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Functional Impairments in Learning and Signal Propagation Following Prenatal Kynurenine Treatment in Mice

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ABSTRACT

The levels of kynurenic acid (KYNA), a metabolite of the kynurenine pathway (KP) of tryptophan degradation, are elevated in the brain of persons with schizophrenia (SZ) and may be linked to cognitive dysfunctions in the disease. Studies in rats indicate that abnormally high fetal brain KYNA may play a pathophysiologically significant role in this context ("EKyn model"). Here, we fed pregnant C57Bl/6J mice with the immediate KYNA precursor kynurenine (10 mg or 30 mg/day; EKyn) or with control chow (ECon) from embryonic day (ED) 11 to ED 18 and assessed offspring postnatally both functionally and biochemically. In adulthood, male, but not female, EKyn mice showed significant impairments in spatial and reversal learning. Moreover, ex vivo recording of evoked local field potentials in coronal brain slices revealed a longer contralateral response latency in EKyn than in ECon animals, suggesting impaired white matter function. However, plasma and brain levels of KYNA and of another KP metabolite, 3-hydroxykynurenine, did not differ between groups on postnatal day (PD) 21, on PD 35 (adolescence), or in adulthood (PD 56–75). Separate mice were fed prenatally with 4-chloro-kynurenine (20 mg/day), which is converted to the selective NMDA receptor antagonist 7-chloro-KYNA in vivo. Offspring did not show electrophysiological impairments in adulthood, indicating that NMDA receptors in the fetal brain were not the sole cause of functional deficits of EKyn mice later in life. The implications of these experiments for the study of psychiatric symptoms, as well as the unexpected differences between rats and mice, are discussed.

Abbreviations: aCSF, artificial cerebrospinal fluid; E-4-Cl-Kyn, embryonic 4-chloro-kynurenine; ECon, embryonic control; ED, embryonic day; EKyn, embryonic kynurenine; eLFP, evoked local field potentials; KYNA, kynurenic acid; KP, kynurenine pathway; NMDA, N-methyl-D-aspartate; PD, postnatal day; SZ, schizophrenia; 3-HK, 3-hydroxykynurenine; 4-Cl-Kyn, 4-chloro-kynurenine; 7-Cl-KYNA, 7-chloro-kynurenic acid; α7nACh, alpha-7 nicotinic acetylcholine.

Sarah Beggiato and P. Leon Brown are co-first authors.

1 | Introduction

The etiology of schizophrenia (SZ) is complex but likely results from a combination of environmental and genetic factors that influence early neurodevelopment (Schmidt and Mirnics 2014; Burrows and Hannan 2016; Bergdolt and Dunaevsky 2019; Murlanova and Pletnikov 2023). Attempts to model such alterations in animals include modulation of the expression of high-risk genes and various experimental insults during the perinatal period (Flores et al. 2016; Brown and Meyer 2018; Bergdolt and Dunaevsky 2019; Eyles 2021). Analyses of cerebrospinal fluid and post-mortem brain tissue of clinical populations have shown that the levels of metabolites of the kynurenine pathway (KP) of tryptophan degradation (Figure 1A), specifically kynurenic acid (KYNA), are elevated in individuals with SZ (Schwarcz et al. 2001; Nilsson et al. 2005; Miller et al. 2006; Linderholm et al. 2010; Sathyasaikumar et al. 2010). KYNA is an endogenous antagonist of alpha-7 nicotinic acetylcholine (α7nACh) and N-methyl-D-aspartate (NMDA) receptors, both of which play major roles in cognitive function and have been implicated in the pathophysiology of SZ (Bickel and Javitt 2009; Wonodi and Schwarcz 2010; Araud et al. 2011). Notably, work in experimental animals has consistently demonstrated that a number of perinatal challenges result in increased peripheral formation of the pivotal KP metabolite kynurenine, in turn raising KYNA levels in the brain (Notarangelo and Schwarcz 2016; Honorio de Melo Martimiano et al. 2017; Baratta et al. 2020).

