HHS Public Access

Author manuscript

Eur J Neurosci. Author manuscript; available in PMC 2022 May 02.

Published in final edited form as:

Eur J Neurosci. 2021 September ; 54(6): 5968–5981. doi:10.1111/ejn.15416.

A single prenatal lipopolysaccharide injection has acute, but not long-lasting, effects on cerebral kynurenine pathway metabolism in mice

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Abstract

In rodents, a single injection of lipopolysaccharide (LPS) during gestation causes chemical and functional abnormalities in the offspring. These effects may involve changes in the kynurenine pathway (KP) of tryptophan degradation and may provide insights into the pathophysiology of psychiatric diseases. Using CD1 mice, we examined acute and long-term effects of prenatal LPS treatment on the levels of kynurenine and its neuroactive downstream products kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK) and quinolinic acid. To this end, LPS (100 μg/kg, i.p.) was administered on gestational day 15, and KP metabolites were measured 4 and 24 h later or in adulthood. After 4 h, kynurenine, KYNA and 3-HK levels were elevated in the fetal brain, 3-HK and KYNA levels were increased in the maternal plasma, and kynurenine was increased in the maternal brain, whereas no changes were seen in the placenta. These effects were less prominent after 24 h, and prenatal LPS did not affect the basal levels of KP metabolites in the forebrain of adult animals. In addition, a second LPS injection (1 mg/kg) in adulthood in the offspring of prenatally saline- and LPS-treated mice caused a similar elevation in 3-HK levels in both groups after 24 h, but the effect was significantly more pronounced in male mice. Thus, acute immune activation during pregnancy has only short-lasting effects on KP metabolism and does not cause cerebral KP metabolites to be disproportionally affected by a second immune challenge in adulthood. However, prenatal KYNA elevations still contribute to functional abnormalities in the offspring.

Keywords

Development; 3-Hydroxykynurenine; Kynurenic acid; Schizophrenia

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Author Contributions

FMN performed the experiments and drafted the manuscript. RS designed the study and revised the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Introduction

Adverse events during pregnancy have significant effects on brain development and can in turn lead to pathological consequences in the offspring (Debnath et al., 2015; Haddad et al., 2020; Seidman et al., 2000; Stolp et al., 2012). More specifically, epidemiological studies suggest that prenatal exposure to infectious agents can be linked to an increased risk to develop a variety of psychiatric disorders, including depression and schizophrenia (Borrell et al., 2002; Brown, 2012; Conway & Brown, 2019; Kirkbride et al., 2012; Rapoport et al., 2005; Stower, 2019). This concept is supported by a substantial number of experiments in rodents, which showed that maternal immune activation leads to several neurochemical and behavioral abnormalities in the adult offspring, which are similar to those observed in patients (Meyer & Feldon, 2010, 2012; Ozawa et al., 2006; Romero et al., 2007; Smith et al., 2007). One of the most widely used experimental approaches in this respect involves maternal exposure to the prototypical endotoxin lipopolysaccharide (LPS), which triggers an immune response by interacting with Toll-like receptors of the innate immune system (Janssens & Beyaert, 2003; Park & Lee, 2013). This treatment results in distinct deficits in the progeny later in life, including decreased hippocampal neurogenesis, dysfunctional synaptic transmission, and a number of behavioral abnormalities, including cognitive impairments (Chlodzinska et al., 2011; Coyle et al., 2009; Depino, 2015; Escobar et al., 2011; Fernandez de Cossio et al., 2017; Lin & Wang, 2014). Converging evidence suggests a *causal* relationship between the prenatal bacterial infection and the deficits seen in the adult offspring. However, in spite of their possible relevance for the pathophysiology of major psychiatric diseases, the mechanisms underlying the untoward long-term consequences of maternal LPS administration have not been clarified so far.

Activation of the kynurenine pathway (KP) of tryptophan degradation may be a significant factor in this context. In particular, in adult animals, the initial, rate-limiting enzyme of the KP, indoleamine-2,3-dioxygenase (IDO), is readily induced by LPS and other inflammatory stimuli (Lestage et al., 2002; O'Connor et al., 2009), resulting in an increased conversion of tryptophan to kynurenine (Figure 1). Downstream, the KP contains several neuroactive metabolites, including the free radical generator 3-hydroxykynurenine (3-HK), the NMDA receptor agonist quinolinic acid (QUIN), and, in a competing branch, kynurenic acid (KYNA), an antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptor function (Schwarcz & Stone, 2017). Focusing on KYNA because of its ability to affect these two receptors, which are both believed to be critically involved in the pathophysiology of psychiatric disorders (Lakhan et al., 2013; Olincy & Freedman, 2012), a series of studies demonstrated that even moderate increases in the brain levels of this metabolite cause disease-relevant cognitive deficits in animals (Pocivavsek et al., 2016). Notably, abnormal *prenatal* increases in KYNA levels, which can be produced experimentally in rodents by administering kynurenine or a kynurenine 3-monooxygenase (KMO) inhibitor to the dam during the last week of gestation (cf. Figure 1), lead to chemical, structural and electrophysiological abnormalities reminiscent of psychiatric disorders in the adult offspring (Alexander et al., 2013; Forrest, Khalil, Pisar, Darlington, et al., 2013; Khalil et al., 2014; Pershing et al., 2015; Pisar et al., 2014; Pocivavsek et al., 2014). Interestingly, these long-term effects include functionally relevant increases in KYNA levels in the adult brain

(Pershing et al., 2016; Pocivavsek et al., 2019; Pocivavsek et al., 2014). In view of the fact that the concentration of KYNA is significantly elevated in the brain and cerebrospinal fluid of persons with schizophrenia (Erhardt et al., 2001; Linderholm et al., 2012; Sathyasaikumar et al., 2011; Schwarcz et al., 2001), these findings jointly raised the possibility that an impairment in KP metabolism during the prenatal period may play a role in the emergence of psychiatric symptoms later in life (Notarangelo & Pocivavsek, 2017).

Despite the fact that both prenatal LPS administration and KYNA up-regulation cause deficits on mature animals, and that the acute consequences of these two interventions during pregnancy have been described individually, the possible relationship between prenatal LPS exposure and KP metabolism has not been directly examined so far. This is particularly relevant as the regulation of the KP differs both qualitatively and quantitatively at various stages of development (Gramsbergen et al., 1997; Notarangelo et al., 2019; Notarangelo & Pocivavsek, 2017).

