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Maternal immune activation in rats blunts brain cytokine and kynurenine pathway responses to a second immune challenge in early adulthood.

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Abstract

Maternal immune activation (MIA) with the viral mimic poly I:C provides an established rodent model for studying schizophrenia (SZ) and other human neurodevelopmental disorders. Postnatal infections are additional risk factors in SZ and may cumulatively contribute to the emergence of pathophysiology. Underlying mechanisms may involve metabolites of the kynurenine pathway (KP) of tryptophan degradation, which is readily induced by inflammatory stimuli. Here we compared the expression of selected cytokines and KP enzymes, and the levels of selected KP metabolites, in the brain of MIA offspring following a second, acute immune challenge with lipopolysaccharides (LPS) on postnatal day (PND) 35 (adolescence) or PND 60 (early adulthood). Assessed in adolescence, MIA did not alter the expression of proinflammatory cytokines (except TNF-α) or KP metabolite levels compared to controls, but substantially reduced the expression of the anti-inflammatory cytokines IL-4 and IL-10 and influenced the expression of two of the four KP enzymes examined (IDO1 and TDO2). LPS treatment caused distinct changes in the expression of pro- and anti-inflammatory cytokines, as well as KP enzymes in MIA offspring, but had no effect on KP metabolites compared to control rats. Several of these effects were blunted in MIA offspring receiving LPS on PND 60. Notably, LPS caused a significant reduction in brain kynurenine levels in these animals. Of relevance for SZ-related hypotheses, these results indicate that MIA leads to an increasingly defective, rather than an overactive, immune regulation of cerebral KP metabolism during the postnatal period.

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Ethical Satement

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Keywords

poly I:C; lipopolysaccharides; cytokines; kynurenic acid; two hit models

1. Introduction

Recent advances in schizophrenia (SZ) research suggest that some of the severe deficits characteristic of the disease may result, at least in part, from neurodevelopmental abnormalities caused by environmental insults. Clinical and epidemiological studies have shown that immunological stressors during pregnancy and/or early postnatal development, including bacterial, viral and parasitic infections, as well as autoimmune and allergic disorders such as hypersensitivity to gluten, are significant risk factors for the development of the disease (Benros et al., 2014, Brown, 2012, Brown and Susser, 2002, Kneeland and Fatemi, 2013, Lionetti et al., 2015, Severance et al., 2016). As genetic vulnerabilities play a significant role in the etiology of SZ (Birnbaum and Weinberger, 2017), it is especially interesting in this context that alleles which are consistently associated with SZ risk are located within regions of the human leukocyte antigen [HLA; also known as major histocompatibility complex (MHC)] locus on chromosome 6p21.1–21.3 (Agartz et al., 2011, Bergen et al., 2012, Purcell et al., 2009). This region codes for several molecules involved in immune function, including Class I and II HLA proteins, complement C2 and C4, and the cytokine tumor necrosis factor-alpha (TNF-α). Consequently, there is considerable consensus that the pathophysiology of SZ may involve early, abnormal immunological processes, which profoundly affect the development of the central nervous system (CNS), leading to permanent deficits in both CNS and immune function (Brown, 2011, Horvath and Mirnics, 2014, Khandaker et al., 2015, McAllister, 2014, Meyer, 2013). Notably, additional inflammatory challenges in adolescence or early adulthood may have cumulative adverse effects on the illness (“multiple hit hypothesis”)(Bayer et al., 1999, Maynard et al., 2001, Walker et al., 2009).

Kynurenic acid (KYNA), a metabolite of the kynurenine pathway (KP) of tryptophan degradation, is an endogenous antagonist of NMDA and α7 nicotinic receptors and has been repeatedly implicated in the pathophysiology of SZ (Albuquerque and Schwarcz, 2013, Erhardt et al., 2017, Schwarcz and Stone, 2017). Increased KYNA levels in the brain and cerebrospinal fluid have been reported in several different SZ patient populations (Erhardt et al., 2001, Linderholm et al., 2012, Miller et al., 2006, Nilsson et al., 2005, Sathyasaikumar et al., 2011, Schwarcz et al., 2001). Moreover, and unrelated to medication (Ceresoli-Borroni et al., 2006, Nilsson, Linderholm, 2005, Sathyasaikumar, Stachowski, 2011), abnormalities in peripheral KP measures may be associated with specific symptoms of the disease (Chiappelli et al., 2014). One plausible hypothesis posits that the phenomenon is causally linked to an activation of the immune system, which reliably induces the enzyme indoleamine 2,3-dioxygenase (IDO1), resulting in an increased conversion of tryptophan to kynurenine (KYN) and downstream metabolites, including KYNA (Dantzer et al., 2008, Maes et al., 2007, O’Connor et al., 2009, Saito et al., 1992). Additional KP metabolites (jointly termed “kynurenines”) include the free radical generator 3-hydroxykynurenine (3-HK) and the excitotoxic NMDA receptor agonist quinolinic acid (QUIN), both of which are

also formed during immune activation and could possibly play a role in SZ dysfunction (Kanchanatawan et al., 2017, Muller, 2017).

Maternal immune activation (MIA) in rodents, caused by viral or bacterial mimetic agents, provides useful models of neurodevelopmental disorders and has generated critical information about abnormal long-term effects in the progeny (Boksa, 2010, Estes and McAllister, 2016, Meyer and Feldon, 2009, Shi et al., 2003). Specifically, prenatal immune challenge with the synthetic viral RNA polyinosinic:polycytidylic acid (poly I:C) has considerable validity for modeling onset and course of SZ, as well as specific behavioral endophenotypes, neurochemical impairments and changes in gene expression in the disease (Meyer and Feldon, 2010, 2012, Ozawa et al., 2006, Smith et al., 2007). However, the use of MIA to explore possible disease-relevant links between activation of the immune system and the KP has so far focused only on peripheral KP metabolism (Zavitsanou et al., 2014) and may not be sufficiently informative with regard to events in the central nervous system (Notarangelo et al. 2014). The present study was therefore designed to investigate the effects of MIA on the expression of canonical cytokine markers of brain immune function, and on the expression of selected KP enzymes and the levels of selected KP metabolites, in the brain of adolescent and young adult rats. This species was selected because modulation of kynurenic acid levels in the brain by the enzyme KAT II are more similar between the human and the rat than the human and the mouse brain (Guidetti et al., 2007). In addition, we examined these outcome measures in MIA offspring 24 h after a second immune challenge (“second hit”) with lipopolysaccharides (LPS) on postnatal day (PND) 35 or 60. Our results showed several effects of this second immune challenge on the immune system and the KP in MIA rats, and revealed, unexpectedly, an overall blunted response when LPS was administered later in the postnatal period.

