Acute sleep deprivation during pregnancy in rats: Rapid elevation of placental and fetal inflammation and kynurenic acid

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ABSTRACT

The kynurenine pathway (KP) is the dominant pathway for tryptophan degradation in the mammalian body and emerging evidence suggests that acute episodes of sleep deprivation (SD) disrupt tryptophan metabolism via the KP. Increases in the neuroactive KP metabolite kynurenic acid (KYNA) during pregnancy may lead to a higher risk for disrupted neurodevelopment in the offspring. As pregnancy is a critical period during which several factors, including sleep disruptions, could disrupt the fetal environment, we presently explored the relationship between maternal SD and KP metabolism and immune pathways in maternal, placenta, and fetal tissues. Pregnant Wistar rat dams were sleep deprived by gentle handling for 5 h from zeitgeber time (ZT) 0 to ZT 5. Experimental cohorts included: i) controls, ii) one session of SD on embryonic day (ED) 18 or iii) three sessions of SD occurring daily on ED 16, ED 17 and ED 18. Maternal (plasma, brain), placental and fetal (plasma, brain) tissues were collected immediately after the last session of SD or after 24 h of recovery from SD. Respective controls were euthanized at ZT 5 on ED 18 or ED 19. Maternal plasma corticosterone and fetal brain KYNA were significantly elevated only after one session of SD on ED 18. Importantly, maternal plasma corticosterone levels correlated significantly with fetal brain KYNA levels. In addition, placental levels of the proinflammatory cytokines interleukin-1β (IL-1β) and interleukin-6 (IL-6) were increased following maternal SD, suggesting a relationship between placental immune response to SD and fetal brain KYNA accumulation. Collectively, our results demonstrate that sleep loss during the last week of gestation can adversely impact maternal stress, placental immune function, and fetal brain KYNA levels. We introduce KYNA as a novel molecular target influenced by sleep loss during pregnancy.

1. Introduction

Insufficient sleep has become a persistent problem in our society. Sleep deprivation (SD) and inadequate sleep duration can lead to cognitive impairments and negatively impact daily function (Banks and Dinges, 2007; Strine and Chapman, 2005). While much research has been done on the effects of sleep loss on adult men and women, inadequate sleep during pregnancy has not been thoroughly studied. Physical and hormonal changes women undergo while pregnant have been shown to disrupt normal sleep patterns, with studies demonstrating an increase in sleep duration during the first trimester, followed by a shortening of sleep time and more disrupted sleep in the subsequent trimesters (Brunner et al., 1994; Hedman et al., 2002). While the complete impact of these sleep disruptions on fetal development remains largely undefined, rodent experimental systems have been used to assess the impact of SD during pregnancy on fetal outcomes. Emerging studies have assessed in utero conditions, early postnatal changes, and even long-lasting phenotypic differences in the offspring. Examples include increased ultrasonic vocalizations and low birth weight in pups before weaning (Gulia et al., 2015; Radhakrishnan et al., 2015), increased risk-taking behavior and hyperactivity during adolescence, and impaired memory task performance during adulthood.
metabolism in the maternal tissue, including the brain. While levels of adult female rats (Baratta et al., 2018), pregnant rats showed a significant increase in interleukin (IL)-1β and IL-6 were found. Importantly, as hypothesized, fetal brain KYNA was increased following a single session of SD. Taken together, our results indicate a relationship between the maternal stress response, placental immune reactivity and increased formation of KYNA in the fetal brain, introducing the KP as a novel molecular pathway influenced by prenatal sleep loss.

Recent work connects the kynurenine pathway (KP) of tryptophan degradation to the regulation of sleep (Pocivavsek et al., 2017) and with increased catabolism towards the neuroactive metabolite kynurenic acid (KYNA) with sleep loss (Baratta et al., 2018; Kuhn et al., 1968; Yamashita and Yamamoto, 2014, 2017). Tryptophan is elevated in the serum and brain following periods of prolonged wakefulness (Davies et al., 2014; Toru et al., 1984). Most of tryptophan is metabolized by tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) into kynurenine (Leklem, 1971). The subsequent downstream metabolite KYNA is of particular interest in the brain where it is specifically astrocyte-derived (Guidetti et al., 2007), acts as an antagonist at N-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine (α7nACh) receptors, and has been shown to modulate neurotransmission with pathologically relevant up or down fluctuations (Pocivavsek et al., 2016; Schwarcz et al., 2012).

Presently, we hypothesized that SD would act as a maternal stressor and influence catabolism of the KP thereby elevating KYNA (Notarangelo and Schwarcz, 2016). Sleep loss can innately be stressful and rodents exposed to periods of SD show increased corticosterone levels, a key glucocorticoid released by the hypothalamic-adrenal-pituitary (HPA) axis, in serum and brain (Baratta et al., 2018; Brianza-Padilla et al., 2018; Leproult et al., 1997). These alternations are also accompanied by altered cytokine release in the same tissues (Wright Padilla et al., 2018; Leproult et al., 1997). These alternations are also accompanied by altered cytokine release in the same tissues (Wright Padilla et al., 2018; Leproult et al., 1997). These alternations are also accompanied by altered cytokine release in the same tissues (Wright Padilla et al., 2018; Leproult et al., 1997). These alternations are also accompanied by altered cytokine release in the same tissues (Wright Padilla et al., 2018; Leproult et al., 1997).

To test our hypothesis, pregnant rat dams were sleep deprived during the last week of gestation for either one session on embryonic day (ED) 18 or three sessions in a row from ED 16 to ED 18. This gestational time frame, late pregnancy, was chosen as it has been extensively shown to be a critical period sensitive to stress, inflammatory stimuli or kynurenic challenge in utero (Cui et al., 2009; Lemaire et al., 2000; Pocivavsek et al., 2014). Maternal, placental and fetal tissues were collected and levels of corticosterone, cytokines and KP metabolites were assessed. Consistent with our previous findings in adult female rats (Baratta et al., 2018), pregnant rats showed a significant increase in corticosterone following SD with little effect on KP metabolism in the maternal tissue, including the brain. While levels of KP metabolites were also unchanged in placental tissue, significant increases in interleukin (IL)-1β and IL-6 were found. Importantly, as hypothesized, fetal brain KYNA was increased following a single session of SD. Taken together, our results indicate a relationship between the maternal stress response, placental immune reactivity and increased formation of KYNA in the fetal brain, introducing the KP as a novel molecular pathway influenced by prenatal sleep loss.