Because of its possible translational significance, the present study was designed to address this question by examining the short- and long-term effects of a single prenatal injection of LPS on KP metabolism in mice. Using a dose known to raise cytokine levels while minimizing the risk of preterm delivery and maternal mortality (Fricke et al., 2018), we administered 0.1 mg/kg LPS intraperitoneally (i.p.) on gestational day (GD) 15. Since activation of the immune system later in life is also considered to play a role in the pathophysiology of major psychiatric disorders including depression and schizophrenia (Benros et al., 2011; Dantzer et al., 2008), the offspring of prenatally treated animals received an additional LPS injection in adulthood. In all animals, the levels of pivotal KP metabolites were analyzed both during pregnancy and in adulthood.

Materials and Methods

Chemicals

Kynurenic acid (KYNA), 3-hydroxy-DL-kynurenine (3-HK), quinolinic acid (QUIN), [$^2H_6$]L-kynurenine, pentafluoropropionic anhydride and 2,2,3,3,3-pentafluoro-1-propanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-Kynurenine sulfate (“kynurenine”; purity: 99.4%) was obtained from Sai Advantium (Hyderabad, India). [$^2H_3$]Quinolinic acid was purchased from Synfine Research (Richmond Hill, Ontario, Canada). LPS from *E. coli* (L-3129, serotype 0127.B8) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

All other chemicals were obtained from various commercial suppliers and were of the highest available purity.

Mice

Pregnant CD-1 mice (2–3 month-old; gestational age: 2 days) were obtained from Charles River Laboratories (Frederick, MD, USA) and were individually housed upon arrival. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Maryland School of Medicine. Mice were maintained on a 12 h light/dark cycle in a temperature-controlled room with *ad libitum* access to food and water. For all

prenatal studies, 2–4 embryos per litter were used, and the data were expressed as averages of each litter. For studies in adult animals, only one male and one female mouse was used for each experimental condition.

Lipopolysaccharide injection

Pregnant dams were injected i.p. with 0.1 mg/kg LPS on GD 15. Control mice received an i.p. injection of a sterile saline solution. To evaluate the acute consequences of LPS, dams (n = 3–4 per group) were euthanized using carbon dioxide either 4 or 24 h later, and maternal brain ("forebrain": whole brain without cerebellum) and plasma (collected in EDTA-containing tubes and centrifuged at 6,000 × g for 10 min) as well as placenta and fetal brain were collected and stored at −80°C until analysis. To investigate long-term effects, separate dams were left undisturbed after the prenatal administration of LPS or saline (n = 7–8 per group), and male and female offspring were weaned on postnatal day (PND) 21. At PND 60, mice were then injected i.p. with either LPS (0.1 or 1 mg/kg, i.p.) or saline and euthanized using carbon dioxide 24 h after the treatment. Forebrains of these animals were rapidly collected and stored at −80°C until analysis.

Kynurenic acid and 3-hydroxykynurenine measurement

Ultrapure water was used for all tissue homogenizations and dilutions. Fetal brain tissue was homogenized by sonication (1:10, w/v), and the homogenate was further diluted for the measurement of 3-HK (1:20 final). Placenta was homogenized (1:10, w/v) and then diluted further (1:20 final for 3-HK, 1:100 final for KYNA). Maternal and offspring brain tissue (whole forebrain) was sonicated (1:5, w/v), and maternal plasma was diluted (1:2, v/v for 3-HK and 1:10, v/v for KYNA).

Twenty-five µl of 6% perchloric acid were added to 100 µl of each sample, and precipitated proteins were removed by centrifugation (16,000 × g, 10 min). For KYNA determination, 20 µl of the resulting supernatant were injected onto a 3 µm C18 reverse phase HPLC column (100 mm × 4 mm; Dr. Maisch GmbH, Ammerbuch, Germany), using a mobile phase containing 50 mM sodium acetate and acetonitrile (3% for brain and 6% for placenta and plasma; pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 0.5 ml/min. Zinc acetate (0.5 M; not pH adjusted), was delivered post-column by a peristaltic pump (Dionex AXP, Thermo Fisher, Waltham, MA, USA) at a flow rate of 0.1 ml/min. In the eluate, KYNA was detected fluorimetrically (excitation: 344 nm, emission: 398 nm; S200a fluorescence detector; Perkin Elmer, Waltham, MA, USA).

For 3-HK determination, 20 µl of the supernatant were applied to a 3 µm HPLC column (HR-80; 80 mm × 4.6 mm; ESA, Chelmsford, MA, USA), using a mobile phase consisting of 1.5 % acetonitrile, 0.9 % triethylamine, 0.59 % phosphoric acid, 0.27 mM EDTA and 8.9 mM sodium heptane sulfonic acid, and a flow rate of 0.5 ml/min. In the eluate, 3-HK was detected electrochemically using a HTEC 500 detector (Eicom Corp., San Diego, CA; oxidation potential: +0.5 V).

Kynurenine and quinolinic acid measurement

To measure kynurenine and QUIN in tissue, the original homogenates were further diluted (v/v) in 0.1% ascorbic acid (1:20 final for maternal and offspring brain, 1:50 final for fetal brain, 1:100 final for placenta). For the determination of QUIN in maternal plasma, samples were diluted in 0.1% ascorbic acid (1:10, v/v). Fifty µl of an internal standard mix ([$^2H_3$]quinolinic acid, [$^2H_6$]L-kynurenine) were added to 50 µl of the samples, and proteins were precipitated with 50 µl of acetone. After centrifugation (13,700 × g, 5 min), 50 µl of methanol:chloroform (20:50) were added to the supernatant, and the samples were centrifuged (13,700 × g, 10 min). The upper layer was added to a glass tube and dried down for 90 min. The samples were then derivatized with 120 µl of 2,2,3,3,3-pentafluoro-1-propanol and 130 µl of pentafluoropropionic anhydride at 75°C for 30 min, dried down and reconstituted in 50 µl of ethyl acetate. One µl was injected in the GC/MS (Notarangelo et al., 2012).

For determination of kynurenine in maternal plasma, 25 µl of 6% perchloric acid were added to 100 µl of the sample. Precipitated proteins were removed by centrifugation (16,000 × g, 10 min), and 20 µl of the resulting supernatant were injected to a 3 µm C18 reverse phase HPLC column (100 mm × 4 mm; Dr. Maisch GmbH), using a mobile phase containing 50 mM sodium acetate and 6% acetonitrile (pH adjusted to 6.2 with glacial acetic acid) at a flow rate of 0.5 ml/min. Zinc acetate (0.5 M; not pH adjusted), was delivered post column by a peristaltic pump (Dionex AXP, Thermo Fisher) at a flow rate of 0.1 ml/min. In the eluate, kynurenine was detected fluorimetrically (excitation: 365 nm, emission: 480 nm; S200a fluorescence detector; Perkin Elmer).

