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Indoleamine 2,3-dioxygenase-dependent neurotoxic kynurenine metabolism mediates inflammation-induced deficit in recognition memory

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Abstract

Cognitive dysfunction in depression is a prevalent and debilitating symptom that is poorly treated by the currently available pharmacotherapies. Research over the past decade has provided evidence for proinflammatory involvement in the neurobiology of depressive disorders and symptoms associated with these disorders, including aspects of memory dysfunction. Recent clinical studies implicate inflammation-related changes in kynurenine metabolism as a potential pathogenic factor in the development of a range of depressive symptoms, including deficits in cognition and memory. Additionally, preclinical work has demonstrated a number of mood-related depressive-like behaviors to be dependent on indoleamine 2,3-dioxygenase-1 (IDO1), the inflammation-induced rate-limiting enzyme of the kynurenine pathway. Here, we demonstrate in a mouse model, that peripheral administration of endotoxin induced a deficit in recognition memory. Mice deficient in IDO were protected from cognitive impairment. Furthermore, endotoxin-induced inflammation increased kynurenine metabolism within the perirhinal/entorhinal cortices, brain regions which have been implicated in recognition memory. A single peripheral injection of kynurenine, the metabolic product of IDO1, was sufficient to induce a deficit in recognition memory in both control and IDO null mice. Finally, kynurenine monooxygenase (KMO) deficient mice were also protected from inflammation-induced deficits on novel object recognition. These data implicate IDO-dependent neurotoxic kynurenine metabolism as a pathogenic factor for cognitive dysfunction in inflammation-induced depressive disorders and a potential novel target for the treatment of these disorders.

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Conflict of Interest

None of the authors have any conflicts of interest to disclose

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Keywords

neuroinflammation; recognition memory; novel object recognition; kynurenine; indoleamine 2, 3-dioxygenase; kynurenine monooxygenase; neuropsychiatric symptom; perirhinal cortex; mouse; behavior

1. Introduction

Activation of the peripheral innate immune system and the production of proinflammatory cytokines are now recognized as important contributors to neuropsychiatric illnesses, specifically in the development of depressive symptoms including aspects of cognitive impairment (Reichenberg, Yirmiya et al. 2001, Dantzer, O'Connor et al. 2008). Depressed patients often exhibit increased plasma levels of proinflammatory cytokines compared to healthy controls including tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1β (Dantzer, O'Connor et al. 2008, Dowlati, Herrmann et al. 2010). Further, administration of cytokines, like interferon (IFN)-α, for the treatment of some cancers and viral infections has been shown to induce symptoms of major depression in more than half of the patients undergoing treatment (Capuron and Miller 2004). More recently, the administration of bacterial endotoxin (lipopolysaccharide or LPS) has become a reliable experimental method to activate the peripheral immune system in both human and animal studies to explore the mediators linking a proinflammatory milieu to resultant depressive symptoms. A low dose of endotoxin in healthy human volunteers was sufficient to increase TNF-α and IL-6 plasma levels up to 4 hours after administration which coincided with an increase in depressed mood and a decrease in memory functions in short term recall tests (Reichenberg, Yirmiya et al. 2001). Furthermore, in rodents, administration of moderate doses of LPS (<1.0 mg/kg) results in a characteristic sickness response including decreased food intake and reduced locomotion which subsides by approximately 12–18 hours post-administration followed by the development of depressive-like behaviors measured 24–28 hours post-LPS (O'Connor, Lawson et al. 2009, Salazar, Gonzalez-Rivera et al. 2012, Walker, Budac et al. 2013).

There is growing evidence implicating the kynurenine pathway (KP) of tryptophan metabolism as a key mediator of inflammation-related depressive symptoms (Fig. 1); (Raison, Borisov et al. 2010, Dantzer, O'Connor et al. 2011). The majority of dietary tryptophan is metabolized through the KP, and under certain pathological conditions neurochemically active metabolites can be generated (Schwarcz, Bruno et al. 2012). During normal homeostatic conditions, the predominantly hepatic enzyme tryptophan 2,3-dioxygenase (TDO) is responsible for the initial step of the kynurenine pathway, metabolizing tryptophan to N-formylkynurenine which is subsequently metabolized to kynurenine. However, during proinflammatory conditions or after experimental administration of LPS, the extra-hepatic enzyme indoleamine 2,3-dioxygenase (IDO) is upregulated both in the periphery and the brain, and increases the production of kynurenine (Andre, O'Connor et al. 2008). Kynurenine can subsequently be metabolized via two distinct routes: via the kynurenine aminotransferases (KAT) yielding kynurenic acid (KYNA) or via kynurenine monooxygenase (KMO), kynureninase (KYNU), and 3-hydroxyanthranilic acid 3,4-dioxygenase (HAAO) to produce quinolinic acid (QUIN). QUIN acts at N-methyl-D-aspartate (NMDA) receptors as an agonist and in the brain, KMO

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activity in microglia is the rate limiting step for the generation of QUIN (Schwarcz, Bruno et al. 2012). KYNA, in addition to acting at NMDA receptors as an antagonist, also has inhibitory activity at the α7 nicotinic acetylcholine receptor (α7nAChR) (Schwarcz, Bruno et al. 2012). Peripherally produced kynurenine readily crosses the blood brain barrier via large neutral amino acid transporters providing the substrate for the production of these neuroactive metabolites which could result in altered brain function.

Interestingly, cytokine induced depression was associated with reduced levels of tryptophan and increased levels of kynurenine measured in serum (Capuron, Ravaud et al. 2002). Furthermore, concentrations of kynurenine, QUIN and KYNA were increased in the cerebrospinal fluid of patients receiving IFN-α therapy, and QUIN was positively correlated with Montgomery-Asberg Depression Rating Scale scores (Raison, Dantzer et al. 2010). Similarly, many LPS-induced depressive-like behaviors in mouse models are mediated by IDO (O'Connor, Lawson et al. 2009, Salazar, Gonzalez-Rivera et al. 2012), and while both peripheral immune challenge and disruption of the KP have been independently reported to impair cognitive processes (Haba, Shintani et al. 2012, Pocivavsek, Wu et al. 2012), the mechanistic role of IDO in mediating inflammation-induced cognitive dysfunction has not been investigated.

The novel object recognition task assesses various aspects of learning and memory. Experimental manipulations allow for investigations of encoding, consolidation and retrieval (Ennaceur and Delacour 1988, Winters and Bussey 2005). Importantly, the perirhinal cortex, a subdivision of the parahippocampal region, is the primary region responsible for facilitating recognition memory. Lesion studies which ablate the function of the perirhinal cortex have demonstrated that this region is necessary for normal performance of the novel object recognition task (Ennaceur and Aggleton 1997, Mumby, Glenn et al. 2002, Norman and Eacott 2004). Additionally, the entorhinal cortex (also a subdivision of the parahippocampal region), the hippocampus and the medial prefrontal cortex, have all been identified as important regulators of object recognition. Both hippocampus and medial prefrontal cortex (mPFC) appear to be involved in object recognition tasks with a temporal factor (Hammond, Tull et al. 2004, Cross, Brown et al. 2012). Hammond et al. found that lesions to the hippocampus interfered with object recognition at a long delay (24h) but not a short delay (5 min) (Hammond, Tull et al. 2004). Lesions to the mPFC do not affect a simple object recognition task, however when several objects are presented in succession, lesions to the mPFC interfere with discrimination of recency (i.e. which object did they encounter most recently) (Cross, Brown et al. 2012). Here, we employed the novel object recognition test to assess the effect of peripheral immune challenge on performance in a cognitive task. We utilized mice with a targeted deletion of the IDO1 or KMO gene to determine the mechanistic role of inflammation-induced upregulation of IDO1 and neurotoxic kynurenine metabolism on cognitive performance in this task. These data are the first to demonstrate a decrease in cognitive performance induced by peripheral immune challenge which is mediated by IDO1 and downstream neurotoxic kynurenine metabolism.