2. Methods

2.1. Animals

Pregnant, female Wistar rats were obtained from Charles River Laboratories (Frederick, MD) on ED 2. Animals were singly housed in a temperature-controlled facility, fully accredited by the American Association for the Accreditation of Laboratory Animal Care, at the Maryland Psychiatric Research Center. Rats were kept on a 12/12 h light-dark cycle, where lights on corresponded to ZT 0 and lights off to ZT 12, and had ad libitum access to food and water. All experimental protocols were approved by the Institutional Care and Use Committee at the University of Maryland School of Medicine and followed the “Principles of Laboratory Animal Care” (NIH publication No. 86–23, 1996).

2.2. Sleep deprivation, study design and experimental cohorts

Rats were sleep deprived with the gentle handling method. Animals remained in their home cages and were presented with objects (i.e. wooden blocks, paper towels, cotton swabs) approximately every 30 min to disrupt sleep. When necessary, animals were gently stimulated with a long cotton tip applicator and cages were shifted to stop the initiation of sleep. Controls remained in their home cages for the duration of the experiment.

The experimental design is shown in Fig. 1. Upon arriving in our animal facility, pregnant dams were randomly assigned to the various experimental groups. The samples were processed randomly and the experimenter was blind to group assignment during the biochemical analysis. SD was performed for 5 h from ZT 0 to ZT 5 for one session on ED 18 or for three consecutive daily sessions on ED 16, ED 17, and ED 18. Maternal blood and brain, placenta, and fetal blood and brain were collected.

Fig. 1. Schematic representation of sleep deprivation (SD) experimental design. Pregnant rat dams were sleep deprived for 5 h from Zeitgeber time (ZT) 0 to ZT 5 for either 1 session on embryonic day (ED) 18 or 3 consecutive daily sessions on ED 16, ED 17, and ED 18. Maternal blood and brain, placenta, and fetal blood and brain were collected.
18. The experimental cohorts of dams included i) control, 0 h after SD, ED 18 (N = 6), ii) SD: 1 session, 0 h after SD, ED 18 (N = 5), iii) SD: 3 sessions, 0 h after SD, ED 18 (N = 3), iv) control, 24 h after SD, ED 19 (N = 4), v) SD: 1 session, 24 h after SD, ED 19 (N = 4), and vi) SD: 3 sessions, 24 h after SD, ED 19 (N = 3). A maximum of 10 fetuses and corresponding placenta were dissected from each dam. Fetal blood was pooled among subjects from a single litter. Tissues from both sexes were used without regard to sex.

2.3. Tryptophan, kynurenine, and KYNA biochemical analysis

Animals were euthanized by CO₂ asphyxiation at ZT 5 on ED 18 or ED 19. Maternal and fetal blood was collected in tubes containing K₃-EDTA (0.15%). Brains and placenta were promptly removed and snap-frozen on dry ice. To separate plasma from blood cells, blood was centrifuged (300×g, 10 min). The supernatant was removed and stored at −80 °C along with the maternal and fetal brains and placenta. On the day of the assays, fetal brains were weighed and sonicated (1:10 w/v) in ultrapure water for determination of KYNA. Placenta samples were weighed and sonicated (1:10 w/v) in ultrapure water, then further diluted to 1:50 and 1:200 to determine kynurenine and KYNA, respectively. Maternal and fetal plasma was thawed and diluted in ultrapure water to quantify tryptophan (1:100), kynurenine (1:2) and KYNA (1:10). One-hundred μl of sample was acidified with 25 μl of 6% perchloric acid for plasma or 25% perchloric acid for tissue. Samples were centrifuged (12,000×g, 10 min) and 20 μl of supernatant was subjected to high-performance liquid chromatography (HPLC). Tryptophan, kynurenine and KYNA were isocratically eluted from a Reprosil-Pur C18 column (4 × 150 mm; Dr. MaischGmbH, Ammerbuch, Germany), using mobile phase containing 50 mM sodium acetate and 5% acetonitrile (pH adjusted to 6.2 using glacial acetic acid) at a flow rate of 0.1 mL/min. Tryptophan (excitation 285 nm, emission 365 nm), kynurenine (excitation 365 nm, emission 480 nm) and KYNA (excitation 344 nm, emission 398 nm) were detected fluorometrically (Waters Alliance, 2475 fluorescence detector) in the eluate at a retention time of 11 min, 6 min and 11 min respectively.

2.4. Corticosterone measurement

Maternal and fetal plasma corticosterone levels were determined using a radioimmunoassay (MP Biomedicals, Solon, OH, USA). The sensitivity threshold for this assay was 5 ng/mL. Inter- and intra-assay coefficients of variance were less than 10%. All samples were run in duplicate, with 5 μl of sample used in each tube.

2.5. Cytokine quantification

As previously described (Deslauriers et al., 2017), placenta and fetal brain tissues were homogenized in RIPA buffer containing protease inhibitors cocktail (Life Technologies, CA, USA). Samples were centrifuged for 15 min at 15,000 rpm and 4 °C, and supernatants were collected. Protein samples from placenta and fetal brain tissues were tested for proinflammatory cytokines IL-1 β, IL-6 and tumor necrosis factor-α (TNF-α) using ELISA duoset kits (R&D Systems, MN, US) following manufacturer instructions.