2. Methods

2.1 Animals and treatments

Timed pregnant Wistar rat dams (Charles River; Wilmington, MA, USA) were received on gestational day (GD) 2 and allowed to acclimate for three days. The dams were then handled daily and monitored for pregnancy progression and general health until they were administered either poly I:C (5 mg/kg) or saline via tail vein injection on GD 15 (saline: n = 10, poly I:C: n = 11). In brief, dams were lightly anesthetized with isoflurane and placed into a restrainer to collect blood from one of the lateral tail veins. Poly I:C (Sigma-Aldrich; lot# 116M4039V; St. Louis, MO, USA), or sterile saline was then injected into the opposing lateral tail vein (2 μl/g of body weight) and the dams were allowed to recover in their home cages. Temperature, weight and sickness behavior were recorded at 1, 3, 6 and 24 h post injection. Sickness behavior was quantified according to a 4 point scale as follows: rats were checked for lethargy (demonstrated by diminished locomotion after prompting and curled body posture), ptosis (drooping eyelids), and piloerection (ruffled, puffy fur) with each symptom equal to 1 point resulting in a scale of 0 to 3 with 0 = no symptom and 3 = all symptoms present. Additionally, blood was collected from the tail vein 3 h post injection using the same procedure as above. Dams were allowed to come to term and were housed with their own litters until offspring were weaned. Upon weaning, a pair of littermate males

from each treated dam were individually assigned to receive either saline or LPS (2 mg/kg; Sigma-Aldrich, serotype 055:B5) on PND 35. When available, additional males from each litter were selected to receive saline or LPS injections on PND 60. Offspring from different litters were housed by treatment group, 2–3 per cage. After administration of LPS, sickness behaviors were recorded as described above, with the exception that no blood was collected. Twenty-four hours post-injection, rats were euthanized via an overdose of chloral hydrate and the anesthetic isoflurane, concurrent with a cardiac transfusion of PBS containing 0.1% 0.5M EDTA to flush blood from the brain prior to dissection. The brain was then rapidly dissected, separating the two hemispheres of the forebrain, and removing the cerebellum and olfactory bulbs. Individual hemispheres were fresh frozen in isopentane on dry ice and stored at −80°C until processed for analysis. Biochemical analyses, which included several forebrain brain regions at 24 h after LPS were chosen based on the study of Walker et al (Walker et al., 2013) on the effects of LPS on KP metabolites. Each individual measure represents the offspring from an individual dam. All procedures complied with institutional and IACUC guidelines at the University of Maryland School of Medicine.

2.2 Real-Time RT-PCR

Total RNA was extracted from randomly assigned left or right hemispheres as described (Clark et al., 2016). Real-time RT-PCR was conducted using the iQ SYBR Green Supermix (Bio-Rad; Hercules, CA, USA) in 25 μl total volume run on a MyiQ instrument (Bio-Rad) with a 3 step cycling program using the set of primers listed in Supplemental Table 1. Relative expression was determined using the $2^{-\Delta\Delta Ct}$ method described by Schmittgen and Livak (Schmittgen and Livak, 2008) (see Suppl. Methods). The selected cytokine panel focused on cytokines known to respond with “de novo” transcription in the brain by peripheral administration of LPS and mediate physiological and behavioral symptoms (Quan et al., 1999, Quan et al., 1998, Tonelli et al., 2003, Tonelli and Postolache, 2005), and cytokines known to affect or induce activation of the KP (O’Connor, Andre, 2009, Walker, Budac, 2013).

2.3 KP metabolite determinations

KP metabolites were measured using the other forebrain hemisphere. KYNA and 3-HK were analyzed by HPLC and detected fluorometrically and electrochemically (Notarangelo et al. 2012), respectively (see Suppl. Methods). Tryptophan (TRP), KYN and QUIN levels in the brain were quantified by gas chromatography/mass spectrometry (GC/MS) using a 7890A GC coupled to a 7000 MS/MS (Agilent Technologies, Wilmington, DE, USA), using electron capture negative chemical ionization as described (Notarangelo, Wu, 2012) (see Suppl. Methods).

2.4 Statistics

Regression analyses were used to determine if there was a two-hit effect of MIA and LPS treatments (interaction: MIA × LPS) and/or main effect of either MIA or LPS using R Studio (Boston, MA, USA). If an interaction or a main effect for both MIA and LPS was detected, then post-hoc Holm-Sidak multiple comparison tests were conducted to determine significant differences between treatment groups. These results are shown in figures 1–3; main effects are described in the text. Two-way ANOVAs for the analysis of the effect of age

(Fig. 4) and two-way repeated measures ANOVAs for sickness responses (Suppl. Fig. 1), as well as all post-hoc tests were conducted using GraphPad Prism (v. 6.07; La Jolla, CA, USA). Data are presented as the mean ± SEM. $p < 0.05$ was considered significant.

3. Results

3.1 Biobehavioral responses to immune challenge

Pregnant dams administered poly I:C on GD 15 showed typical biobehavioral responses to an inflammatory challenge including reduced temperature [Time x treatment interaction: $F_{(4,120)} = 2.917$, $p = 0.024$] and overt symptoms of sickness behavior that resolved over the course of 24 h [interaction: $F_{(3, 69)} = 21.75$, $p = < 0.0001$; Time: $F_{(3, 69)} = 21.75$, $p = < 0.0001$; Poly I:C: $F_{(1, 23)} = 82.07$, $p = < 0.0001$] (Suppl. Fig. 1A-C). Furthermore, increased circulating IL-6 levels were verified 3 h post injection [$t_{(14)} = 4.539$, $p = 0.0005$; Supp. Fig. 1D]. Effects of postnatal treatment of offspring with LPS are detailed in Suppl. Results and Suppl. Fig. 1E-J.

3.2 Expression of pro- and anti-inflammatory cytokines

Evaluation of brain cytokine mRNA expression revealed differential effects of MIA and LPS on specific cytokine subsets. Descriptive statistics for main effects of MIA and LPS, as well as their interaction, are provided in Table 1. As shown in Figure 1A-D, MIA alone was not sufficient to alter basal expression of pro-inflammatory cytokines on PND 35, with the exception of an increase in TNF-α. Nevertheless, MIA exacerbated expression of IL-1β and reduced levels of IL-6 in response to LPS as a "second hit," with no effect on TNF-α, compared to control offspring that received only LPS (Fig 1A-C). Expression of IFN-γ was not altered by either MIA or LPS (Fig. 1C). Analysis of the anti-inflammatory cytokines IL-4 and IL-10 revealed a MIA induced reduction in expression that was further reduced by LPS (Fig. 1E, F).

At PND 60, no basal differences were detected for MIA offspring for either pro- or anti-inflammatory cytokine expression. Remarkably however, while LPS elicited similar increases in IL-1β in control and MIA offspring, mRNA expression of TNF-α, IL-6 and IFN-γ were all blunted in MIA offspring following LPS treatment (Fig. 1 G-J; Table 1). Furthermore, IL-10, but not IL-4, also showed a blunted response to LPS in MIA offspring (Fig. 1K, L).