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2. Methods

2.1. Mouse housing and treatments

All animal care and use was carried out in accord with the Guide for the Care and Use of Laboratory Animals, 8th edition (NRC) and approved by the Institutional Animal Care and Use Committee. Wild-type control C57BL/6J (Jax Strain # 000664) mice and indoleamine 2,3 dioxygenase null (IDO –/–; B6.129-Ido1^{tm1Alm}/J, Jax Strain #005867) mice originated from The Jackson Laboratory (Bar Harbor, ME). KMO transgenic mice (Kmo$^{tm1a(KOMP)Wtsi}$) were obtained through the NIH knockout mouse program, University of California-Davis repository, using a knockout first, conditional-ready design on a C57BL6 background. They were maintained as heterozygous breeding pairs in an in-house breeding colony to provide KMO$^{+/+}$, KMO$^{+/-}$ and KMO$^{-/-}$. Wild type mice from Jackson Laboratory were used to supplement the control group after baseline behavioral phenotypes were confirmed to be no different (data not shown). The presence of the Kmo transgene (KMO) was confirmed using PCR amplification of genomic DNA with the following primers: 5′-ACC AGT CAG CAG GTC CTT GTT T-3′, 5′-CGC GTC GAG AAG TTC CTA TTC C-3′, 5′-AAC CCA TGT TAC CGT CAC ACA C-3′. All experimental mice in the present series of experiments were 12–16 week old males and were individually housed in standard microisolator-topped shoebox cages within a temperature and humidity controlled facility with a modified 12:12 h reverse light cycle (lights on 23:00–11:00) and were allowed ad libitum access to food and water. LPS isolated from E. coli (cat# L-3129, serotype 0127:B8) was purchased from Sigma (St. Louis, MO) and injections were prepared from 1 mg/ml stock solutions on the morning of injections. LPS was dissolved in sterile, endotoxin-free 0.09% saline and injected i.p. at a dose of 0.5 mg/kg. LPS acutely induces a transient sickness behavior response(O'Connor, Satpathy et al. 2005) followed by the development of a distinct depressive-like behavioral phenotype in the forced swim test and tail suspension test (O'Connor, Lawson et al. 2009, Corona, Huang et al. 2010). LPS was administered immediately following the second day of habituation to the open field chamber, 24 hours prior to training with the familiar objects. L-kynurenine (cat# K8265) was purchased from Sigma (St. Louis, MO) and prepared on the morning of injections. Mice received a single i.p. injection of 100 mg/kg L-kynurenine 30 minutes prior to training. This interval is sufficient to allow for an increase in peripheral and central kynurenine metabolites using peripherally administered L-kynurenine, as previously reported (Chiarugi, Carpenedo et al. 1996).

2.2. Novel Object Recognition (Fig. 2A)

All behavioral testing was performed during the first 4 hours of the dark phase of the light cycle in a dimly lit behavioral testing room. Beginning 4 days prior to training, mice were handled for several minutes each day to acclimate to experimental manipulation and body weights were recorded. Mice were habituated to the open field chamber used for both training and testing to reduce any stress associated with being placed in a new environment. Mice were allowed to explore the empty open field chamber for 10 minutes each of the 2 days prior to training. Twenty four hours after the second day of habituation, mice were placed in the open field with two identical objects (amber 50 mL conical vials weighted with clear plastic beads) placed an equal distance from the wall of the open field and

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approximately 6 inches from each other. Mice were allowed to explore the objects for 7 minutes. Twenty four hours after training, mice were placed in the open field with one familiar object (amber 50 mL conical) and one novel object (clear 50 mL conical weighted with clear plastic beads). Both training and testing trials were recorded using an overhead camera. Exploratory behavior was manually scored in a blinded manner, and interaction was defined as direct sniffing of the object or touching the object with forepaws. The time spent exploring each object was recorded for each animal during the 7 minute testing trial and the discrimination index was calculated as: ([time exploring novel object] / [time exploring novel object + time exploring familiar object]*100).

2.3. Tissue Collection

A separate group of mice was administered either saline or LPS (0.5 mg/kg) i.p., and tissues were collected 24 or 48 hours later. The saline animals were divided into two groups, half collected at 24 hours and half collected at 48 hours. Perirhinal/entorhinal cortices were microdissected from two consecutive 1mm coronal sections 1.56–3.56 mm caudal to Bregma, according to the coordinates of The Mouse Brain in Stereotaxic Coordinates (Paxinos and Franklin 2004). Briefly, perirhinal/entorhinal cortices were taken together in a wedge, dissected from the lateral margin of the brain to the lateral boundary of the lateral ventricle, beginning 1mm from the base of the slab (Fig. 3A). Tissues were flash frozen in liquid nitrogen and stored at −8 °C until processing for RT-PCR.

2.4. RNA Isolation and Reverse Transcription

Total RNA from perirhinal/entorhinal cortices was isolated using a PureLink RNA MiniKit (Ambion; cat. 12183618A) according to the manufacturer's instructions. Reverse transcriptase reaction was performed in a BioRad C1000 Thermal Cycler using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems; cat. 4368814) without RNase inhibitor according to the manufacturer's instructions.

2.5. Real-time RT-PCR

All RT-PCR reactions were performed on a BioRad CFX384 Real-time System using Taqman gene expression assays for allograft inflammatory factor 1 (Iba1/Aif1; cat. Mm00479862), glial fibrillary acidic protein (GFAP, cat. Mm01253033), interleukin (IL)-1β (cat. Mm 1336189), tumor necrosis factor (TNF)-α (cat. Mm 443258), IL6 (cat. Mm00446190), IL10 (cat. Mm00439614), Indoleamine 2,3-dioxygenase 1 (IDO1, cat. Mm00492586), Kynurenine aminotransferase II (KATII/Aadat; cat. Mm00496169), Kynurenine 3-monooxygenase (KMO, cat. Mm00505511), Kynureninase (KYNU, cat. Mm00551012), 3-hydroxyanthranilate 3,4-dioxygenase (HAAO, cat. Mm00517945), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; cat. Mm99999915). All samples were run in duplicate. Data were analyzed using the $2^{- \quad CT}$ method with GAPDH serving as the house keeping control gene as previously described (O'Connor, Satpathy et al. 2005). For samples with undetectable targets, the C_t value was recorded as 40, the maximum number of cycles, for calculation purposes (Supplementary Table 1 shows average C_t values for each target prior to calculation of relative expression changes for an approximation of overall expression levels between targets.

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2.6. HPLC analysis of Kynurenine

Plasma and whole brain kynurenine levels were measured by HPLC with electrochemical detection as previously described (O'Connor, Lawson et al. 2009) with minor modifications. Briefly, following homogenization of frozen brain tissue, plasma and brain were deproteinated in a 5X volume of methanol at 4 ° C, followed by centrifugation through a 0.2 μm filter column. The resulting supernatants were further diluted in 0.435 mM perchlorate in 13% methanol and analyzed as previously described (O'Connor, Lawson et al. 2009). Peaks were detected and integrated using a BAS 502 isocratic liquid chromatographic system (Bioanalytical Systems, West Lafayette, IN) with an Ag/AgCl reference electrode and 3 mm glassy carbon electrode at + 950 mV.

2.7. Statistical Analysis

Two factor (genotype vs. treatment) ANOVA was performed for all comparisons of wild-type and IDO$^{-/-}$, for both mRNA expression and performance in the novel object recognition test. When significant interactions were identified, post-hoc analysis was performed using Tukey's multiple comparisons test. Student's t-test was performed for comparisons of novel object recognition performance after L-kynurenine administration. Outlier analysis was performed on groups with n>8 using the Chauvenet test (Taylor 1997).