2.6. Data handling and statistical analysis

Each maternal sample was an independent biological data point. To minimize the contribution of individual litters, placental and fetal samples were handled such that randomized (2–5) samples from each litter were analyzed and considered as one subject in all biochemical experiments. Insufficient amount of fetal plasma was collected from two litters of 1 session, 0 h after SD, thus fetal corticosterone was analyzed in only 3 of these litters. Fetal brain TNFαs were below the limit of detection in many cases, resulting in exclusion from analysis. All data are expressed as the mean ± SEM. Non-parametric two-way analyses of variance (Aligned Ranks Transformation ANOVA, or ART ANOVA) were performed to assess the main effects and interaction of SD and recovery. When appropriate, significant main effects were followed by a Bonferroni post hoc test. Spearman correlational analysis was performed to examine relationships between fetal brain KYNA, placental cytokines, and plasma corticosterone levels. Analysis was performed in R (version 3.6.1) using the ARTool and emmeans packages and a P value of < 0.05 was considered significant.

3. Results

3.1. Increase in maternal, but not fetal, corticosterone after sleep deprivation

To determine the impact of SD on the stress response, corticosterone was measured in maternal and fetal plasma. Maternal plasma corticosterone was significantly impacted by sleep condition (F2,19 = 7.1, P < 0.01), recovery time (F1,19 = 10.7, P < 0.01) as well as a sleep condition X recovery time interaction (F2,19 = 5.1, P < 0.05) (Fig. 2A). In the maternal plasma, corticosterone was increased by 8-fold immediately after one session (P < 0.001), by 6-fold after three sessions (P < 0.05) of SD, and returned to baseline levels after 24 h of recovery from SD. While fetal plasma corticosterone levels were not impacted by SD (F2,17 = 0.6, P = 0.58), a significant effect of recovery time (F1,17 = 22.9, P < 0.001) was observed, with a trend toward a
meaningful sleep condition X recovery time interaction (F2,17 = 3.3, P = 0.06) (Fig. 2B). Of note, fetal plasma corticosterone levels were significantly higher on ED 19 compared to ED 18.

3.2. Impact of sleep deprivation on KP metabolites in maternal, fetal and placental tissue

Maternal brain KYNA levels were not significantly changed in response to SD (sleep condition: F2,19 = 0.4, P = 0.65; recovery time: F1,19 = 0.2, P = 0.64; sleep condition X recovery time: F2,19 = 0.2, P = 0.86) (Fig. 3A). Interestingly, fetal brain KYNA was significantly impacted by sleep condition (F2,19 = 8.2, P < 0.01), but not recovery time (F1,19 = 3.2, P = 0.09) and there was no significant sleep condition X recovery time interaction (F2,19 = 1.6, P = 0.22) (Fig. 3B). Post hoc analysis indicated that immediately following one session of SD, fetal brain KYNA was significantly elevated by 2.1-fold compared to controls (P < 0.001) and this change persisted on ED 19 (P < 0.05).

To understand the association of increased fetal brain KYNA, tryptophan and KP metabolites in the maternal plasma were assessed. Tryptophan levels in maternal plasma were significantly affected by sleep condition (F2,19 = 4.5, P < 0.05), but not recovery time (F1,19 = 0.23, P = 0.63) and there was a slight trend towards an interaction between sleep condition X recovery time (F2,19 = 3.0, P = 0.07) (Fig. 4A). Per post hoc assessment, we found that maternal plasma tryptophan was significantly elevated after one session of SD (P < 0.05) without recovery and also after one session or three sessions of SD followed by 24 h of recovery when compared to respective controls (P < 0.05). Maternal plasma kynurenine (sleep condition: F2,19 = 3.2, P = 0.06; recovery time: F1,19 = 0.2, P = 0.66; sleep condition X recovery time: F2,19 = 1.1, P = 0.36) (Fig. 4B) and KYNA (sleep condition: F2,19 = 2.0, P = 0.16; recovery time: F1,19 = 0.23, P = 0.64; sleep condition X recovery time: F2,19 = 0.12, P = 0.89) (Fig. 4C) were unchanged with SD. Similarly, placental KP metabolite levels were unaffected by SD. Neither kynurenine (sleep condition: F2,19 = 0.2, P = 0.79; recovery time: F1,19 = 0.2, P = 0.63; sleep condition X recovery time: F2,19 = 0.1, P = 0.90) (Fig. 5A) nor KYNA (sleep condition: F2,19 = 2.6, P = 0.10; recovery time: F1,19 = 1.8, P = 0.19; sleep condition X recovery time: F2,19 = 1.1, P = 0.37) (Fig. 5B) levels in the placental tissue were altered following deprivation and/or recovery.

3.3. Sleep deprivation elevates placental and fetal brain cytokines

As KP metabolism can be initiated by elevated proinflammatory cytokines (Fujigaki et al., 2006), we measured levels of IL-1β, IL-6 and TNFα, in placental and fetal brain tissues. Placental IL-1β levels were significantly impacted by an interaction in sleep condition X recovery time (F2,19 = 3.2, P = 0.06; sleep condition: F2,19 = 2.6, P = 0.05; recovery time: F1,19 = 0.2, P = 0.64; sleep condition X recovery time: F2,19 = 1.1, P = 0.37) (Fig. 5B) levels in the placental tissue were altered following deprivation and/or recovery. Of note, three sessions of SD did not impact IL-1β levels. Placental IL-6 levels were significantly impacted by an interaction in sleep condition X recovery time (F2,19 = 3.0, P = 0.05). One session of SD was sufficient to elevate placental IL-1β by 3-fold compared to controls (P < 0.05), and then levels of the cytokine returned after recovery. Of note, three sessions of SD did not impact IL-1β levels. Placental IL-6 levels were significantly impacted by an interaction in sleep condition X recovery time (F2,19 = 3.0, P = 0.05) and sleep condition (F2,19 = 7.3, P < 0.01), but not recovery time (F1,19 = 3.8, P = 0.07) (Fig. 6B) alone. Placental IL-6 levels were also elevated by 3-fold after one session of SD (P < 0.05), but actually dipped significantly below baseline with three session of SD (P < 0.05) and after 24 of recovery of SD (P < 0.05). Placental TNFα analysis revealed no significant sleep condition X recovery time interaction (F2,19 = 1.3, P = 0.29), and no individual main effect of sleep condition (F2,19 = 1.0, P = 0.41) or recovery time (F1,19 = 0.7, P = 0.43) (Fig. 6C).