Sex determination

Embryonic tissue was retained for determination of sex by genotyping, using primers specific to Jarid1 (5′-CTGAAGCTTTTGGCTTTGAG-3′ and 5′-CCGCTGCCAAATTCTTTGG-3′; Invitrogen, Carlsbad, CA, USA) as previously described (Clapcote & Roder, 2005). One or two male and female embryos per litter were used for the analyses.

Protein determination

Protein was determined according to Lowry et al. (Lowry et al., 1951), using bovine serum albumin as a standard.

Statistical analysis

All results are expressed as the mean ± SEM. Statistical analyses were performed with Graphpad Prism 9 (San Diego, CA, USA) and two-way ANOVA followed by Bonferroni's post-hoc test was used to determine significance in all experiments. A p value of <0.05 was considered significant.

Results

Acute effect of prenatal LPS treatment on maternal brain and plasma

To evaluate the effect of moderate immune activation on KP metabolites during pregnancy, mice received a single injection of 0.1 mg/kg LPS on GD15. Assessed 24 h later, this treatment did not influence maternal body weight or the number of embryos (data not shown), confirming that the dose of the endotoxin used does not affect pregnancy (Chlodzinska et al., 2011).

Kynurenine, KYNA, 3-HK and QUIN were analyzed in the maternal brain 4 and 24 h after LPS treatment (Figure 2). Kynurenine levels increased after 4 h (12.8 ± 0.3 vs. 7.4 ± 0.3 pmol/mg protein in saline-treated mice; $p < 0.05$) but returned to control levels after 24 h (main effect of treatment: $F_{(1,10)} = 5.81$, $p < 0.05$; interaction: $F_{(1,10)} = 6.17$, $p < 0.05$). No significant changes in the maternal brain levels of 3-HK, KYNA or QUIN were observed at either time point, though the elevation in 3-HK (from 536.8 ± 29.9 in control mice to 901.1 ± 157.6 fmol/mg protein) 4 h following LPS administration approached statistical significance ($p = 0.07$).

LPS treatment induced no significant changes in kynurenine levels in maternal plasma after either 4 or 24 h. In contrast, we observed significant increases in the circulating levels of both KYNA and 3-HK after 4 h ($p < 0.05$). Although the concentration of both metabolites tended to remain higher than endogenous levels after 24 h (main effect of treatment: $F_{(1,10)} = 10.54$, $p < 0.01$ for KYNA; $F_{(1,10)} = 9.65$, $p < 0.05$ for 3-HK), neither effect reached statistical significance after post-hoc analysis. No significant changes in QUIN levels were observed in maternal plasma at either timepoint (Figure 3).

Acute effects of prenatal LPS treatment on placenta and fetal brain

Prenatal LPS administration on GD 15 did not cause significant changes in the levels of kynurenine, KYNA, 3-HK or QUIN after 4 or 24 h in the placenta (Suppl. Figure 1).

However, prenatal LPS treatment affected KP metabolism in the fetal brain (Figure 4). Compared to the control group, the tissue levels of kynurenine were elevated after 4 h ($p < 0.01$). A trend in the same direction was also seen at 24 h, although the effect did not reach statistical significance after post-hoc analysis (main effect of treatment: $F_{(1,10)} = 17.47$, $p < 0.01$). The levels of KYNA and 3-HK, too, increased 4 h after the administration of LPS ($p < 0.01$ and $p<0.05$, respectively), and trended back toward endogenous levels by 24 h (main effect of treatment: $F_{(1,10)} = 22.90$, $p < 0.001$ for KYNA; $F_{(1,10)} = 15.19$, $p < 0.01$ for 3-HK). No significant changes in QUIN levels were observed at either time point. Notably, prenatal LPS induced similar changes in the brain of male and female embryos (Suppl. Figure 2).

Long-term effects of prenatal LPS treatment and effects of an additional LPS challenge in adulthood

Body weight—The long-term effects of prenatal LPS administration were studied in separate cohorts of animals. Compared to control dams, treatment with 0.1 mg/kg LPS on GD 15 did not induce significant changes in maternal body weight until birth or alter the

number of pups (data not shown). Moreover, prenatal LPS treatment did not affect the body weight of the offspring at PND 1, 21 or 56 (data not shown).

To investigate if prenatal treatment altered the response to an acute LPS challenge in adulthood, we administered 0.1 or 1 mg/kg LPS to offspring of dams treated prenatally with saline ("prenatal saline") or LPS ("prenatal LPS"). Irrespective of prenatal treatments, we observed a significant decrease in body weight in both male and female mice receiving 1 mg/kg LPS 24 h later ($p < 0.0001$ and $p < 0.01$, respectively; Two-way Anova followed by Bonferroni's post-hoc test). The lower dose of LPS (0.1 mg/kg) did not affect body weight in either group (data not shown).

KP metabolites in the brain—No significant differences in the basal brain tissue levels of kynurenine, KYNA, 3-HK or QUIN were seen between either male or female offspring of dams which had received saline or LPS injections on GD15 (Figures 5 and 6). Analysis of the effect of an i.p. injection of LPS in adulthood revealed a main effect of treatment ($F_{(2,18)} = 4.65$, $p < 0.05$ for males; $F_{(2,24)} = 6.15$, $p < 0.01$ for females), but no significant changes after post-hoc analysis in the tissue levels of kynurenine 24 h following the injection of either 0.1 or 1 mg/kg LPS. KYNA and QUIN levels remained unaffected 24 h after the acute administration of either dose of LPS. In the same tissues, 3-HK levels were found to increase significantly after the administration of 1 mg/kg (but not after 0.1 mg/kg) LPS in male ($p < 0.001$ and $p < 0.0001$) and female (both $p < 0.0001$) offspring of both "prenatal saline" and "prenatal LPS" animals. Interestingly, however, while no sex differences were observed for kynurenine, KYNA and QUIN, the acute LPS challenge in adulthood raised 3-HK levels more in the brain of male than in female offspring of both "prenatal saline" and "prenatal LPS" dams ($p < 0.001$ and $p < 0.0001$, respectively). Importantly, no significant differences in the levels of any of the KP metabolites were seen between adult offspring of "prenatal saline" and "prenatal LPS" dams after the administration of either dose of LPS in adulthood.