In rats, systemic administration of the immediate KYNA precursor kynurenine has several adverse consequences, which can be documented using a variety of informative biochemical, electrophysiological, and behavioral outcome measures (Shepard et al. 2003; Erhardt et al. 2004; Chess et al. 2007; Chess et al. 2009; Pocivavsek et al. 2011; Alexander et al. 2013). Interestingly, and of possible translational relevance with regard to the pathophysiology of SZ, administration of a "high kynurenine" diet to pregnant dams causes not only acute elevations of KYNA levels in the fetal brain but also results in long-lasting behavioral and biochemical impairments in the adult offspring (the "EKyn model") (Pocivavsek et al. 2014; Pershing et al. 2015; Pershing et al. 2016; Notarangelo and Pocivavsek 2017; Pocivavsek et al. 2019; Buck et al. 2020; Rentschler et al. 2021; Wright et al. 2021).

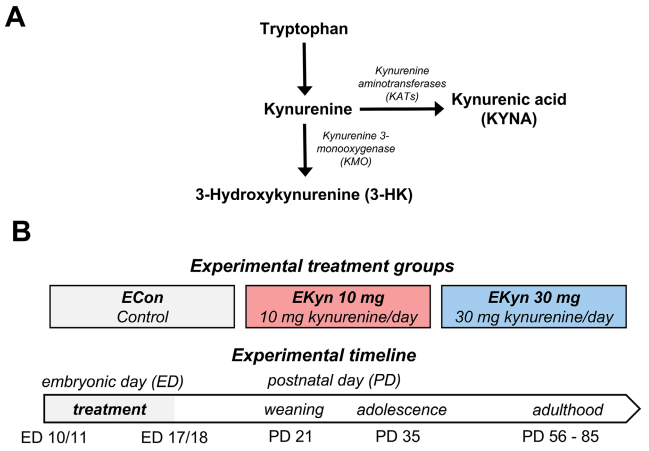

FIGURE 1 | (A) Neosynthesis of the kynurenine pathway metabolites KYNA and 3-HK from their common bioprecursor kynurenine. (B) Summary of the experimental design of the study. EKyn, embryonic kynurenine treatment.

While the acute effect of prenatal kynurenine treatment on fetal brain KYNA has been verified in mice (Beggiato et al. 2018), long-term consequences in the offspring have not been examined so far. To fill this void, and in anticipation of planned follow-up studies with genetically modified mice, the present study was designed to assess possible changes in selected functional (behavioral and electrophysiological) and biochemical (tissue levels of KYNA and the KP metabolite 3-hydroxykynurenine [3-HK]) outcome measures in the adult progeny of wild-type dams that received a high kynurenine diet during the last week of gestation. Using electrophysiological assessment, we also examined the specific role of prenatal NMDA receptor antagonism in this context by substituting kynurenine with 4-chloro-kynurenine (4-Cl-Kyn), which is converted to the selective NMDA receptor antagonist 7-chloro-KYNA (7-Cl-KYNA) in vivo (Wu et al. 1997). Although differences between mice and rats were noted, our results revealed clear functional impairments in adult EKyn mice. Moreover, the study showed that the detrimental long-term effects of prenatal kynurenine treatment in mice cannot be attributed solely to NMDA receptor dysfunction in the fetal brain.

2 | Materials and Methods

2.1 | Animals

Male and female C57Bl/6J mice (Jackson Laboratories; Bar Harbor, Maine, United States) were obtained for on-site breeding. Breeding pairs were housed together for 48 h before removal of the male mouse. Female mice were then monitored daily for signs of pregnancy. Following parturition, the health of the pups was examined each day, and the animals remained housed with the dam until weaning on postnatal day (PD) 21. Pups were then separated by sex and group-housed (3–5 mice per cage) for the remainder of the study. Food and water were provided ad libitum. In adulthood, experiments with animals were performed and analyzed by individuals who were blind to the prenatal treatment condition. Randomization of animals was ensured by distributing offspring from prenatal treatment litters across experiments in adulthood. All experiments were approved by local Institutional Animal Care and Use Committees.

2.2 | Chemicals

L-Kynurenine sulfate ("kynurenine"; purity: 99.4%) was obtained from Sai Advantium (Hyderabad, India). 4-Cl-Kyn was kindly provided by VistaGen Therapeutics (South San Francisco, California, United States). KP metabolites were purchased from Sigma Chemical Co. (St. Louis, Missouri, United States). All other chemicals were obtained from various suppliers and were of the highest commercially available purity.