Assessment of fetal brain cytokines showed that levels of IL-1β were significantly affected by a sleep condition X recovery time interaction (F2,19 = 6.5, P < 0.01), but no main effect of sleep condition (F2,19 = 1.9, P = 0.18) or recovery (F1,19 = 1.3, P = 0.09) (Fig. 7A). Consistent with the placental results, fetal brain IL-1β levels were elevated by almost 2-fold after one session of SD (P < 0.05) and returned to baseline following 24 h of recovery. Fetal brain IL-6 levels were also significantly affected by an interaction between sleep condition X recovery time (F2,19 = 5.0, P < 0.05), however sleep condition (F2,19 = 2.0, P = 0.17) and recovery time (F1,19 = 1.6, P = 0.23) alone did not have a significant effect on fetal brain IL-6 levels (Fig. 7B). Levels of TNFα were measured in fetal brain samples but were undetectable in many cases (data not shown).

3.4. Correlational analysis

Given the findings described presently, we sought to explore possible relationships between fetal brain KYNA, placental cytokines, and plasma corticosterone levels. A positive correlation was observed between maternal corticosterone and fetal brain KYNA (r = 0.50, P < 0.05) and we also observed a positive correlation between maternal corticosterone and placental IL-1β (r = 0.46, P < 0.05). Importantly no relationship was observed between fetal brain KYNA and fetal corticosterone (r = 0.01, P = 0.95). Taken together, these data highlight a relationship between maternal stress reactivity, placental immune response and fetal KP metabolism.

3.5. Data availability

The data generated in the present study are available from the corresponding author upon reasonable request.

![Fig. 3. Increase in fetal, but not maternal, brain kynurenine acid (KYNA) with sleep deprivation (SD).](image-url)
4. Discussion

Our study sought to determine the impact of maternal SD on HPA axis regulation, inflammatory response and KP metabolism. Despite no indication of KP dysfunction in maternal tissues, a significant increase in fetal brain KYNA was observed following a single day of SD. This was accompanied by an increase in maternal corticosterone and elevated proinflammatory cytokine levels, namely IL-1β and IL-6, in placenta and fetal brain. Taken together, these results support the notion that in utero stress and immune reactivity may drive changes in fetal brain KYNA and inflammation. Our findings implicate KYNA as a novel molecule by which maternal sleep loss during pregnancy could impact fetal neurodevelopment.

In rodents and humans, healthy fetal brain development is critically linked to endocrine and immunological homeostasis between the mother and fetus. The significance of the placenta’s fundamental role in modulating pathophysiological responses to maternal insults is well established (Bronson and Bale, 2014; Hsiao and Patterson, 2012; O’Donnell et al., 2009). Fetal exposure to maternal glucocorticoids or placental proinflammatory cytokines can exert detrimental effects on brain development (Bitanihirwe et al., 2010; Bock et al., 2011; Fatemi et al., 2002; Mychasiuk et al., 2012; Nyffeler et al., 2006; Schepanski et al., 2018). In relation to the KP, a recent study demonstrated that acute restraint stress transiently increased fetal brain KYNA (Notarangelo and Schwarcz, 2016). Our study bridges several of these concepts by demonstrating for the first time that increased maternal corticosterone due to sleep loss can adversely impact the endocrine and immunological homeostasis in utero and thereby significantly alter fetal brain KYNA.

Herein, SD in pregnant rats was induced by the gentle handling method, which we have previously shown to effectively eliminate rapid eye movement (REM) sleep and substantially (> 95%) reduce non-REM (NREM) sleep (Baratta et al., 2018). In line with our findings of increased circulating maternal, but not fetal, levels of corticosterone, other studies have shown elevated corticosterone levels following sleep loss (Baratta et al., 2018; Calegare et al., 2010). The sleep loss-induced elevation in circulating corticosterone can subsequently activate the enzyme TDO, which converts tryptophan to kynurenine (Gibney et al., 2014; Ohta et al., 2017). Stress-induced increases in TDO have been shown to elevate kynurenine and downstream metabolites in the brain and periphery (Miura et al., 2008a, 2008b; Pawlak et al., 2000). IDO, which also converts tryptophan to kynurenine, is alternatively induced by immunological activation and elevated cytokines (Dantzer et al., 2011; Gibney et al., 2013). While we presently demonstrate that maternal SD elevated plasma corticosterone and tryptophan, this elevation did not result in higher maternal or placenta KP metabolism. Instead,
we find that maternal corticosterone correlated highly with placental cytokine levels, as in other models of prenatal stress (Bronson and Bale, 2014; Gur et al., 2017). To our knowledge, our findings are the first to demonstrate a within subject correlation among these factors in a prenatal sleep manipulation study.

The observed increase in proinflammatory cytokine levels in the placenta following SD may indicate a mechanism by which maternal physiological alterations in response to stress could directly or indirectly affect the fetus. Ex vivo placental perfusions of full-term placentas have demonstrated that cytokines are not transferred across the placenta (Aaltonen et al., 2005), but it is able to produce and release cytokines independently (Bowen et al., 2002) and their release can be increased by external factors such as stress or infection (Ashdown et al., 2006). Such elevations in proinflammatory cytokines are also associated with placental damage (Silen et al., 1989) and intrauterine TNF-α and IL-1β in late pregnancy have also been shown to stimulate preterm labor (Sadowsky et al., 2006), identified as a risk factor for the development of cognitive dysfunction and altered neurodevelopmental outcomes (Moster et al., 2008; Sadowsky et al., 2006). Additionally, fetal brain inflammation may detrimentally impact neurodevelopment, such as the establishment of dendritic processes and the growth of neurons (Burd et al., 2011) as well as cognitive functions later in life (Smith et al., 2007). As observed in our present study, increased fetal brain cytokines may directly stimulate IDO activity (Dantzer et al., 2011; Gibney et al., 2013), leading to the observed overproduction of KYNA.