Discussion

In light of increasing evidence supporting a role of the KP in the pathophysiology of schizophrenia and other psychiatric diseases (Bryleva & Brundin, 2017; Erhardt et al., 2017; Ogyu et al., 2018; Schwarcz et al., 2012; Zavitsanou et al., 2014) and the consensus that both prenatal infections and immune activation later in life are major risk factors in these disorders (Borrell et al., 2002; Brown, 2012; Conway & Brown, 2019; Kirkbride et al., 2012; Rapoport et al., 2005; Stower, 2019), the present study was designed to provide translationally relevant new insights, using mice as the experimental animals. By measuring the levels of the key KP metabolites kynurenine, KYNA, 3-HK and QUIN, we first examined the short- and long-term effects of a single prenatal injection of the immunogen LPS on KP metabolism, and then investigated whether an additional LPS injection in the offspring of these prenatally treated animals has disproportionate, and possibly sex-specific, acute effects on cerebral KP metabolism. Applied at doses which are widely used in preclinical studies (Chlodzinska et al., 2011; Fricke et al., 2018), a single injection of LPS during pregnancy induced transient increases in the levels of kynurenine, KYNA and 3-HK – but not QUIN - in the fetal brain. However, this prenatal LPS treatment did not affect

basal KP metabolite levels and did not influence the response to a second LPS injection in the brain of the adult offspring, possibly due to a blunted immune response to the repeated stimulus, as previously observed (Clark, Notarangelo, et al., 2019). Interestingly, an acute challenge with LPS (1 mg/kg) in adulthood raised cerebral 3-HK levels significantly more in male than in female mice.

In experimental animals, systemic application of LPS or other immunostimulants rapidly promotes the formation of cytokines, which in turn activate IDO activity (Campbell et al., 2014; Williams et al., 2017). In the present study, this effect likely accounted for the prompt, LPS-induced increase in cerebral kynurenine levels, and the rise in 3-HK and KYNA concentrations in the plasma, in the pregnant mouse following a single systemic injection of 0.1 mg/kg LPS on GD15. As the plasma levels of kynurenine, which readily enters the fetus from the maternal circulation (Goeden et al., 2017), did not change significantly in response to LPS, since KYNA does not cross the placental barrier (Goeden et al., 2017), and KP metabolites were not affected in the placenta (despite containing various KP enzymes (Manuelpillai et al., 2005; Murthi et al., 2017; Suzuki et al., 2001), the observed increase in the levels of these two metabolites in the fetal brain was probably due to *local events within the fetus*. Although controversial (Brown et al., 2019; Fricke et al., 2018), this may have involved the transfer of LPS itself into the embryo or, more likely, the trans-placental influx of LPS-induced maternal cytokines and the subsequent stimulation of fetal KP metabolism (Oskvig et al., 2012; Simoes et al., 2018; Williams et al., 2017). This would also explain the observed increase in 3-HK levels in the fetal brain, though enhanced influx from the maternal blood, where 3-HK was elevated following the LPS treatment, may have played a role as well (Goeden et al., 2017). The molecular dynamics of these processes, and the finding that QUIN levels in the fetal brain remained unaffected by LPS, clearly need to be elaborated in greater detail, keeping in mind qualitative differences in the regulation of cerebral KP metabolism at different stages of early development and lifespan (Gramsbergen et al., 1997; Notarangelo et al., 2019; Notarangelo & Pocivavsek, 2017; Walker et al., 1999). Moreover, brain cytokines were not measured in this study and further experiments are needed to clarify their specific relationship with KP metabolism activation and behavior.

In line with previous studies in mice, we observed a significant increase in brain 3-HK levels, but no changes in KYNA, after a single administration of LPS in adulthood (Larkin et al., 2016; Larsson et al., 2016; Walker et al., 2013). Moreover, as in the fetal brain, and possibly due to its rapid conversion to NAD+ (Moffett et al., 2020), LPS treatment did not raise brain QUIN levels acutely in the adult animals, as also previously reported (Clark, Notarangelo, et al., 2019). However, in contrast to other reports, and possibly related to strain differences, the serotype of the LPS used, and/or the fact that only a single timepoint (24 h) following LPS administration was examined (Migale et al., 2015; Murakami & Saito, 2013; Parrott et al., 2016; Piirsalu et al., 2020), only a non-significant trend toward elevated kynurenine levels was detected. Also of possible relevance in this context, the effect of systemically applied LPS on KP metabolites differs at various doses and between brain regions (Parrott et al., 2016; Tao et al., 2020). These variables should be evaluated to fully characterize the role of altered KP metabolism on cognitive function and kept in mind in the design of follow-up studies exploring the role of KP-related redox processes (Gonzalez Esquivel et al., 2017) and the formation of the neuroactive downstream KP metabolites

xanthurenic acid and cinnabarinic acid (Fazio et al., 2017), which were not examined in the present study. Moreover, genetic vulnerability should be considered (Beggiato et al., 2018), and *repeated* or *chronic* immune activation either during pregnancy or later in life, too, may have functionally relevant, detrimental impacts on brain KP metabolism (Larsson et al., 2016; Saito et al., 1992).

These considerations could also be relevant for explaining the significantly larger increase in brain 3-HK levels that we observed in male compared to female animals. Though sex differences in LPS-induced immune responses and behavior have been documented in rodents (Cai et al., 2016; Chlodzinska et al., 2011; Foley et al., 2015; Kuo, 2016), it is worth noting that links to cerebral KP metabolism have so far been predominantly examined in males (Larkin et al., 2016; Larsson et al., 2016; Tao et al., 2020; Walker et al., 2013), and that greater effects of immune activation in male mice (Cai et al., 2016; Kuo, 2016) may be related to the fact that females are more resilient due to the modulating effect of estrogen on cytokine gene expression (Dimayuga et al., 2005).

Our study design was based on – and is in line with – an extensive body of literature *separately* linking immune changes or abnormal KP metabolism during the prenatal period with adverse consequences in adulthood (Haddad et al., 2020; Notarangelo & Pocivavsek, 2017). Specifically, and in accordance with the popular “two-hit” hypothesis of psychiatric disorders (Maynard et al., 2001), our goal here was to evaluate the relationship between these two phenomena by focusing on *acute* immune-stimulations and their short-term effects on KP metabolism. In this context, we were especially interested in a possible role of KYNA, an established neuromodulator, which can inhibit α7nACh and NMDA receptor function in the adult brain (Pocivavsek et al., 2016) and has been shown to significantly affect progenitor cell proliferation, differentiation, and survival of human cortical cells (Bagasrawala et al., 2016). Thus, acute prenatal elevation in KYNA levels can apparently affect normal brain development and, consequently, influence behavior later in life. Notably, even the normal, i.e. endogenous, brain concentration of KYNA, like that of several other KP metabolites, is substantially higher prenatally than postnatally (Beal et al., 1992; Beggiato et al., 2018; Cannazza et al., 2001; Ceresoli-Borroni & Schwarcz, 2001; Walker et al., 1999), and the fetal brain produces more KYNA from kynurenine than the maternal brain under *ex vivo* conditions (Notarangelo et al., 2019). Of special interest in the context of dysfunctions triggered by prenatal immune activation, the α7nACh receptor *agonist* choline (Albuquerque et al., 1998; Alkondon et al., 1999; Fayuk & Yakel, 2004), possibly by counteracting the adverse consequences of enhanced KYNA inhibition of this receptor, attenuates the undesirable long-term effects of maternal immune activation on anxiety- and cognitive-related behaviors in adulthood (Wu et al., 2015). Finally, and further supporting a physiological role of endogenous KYNA in brain development, KYNA’s synthesizing enzyme kynurenine aminotransferase II (Figure 1) is highly expressed in the germinal zones (Csillik et al., 2002; Song et al., 2018) and mediates oligodendrogenesis as well as cell proliferation in the subventricular zone (Clark, Mou, et al., 2019).