2.3 | Prenatal Treatment

Pregnant mice were fed kynurenine mixed with wet rodent mash (10 or 30 mg/day; "EKyn 10" and "EKyn 30," respectively)

for 8 consecutive days starting on embryonic day (ED) 10/11 (Beggiato et al. 2018). Rodent chow was ground in a food processor, and each pregnant mouse ate approximately 5 g per day. Since the body weight of dams quickly increases during the last embryonic week, chow was laced with a steady amount of kynurenine (10 or 30 mg) for EKyn 10 and EKyn 30, respectively. The doses were selected based on previous studies in rats and mice (Pocivavsek et al. 2014; Beggiato et al. 2018). Control dams (ECon) were fed wet mash alone during the same period (Figure 1B). Some biochemical assays were performed on tissues from animals that were euthanized by CO_2 inhalation on the last day of treatment (ED 17/18). EKyn 10, EKyn 30, and ECon pups were separated at weaning by sex, group-housed (3–5 mice/cage), and then studied at various postnatal ages using behavioral, electrophysiological, and biochemical outcome measures (offspring of EKyn 10 dams were only assessed behaviorally; see below).

Other cohorts of pregnant mice were fed 4-Cl-Kyn (5 or 20 mg/day) mixed with wet rodent mash for 8 consecutive days starting on ED 10/11 ("E-4-Cl-Kyn" treatment). ECon dams were treated during the same period as described above (Figure 5B). At weaning, E-4-Cl-Kyn 5, E-4-Cl-Kyn 20, and ECon pups were group-housed by sex until electrophysiological studies were performed in adulthood (PD 56–85).

2.4 | Barnes Maze

Behavior to investigate learning and memory in the Barnes maze (Rosenfeld and Ferguson 2014) was examined individually in adult offspring of ECon (34 males and 33 females from 12 litters), EKyn 10 (20 males and 19 females from 7 litters), and EKyn 30 (25 males and 21 females from 8 litters) animals. Briefly, mice were habituated to the maze and given 180 s to explore and acclimate to the escape box. On the next day, acquisition training, designed to assess hippocampus-dependent learning, began by placing the animal in the maze for 3 consecutive days (2 trials per day; 180 s maximum per trial; 4 h intertrial interval). Reversal learning, designed to assess frontal cortex-dependent learning, was conducted on the next day, with the escape box location moved by 180°. Search strategies that mice employed navigating the maze were categorized as direct (< 3 errors within 2 holes from the escape or < 3 errors and course correction directly toward escape location), serial (at least 3 errors, with majority sequential, and < 2 changes of direction) and random (at least 3 errors, with minority sequential, or with 2 or more changes of direction) (Rosenfeld and Ferguson 2014). All trials were evaluated with Noldus EthoVision XT video tracking software.

2.5 | Slice Preparation and Electrophysiological Recording

Coronal brain slices of the cingulate cortex (400 μm; 20 slices) from adult ECon ($N = 10$ slices; 4 slices from 4 males, 6 slices from 4 females) and EKyn 30 ($N = 10$ slices; 4 slices from 3 males, 6 slices from 3 females) mice were collected as previously described (Rovira and Geijo-Barrientos 2016). Three slices from each animal (one anterior to, one at, and one posterior to the decussation of the anterior commissure) were prepared for extracellular recording using artificial cerebrospinal fluid (aCSF) formulated for slicing, incubation, and recording (Brown and Shepard 2016), with the addition of 15 μM bicuculline methiodide during recording. In a separate group of untreated adult mice (35 slices; 23 from 18 males, 12 from 8 females), we tested the effect of acute bath exposure to KYNA (0, 1, 10, 100, or 1000 nM) on signal propagation. For the 4-Cl-Kyn experiment, slices were collected from ECon ($N = 9$ slices; 5 slices from 3 males, 4 slices from 3 females) and E-4-Cl-Kyn ($N = 18$ slices; 8 slices from 5 males, 10 slices from 6 females) mice, respectively.