3. Results

3.1. Indoleamine 2,3-dioxygenase 1 mediates a deficit in novel object recognition following peripheral immune challenge

We and others have previously demonstrated that IDO mediates many of the depressive-like behaviors that develop following peripheral immune activation (O'Connor, Lawson et al. 2009, O'Connor, Lawson et al. 2009, Salazar, Gonzalez-Rivera et al. 2012), but its mechanistic role in mediating inflammation-induced cognitive deficits has not been explored. Peripheral administration of LPS induced a deficit in novel object recognition in WT mice as indicated by a significant decrease in their discrimination index (Fig. 2B; Genotype x Treatment Interaction: $F_{(1,36)}=13.00$, $p=0.0009$). Post hoc analysis revealed that IDO$^{-/-}$ mice administered LPS were protected from this deficit as indicated by a significant difference compared to WT mice administered LPS and no difference compared to either saline control group (Fig. 2B; $p<0.001$). Importantly, no baseline differences in discrimination were observed between control mice. Also, overall exploratory activity, as measured by total number of direct object contacts (Fig. 2C) or total duration of direct exploration (Fig. 2D) was not different between any of the experimental groups.

3.2. Peripheral LPS administration increases the mRNA expression of proinflammatory cytokines and markers of glial activation in the perirhinal/entorhinal cortex

Peripheral immune challenge with LPS induces a general upregulation of glial activation and proinflammatory gene transcripts within the brain (Corona, Huang et al. 2010), but the local neuroimmune response in the perirhinal/entorhinal cortex (Fig. 3A), which is a primary brain region mediating recognition memory, has not been characterized following LPS. Steady-state mRNA transcript levels of allograft inflammatory factor 1 (Iba1/Aif1) and glial fibrillary acidic protein (GFAP) were measured in perirhinal/entorhinal cortex 24h or 48h

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after saline or LPS injection as an index of general microglial and astrocyte activity state. Peripheral administration of LPS transiently increased mRNA expression of both microglial Iba1 and astrocytic GFAP at 24 hours post-challenge (Fig. 3B and C). LPS increased the expression of Iba1 mRNA (Fig. 3B; LPS effect: $F_{(2,37)=}15.47$, $p<0.0001$). Similarly, LPS increased the expression of GFAP (Fig 3C; LPS effect: $F_{(2.37)}=15.28$, $p<0.0001$). Peripheral LPS challenge also increased the expression of the proinflammatory cytokines IL-1β (Fig. 3D, LPS effect: $F_{(2,37)}=17.67$, $p<0.0001$) and TNF-α (Fig. 3E, LPS effect: $F_{(2,35)}=56.32$, $p<0.0001$) in both WT and $IDO^{-/-}$ mice. In contrast, mRNA expression of IL-6 was reduced following LPS treatment (Fig. 3F, LPS effect: $F_{(2,37)}=5.724$, $p=0.0068$).

3.3. Indoleamine 2,3-dioxygenase 1 and hydroxyanthranilic acid oxygenase mRNA expression is increased following peripheral LPS challenge

Consistent with our previous data from whole brain tissue analysis, peripheral LPS challenge increased mRNA expression of IDO1 in the perirhinal/entorhinal cortices of WT, but not $IDO^{-/-}$ mice (Fig. 4A; Genotype x Treatment Interaction: $F_{(2,37)}=10.82$; $p=0.0002$). Post-hoc analysis revealed a significant increase in IDO1 expression 48 hours post-LPS in WT mice (Fig. 4A; $p<0.0001$). As expected, no IDO1 was detected in $IDO^{-/-}$ mice (Fig. 4A). When downstream KP enzyme mRNA expression was measured, administration of LPS reduced KATII expression (Fig. 4B;LPS effect $F_{(2,37)}=6.09$; $p=0.005$. While LPS challenge did not significantly alter the expression of either KMO (Fig. 4C) or KYNU (Fig. 4D) mRNA transcripts, HAAO was significantly upregulated in both WT and $IDO^{-/-}$ (Fig. 6E; LPS effect: $F_{(2,37)}=18.63$; $p<0.0001$).

3.4. Direct administration of L-kynurenine induces a deficit in Novel Object Recognition

We have previously demonstrated that acute peripheral administration of L-kynurenine precipitates depressive-like behaviors (Salazar, Gonzalez-Rivera et al. 2012), and administration of 100 mg/day to rats impairs spatial learning in the Morris water maze (Pocivavsek, Wu et al. 2012). In order to determine whether peripheral administration of L-kynurenine to naïve WT mice disrupts recognition memory, we first determined the kinetics of kynurenine clearance following ip administration of 100 mg/kg L-kynurenine. Peak levels were observed in both circulation (Fig. 5A, $F_{(3,17)}=145.1$, $p<0.0001$) and in whole brain tissue (Fig. 5B, $F_{(3,16)}=66.67$, $p<0.0001$) 30 minutes post-injection followed by a rapid return to baseline levels by 2h post-injection. To determine the impact of acute elevation of kynurenine levels on recognition memory, 100 mg/kg L-kynurenine or saline was administered 30 minutes prior to training. Both kynurenine treated WT (Fig. 5C; $p<0.05$) and $IDO^{-/-}$ (Fig. 5D); $p<0.05$) mice exhibited a significant deficit in novel object recognition as reflected by a decreased discrimination index. Importantly, L-kynurenine treatment had no effect on overall exploratory activity (e.g. contact time or total number of contacts (data not shown), or on the induction of proinflammatory cytokine mRNA transcripts measured in brain tissue (data not shown).

3.5. LPS-induced deficits in novel object recognition are attenuated in kynurenine monooxygenase deficient mice

Recent data demonstrated that kynurenine metabolism, downstream of IDO, is skewed preferentially toward the generation of potentially neurotoxic metabolites following

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peripheral LPS challenge (Walker, Budac et al. 2013). To determine whether kynurenine 3-monooxygenase (KMO)-dependent (see figure 1 as reference) metabolism of kynurenine mediated the cognitive impairment caused by LPS, the effect of intraperitoneal LPS on performance in the novel object recognition task was measured as in figure 2. Consistent with the previous experiment, peripheral administration of LPS induced a deficit in novel object recognition in WT mice as indicated by a significant decrease in their discrimination index (Fig. 6; Genotype x Treatment Interaction: $F_{(1,29)}=8.23$, $p=0.0076$). Post hoc analysis revealed that $KMO^{-/-}$ mice administered LPS were protected from this deficit as indicated by a significant difference compared to WT mice administered LPS and no difference compared to either saline control group (Fig. 6; $p<0.01$). Importantly, no baseline differences in discrimination were observed between control mice, and overall exploratory activity was not different between any of the experimental groups (data not shown).

4. Discussion

The aim of the present study was to determine if activation of the peripheral innate immune response was sufficient to induce a deficit in cognitive function relevant to depressive symptomology and if IDO-dependent kynurenine metabolism is an important pathogenic mediator. Consistent with our previous findings, peripheral challenge with LPS induced a deficit in novel object recognition memory which was mediated by IDO, the rate-limiting enzyme of the kynurenine pathway (Fig. 1). In the absence of peripheral immune activation, even a single peripheral injection of L-kynurenine, the metabolic product of IDO, was sufficient to induce a deficit in recognition memory. Further, mice with a targeted deletion of the KMO gene were protected from LPS-induced disruption in novel object recognition. These data suggest, for the first time, an important role for the neurotoxic branch of the kynurenine pathway in mediating in inflammation-induced deficits in recognition memory.

Mental illness is a growing problem in the United States, with an approximate lifetime prevalence of 20% for mood disorders in the US adult population, as well as being a leading contributor to disability and economic burden (Kessler, Berglund et al. 2005). Furthermore, there is an increased prevalence of depression associated with chronic diseases, such as obesity, diabetes and heart disease (Evans, Charney et al. 2005), and inflammation has been implicated as a point of pathogenic convergence. Unfortunately, currently available treatment options, which primarily target the monoamine system, are only moderately effective with less than half of those patients with major depressive disorder receiving treatment achieving remission (Trivedi, Hollander et al. 2008). Even among patients achieving "remission", approximately half may still suffer from residual symptoms (Nierenberg, Keefe et al. 1999, Trivedi, Hollander et al. 2008, Hasselbalch, Knorr et al. 2011). The presence of residual symptoms greatly increases the risk of relapse compared to remitted patients without residual symptoms (Paykel 2008). Furthermore, these difficulties are not unique to major depressive disorder. Several studies suggest inter-episode residual symptoms in bipolar disorder are associated with poorer outcomes and a substantially higher risk of relapse to a full blown episode (Keller, Lavori et al. 1992, Morriss 2002, Paykel 2008). In a 3-year prospective study, one of the most common residual symptoms in a group of major depressive disorder patients was cognitive impairment, which persisted in nearly half of the 267 patients followed (Conradi, Ormel et al. 2011). Additionally, the persistence

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of cognitive impairment significantly contributed to the rate of functional disability experienced in major depressive disorder, and improvement in these cognitive symptoms is associated with a greater chance of recovery of life functioning (Jaeger, Berns et al. 2006). Taken together, these studies suggest the cognitive symptoms of mood disorders are inadequately treated by currently available therapeutics and the persistence of these symptoms constitute a major obstacle to successful treatment. The data in the present study are the first to suggest a mechanistic role for the kynurenine pathway in inflammation-induced deficits of recognition memory. Targeting the kynurenine pathway could represent a novel target for treating the cognitive deficits, particularly as related to aspects of memory, of mood disorders, although similar experiments designed to probe distinct cognitive processes remain to be performed.