Like acute prenatal immune activation (see Introduction), experimentally induced increases in the levels of KP metabolites in the fetal brain have been consistently shown to be associated with dysfunctions later in life. These impairments, which include abnormalities

in synaptic transmission, imbalanced neurotransmitter functions and several translationally relevant behavioral deficits (Alexander et al., 2013; Forrest, Khalil, Pisar, Darlington, et al., 2013; Forrest, Khalil, Pisar, McNair, et al., 2013; Khalil et al., 2014; Pershing et al., 2015; Pisar et al., 2014; Pocivavsek et al., 2014), may increase the risk of the offspring for developing major psychiatric disorders, including depression and schizophrenia.

The present results show that brief, transient maternal immune activation, while stimulating KP metabolism in the fetal brain significantly and rapidly, does not cause long-lasting changes in cerebral KP metabolism or disproportionate acute vulnerability of cerebral KP metabolism to a second immune challenge. Similar conclusions were recently drawn from experiments using prenatal treatment with poly I:C, a classic experimental tool for studying translationally relevant long-term effects of immune dysfunctions during pregnancy (Clark, Notarangelo, et al., 2019; Estes et al., 2020; Haddad et al., 2020).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Kevin Wons and Ashley Holmes for excellent technical assistance. This work was supported by USPHS grant P50 MH103222 (Conte Center for Translational Mental Health Research)

Data Availability Statement

Data will be made available upon reasonable request to the corresponding author.

Abbreviations:

GD	Gestational day
3-HK	3-Hydroxykynurenine
IDO	Indoleamine-2,3-dioxygenase
KP	Kynurenine pathway
KYNA	Kynurenic acid
LPS	Lipopolysaccharide
α7nACh	α7 Nicotinic acetylcholine
NMDA	N-Methyl-D-aspartate
PND	Postnatal day
QUIN	Quinolinic acid

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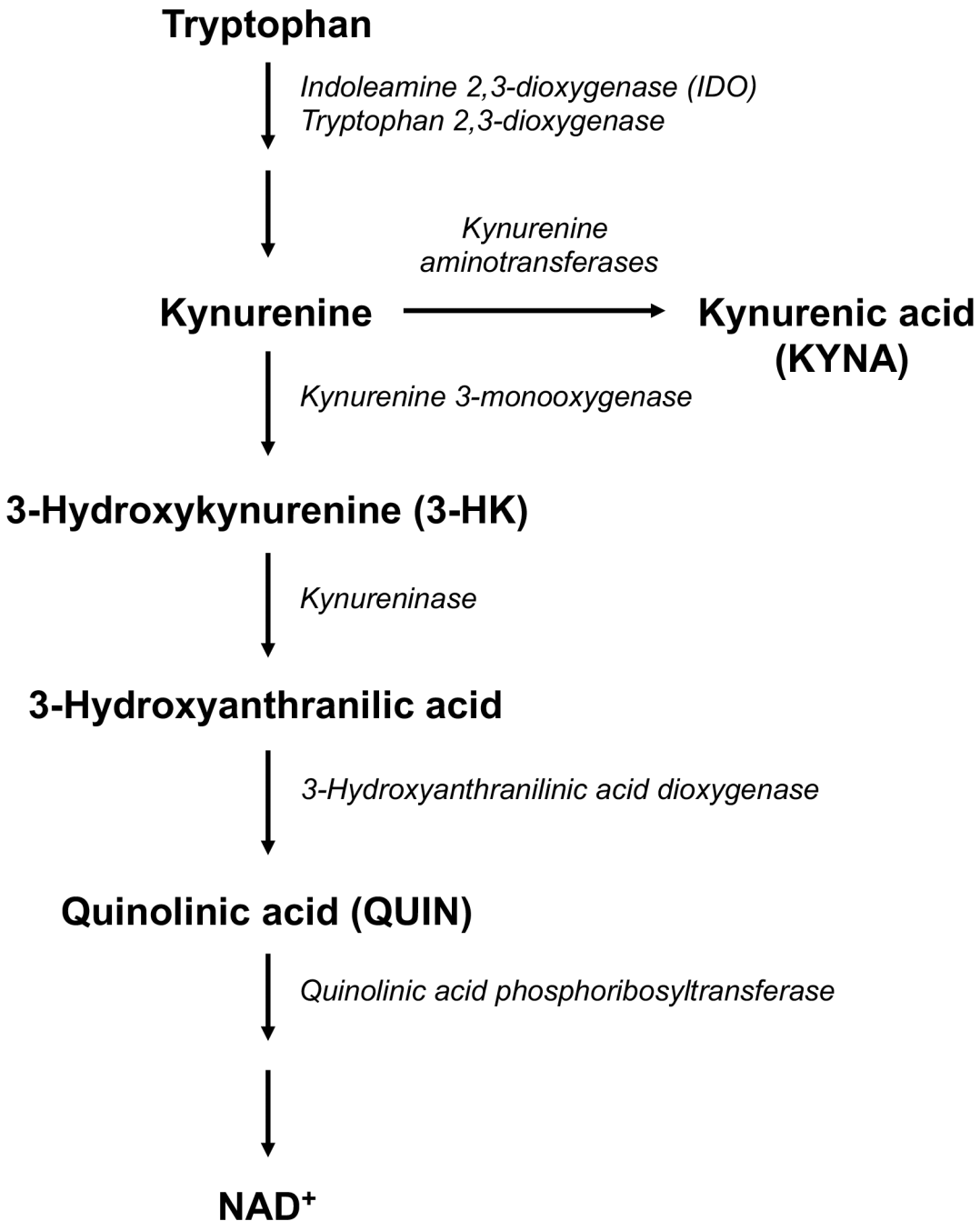