3.3 Expression of kynurenine pathway enzymes

Assessed on PND 35, both MIA and LPS affected the expression of IDO1and TDO2 (Fig. 2 A, B; Table 2). MIA induced opposing effects on basal levels of IDO1, which were increased, and TDO2, which were decreased, compared to controls (Fig. 2A, B). LPS as a second hit resulted in an overall increase in IDO1, an effect that was not exacerbated by MIA. In contrast, TDO2 levels were further reduced to almost non-detectable levels in MIA offspring following LPS administration. While there was no effect of MIA on basal levels of KAT II, MIA offspring showed a blunted LPS response (Fig. 2C). Finally, KMO expression was not altered by MIA, but significantly increased by LPS (Fig. 2D).

Examination of these enzymes at PND 60 revealed a limited effect of MIA on basal expression with blunted effects on mRNA levels in MIA offspring after LPS. Of the four enzymes analyzed, only IDO1 showed an interaction between MIA and LPS (Table 2), with MIA offspring showing an abrogated response to LPS (Fig. 2E). Consistent with PND 35, TDO2 levels were also reduced by MIA (Fig. 2F), while KAT II and KMO were both increased by LPS (Fig. 2G, H).

3.4 Kynurenine pathway metabolites

Consistent with previous results, MIA was not sufficient to alter basal expression of any of the metabolites measured during adolescence. Furthermore, no interactions between MIA and LPS were detected for any of the KP metabolites at this age. Nevertheless, there were LPS-induced increases detected for KYN, the KYN:TRP ratio, KYNA and 3-HK, while no changes in expression were seen for TRP or QUIN (Fig. 3A-F; Table 3).

Though MIA also failed to influence basal levels of KP metabolites in animals examined on PND 60 (Fig. 3G-L; Table 3), an interaction between MIA and LPS was detected for TRP and KYN, revealing blunted responses to the LPS challenge in MIA offspring (Fig 3G, H). While a main effect of LPS was detected for KYN formation (KYN:TRP ratio), as well as KYNA and 3-HK, only the KYN:TRP ratio was increased in both control and MIA offspring (Fig. 3I). Post hoc analysis for both KYNA and 3-HK showed significant increases only within control offspring treated with LPS (Fig. 3J, K), further supporting blunted responses to LPS in MIA offspring. Neither MIA nor LPS impacted QUIN levels (Fig. 3L). These effects are consistent with the altered expression of KP enzymes following LPS in MIA offspring detected at this developmental stage.

3.5 Age dependent differences in cytokines and the KP in MIA offspring.

Inspection of data obtained on PND 35 and PND 60 suggested an effect of postnatal age on several outcome measures. The fold change differences for saline- and LPS-treated MIA offspring relative to their control groups were therefore analyzed across age by two-way ANOVA. Significant differences between age groups were detected for the cytokines TNF-α ($p = 0.002$), IFN-γ ($p < 0.0001$), IL-4 ($p < 0.0001$) and IL-10 ($p < 0.0001$), the enzymes IDO1 ($p < 0.0001$), TDO2 ($p = 0.026$) and KMO ($p = 0.042$), and the KP metabolites KYN ($p = 0.033$), KYNA ($p = 0.005$) and QUIN ($p = 0.004$). A significant interaction between age and treatment was observed for IL-1β ($p = 0.003$), reflecting the lack of MIA effect at PND 60. Post-hoc analyses for MIA treatment (single hit) or MIA plus LPS (two-hit) along with the direction of changes are summarized in Fig. 4. These results, comparing the effects between age groups, suggest an age-dependent progression in alterations to immune function and KP activation induced by MIA. PND 60 MIA offspring displayed significant differences compared to PND 35 MIA offspring in cytokine expression and KP function, notably showing LPS-induced reductions in IDO1 expression, as well as KYN and KYNA levels. Finally, Fig. 5 outlines the hypothesized direction of events, the relationship between the cytokines and KP molecules analyzed in our study, and the end-points related to KP regulation. The model also depicts the main effects reported in the study at different developmental end-points.

4. Discussion

The present study, employing the established MIA model of neurodevelopmental disorders in rats, revealed that an inflammatory insult *in utero* affects the regulatory mechanisms of brain cytokines and KP enzymes in the offspring. Specifically, MIA resulted in a heightened inflammatory status in adolescent (i.e. PND 35) rats, which was characterized by increased expression of the pro-inflammatory cytokine TNF-α and reduced expression of the anti-inflammatory cytokines IL-4 and IL-10. These changes were accompanied by elevated IDO1 and decreased TDO2 expression, but not by changes in the brain levels of the four KP metabolites tested. Notably, MIA alone had no apparent long-lasting effects on any of the analytes by PND 60, i.e. in young adulthood. However, we observed a blunted response of several cytokines and KP measures to a second immune challenge with LPS in young adulthood, compared to adolescence, in the MIA offspring. These results suggest a progressive impairment of the inflammatory and KP response as the brain matures.

Postnatal brain cytokine abnormalities using *in utero* poly I:C challenge have been reported by relatively few studies (Garay et al., 2013, Giovanoli et al., 2016, Meyer et al., 2006, Pendyala et al., 2017), all of them performed in mice. Remarkably, the effects on specific cytokines appear to largely depend on the developmental stage and brain region analyzed (Garay, Hsiao, 2013, Pendyala, Chou, 2017). While some discrepancies exist, there is consensus of a pro-inflammatory effect during postnatal development. Overall, the brain cytokine abnormalities reported here in rats are consistent with these studies and, taken together, show that poly I:C-induced MIA clearly causes an overall pro-inflammatory state in the brain of the adolescent offspring. Interestingly, this presumably detrimental condition may be a consequence of the reduced expression of the anti-inflammatory cytokines IL-4 and IL-10. Studies have shown that IL-4 in the CNS stimulates the production of brain derived neurotrophic factor (BDNF) and has pro-cognitive and protective effects (Derecki et al., 2010, Gadani et al., 2012). A deficit in the immunoregulatory/anti-iflammatory actions of these cytokines in MIA animals, in addition to the protective actions of IL-4, may eventually lead to synaptic changes mediated by overactive pro-inflammatory cytokines such as TNF-α. This cytokine, in particular, is known to have important homeostatic roles in glutamatergic and GABAergic neurotransmission (Beattie et al. 2002, Lewitus et al., 2014, Stellwagen, 2011, Stellwagen et al., 2005), and imbalanced expression may lead to pathophysiologically significant developmental deficits. Whether impairments in IL-4 and IL-10 function at this developmental stage are responsible for MIA-related neural abnormalities has not been directly examined thus far.