Our group and others have shown IDO1 mediates a number of depressive-like behaviors in rodent models. Pretreatment with 1-methyltryptophan (1-MT), a tryptophan analog and competitive inhibitor of IDO, blocked the increase in immobility 24 hours after LPS administration in both the forced swim test (FST) and tail suspension test (TST) (O'Connor, Lawson et al. 2009). Behavioral measures of anhedonia are also induced by administration of peripheral LPS up to 48 hours, as reflected by a decrease in preference for a sweetened solution (sucrose preference, SP), and blockade of IDO attenuates this response (Frenois, Moreau et al. 2007, Salazar, Gonzalez-Rivera et al. 2012). The anxiogenic properties of LPS as measured by both the open field test and the light-dark box test were shown to be mediated by IDO (Salazar, Gonzalez-Rivera et al. 2012). Finally, direct peripheral administration of kynurenine increases anxiety- and depression-like behaviors in the FST, TST, SP, and open field tests (O'Connor, Lawson et al. 2009, Salazar, Gonzalez-Rivera et al. 2012). These data implicate IDO and the kynurenine pathway as critical mediators of inflammation-induced depression-like behaviors; however their role in cognitive symptoms of depressive behavior had not yet been explored. Here, we have shown the first evidence to suggest a role for both IDO and kynurenine in mediating LPS –induced deficits in a measure of learning and memory, and we provide the first direct experimental evidence that KMO-dependent neurotoxic kynurenine metabolism mediates LPS-induced cognitive impairment. Using the novel object recognition test, we have shown that induction of IDO via peripheral administration of LPS, during the training period, induces a deficit in recognition of a novel object 24 h later, which is ablated by targeted deletion of the IDO1 or KMO genes. Furthermore, we show that direct peripheral administration of kynurenine induces a similar deficit in recognition memory in wild type mice.

While various stages of learning and memory can be assessed using the novel object recognition task (Dere, Huston et al. 2007), the long term (up to 48 hours shown here) local molecular consequences of LPS administration make it difficult to tease apart the effects on encoding, consolidation and retrieval. Peripheral LPS challenge induced significant increases in both markers of glia and proinflammatory cytokines in the perirhinal/entorhinal cortices at 24 hours, which coincides with the training phase of our test. The neuroinflammatory signature had resolved by 48 hours; the time at which mice were tested on the ability to recall a familiar object. Additionally, expression of IDO mRNA is increased at 24 hours and significantly increased at 48 hours in wild type mice. Our previously published reports have shown that kynurenine levels within the brain are increased at this

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time, and a recent report by Walker et. al. suggests that the balance of kynurenine metabolism is skewed toward KMO-dependent increases in the so-called neurotoxic branch of the kynurenine pathway (Walker, Budac et al. 2013). Data in figure 6 support the notion that a shift toward neurotoxic kynurenine metabolism may be the pathogenic precipitant of cognitive impairment. However, in all cases, the data are cross-sectional in nature, and additional experimental investigation to characterize the dynamic changes of kynurenine metabolism in the brain following peripheral immune challenge would be enlightening. Increased proinflammatory activation and induction of neurotoxic kynurenine metabolism at 24 hours, during the training session, could have effects on the encoding and consolidation of object characteristics, resulting in a decreased preference for the novel object during testing because both objects may be perceived as novel. Several downstream metabolites of the kynurenine pathway act on NMDA receptors, which have long been known to play a critical role in synaptic plasticity and therefore in the processes of learning and memory (Collingridge, Volianskis et al. 2013). At 48 hours post-LPS, there is a significant increase in HAAO mRNA in both wild type and IDO −/− mice, suggesting perhaps that increased synthesis of QUIN could be involved in directly mediating the behavioral effects observed in this study. Although QUIN levels were not directly measured in the present study, increased expression of HAAO in wild type mice could lead to increased production of its enzymatic product, QUIN, an NMDA receptor agonist. However, in IDO−/− and KMO −/−mice, this metabolic process would be stifled due to the absence of the rate limiting enzymes.

NMDA receptor blockade has been shown to interfere with memory retrieval in recent studies (Jo and Choi 2014, Zarrindast, Ownegh et al. 2014), and as the downstream metabolite KYNA exhibits NMDAR antagonist properties, it is possible that local accumulation of this metabolite contributes to the functional impairment. Additional studies are required to fully tease apart the mechanistic contributions of downstream KP metabolites to LPS-induced cognitive impairment. Since KMO−/− mice were protected from the effects of LPS, we speculate that neurotoxic kynurenine metabolism drives the cognitive impairment caused by LPS, and this interpretation is consistent with prior metabolite measurements showing a LPS-driven shift towards KMO-dependent metabolism. However, kynurenine metabolites were not measured in the present study, so it is possible that deletion of KMO during LPS challenge results in an accumulation of kynurenine and kynurenic acid. Additional experiments in the future should be aimed at addressing this possibility. de Lima et al. showed that both pre- and post-training administration of an NMDA receptor antagonist disrupted performance in novel object recognition indicating an important role for the NMDA receptor in both encoding and consolidation (de Lima, Laranja et al. 2005). It should be noted, however, that Walker et. al. recently reported that peripheral administration of LPS to CD-1 outbred mice resulted in elevated QUIN levels within the brain, while KYNA levels remained unchanged (Walker, Budac et al. 2013). Given the critical role for the normal function of these receptors in all of the stages necessary for the performance of the novel object task, overproduction of QUIN and thus aberrant overactivation of these receptors could be just as detrimental to performance.

While the regional focus of the present study was restricted to the perirhinal/entorhinal cortex, the interesting possibility exists that IDO-mediated changes in other brain regions

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responsible for regulating recognition memory also contribute to the deficits observed in figure 3. Andre et al. observed a significant increase in proinflammatory cytokine expression that preceded increases in IDO mRNA expression after peripheral LPS administration in the hippocampus, a region that has been implicated in performance of the novel object recognition task (Hammond, Tull et al. 2004, Andre, O'Connor et al. 2008). Furthermore, unpublished data generated by our lab has also shown that the mPFC, another region potentially involved in regulating recognition memory, has a similar response profile to peripheral immune activation in terms of kynurenine pathway metabolism which is also related to other aspects of cognitive dysfunction. Of note, while KMO mRNA was not increased by LPS at 24 or 48 h (figure 4C), unpublished data from our laboratory indicate that KMO mRNA is increased in whole brain tissue analysis at 6h post-LPS, which more closely resembles the expression pattern of proinflammatory cytokines.