A lab-prepared stimulating electrode was lowered into layer 1 of the motor cortex, and an aCSF-filled glass recording electrode (~1.0 MΩ; range 0.6–1.8 MΩ; 1B150–4, WPI; Sarasota, Florida) was lowered 100 μm below the surface of layer 2/3 of the cingulate cortex, 400 μm dorsal to the basal part of the interhemispheric fissure. Voltage recordings of evoked local field potentials (eLFP) were obtained using an Axoclamp 2B amplifier (Molecular Devices; Sunnyvale, California), amplified, band-pass filtered (1 Hz to 1 kHz), digitized at 10 kHz with a Micro 1401 laboratory interface (CED; Cambridge, England), and stored for off-line analysis (Spike 2, CED). All recordings were performed at 30°C unless stated otherwise. Square electrical pulses (100 μs, mean 320 μA, range 200–700 μA, 0.033 Hz (Model 2100 Isolated Pulse Stimulator, A-M Systems; Carlsberg, WA) were delivered 10 times per recording on the ipsilateral and contralateral side with a 5-min rest between recordings on each side. Latency was calculated as the average time from stimulation to eLFP across all 10 pulses.

2.6 | Biochemical Analyses

Offspring of ECon (29 males and 28 females from 14 litters) or EKyn 30 (29 males and 29 females from 16 litters) mice were euthanized by CO_2 inhalation. Plasma was collected from all animals, and the brain was quickly removed to dissect out forebrain (PD 21), cortex (PD 35), and cortex and hippocampus (PD 56–75), respectively. All samples were then stored at −80°C. Special care was taken to ensure across-litter representation in postnatal time points. On the day of the assays, 2–3 same-sex samples were pooled for analysis.

Pregnant ECon, EKyn 30, E-4-Cl-Kyn 5, or E-4-Cl-Kyn 20 mice ($N = 3$ per group) were euthanized by CO_2 inhalation on ED 17/18. The fetal brains were quickly removed and stored at −80°C. On the day of the assays, 3–4 fetal brains per litter were homogenized by sonication in ultrapure water (1:5, w/v) and used for the analysis of KYNA and 7-Cl-KYNA, respectively, as described below.

2.6.1 | KYNA

Plasma was diluted (1:10, v/v), and the brain was homogenized (1:5, w/v) in ultrapure water. Twenty-five microliters of 6% or 25% perchloric acid were added to 100 μL of plasma and brain homogenate, respectively, and precipitated proteins were removed by centrifugation (16,000 x g, 15 min). Twenty

microliters of the supernatant were then applied to a 3 μm ReproSil C18 column (100 mm × 4 mm; Dr. Maisch GmbH, Ammerbuch, Germany). KYNA was isocratically eluted at a flow rate of 0.5 mL/min, using a mobile phase containing 50 mM sodium acetate and 3% acetonitrile (pH adjusted to 6.2 with glacial acetic acid). After post-column derivatization with 500 mM zinc acetate, delivered at a flow rate of 0.1 mL/min, KYNA was determined in the eluate by fluorometric detection (excitation 344 nm, emission 398 nm; Perkin-Elmer series 200; Waltham, Massachusetts). The retention time of KYNA was ~18 min.

2.6.2 | 3-HK

Plasma was diluted (1:2, v/v), and the brain was homogenized (1:5, w/v) in ultrapure water. Twenty-five microliters of 6% perchloric acid were each added to 100 μL of plasma and brain homogenate. Precipitated proteins were removed by centrifugation (16,000×*g*, 15 min). Twenty microliters of the supernatant were then applied to a 3 μm HR80 column (80 × 4.6 mm, Thermo-Fisher Scientific, Waltham, Massachusetts, United States), using a mobile phase consisting of 1.5% acetonitrile, 0.9% triethylamine, 0.59% phosphoric acid, 0.27 mM sodium EDTA, and 8.9 mM heptane sulfonic acid at a flow rate of 0.5 mL/min. In the eluate, 3-HK was detected electrochemically (Eicom HTEC-500; San Diego, California, United States) at an oxidation potential of +0.5 V. The retention time of 3-HK was ~11 min.

2.6.3 | 7-Cl-KYNA

Twenty-five microliters of 25% perchloric acid were added to 100 μL of the original brain homogenate, and precipitated proteins were removed by centrifugation (16,000 x *g*, 15 min). Twenty microliters of the supernatant were then applied to a 3 μm BDS Hypersil C18 column (100 mm × 4.6 mm; Thermo-Fisher Scientific). 7-Cl-KYNA was eluted isocratically at a flow rate of 1 mL/min, using a mobile phase containing 50 mM sodium acetate, 250 mM zinc acetate, and 10% acetonitrile (pH adjusted to 6.2 with glacial acetic acid). 7-Cl-KYNA was determined in the eluate by fluorometric detection (excitation 344 nm, emission 398 nm; Perkin-Elmer series 200). The retention time of 7-Cl-KYNA was ~6 min.