In agreement with a large number of studies, the expression of brain cytokines, and especially of IL-1β, was significantly increased 24 h after the administration of LPS in normal animals in both adolescence and young adulthood (Quan, Stern, 1999, Quan, Whiteside, 1998, Tonelli, Maeda, 2003). In MIA animals tested on PND 35, the LPS challenge caused an exacerbated IL-1β increase yet resulted in substantially smaller responses of IFN-γ and IL-6, and in further decreases in IL-4 and IL-10 expression compared to controls. Notably, with the exception of IL-1β, MIA rats at PND 60 showed a blunted cytokine response to LPS, suggestive of a defective innate immune activation. These results indicate that the dysregulated inflammatory response to LPS in adolescent MIA

animals is further impaired by the time when these animals reach young adulthood. Of relevance for interpretation of these results is a consideration for the cellular source of these cytokines. Most of the cytokine production in the brain during an LPS challenge, which is dominated largely by IL-1 β production, is mediated by perivascular and microglial cells (Buttini and Boddeke, 1995, Quan, Stern, 1999, Quan, Whiteside, 1998). To a lesser extent, cytokines such as IL-4 and IFN-γ are likely produced in the brain by meningeal T cells (Filiano et al., 2017). Thus, developmental differences in cytokine production by MIA may be also related to different effects in different cell types by the *in utero* insult.

Assessment of the expression of KP enzymes and of the levels of KP metabolites in the same brains used for the profiling of cytokines revealed mechanistically meaningful links. Although we demonstrated that cerebral IDO1 expression was increased in offspring of MIA rats, and despite the fact that TNF-α is actively involved in the activation of this enzyme in the brain (O'Connor, Andre, 2009), neither TRP nor KYN levels were altered in the brain of MIA rats in either adolescence or young adulthood. This was most likely related to the unexpected simultaneous reduction in TDO2 expression, which was especially remarkable in the adolescent MIA brain. Thus, though the molecular events causing decreased TDO2 expression in the brain of MIA offspring remain to be elucidated, our results suggest that TDO2 plays an essential role in the regulation of cerebral KP metabolism (Larkin et al., 2016, Miller et al., 2004). Moreover, the comparatively small changes in KAT II and KMO mRNA seen after MIA at both PND 35 and PND 60 are in line with the fact that the basal brain levels of downstream metabolites in both arms of the KP (KYNA, 3-HK and QUIN) were minimally influenced by the prenatal immune challenge.

As in the case of cytokine expression, the effects of LPS on KP metabolism differed when rats were treated on PND 35 and PND 60. Although the bacterial toxin increased the expression of IDO1, KAT II and KMO in the brain of control rats of both ages, TDO2 expression was only reduced in adolescence. On the other hand, LPS administration increased the tissue levels of KYN, KYNA and 3-HK (Larsson et al., 2016, Walker, Budac, 2013), again without effect on QUIN. Interestingly, however, LPS did not cause further increases in any of the KP measures in MIA rats at PND 35, and, in fact, led to a further reduction in TDO2 expression and diminished the increase in KAT II mRNA in these animals. Blunted responses were even more apparent when MIA rats were treated with LPS on PND 60. In those animals, all KP enzyme mRNA expressions and KP metabolite levels tended to be lower or showed significant reductions compared to LPS-treated controls.

Taken together, our results demonstrate that although MIA influences the basal expression of pro- and anti-inflammatory cytokines in the adolescent brain, these effects are transient. Moreover, while we also confirmed that an acute administration of LPS reliably affects the cerebral expression of several cytokines in normal adolescent and young adult rats (Quan, Stern, 1999, Tonelli et al., 2008), aggravation of this effect in MIA animals was limited to IL-1β, and was seen only in adolescence. Unexpectedly, MIA attenuated the effect of LPS on cytokine expression in several cases at PND 60.

While the present studies did not examine causal effects between cytokine expression and MIA-induced KP abnormalities, they clearly demonstrate that MIA alone is not sufficient to

induce persistent overactivity in cerebral KP metabolism and mostly does not aggravate the effects of an acute immune challenge on KP metabolism during the adolescent period. On the contrary, it appears that compensatory or impaired mechanisms operate in the brain of MIA animals during postnatal brain development and beyond, eventually leading to an increasingly hypo-reactive, rather than the expected hyper-reactive, state of the cerebral KP when the organism experiences a renewed, acute distress of the immune system.

The present study has implications for the conceptualization of the proposed role of abnormal cerebral KP metabolism in several major brain diseases with a presumed neurodevelopmental origin. Thus, our results argue against our initial hypothesis that prenatal infections, which are widely regarded as significant risk factors (Brown, 2006), stimulate cerebral KP function during the critical early phases of pathophysiology. We demonstrated, however, that KP metabolism in the postnatal brain is indeed affected, namely down-regulated, by MIA, in line with effects in the periphery (Zavitsanou, Lim, 2014). Studies exploring the underlying mechanisms and functional significance of these MIA-related KP changes and, in particular, how they might be linked to the KP impairments which have been repeatedly documented in the brain and cerebrospinal fluid of SZ patients (Erhardt, Blennow, 2001, Linderholm, Skogh, 2012, Miller, Llenos, 2006, Nilsson, Linderholm, 2005, Sathyasaikumar, Stachowski, 2011, Schwarcz, Rassoulpour, 2001) are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Maternal immune activation exacerbates brain inflammatory responses to an LPS challenge in adolescent rats
- Maternal immune activation exacerbates brain indoleamine-2,3-dioxygenase expression to an LPS challenge in adolescent rats
- These effects are blunted in young adult rats
- Brain Kynurenine responses to an LPS challenge are blunted in young adult rats exposed to maternal immune activation