Together, the present study suggests a critical role for IDO and subsequent KMO-dependent kynurenine metabolism in mediating inflammation-induced deficits in recognition memory. As cognitive symptoms are among the most poorly treated by standard monoaminergic antidepressant therapies, this initial discovery of a distinct pathogenic mechanism underlying inflammation-induced cognitive impairment may be transformative. Although, follow-up studies using alternative cognitive paradigms and those aimed and comprehensively delineating the contribution of downstream kynurenine metabolites will be necessary.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Peripheral immune challenge with LPS impairs novel object recognition (NOR)
- IDO mediates impaired recognition memory caused by LPS
- Direct administration of L-kynurenine recapitulates the effect of LPS
- KMO deficient mice are protected from LPS-induced disruption of NOR

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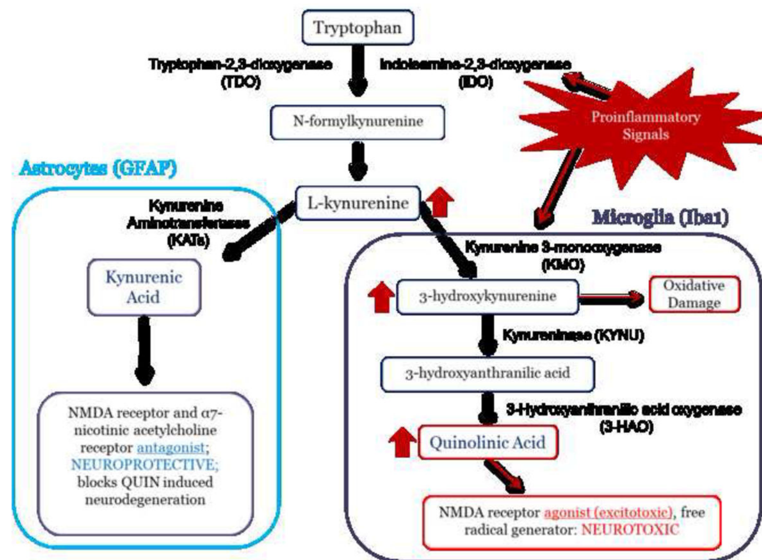

Figure 1.

The kynurenine pathway of tryptophan metabolism. In the brain, kynurenine metabolism can begin with the catabolism of tryptophan to N-formylkynurenine via either tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO), expressed in both neurons and glia. Kynurenine is metabolized either via kynurenine aminotransferases (KATs) to kynurenic acid (KYNA) in astrocytes, or via kynurenine monooxygenase (KMO), kynureninase, and 3-hydroxyanthranilic acid oxygenase (3-HAO) to quinolinic acid (QUIN). KYNA and QUIN act at the NMDA receptor as an antagonist and agonist, respectively.

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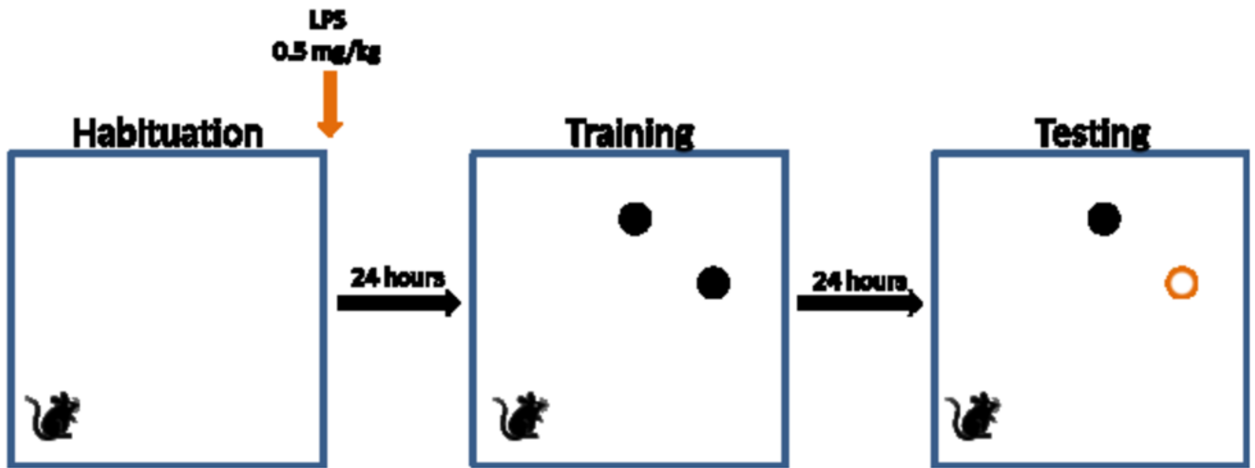

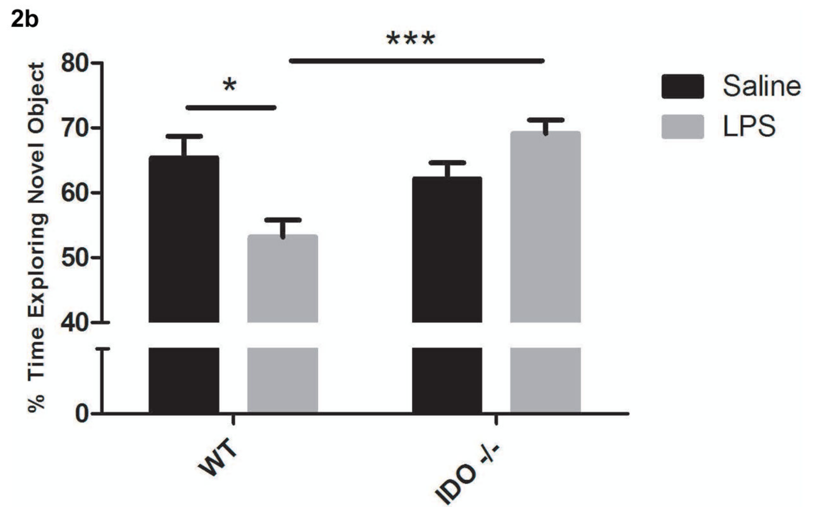

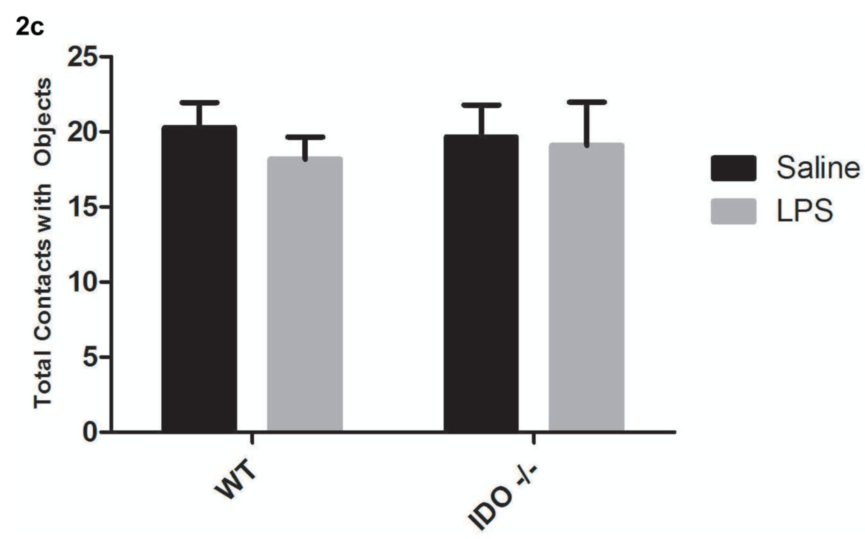

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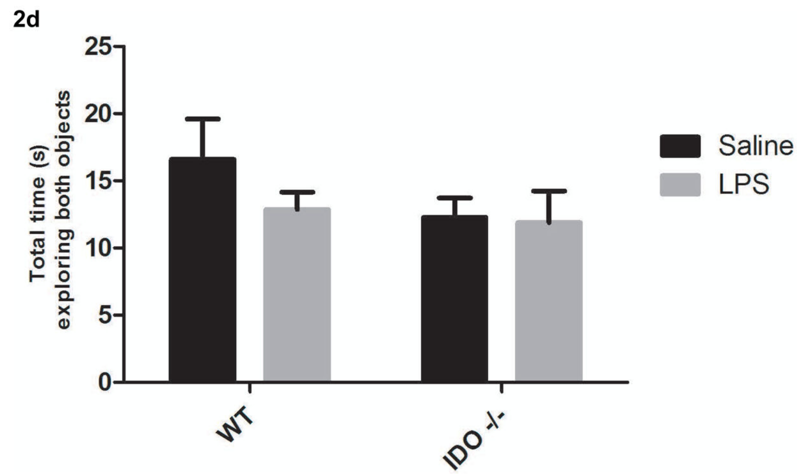

Figure 2.