2.6.4 | Protein

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

2.7 | Statistical Analysis

Analyses were performed using Prism 9 (GraphPad, San Diego, California, United States) or SigmaPlot (Systat Software, Inc., Palo Alto, California, United States; SPSS, Chicago, Illinois, United States). Data were visually inspected using Q-Q plots to confirm a relative bell-shaped distribution and the absence of outliers. The type of parametric analysis, post hoc tests, and correction factors used are listed below in each sub-section of the Results.

3 | Results

3.1 | Offspring Number and Body Weight

The number of pups per litter (ECon 6.5 ± 0.3; EKyn 10 6.4 ± 0.6; EKyn 30 6.6 ± 0.4) was not impacted by prenatal treatment across study sites (ANOVA; $F_{2,84} = 0.021$, $p = 0.98$). Evaluation at PD 56 revealed that female mice, across prenatal treatment conditions, had lower body weights than males (two-way ANOVA; sex: $F_{1,81} = 255.2$; $p < 0.0001$). EKyn treatment did not impact body weight in either male (ECon: 24.4 ± 0.3 g, $N = 12$; EKyn 10: 24.2 ± 0.4 g, $N = 7$; EKyn 30: 24.4. ±0.5 g, $N = 8$) or female (ECon: 19.6 ± 0.3 g, $N = 11$; EKyn 10: 19.3 ± 0.3 g, $N = 7$: EKyn 30: 20.1 ± 0.4 g, $N = 8$) mice.

3.2 | Impaired Spatial and Reversal Learning in Adult Male EKyn 30 Mice

The latency to enter the escape box across acquisition training days differed significantly between adult male offspring of ECon and EKyn 30 mice (two-way ANOVA; $F_{1,52} = 4.386$, $p = 0.04$) (Figure 2A). On the third acquisition day, adult EKyn 30 animals (but not EKyn 10 mice; $p = 0.4$) took significantly more time to find the escape box compared to ECon mice (Fisher's LSD, $p < 0.05$). Velocity of movement in the maze did not differ significantly between the experimental groups ($F_{2,71} = 0.2175$, $p = 0.81$), i.e., EKyn treatment had no adverse effect on locomotor activity in adulthood. During reversal learning, male EKyn 30 offspring again took longer to find the escape box (one-way ANOVA; $F_{2,69} = 3.545$, $p = 0.03$; Fisher's LSD, $p < 0.05$) (Figure 2B) and revisited the previous escape box location more frequently than ECon males (one-way ANOVA; $F_{2,69} = 3.805$, $p = 0.03$; Fisher's LSD, $p < 0.05$) (Figure 2C). No significant impairments in reversal trial escape latency were noted in EKyn 10 mice ($p = 0.13$), but the number of revisits was more frequent in EKyn 10 mice compared to controls ($p < 0.05$). As mice learn, the distribution of search strategies normally changes across testing days so that ECon males employed random searching less than 25% by day 3. In contrast, EKyn 30 animals failed to abandon random searching for the escape box, and Chi-squared comparison revealed a significant difference in search strategy distribution compared to ECon mice (C^2 (2) = 7.287, $p = 0.02$) (Figure 2D). Notably, using the same assessments, we did not observe any learning and memory impairments in female EKyn offspring (Figure 2E–H).