The implications of sleep loss during pregnancy are multifaceted, but have been poorly investigated. Sleep loss during embryogenesis is a risk factor for pregnancy loss (Calegare et al., 2010), poor fetal growth and preterm delivery (Chang et al., 2010; Okun et al., 2011; Palagini et al., 2014). Lasting negative impacts of SD during pregnancy, investigated in the developing offspring, include greater amounts of active sleep and less quiet sleep compared to controls, indicative of immature brain development (Aswathy et al., 2018), and increased ultrasonic vocalizations (Gulia et al., 2015), indicative of higher sensitivity to maternal separation. As pre-adolescents, offspring display increased risk-taking behavior (Gulia et al., 2015; Radhakrishnan et al., 2015) and deficits in hippocampal-dependent learning and memory (Zhao et al., 2014, 2015). Pre-adolescent pups also show decreased hippocampal neurogenesis and increased neuroinflammation (Zhao et al., 2014, 2015). Adult offspring of SD mothers present with many of the same behavioral changes, increased anxiety and impaired cognitive performance, indicating that these changes are long-lasting (Peng et al., 2016). Additionally, adult offspring have higher body weights, increased visceral adipose tissue and display insulin resistance, indicating metabolic changes following maternal SD in addition to persistent

![Fig. 6.](image1) Sleep deprivation (SD) elevates proinflammatory cytokines in placental tissues. Pregnant rat dams were sleep deprived for 5 h from ZT 0 to ZT 5 for either 1 session on ED 18 or 3 consecutive daily sessions on ED 16, ED 17, and ED 18. (A) IL-1β, (B) IL-6 and (C) TNFα in the placenta immediately after SD (0 h after SD), or after recovery (24 h after SD). All data are mean ± SEM. *P < 0.05 versus corresponding control. $P < 0.05 versus corresponding 0 h group. N = 3–6 per group.

![Fig. 7.](image2) One session of sleep deprivation (SD) elevates proinflammatory cytokines in the fetal brain. Pregnant rat dams were sleep deprived for 5 h from ZT 0 to ZT 5 for either 1 session on ED 18 or 3 consecutive daily sessions on ED 16, ED 17, and ED 18. (A) IL-1β and (B) IL-6 in the fetal brain immediately after SD (0 h after SD), or after recovery (24 h after SD). All data are mean ± SEM. *P < 0.05 versus control. N = 3–6 per group.
cognitive deficits (Khalyfa et al., 2015; Trzepizur et al., 2017). While there have been few studies attempting to elucidate the mechanism behind these changes, our findings introduce elevated KYNA as a molecular underpinning.

Elevations in fetal brain KYNA disrupt normal neurodevelopment (Notarangelo and Pocivavsek, 2017). Adult offspring from rat dams fed a kynurenine-laced diet, resulting in higher fetal brain KYNA levels, display cognitive deficits, morphological, and biochemical alterations (Hahn et al., 2018; Pershing et al., 2015, 2016; Pocivavsek et al., 2014, 2019). Elevations in fetal brain KYNA by a pharmacological inhibitor of kynurenine-3-monoxygenase (KMO), the enzyme responsible for metabolizing kynurenine into 3-hydroxykynurenine (3-HK), resulted in functional consequences on synaptic transmission in adulthood (Forrest et al., 2013a, 2013b, 2015; Khalil et al., 2014). These studies together support the notion that the KP plays a role in neurodevelopment and altering levels of fetal brain KYNA, as we induced with maternal SD, may also lead to long-lasting functional changes, to be explored in the future.

Moreover, the KP has been found to play many roles during pregnancy (Badawy, 2015; Keaton et al., 2019), including supporting fetal implantation and neural protection, which are most likely due to an interplay between maternal, placental and fetal metabolite levels. The placenta can metabolize tryptophan (Ligam et al., 2005) and thus act as a source to elevate KP metabolites, namely kynurenine, in the fetus without elevations in maternal plasma. Since the placenta is highly vascularized (Rennie et al., 2014), sources of tryptophan, kynurenine, and KYNA within the placenta could be partly due to residual blood, as we previously investigated by perfusing pregnant dams (Goeden et al., 2017). A limitation presently is that we were unable to measure placental tryptophan after SD in utero. However, we were able to determine no alterations in placental kynurenine and KYNA after SD. Considering the impact of placental sources on the fetal brain KP metabolites, we recognize that tryptophan and kynurenine could readily cross the placental barrier, but even vascular components of KYNA within the placenta are not likely to contribute to KYNA levels in the fetus as this metabolite does not readily cross the placental barrier (Goeden et al., 2017; Notarangelo et al., 2019). While the fetal tissues themselves can take maternal or fetal derived kynurenine and de novo synthesize KYNA, via transamination by kynurenine aminotransferases (KATs) (Goeden et al., 2017; Notarangelo et al., 2019), the exact source of fetal derived brain KYNA remains elusive in vivo since the fetal liver also has a great ability to metabolize kynurenine (Real et al., 1992; Cannazza et al., 2001; Ceresoli-Borroni and Schwarz, 2000; Nicholls et al., 1999; Notarangelo and Pocivavsek, 2017). Recent evidence supports the notion that the fetal rodent brain has the necessary enzymatic machinery to synthesize KYNA (Notarangelo et al., 2019), and this source of KYNA is hypothesized to be responsible for its neurodevelopmental impacts (Notarangelo and Pocivavsek, 2017).