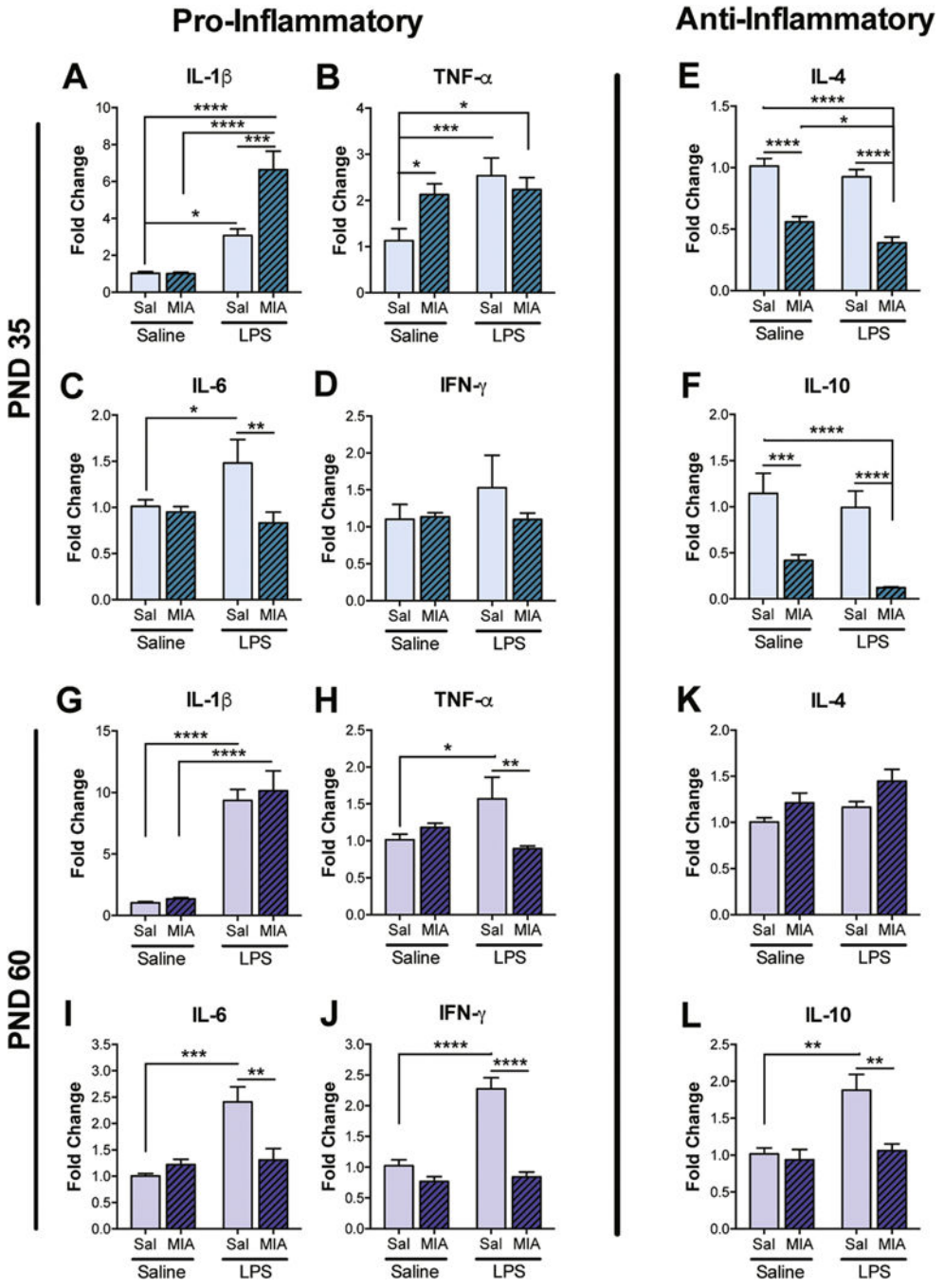

Figure 1.

Brain cytokine mRNA levels after maternal immune activation (MIA) with poly I:C on GD15 followed by a postnatal challenge with LPS on PND 35 (A-F) or PND 60 (G-L). PND 35 pro-inflammatory cytokines: A) IL-1β (interaction: $p = 0.005$; MIA: p = 0.005; LPS: $p < 0.0001$), B) TNF-α (interaction: $p = 0.030$; LPS: $p = 0.043$), C) IL-6 (interaction: $p = 0.038$; MIA: $p = 0.017$), and D) IFN-γ showed no effect of either MIA or LPS. PND 35 anti-inflammatory cytokines: E) IL-4 (MIA: $p < 0.001$; LPS: $p = 0.011$) and F) IL-10 (MIA: $p < 0.001$; LPS: $p = 0.047$). PND 60 pro-inflammatory cytokines: G) IL-1β (LPS: $p < 0.0001$),

H) TNF-α (interaction: $p = 0.006$), I) IL-6 (interaction: $p = 0.001$; MIA: $p = 0.022$; LPS: $p = 0.006$), and J) IFN-γ (interaction: $p < 0.0001$; MIA: $p < 0.0001$; LPS: $p < 0.001$). PND60 anti-inflammatory cytokines: K) IL-4 (no significant effects) and L) IL-10 (interaction: $p = 0.012$; MIA: $p = 0.003$; LPS: $p = 0.001$). Main and interaction effects were modeled through regression analyses followed by Holm-Sidak multiple comparisons post-hoc tests. PND 35: n = 7–11/group. PND 60: n = 5–6/group. Mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

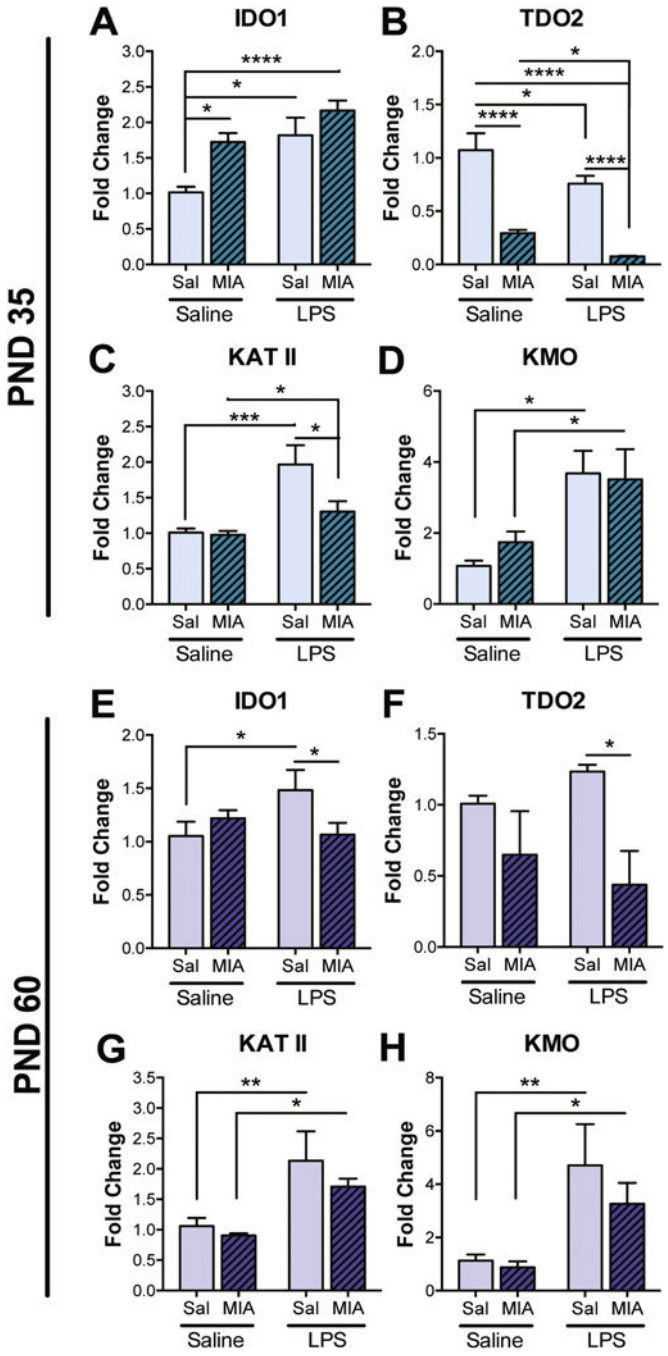

Figure 2.

