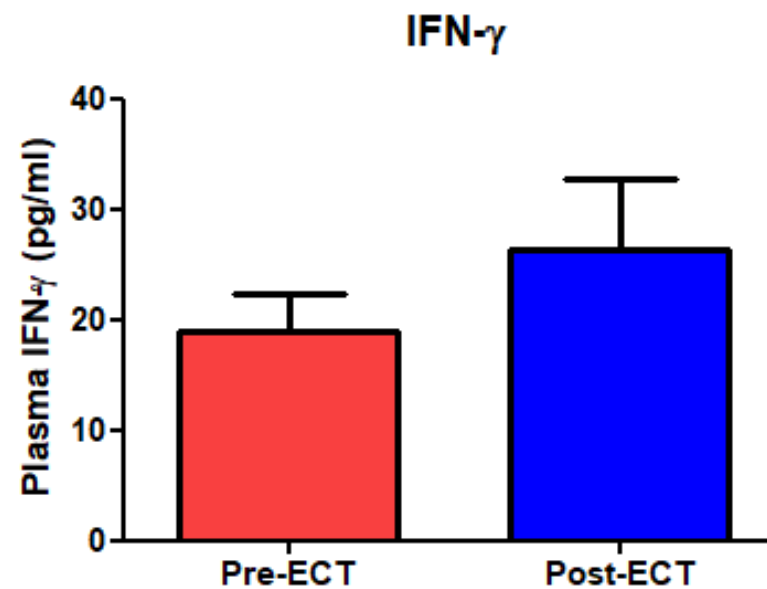
B.



C.



D.



Research highlights

- Plasma kynurenine was not altered following successful ketamine response
- Kynurenic acid was reduced in patients with treatment-resistant depression
- In patients who responded to ECT, the cortisol awakening response was decreased

Conflict of interest

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