Mechanistically, we speculate that the impact of fetal brain KYNA on neurodevelopment may be via its antagonism of NMDA and α7nACh receptor function. Both receptor classes have been implicated in regulating neurodevelopment, including the development and morphology of dendritic spines, maintaining the stability of synapses, survival of neurons, and modulation of excitatory-inhibitory circuits during development (Alvarez et al., 2007; Ikonomidou et al., 1999; Liu et al., 2006, 2007; Rajan and Cline, 1998; Ulation et al., 2007). However, we also consider that KYNA has been found to activate signaling receptors, including G protein-couple receptor (GPR) 35 and the aryl hydrocarbon, that are functional in both the brain and periphery (Pocivavsek et al., 2016). Additionally, while the focus of this study was on KYNA formation in fetal tissue, it is important to consider the alternative branch of the KP that generates 3-HK, 3-hydroxyanthranilic acid and quinolinic acid (QUIN), which may induce generation of free radicals and downstream neurotoxic effects (Foster et al., 1984; Guillemin, 2012; Schwarz et al., 2012). From our previous SD experiments in adult rats, we determined that acute loss of sleep does not alter 3-HK formation in the adult rat brain (Baratta et al., 2018). Given that SD triggers oxidative processes (Calegare et al., 2010; D’Almeida et al., 1998; Everson et al., 2005; Gopalakrishnan et al., 2004; Rajendiran et al., 2015) it remains to be seen if maternal SD could in part also lead to an increased production of KP metabolites through both enzymatic and oxidative mechanisms (Blanco Ayala et al., 2015; Notarangelo et al., 2019).

Assessing our findings after one session of SD between animals that were euthanized immediately or allowed 24 h of recovery, we determined that some of the striking effects were normalized with recovery sleep. This is most likely due to the phenomenon of rebound sleep, where animals exposed to acute SD exhibit increases in sleep duration during the recovery period (Rechtschaffen et al., 1999; Tobler and Borbely, 1990). We speculate that this increase in sleep reinstates homeostatic levels of cytokines and corticosterone. Unexpectedly, though, we observed very few changes following three sessions of SD. It remains to be seen if this may be due to compensatory mechanisms that, after multiple exposures to SD, help to initiate an allostatic response (Kim et al., 2013; Wallingford et al., 2014).

Our present findings place new attention on the KP as a possible mechanism by which maternal stress, in the form of sleep disturbances, can negatively impact fetal neurodevelopment. It is also enticing to speculate that a rise in prenatal KP metabolism described here may be a physiologically protective response in utero. This could be considered the fetal brain’s attempt to protect against excitotoxic damage during development (Ceresoli-Borroni and Schwarz, 2000). The exact process by which maternal glucocorticoids and placental inflammation regulate KP metabolism has yet to be elucidated, including further investigation into the activity of KP enzymes. Future studies investigating the relationship between the maternal-placental-fetal interplay, will provide valuable understanding of the impacts of prenatal sleep loss on KYNA formation and defining a causal relationship on long-term behavioral outcomes. As we have demonstrated in adult rodents that acute sleep loss differentially impacts brain KYNA levels (Baratta et al., 2018), future prenatal studies will also assess the impact of biological sex of the developing embryos. Of note, alterations in KYNA are found in several psychiatric and neurological conditions that are plagued with cognitive dysfunction and sleep abnormalities, highlighting the translational implications of our studies (Cho et al., 2017; Pocivavsek and Rowland, 2018; Schwarz et al., 2012). Leading hypotheses suggest that cognitive impairments and sleep disturbances are interlinked, thus it will be critical to further define the mechanistic contribution of KYNA, and potential therapeutic value of reducing its synthesis via KAT II inhibition (Kozak et al., 2014; Pocivavsek et al., 2019), as a connection in mediating these relationships in future studies.

Author contributions section

Annalisa M Baratta: conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review & editing, visualization. Nickole R Kanyuch: methodology, investigation, writing – original draft, visualization. Casey A. Cole: formal analysis, writing – review & editing. Homayoun Valafar: formal analysis, resources, writing – review & editing, supervision. Jessica Deslauriers: formal analysis, investigation, writing – original draft, writing – review & editing, visualization, project administration. Ana Pocivavsek: conceptualization, methodology, validation, formal analysis, investigation, resources, writing – original draft, writing – review & editing, visualization, supervision, project administration, funding acquisition.

Declaration of competing interest

The authors report no conflicts of interest.
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References