Expression of kynurenine pathway (KP) enzymes in the brain after MIA (poly I:C on GD 15), followed by a postnatal challenge with LPS on PND 35 (A-D) or PND 60 (E-H). Protein levels were determined *via* HPLC. PND 35: Main effects of both MIA and LPS were observed for IDO1 (MIA: $p = 0.002$, LPS: $p < 0.001$), TDO2 (MIA: $p < 0.001$, LPS: $p < 0.0001$) and KAT II (MIA: $p = 0.033$, LPS: $p < 0.001$). An interaction between MIA and LPS was also detected for KAT II ($p = 0.046$). KMO was affected by LPS only ($p = 0.002$). PND 60: an interaction was detected for IDO1 ($p = 0.037$), while a main effect of MIA was

revealed for TDO2 ($p = 0.011$). A main effect of LPS only was found for KAT II ($p < 0.001$) and KMO ($p = 0.001$). Main and interaction effects were modeled through regression analyses followed by Holm-Sidak multiple comparison post-hoc tests. PND 35: n = 7–11/group. PND 60: n = 5–6/group. Data are the mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

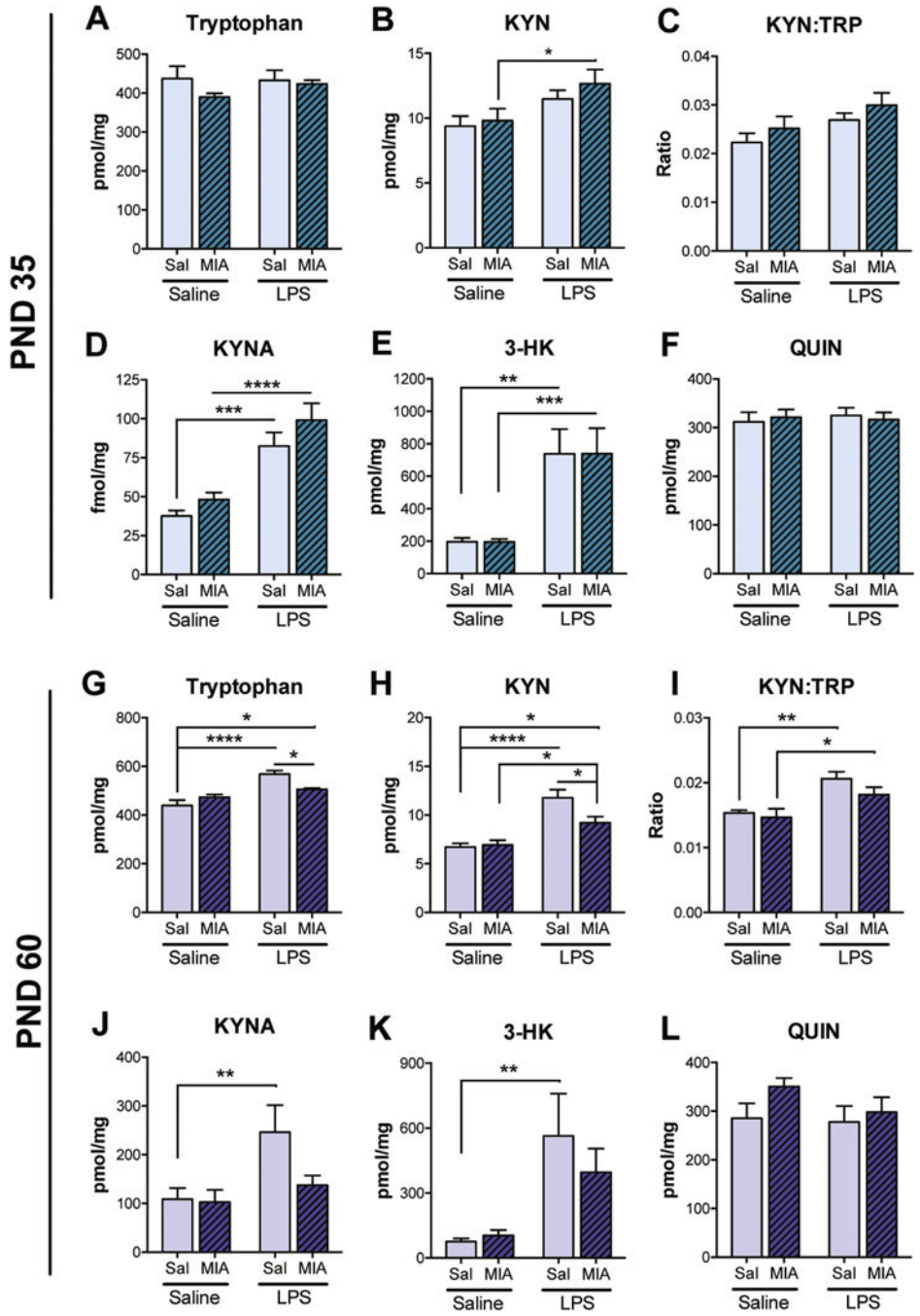

Figure 3.

Levels of cerebral kynurenine pathway metabolites (expressed per mg protein) after MIA (poly I:C on GD 15), followed by a postnatal challenge with LPS on PND 35 (A-F) or PND 60 (G-L). No main effect of MIA was detected for metabolites at either time point. PND 35: A significant main effect of LPS was detected for KYN ($p = 0.008$), the KYN:TRP ratio ($p = 0.039$), KYNA ($p < 0.0001$) and 3-HK ($p < 0.0001$). PND 60: A significant interaction was detected between MIA and LPS for KYN ($p = 0.028$). An LPS effect was revealed for the KYN:TRP ratio ($p < 0.001$), KYNA ($p = 0.013$) and 3-HK ($p = 0.004$). Neither MIA nor

LPS affected QUIN levels at either time point. Main and interaction effects were modeled through regression analyses followed by Holm-Sidak multiple comparison post-hoc tests. PND 35: n = 10–11/group. PND 60: n = 5–6/group. Data are the mean ± SEM. * $p < 0.05$, **** $p < 0.0001$.