Indoleamine 2,3-dioxygenase 1 mediates a deficit in Novel Object Recognition following peripheral immune challenge. (A) Schematic representation of Novel Object Recognition protocol. (B) LPS administration induces a significant reduction in preference for the novel object in WT mice, whereas IDO –/– mice are protected (Genotype x Treatment Interaction: $F_{(1,36)}=13.00$, $p=0.0009$). The total number of contacts with the objects (C) and the total time spent exploring the objects (D) is not different between any of the experimental groups. Data represent mean ± SEM. *$p<0.05$, **$p<0.01$. n=7–12 mice/group.

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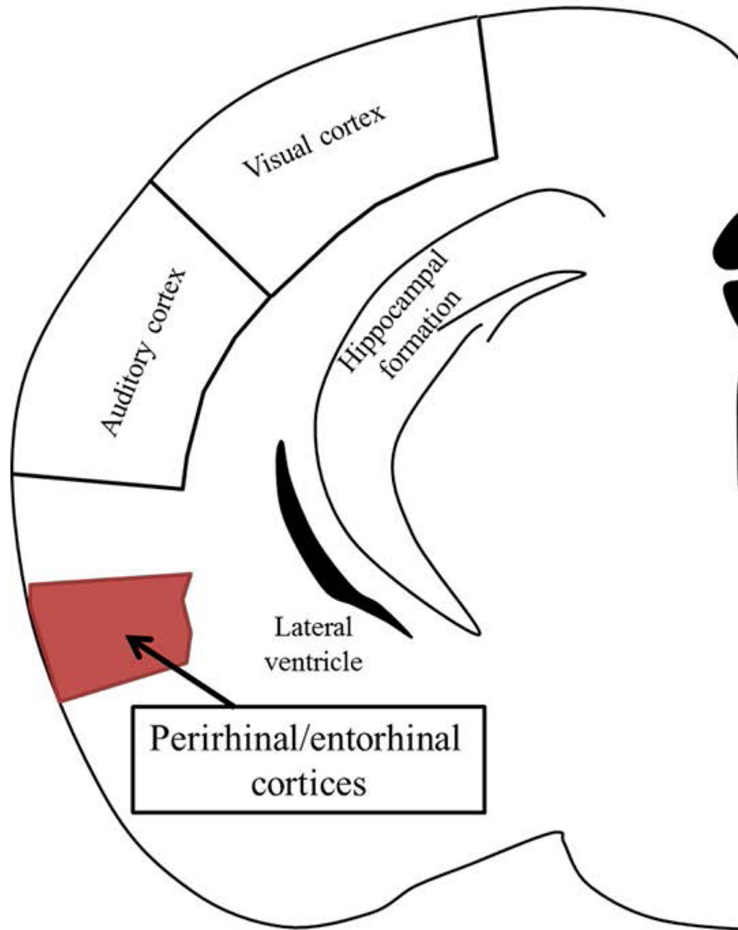

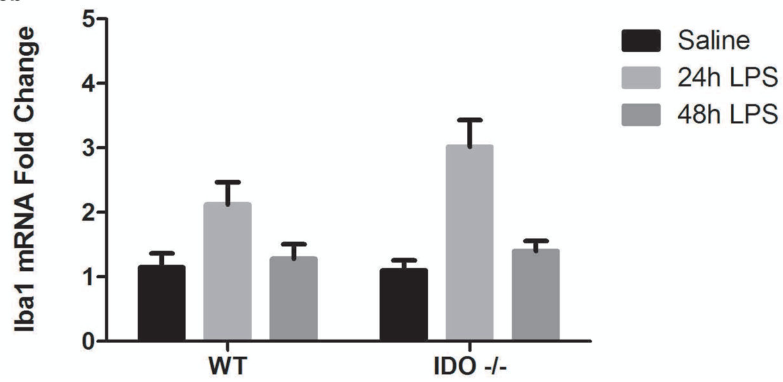

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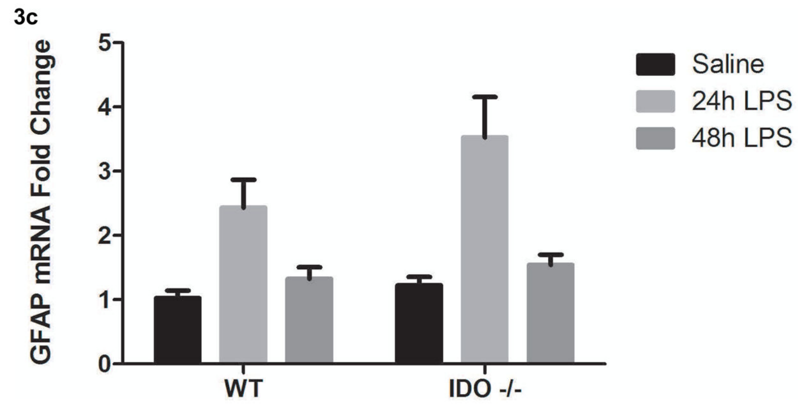

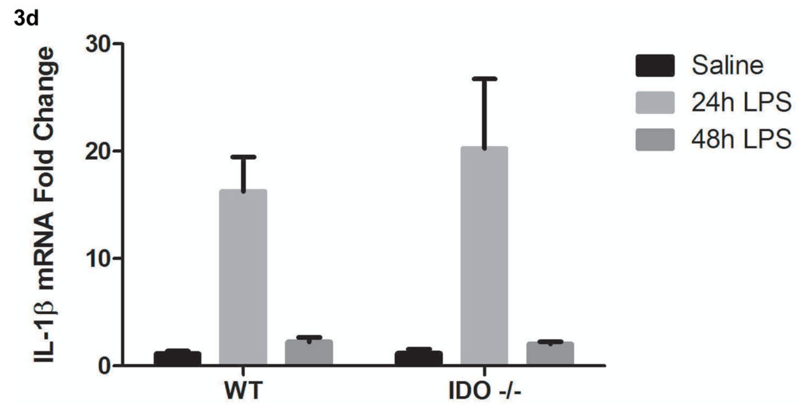

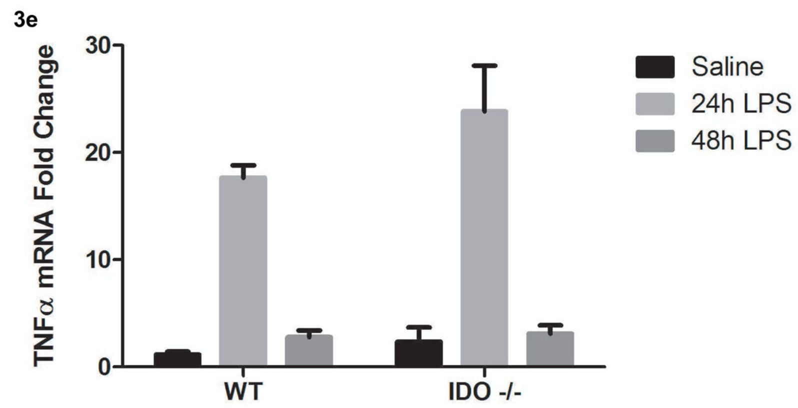

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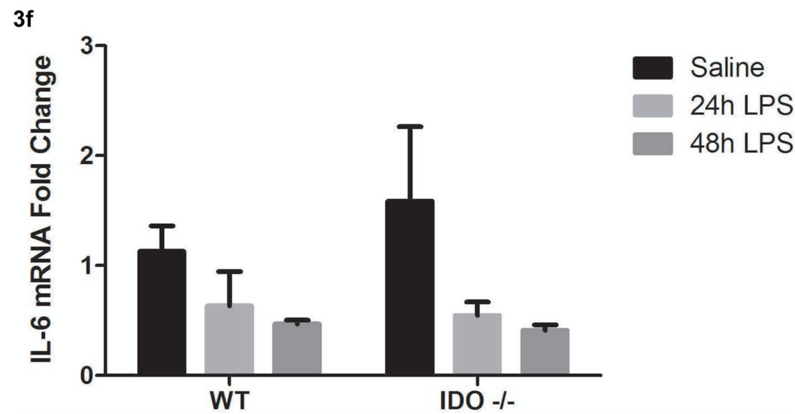

Figure 3.