Aaltonen, R., Heikkinen, T., Hakala, K., Laine, K., Alalen, A., 2005. Transfer of proinflammatory cytokines across term placenta. Obstet. Gynecol. 106, 802–807.
Alvarez, V.A., Ridouen, D.A., Sabatini, B.L., 2007. Distinct structural and isonitrogenous roles of NMDA receptors in controlling spine and synapse stability. J. Neurosci. 27, 7365–7376 the official journal of the Society for Neuroscience.
Ashdown, H., Dumont, Y., Ng, M., Poole, S., Boksia, L., Luheshi, G.N., 2006. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol. Psychiatry 11, 47–55.
Aswathy, B.S., Kumar, V.M., Gulia, K.K., 2018. Immature sleep pattern in newborn rats when dams encountered sleep restriction during pregnancy. Int. J. Dev. Neurosci. 60, 60–67.
Badau, A.A.B., 2015. Tryptophan metabolism, disposition and utilization in pregnancy. Biosci. Rep. 35, e00261.
Banks, S., Dinges, D.F., 2007. Behavioral and physiological consequences of sleep restriction. J Clin Sleep Med 3, 519–528.
Baratta, A.M., Buck, S.A., Buchia, A.D., Fabian, C.B., Chen, S., Mong, J.A., Pocivavsek, A., 2018. Sex differences in hippocampal memory and kynurenine acid formation following acute sleep deprivation in rats. Sci. Rep. 8, 9693.
Beattie, M.E., Szwarta, K.C., 1992. Development changes in brain kynurenine acid concentrations. Brain Res Dev Brain Res 68, 136–139.
Bitanrihime, K.R., Peleg-Raiabin, D., Moutet, F., Feldon, J., Meyer, U., 2010. Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. Neuropsychopharmacology 35, 2462–2478.
Blanco Ayala, T., Lugo Huitron, R., Carmona Aparicio, L., Ramirez Ortega, D., Gonzalez Esquivel, D., Prieda Chevarri, J., Perez de la Cruz, G., Rios, C., Schwarcz, R., Perez de la Cruz, V., 2015. Alternative kynurenic acid synthesis routes studied in the rat cerebellum. Front. Cell. Neurosci. 9, 178.
Bock, J., Murmu, M.S., Biala, Y., Weinstock, M., Braun, K., 2011. Prenatal stress and cytokine release: a study of maternal and fetal cytokines in women. Placenta 23, 239–256.
Briañez-Padilla, M., Sanchez-Munoz, F., Vazquez-Palacios, G., Huang, F., Almanza-Perez, J.C., Bojalil, R., Bonilla-Jaime, H., 2018. Cytokine and microRNA levels during different periods of paradoxical sleep deprivation and sleep recovery in rats. PeerJ 6, e6567.
Brons, S.L., Bale, T.L., 2014. Prenatal stress-induced increases in placental inflammation and sleep recovery behavior are male-specific and ameliorated by maternal antidepressant treatment. Endocrinology 155, 2635–2646.
Bruner, D.P., Munch, M., Biedermann, K., Huch, R., Huch, A., Borbely, A.A., 1994. Changes in sleep and sleep electroencephalogram during pregnancy. Sleep 17, 576–582.
Burd, I., Brown, A., Gonzalez, M.J., Chai, J., Elvovitz, M.A., 2011. A mouse model of term chorionamnionitis: unraveling causes of adverse neurological outcomes. Reprod. Sci. 18, 900–907.
Calegari, B.F., Fernandes, L., Tufik, S., D’Almeida, V., 2010. Biochemical, biometrical and behavioural changes in male offspring of sleep-deprived mice. Psychoneuroendocrinology 35, 775–784.
Cannazza, G., Chiarugi, A., Parenti, C., Zanoli, P., Baraldi, M., 2001. Changes in kynurenic acid formation in the rat hippocampus. Neuroreport 12, 2853–2856.
Guidetti, P., Hoffman, G.E., Melendez-Ferro, M., Albuquerque, E.X., Schwarcz, R., 2007. Oxidative stress. Sleep 30, 960–967.
Guillemin, G.J., 2012. Quinolinic acid, the inescapable neurotoxin. FEBS J. 279, 1356–1365.
Gulia, K.K., Patel, N., Kumar, V.M., 2015. Increased ulcerogenic expressions and risk-taking in rats of sleep-deprived dams. Physiol. Behav. 139, 59–66.
Gu, T.L., Shay, L., Palkar, A.V., Fisher, S., Varalj, A.V., Dowd, S.A., Bulley, M.T., 2017. Prenatal stress affects placental cytokines and neurotrophins, commensal microbes, and anxiety-like behavior in adult female offspring. Brain Behav. Immun. 58, 78–92.
Guillemij, G.I., 2012. Quinolinic acid, the inescapable neurotoxin. FEBS J. 279, 1356–1365.
Hahn, B., Renakki, G., Pocivavsek, A., Schwarz, R., 2016. Prenatal kynurenine treatment in rats causes schizophrenia-like cognitive and monitoring deficits in adulthood. Psychopharmacology (Berl) 235, 651–661.
Hedman, C., Pohjanvirta, V., Balopoulou, M., Almyllinen, S., Mylläri, V.T., 2002. Effects of pregnancy on maternal sleep during pregnancy. Sleep Med. 3, 153–159.
Hisay, E.O., Patterson, P.H., 2012. Prenatal immune activation alters maternal-fetal interactions and brain development. Dev. Neurobiol. 72, 1317–1326.
Ikonomidou, C., Bosch, F., Finka, M., Bitetti, G., Pockler, J., Dzirainis, K., Tenkova, T.I., Stefovska, V., Turski, L., 1994. Blockade of NMDA receptors and antitonic and antipsychotic action in the rat brain. Brain Res. 666, 378–387.
Keaton, S.A., Heilman, P., Bryleva, E.Y., Madaj, Z., Krzyzanowski, S., Grit, J., Miller, E.S.,ия, 2019. Altered tryptophan catabolism in placentas from women with pre-eclampsia. Int. J. Tryptophan Res. 12 11786649198040321.
Khali, O.S., Pocivavsek, A., Racki, O., Mccinnes, D., Abbott, A.L., Roberts, R.M., Conneau, K., Gunawoyski, Y., Young, M.A., Armour, P.A., Wiss, M., 2013. Reduction of brain kynurenine acid improves cognitive function. J. Neurosci. 33, 1598–1601.
Khalyfa, A., Carreras, A., Almendros, I., Hakim, F., Gozal, D., 2015. Sex dimorphism in cytokines in mediating effects of prenatal infection on the fetus: implications for neuroinflammatory disease. Mol. Neurobiol. 52, 170–181.
Khalil, O.S., Pisar, M., Forrest, C.M., Khalil, O.S., Pisar, M., McNair, K., Kornisnik, E., Snitcofsky, M., Gonzalez, A., 2014. Reduction of brain kynurenic acid improves cognitive function. J. Neurosci. 34, 2462–2478.
Koehler, D.H., Earle, J., Kanemoto, E., Patterson, E.P., Cui, N., McClin, B., Ackerman, A.E., Raymond, F.I., Skene, D.J., 2014. Effect of sleep deprivation on the human metabolome. Proc. Natl. Acad. Sci. U. S. A. 111, 10761–10766.
Lemaire, V., Koehl, M., Le Meaux, A., Fagot, E., 2014. Sleep loss produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. Proc. Natl. Acad. Sci. 97, 11032–11037.
Leproult, R., Copinshi, G., Buxton, O., Van Cauter, E., 1998. Sleep loss results in an elevation of cortisol levels the next evening. Sleep 20, 860–865.
Ligam, P., Manelipillai, U., Wallace, E.M., Walker, D. 2005. Localisation of indoleamine 2,3-dioxygenase and kynurenine hydroxylase in the human placenta and decidua: implications for role of the kynurenine pathway in pregnancy. Placenta 26, 498–504.

Liu, Z., Neff, R.A., Berg, D.K. 2006. Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. Science 314, 1610–1613.

Liu, Z., Zhang, J., Berg, D.K. 2007. Role of endogenous nicotinic signaling in guiding neuronal development. Biochem. Pharmacol. 74, 1112–1119.