Figure 1:
The kynurenine pathway of tryptophan degradation

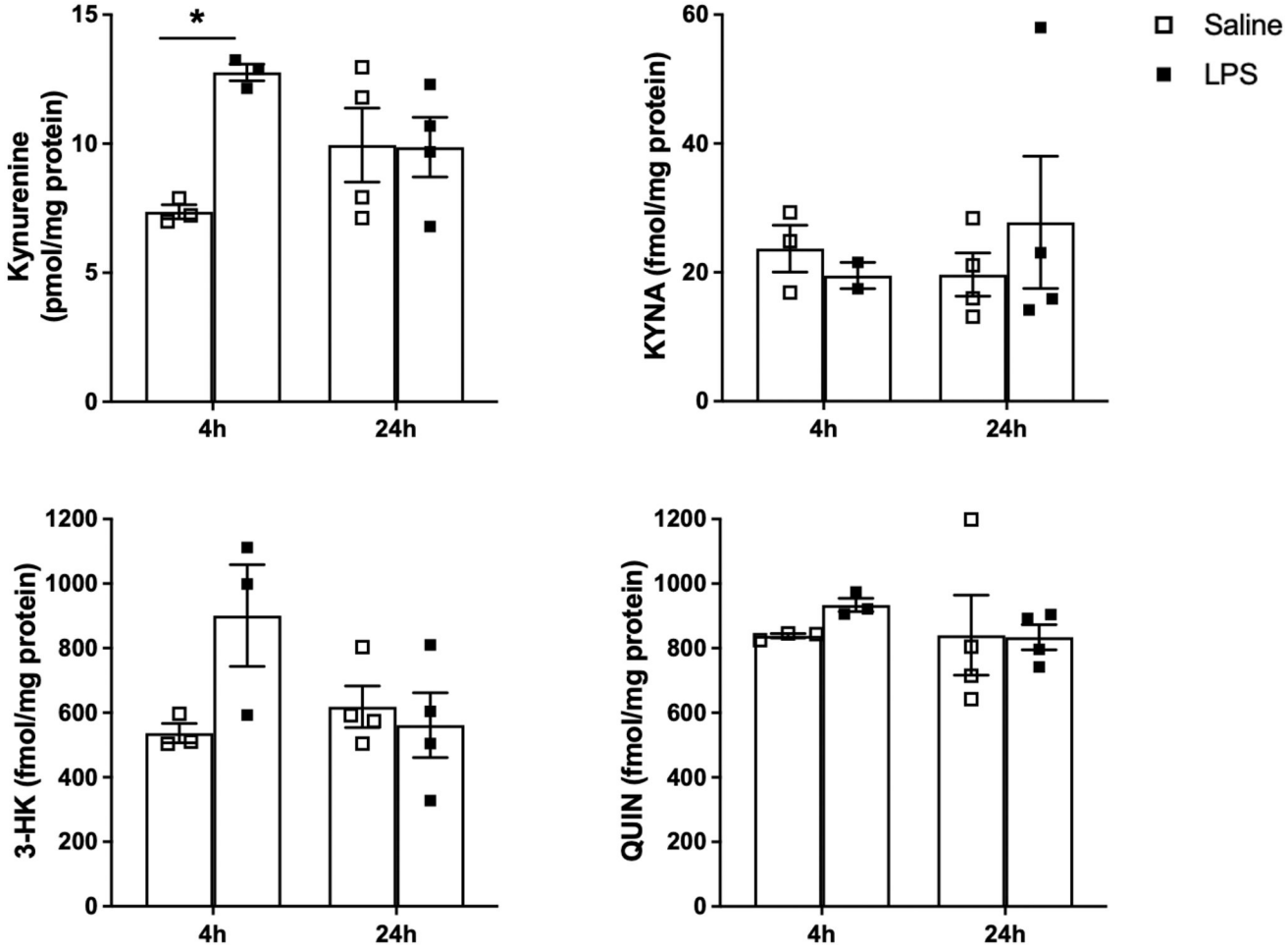

Figure 2:

KP metabolite levels in the maternal brain 4 and 24 h after saline or LPS administration (0.1 mg/kg, i.p.) on GD15. Data are the mean ± SEM (n = 3–4). * $p < 0.05$ (two-way Anova, followed by Bonferroni's post-hoc test).

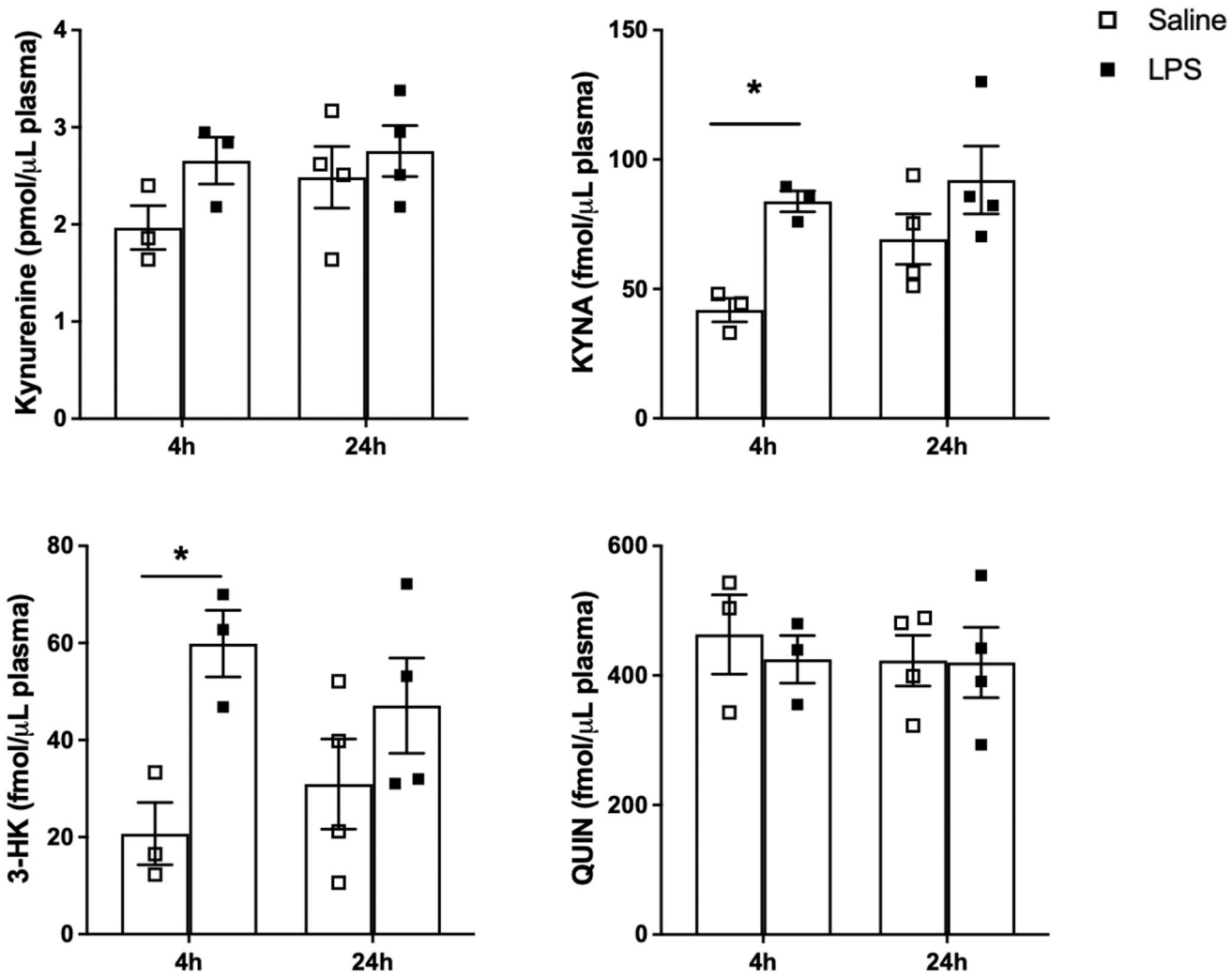

Figure 3:

KP metabolite levels in the maternal plasma 4 and 24 h after saline or LPS administration (0.1 mg/kg, i.p.) on GD15. Data are the mean ± SEM (n = 3–4). * $p < 0.05$ (two-way Anova, followed by Bonferroni's post-hoc test).

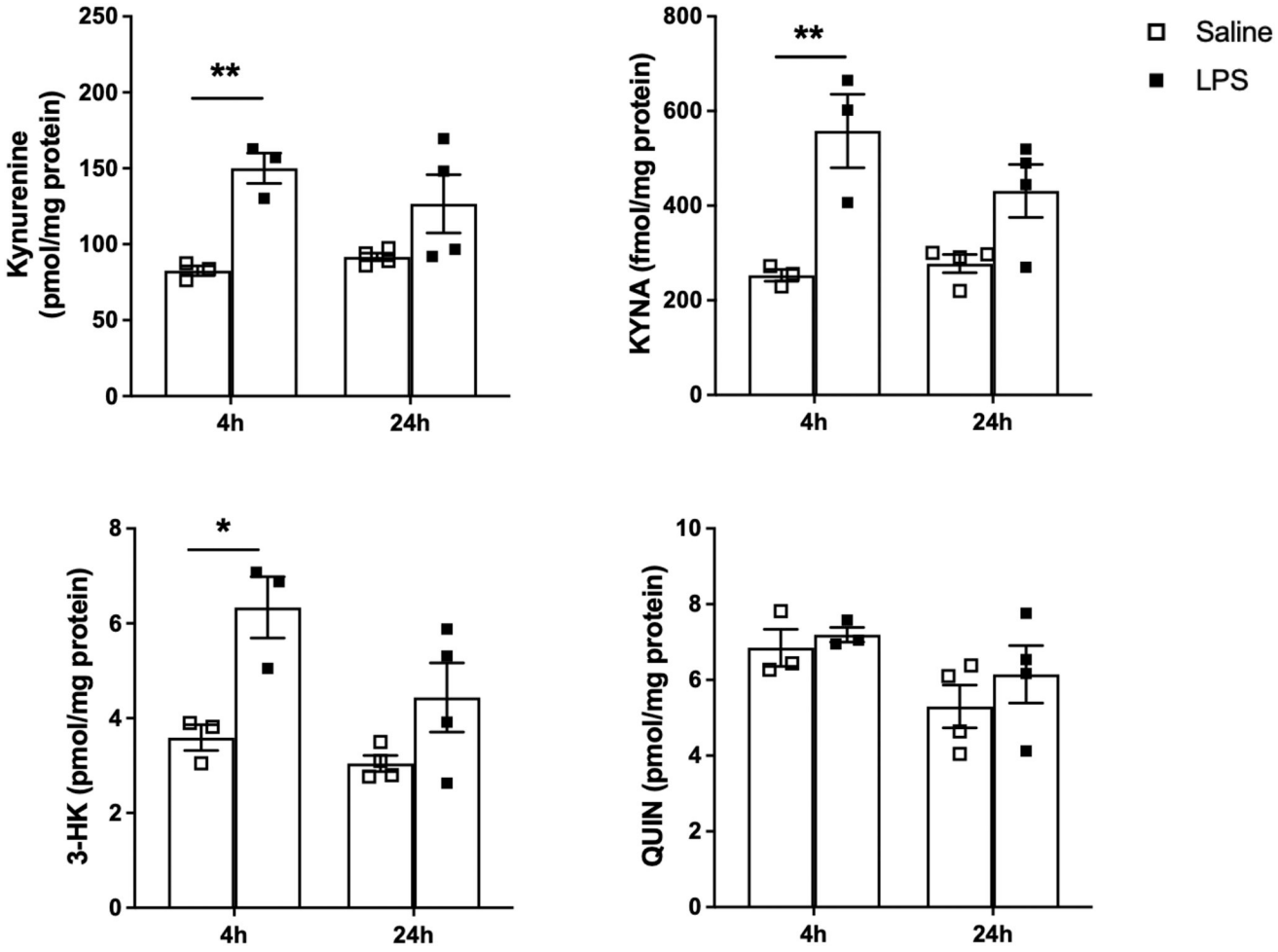

Figure 4:

KP metabolite levels in the fetal brain 4 and 24 h after saline or LPS administration (0.1 mg/kg, i.p.) on GD15. Data are the mean ± SEM (n = 3–4). * $p < 0.05$, ** $p < 0.01$ (two-way Anova, followed by Bonferroni's post-hoc test).