3.3 | Abnormal Interhemispheric Signal Propagation in Adult EKyn 30 Mice

Given the significant behavioral impairment seen in adult EKyn 30 (but not in EKyn 10) mice, we next explored whether early kynurenine exposure may result in altered electrophysiological signals, specifically signal propagation, in the brain of these animals (Figure 3). No main effect of sex was observed by initial

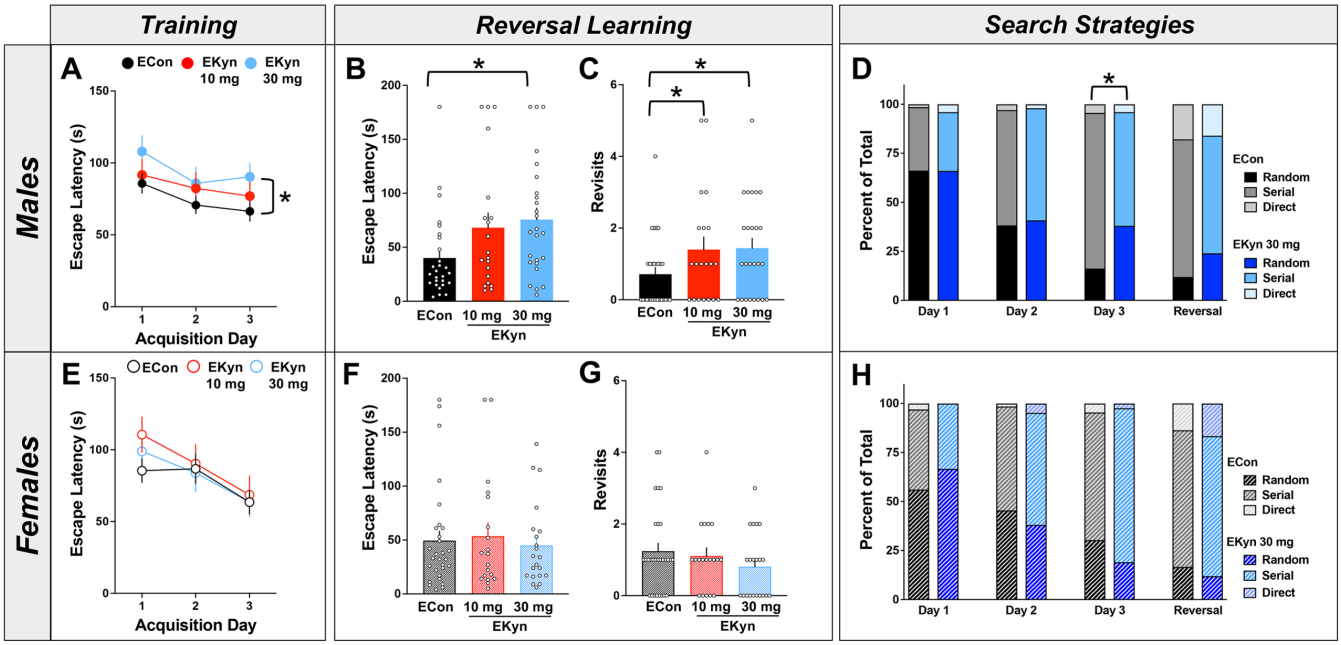

FIGURE 2 | EKyn treatment impairs Barnes maze performance in adult male mice (PD 56–85). Pregnant mice were fed control mash (ECon), kynurenine (10 mg per day—EKyn 10, or 30 mg per day—EKyn 30) during the last week of gestation. Adult male (A–D) and female (E–H) offspring were tested in the Barnes maze. (A,E) Latency to find the escape box during acquisition training. (B,F). Latency during reversal learning. (C,G). Revisits to previous escape box location (D,H). Stacked bar graphs depict the percentage of mice that employed random, serial, or direct search strategies in finding the escape box across testing days. See text for experimental details. Data are the mean ± SEM; $^{*}p < 0.05$.