Miura, H., Ozaki, N., Sawada, M., Isobe, K., Otta, T., Nagatsu, T. 2008a. A link between stress and depression: shifts in the balance between the kynureninestress Int. J. Biol. Stress 31, 198–209.

Miura, H., Ozaki, N., Shirakawa, T., Isobe, K. 2008b. Changes in brain tryptophan metabolism elicited by aging, social environment, and psychological stress in mice. Stressforskningsrapporten 11, 160–169.

Moster, D., Lie, R.T., Markstedt, T. 2008. Long-term medical and social consequences of preterm birth. N. Engl. J. Med. 359, 262–273.

Mychàisík, R., Gibb, R., Kolb, B. 2012. Prenatal stress alters dendritic morphology and synaptic connectivity in the prefrontal cortex and hippocampus of developing offspring. Synapse 66, 308–314.

Nicholls, T., Nitos, I., Walker, D.W. 1999. Tryptophan metabolism in pregnant sheep: increased fetal kynurenine production in response to maternal tryptophan loading. Am. J. Obstet. Gynecol. 181, 1452–1460.

Notarangelo, F.M., Beggisto, S., Schwarz, R. 2013. Assessment of prenatal kynurenine metabolism using tissue slices: focus on the neosynthesis of kynurenic acid in mice. Dev. Neurosci. 41, 102–111. https://doi.org/10.1159/000349736.

Notarangelo, F.M., Pocivavsek, A. 2017. Elevated kynurenine pathway metabolism during neurodevelopment: implications for brain and behavior. Neuropharmacology 112, 275–285.

Notarangelo, F.M., Schwarz, R. 2016. Restraint stress during pregnancy rapidly raises kynurenic acid levels in mouse placenta and fetal brain. Dev. Neurosci. 38, 458–468.

Nyffeler, M., Meyer, U., Yee, B.K., Feldon, J., Knuesel, I. 2006. Maternal immune activation during pregnancy increases limbic GABA receptor immunoreactivity in the adult offspring: implications for schizophrenia. Neuroscience 143, 51–62.

O’Donnell, K., O’Connor, T.G., Glover, V. 2009. Prenatal stress and neurodevelopment of the child: focus on the HPA axis and role of the placenta. Dev. Neurosci. 31, 285–292.

Oba, Y., Kubo, H., Yahiro, K., Ohashi, K., Tsuchi, Y., Wada, N., Yamamoto, Y., Saito, K. 2017. Effect of water-immersion restraint stress on tryptophan catabolism by tryptophan catabolism in the kynurenine pathway in rat tissues. J. Physiol. Sci. 67, 361–372.

Okun, M.L., Schetter, C.D., Glynn, L.M. 2011. Poor sleep quality is associated with increased fetal kynurenine production in response to maternal tryptophan loading. Am. J. Obstet. Gynecol. 195, 1578–1589.

Olszewski, N., Buss, C., Hanagud-Opatz, I.L., Arck, P.C. 2018. Prenatal immune and endocrine modulators of offspring’s brain development and cognitive functions during life. Front. Immunol. 9, 2186.

Schwarz, R., Bruno, J.P., Kisowski, P.J., Wu, H.-Q., 2012. Kynurenines in the mammalian brain: when physiology meets pathology. Nat. Rev. Neurosci. 13.

Silen, M.L., Fifaro, A., Moregelo, S., Lowry, S.F., Frances, T. 1989. Interleukin-1 alpha and tumor necrosis factor alpha cause placental injury in the rat. Am. J. Pathol. 135, 239–244.

Smith, S.E.P., Li, J., Garbett, K., Mirmics, K., Patterson, P.H. 2007. Maternal immune activation alters fetal brain development through interleukin-6. J. Neurosci. 27, 10095–10097.

Strine, T.W., Chapman, D.P. 2005. Associations of frequent sleep insufficiency with health-related quality of life and health behaviors. Sleep Med. 6, 23–27.

Tobler, I., Borbély, A.A. 1990. The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. Behav. Brain Res. 36, 73–78.

Toru, M., Mitsushio, H., Mataga, N., Takashima, M., Arito, H. 1984. Increased brain serotonin metabolism during reboud sleep in sleep-deprived rats. Pharmacol. Biochem. Behav. 20, 757–761.

Trzepiñ, W., Khalyfa, A., Qiao, Z., Popko, B., Gozal, D. 2017. Integrated stress response activation by sleep fragmentation during late gestation in mice leads to emergence of adverse metabolic phenotype in offspring. Metabolism 69, 188–198.

Ulltaiar, S.K., Kim, J.E., Hall, B.J., Dverinck, T., Ellisson, M., Ghosh, A. 2007. Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. Proc. Natl. Acad. Sci. U. S. A. 104, 19553–19558.

Wallingford, J.K., Deverilhe, S., Currie, R.W., Fawcett, J.P., Semba, K. 2014. Increases in inflammatory markers, and cytokine balance. Brain Behav. Immun. 47, 24–34.

Wright Jr., K.P., Drake, A.L., Frey, D.J., Fleshner, M., Desouza, C.A., Gronfier, C., Czeisler, A.A. 2007. Regulation of spine morphology and spine density by NMDA receptor signaling in vivo. Proc. Natl. Acad. Sci. U. S. A. 104, 19553–19558.

Zhao, Q., Xie, X., Fan, Y., Zhang, J., Jiang, W., Wu, Y., Yan, S., Chen, Y., Peng, C., You, Z. 2014. Maternal sleep deprivation inhibits hippocampal neurogenesis associated with inflammatory response in young offspring rats. Neurobiol. Dis. 68, 57–65.

Zhao, Q., Xie, X., Fan, Y., Zhang, J., Jiang, W., Wu, Y., Yan, S., Chen, Y., Peng, C., You, Z., 2015. Phenotypic dysregulation of microglial activation in young offspring rats with maternal sleep deprivation-induced cognitive impairment. Sci. Rep. 5, 9513. 