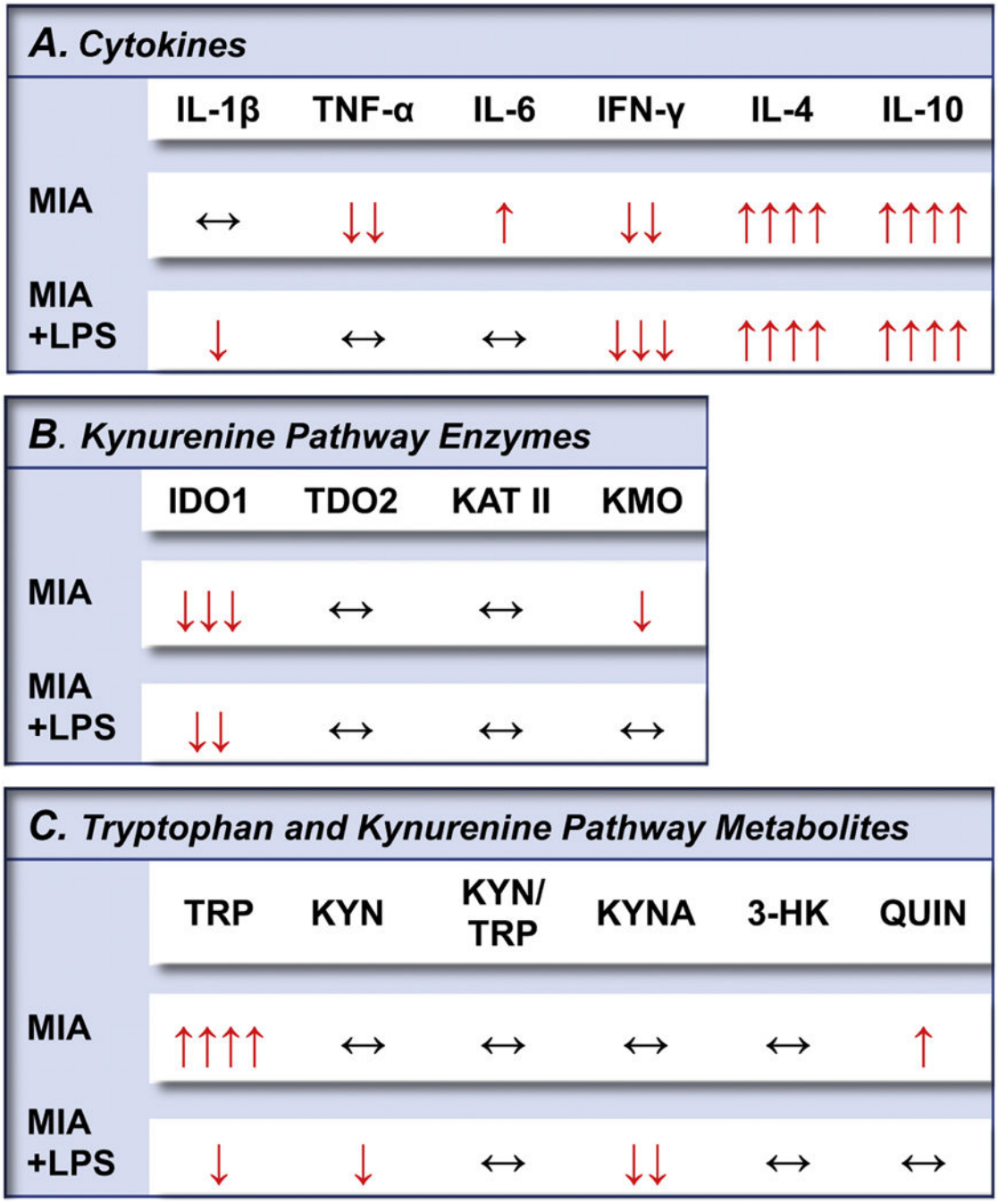

Figure 4.

Age-dependent differences in brain cytokine (A), KP enzyme expression (B) and KP metabolite levels (C) in the offspring of maternal immune activation (MIA) rats. Two-way ANOVAs were conducted between the PND 35 and PND 60 age groups comparing treatment effects (i.e. fold changes with respect to appropriate control groups). Post-hoc analyses for significant effects of age are shown in the tables. Horizontal black arrow = no difference. Red up- and down arrows indicate direction of change in PND 60 with respect to

PND 35. One arrow: $p \leq 0.05$, two arrows: $p \leq 0.01$, three arrows: $p \leq 0.001$, four arrows: $p \leq 0.0001$.

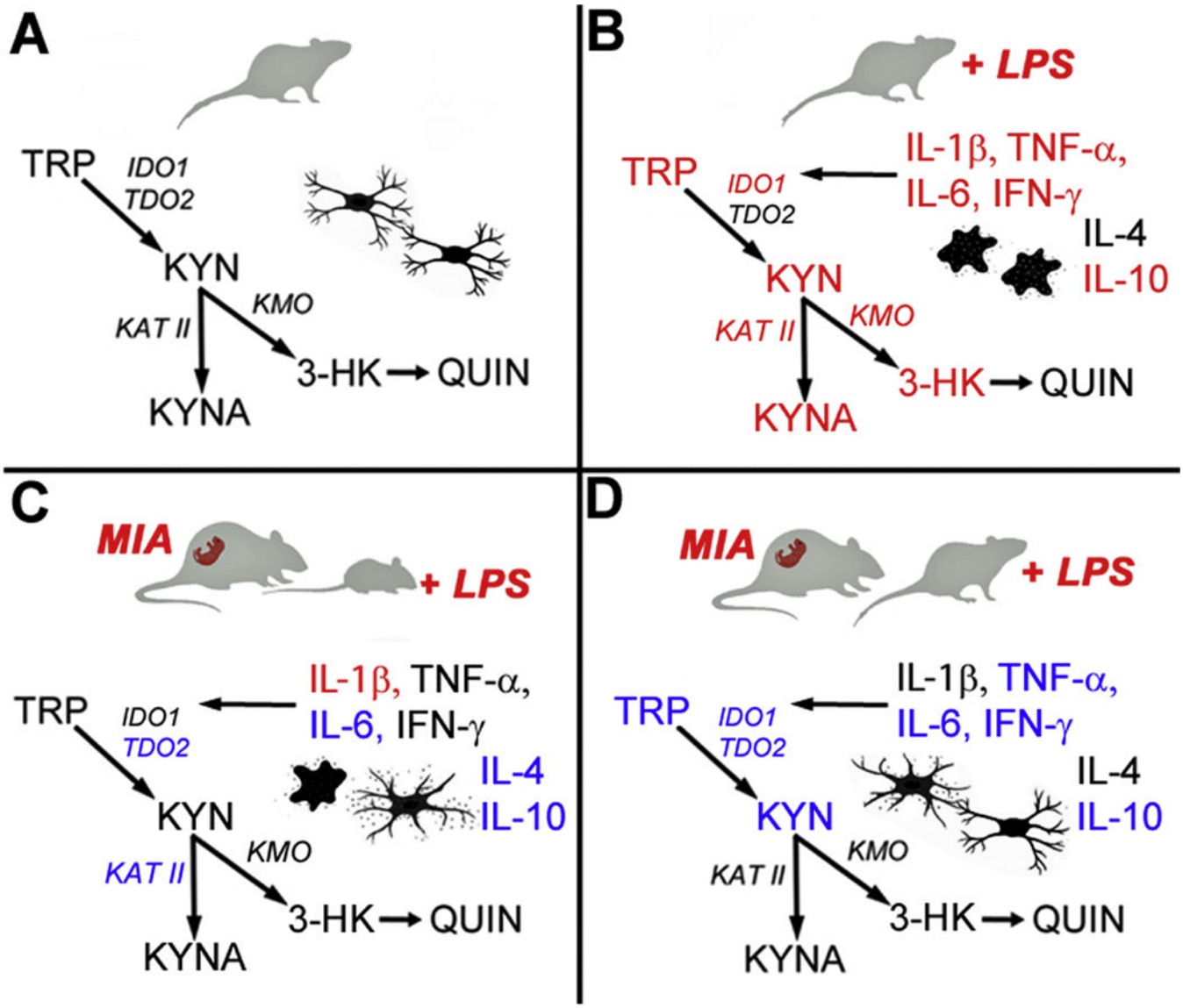

Figure 5.