Peripheral LPS administration increases the mRNA expression of proinflammatory cytokines, markers of glial activation in the perirhinal/entorhinal cortex. (A) Representative area of dissection of perirhinal/entorhinal cortices. Two 1mm sections taken from 1.56mm to 3.56mm caudal to Bregma. (B & C) LPS significantly increases expression of both microglia and astrocyte markers (Iba1: LPS effect: $F_{(2,37)=}15.47$, $p<0.0001$; GFAP: LPS effect: $F_{(2.37)}=15.28$, $p<0.0001$). LPS additionally significantly increases mRNA expression of proinflammatory cytokines (D)(IL-1β: LPS effect: $F_{(2,37)}=17.67$, $p<0.0001$; (E) TNF-α (LPS effect: $F_{(2,35)}=56.32$, $p<0.0001$). There was also a significant main effect of LPS on (F) IL-6 mRNA expression (LPS effect: $F_{(2,37)}=5.724$, $p=0.0068$), however there were no significant between group differences. Data represent mean ± SEM. n=7–12 mice/group.

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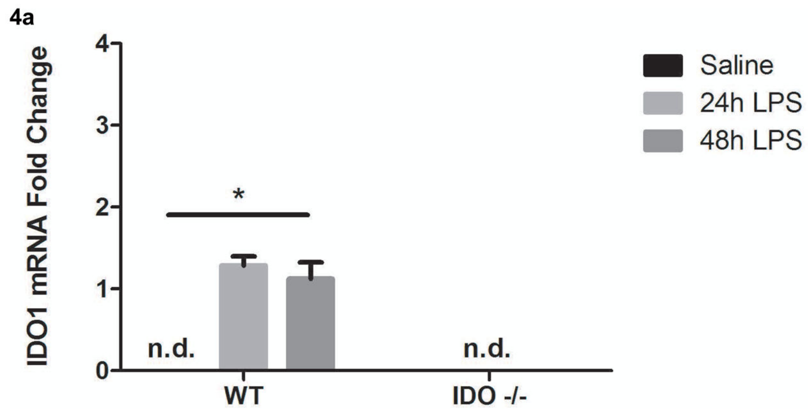

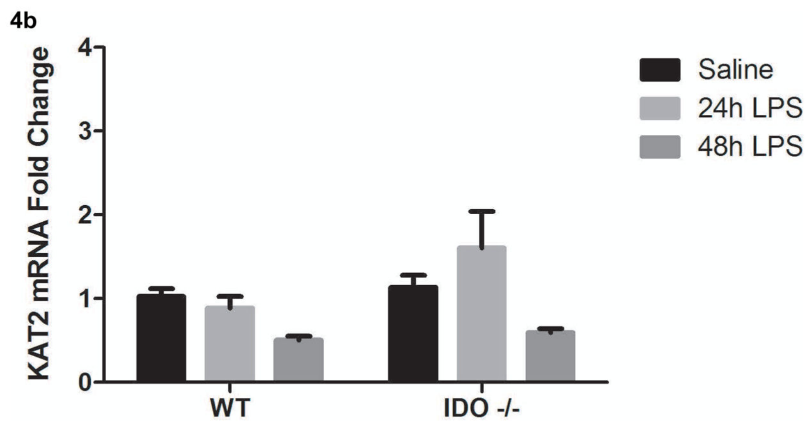

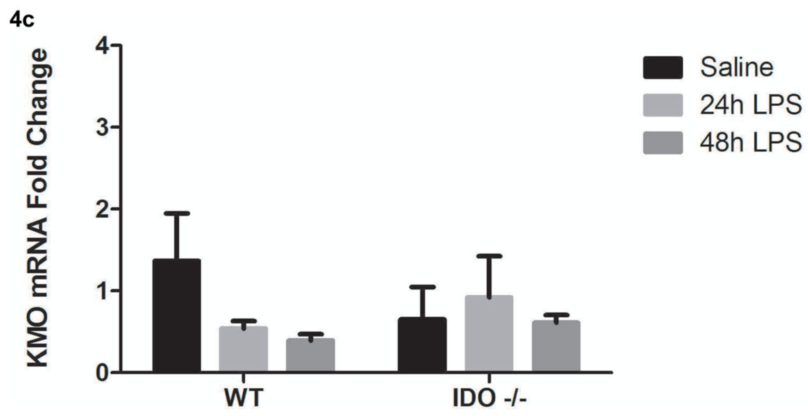

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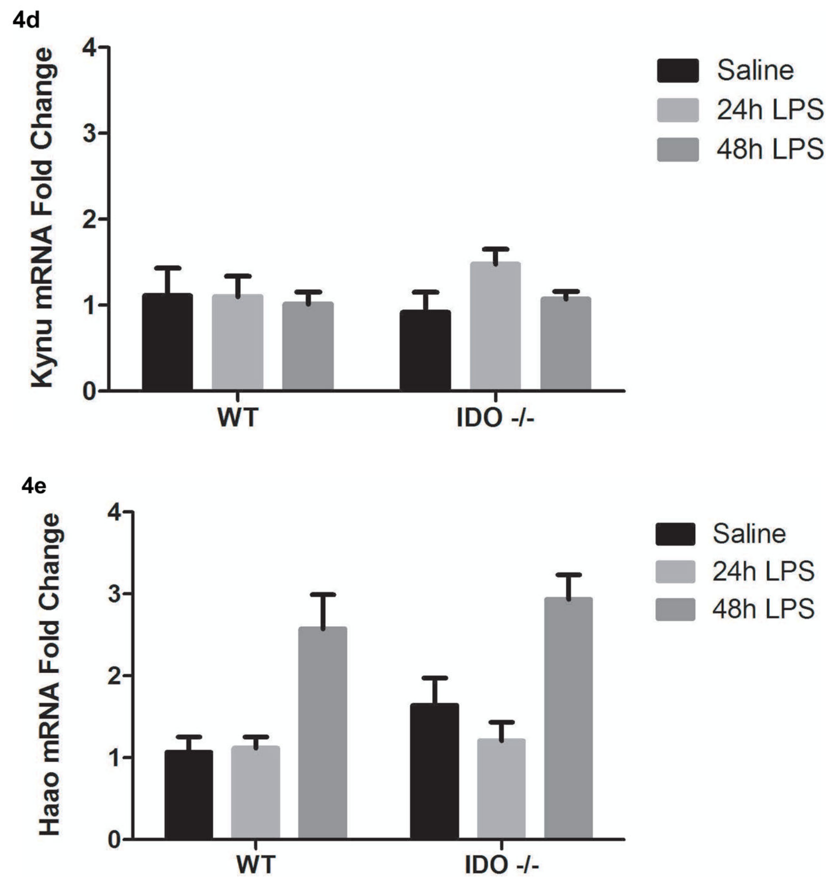

Figure 4.
Indoleamine 2,3-dioxygenase 1 and hydroxyanthranilic acid oxygenase mRNA expression is increased following peripheral LPS challenge. (A) There was a significant effect of LPS and genotype on IDO1 expression (Genotype x Treatment Interaction: $F_{(2,37)}$=10.82; p=0.0002). LPS significantly increased mRNA expression of IDO1 in WT mice 48h post-LPS (p<0.0001). (B) There was a significant effect of LPS on KATII expression (LPS effect: $F_{(2,37)}$=6.09; p=0.005). There was no effect of LPS on mRNA expression of KMO (C) or KYNU (D). LPS significantly increased the mRNA expression of HAAO in both WT and IDO −/− mice (LPS effect: $F_{(2,37)}$=18.63; p<0.0001). Data represent mean ± SEM. *p<0.001, n=7–12 mice/group.