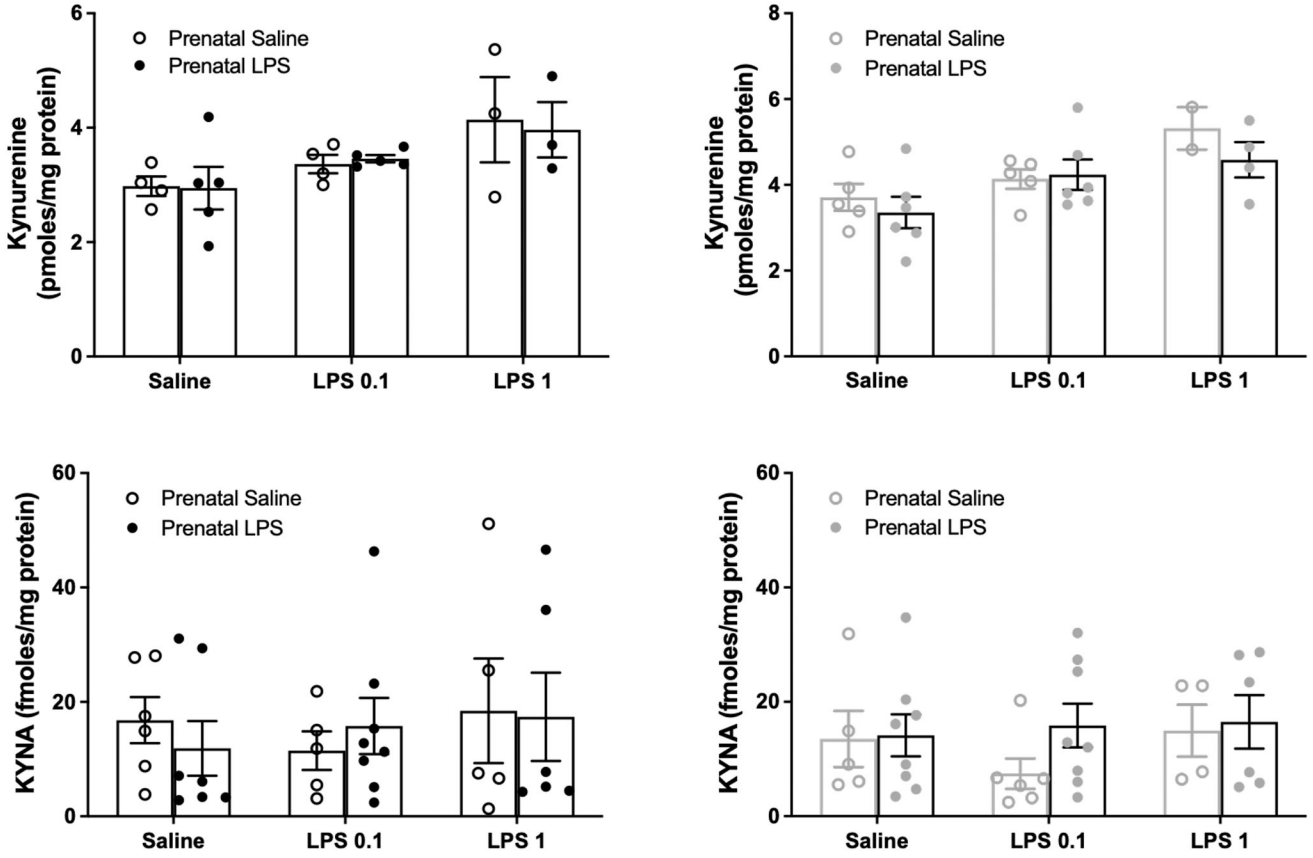

Figure 5:

Brain kynurenine and KYNA levels in male (black) and female (grey) offspring of dams treated prenatally with saline or LPS 24 h after an injection of saline or LPS (0.1 or 1 mg/kg i.p.) in adulthood. See text for experimental details. Data are the mean ± SEM (n = 3–8).

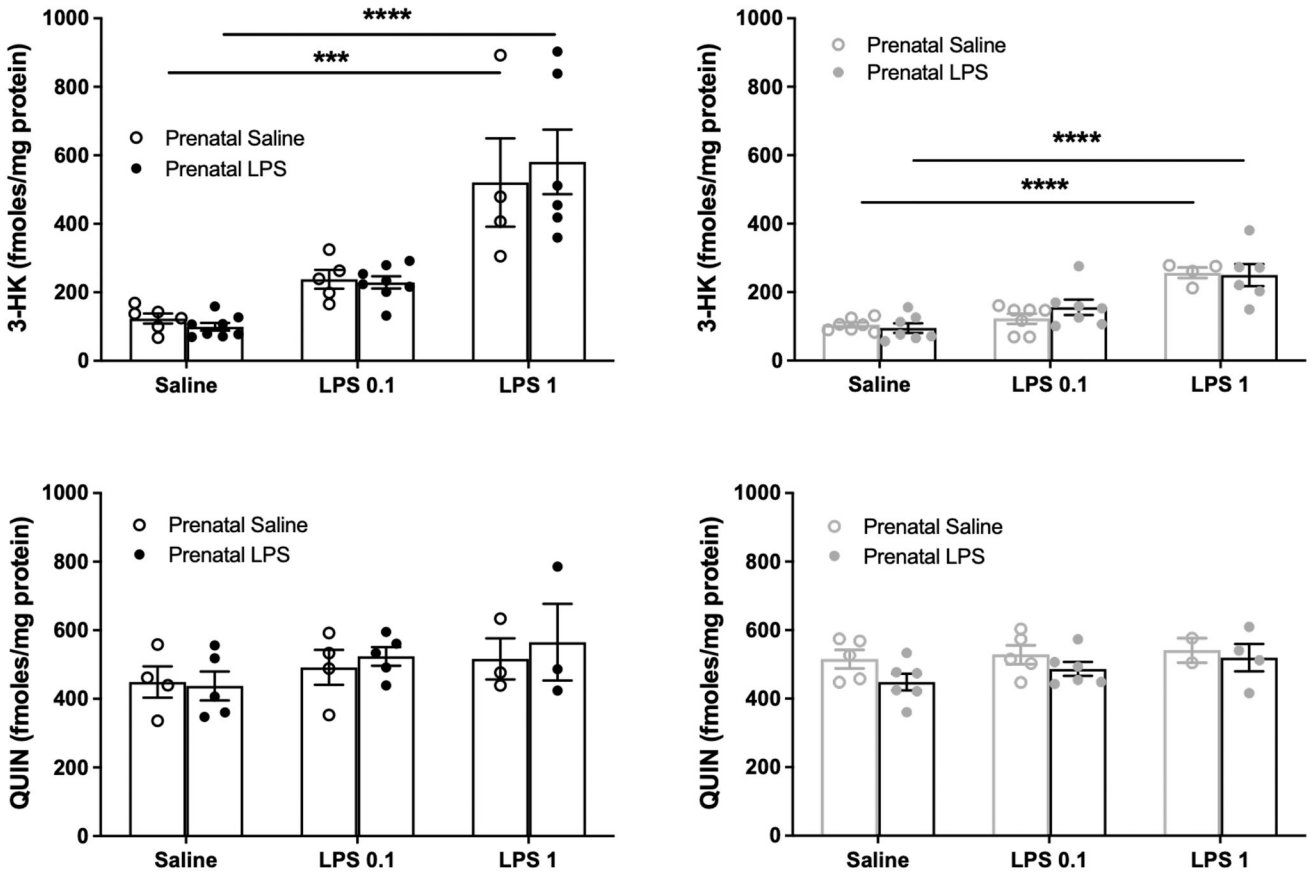

Figure 6:

Brain 3-HK and QUIN levels in male (black) and female (gray) offspring of dams treated prenatally with saline or LPS 24 h after an injection of saline or LPS (0.1 or 1 mg/kg i.p.) in adulthood. See text for experimental details. Data are the mean ± SEM (n = 3–8). *** $p < 0.001$, **** $p < 0.0001$ (two-way Anova, followed by Bonferroni's post-hoc test).