(A) Simplified model of the cerebral kynurenine pathway (KP) under basal conditions in adult rats. B) Exposure to lipopolysaccharides (LPS) induces neuroinflammation, characterized by activation of microglia and increased cytokine expression in the brain. One associated effect is the increased expression of IDO1 and other KP enzymes and metabolites. Maternal immune activation (MIA) leads to dysregulation of the immune system and to altered responses to an immune challenge with LPS later in life. C) Compared to the LPS effect during adolescence MIA causes exacerbated IL-1β and reduced IL-6, IL-4, and IL-10 responses. D) As MIA offspring progress into adulthood, the cytokine responses to LPS become more attenuated and are associated with a blunting of KP metabolism. Increased expression/levels are indicated in red. Decreased expression/levels are indicated in blue. See legends to Figures 1–3 for details of statistical analyses. 3-HK: 3-hydroxy-kynurenine; IDO1: indoleamine-2,3-dioxygenase; IFN-γ: interferon-gamma; IL: interleukins; KAT II: kynurenine aminotransferase-2; KMO: kynurenine 3-monooxygenase;

KYNA: kynurenic acid; QUIN: quinolinic acid; TDO2: tryptophan 2,3-dioxygenase-2; TNF-α: tumor necrosis factor-alpha; TRP: tryptophan.

Table 1.
Summary of interactions and main effects for cytokines.

Multiple linear regression analyses were conducted for mRNA expression of central cytokines to determine if there was an interaction between maternal immune activation (MIA) and lipopolysaccharide (LPS) challenge at postnatal day (PND) 35 and 60. In the absence of an interaction, a main effect of MIA or LPS is also shown if present. Significant results are indicated in bold. Results of post-hoc analyses are shown in Figure 1. n.s. = not significant.

	PND 35			PND 60		
	MIA × LPS	***MIA***	***LPS***	***MIA × LPS***	***MIA***	***LPS***
IL-1β	**$p = 0.005$** $F_{(1, 29)} = 9.49$	-	-	n.s.	n.s.	**$p < 0.0001$** $F_{(1, 19)} = 83.16$
TNF-α	**$p = 0.030$** $F_{(1, 31)} = 5.16$	-	-	**$p = 0.006$** $F_{(1, 19)} = 9.38$	-	-
IL-6	**$p = 0.038$** $F_{(1, 31)} = 4.7$	-	-	**$p = 0.001$** $F_{(1, 19)} = 13.81$	-	-
IFN-γ	n.s	n.s.	n.s.	**$p < 0.0001$** $F_{(1, 19)} = 30.07$	-	-
IL-4	n.s	**$p < 0.001$** $F_{(1, 31)} = 92.62$	**$p = 0.011$** $F_{(1, 31)} = 7.20$	n.s.	n.s.	n.s
IL-10	n.s.	**$p < 0.001$** $F_{(1, 31)} = 45.99$	**$p = 0.047$** $F_{(1, 31)} = 4.24$	**$p = 0.012$** $F_{(1, 19)} = 7.65$	-	-

Table 2.
Summary of interactions and main effects for kynurenine pathway enzymes.

Multiple linear regression analyses were conducted for mRNA expression of enzymes to determine if there was an interaction between maternal immune activation (MIA) and lipopolysaccharide (LPS) challenge at postnatal day (PND) 35 and 60. In the absence of an interaction, a main effect of MIA or LPS is also shown if present. Significant results are indicated in bold. Results of post-hoc analyses are shown in Figure 2. n.s. = not significant.

	PND 35			PND 60		
	MIA × LPS	***MIA***	***LPS***	***MIA × LPS***	***MIA***	***LPS***
IDO1	n.s.	**$p = 0.002$** $F_{(1, 31)} = 11.10$	**$p < 0.001$** $F_{(1, 31)} = 14.46$	**$p = 0.037$** $F_{(1, 19)} = 5.04$	-	-
TDO2	n.s.	**$p < 0.001$** $F_{(1, 31)} = 13.26$	**$p < 0.0001$** $F_{(1, 31)} = 101.95$	n.s.	**$p = 0.011$** $F_{(1, 19)} = 16.05$	*n.s.*
KAT II	**$p = 0.046$** $F_{(1, 31)} = 4.31$	-	-	n.s.	n.s.	**$p < 0.001$** $F_{(1, 19)} = 16.59$
KMO	n.s.	n.s.	**$p = 0.002$** $F_{(1, 31)} = 11.69$	n.s.	n.s.	**$p = 0.001$** $F_{(1, 19)} = 13.95$

Table 3.

Summary of interactions and main effects for kynurenine pathway metabolites.

Multiple linear regression analyses were conducted for protein levels of kynurenine pathway metabolites to determine if there was an interaction between maternal immune activation (MIA) and lipopolysaccharide (LPS) challenge at postnatal day (PND) 35 and 60. In the absence of an interaction, a main effect of MIA or LPS is also shown if present. Significant results are indicated in bold. Results of post-hoc analyses are shown in Figure 3. n.s. = not significant.

	PND 35			PND 60		
	MIA × LPS	***MIA***	***LPS***	***MIA × LPS***	***MIA***	***LPS***
TRP	n.s.	n.s.	n.s.	**$p = 0.003$** $F(1, 19) = 11.17$	-	-
KYN	n.s	n.s.	**$p = 0.008$** $F_{(1, 53)} = 7.46$	**$p = 0.028$** $F_{(1, 19)} = 3.94$	-	-
KYN:TRP	n.s	n.s.	**$p = 0.039$** $F_{(1, 53)} = 4.48$	n.s.	n.s.	**$p < 0.001$** $F_{(1, 19)} = 17.28$
KYNA	n.s	n.s.	**$p < 0.0001$** $F_{(1, 55)} = 38.45$	n.s.	n.s.	**$p = 0013$** $F_{(1, 19)} = 7.50$
3-HK	n.s	n.s.	**$p < 0.0001$** $F_{(1, 55)} = 16.89$	n.s.	n.s.	**$p = 0.004$** $F_{(1, 19)} = 14.42$
QUIN	n.s	n.s.	n.s.	n.s	n.s.	n.s.