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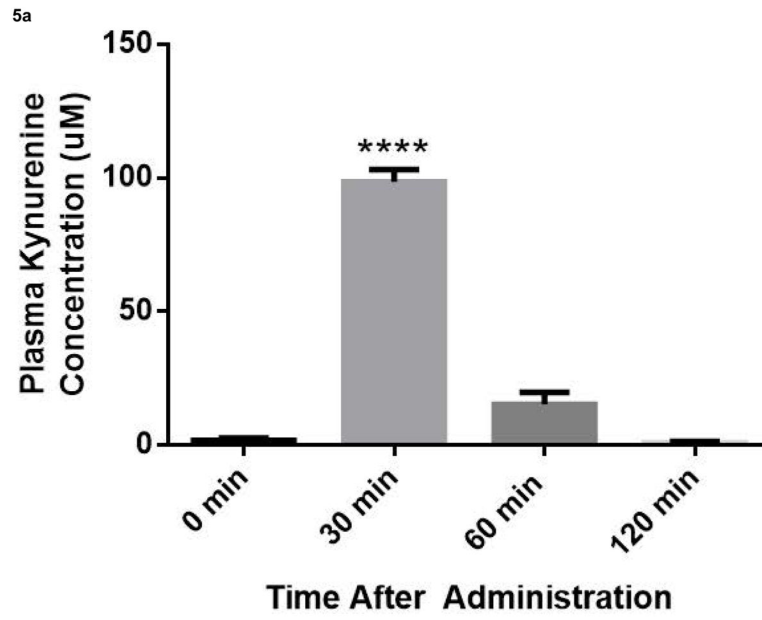

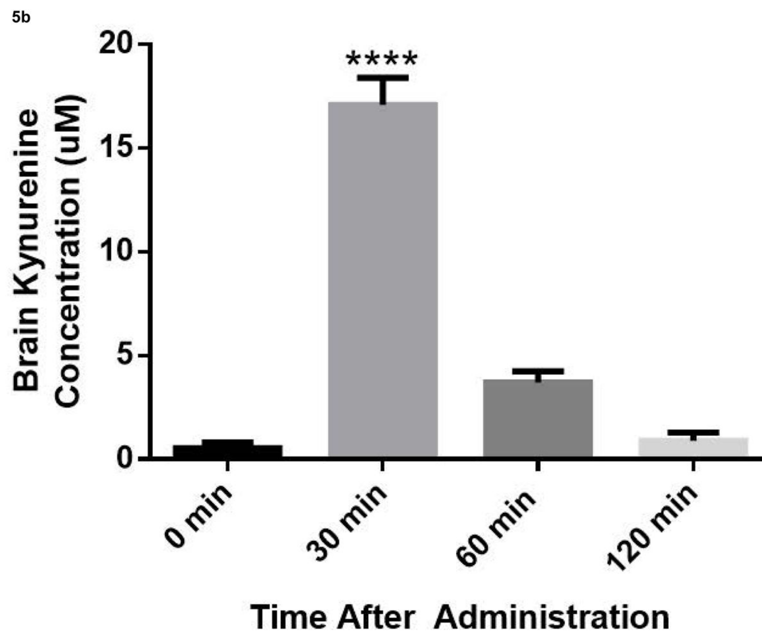

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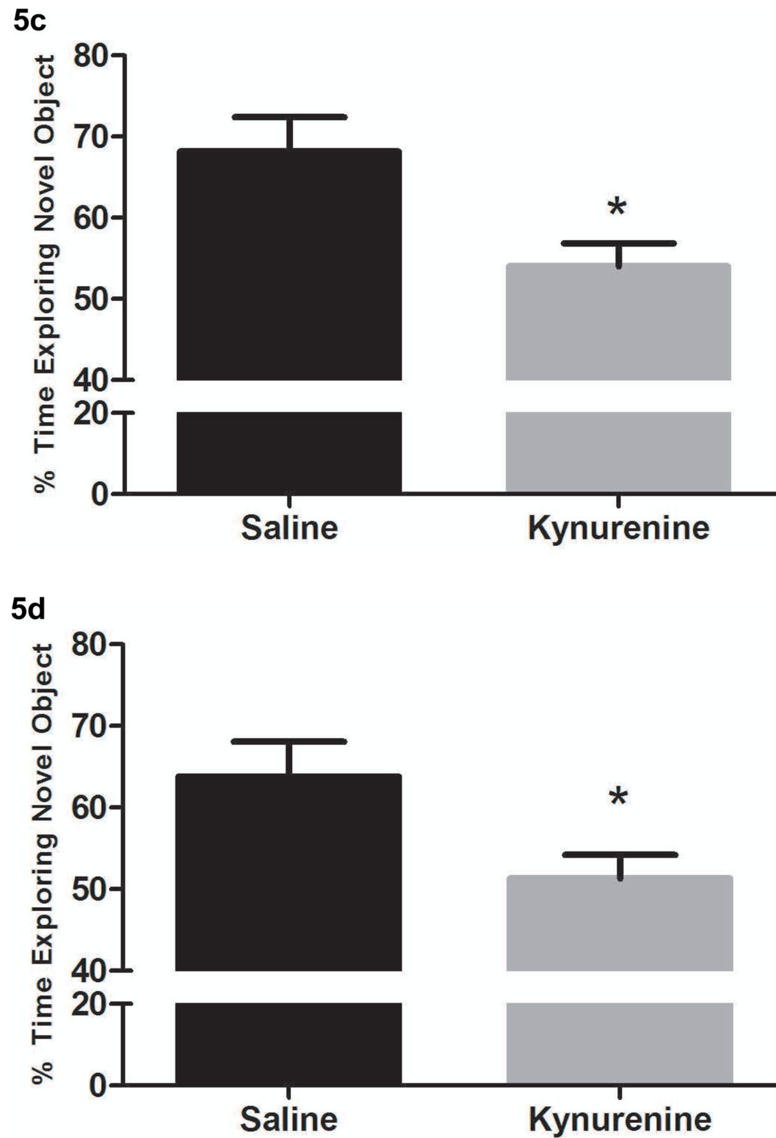

Figure 5.
Direct administration of L-kynurenine induces a deficit in Novel Object Recognition. A significant increase in plasma and brain concentration of kynurenine is observed 30 min post administration of L-kynurenine (A–B). Peripheral administration of kynurenine 30 min prior to training results in a significant reduction in novel object preference during testing, 24 hours post administration in both (C) WT and (D) IDO KO mice. There was no effect on overall exploratory activity (not shown). Data represent mean ± SEM. *p<0.05, ****p<0.0001. n=5–6 mice/group.

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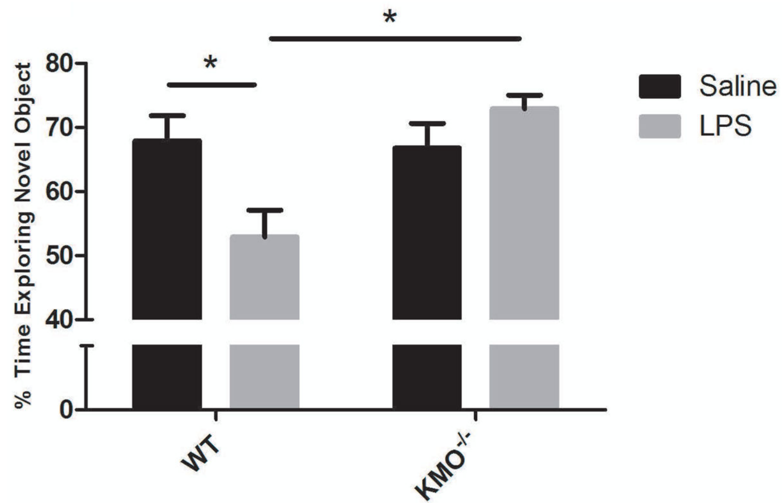

Figure 6.

Kynurenine 3-monooxygenase mediates a deficit in Novel Object Recognition following peripheral immune challenge. LPS administration induces a significant reduction in preference for the novel object in WT mice, whereas KMO−/− mice are protected (Genotype x Treatment Interaction: $F_{(1,29)}$=8.23, p=0.01). Data represent mean ± SEM. *p<0.05. n=7–10 mice/group.