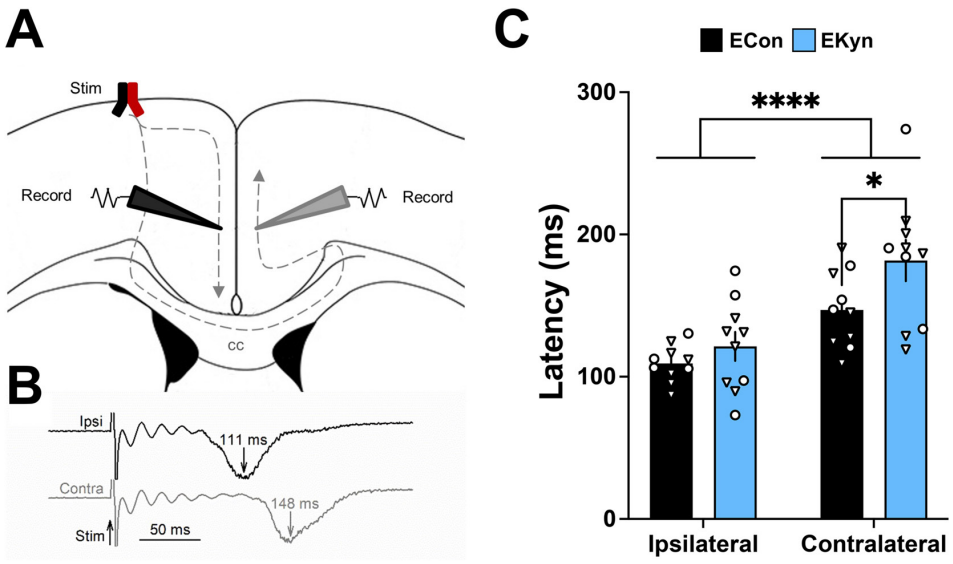

FIGURE 3 | Interhemispheric signal propagation is impaired in adult EKyn mice (PD 56–85). Pregnant mice were fed control mash (ECon) or kynurenine (30 mg per day; EKyn) during the last week of gestation. (A) Schematic of a coronal slice showing the placement of the stimulating electrodes (Stim) and the recording electrodes (Record) for ipsilateral and contralateral eLFP detection. The signal propagates to the contralateral recording site via the corpus callosum (cc). See text for experimental details. (B) The sample traces demonstrate longer contralateral eLFP latency. (C) Quantification of eLFP latency in individual male (circles) and female (triangles) animals. Data are the mean ± SEM; $^{*}p < 0.05$. $^{****}p < 0.0001$. (*Note:* One EKyn contralateral value was high but is included in the analysis as it was exactly 2.0 standard deviations from the mean for that group.)

three-way mixed ANOVA ($F_{1,16} = 0.58$, $p = 0.46$). Comparison of the latency of eLFPs across a major white matter tract, i.e., the corpus callosum, by treatment groups revealed a significant effect of recording location ($F_{1,16} = 84.51$, $p < 0.0001$) and hemisphere x prenatal treatment interaction ($F_{1,16} = 5.173$, $p = 0.037$), but no main effect of prenatal treatment alone ($F_{1,16} = 3.001$, $p = 0.10$). Bonferroni's post hoc analysis showed no latency difference between groups on the ipsilateral side ($p > 0.05$), but a longer eLFP latency on the contralateral side for tissue slices obtained from EKyn animals ($p < 0.05$) (Figure 3C). This suggests that EKyn 30 treatment interferes with white matter integrity, resulting in impaired interhemispheric signal propagation.

To test the potential effect of *acute* KYNA elevation on signal propagation, we performed the same experiments using slices from untreated adult control mice (PD 60–120) using continuous bath application of KYNA at four different concentrations (1, 10, 100, and 1000 nM). Whereas latency was again significantly longer on the contralateral side ($F_{1,30} = 83.27$, $p < 0.001$), KYNA had no effect of its own at any of the concentrations used ($F_{4,30} = 1.76$, $p = 0.16$) irrespective of the recording location ($F_{4,30} = 1.17$, $p = 0.34$). Therefore, the impaired interhemispheric signal propagation seen in EKyn mice cannot be attributed to differences in brain concentrations of KYNA, if any such differences exist. We confirmed that signal propagation can be altered in this assay by physiological parameters, such as temperature, and is dependent upon the presence of bicuculline methiodide and an intact corpus callosum (Figures S1 and S2). Finally, we did not see evidence of reduced myelination in EKyn mice (Figure S3), though this conclusion may be tempered by the limitations of our tissue analysis (see Discussion).

3.4 | Biochemical Effects of EKyn Treatment on the Last Day of Gestation and in Adulthood

On the last day of treatment, KYNA levels in the fetal brain were elevated 3.6-fold in EKyn 30 mice compared to ECon animals (ECon 22.7 ± 1.0 fmoles/mg tissue, $N = 3$; EKyn 30: 81.7 ± 19.9 fmoles/mg tissue, $N = 3$; unpaired t test, $p < 0.05$).

Plasma and forebrain levels of KYNA and 3-HK in ECon and EKyn 30 animals showed no differences between ECon and EKyn 30 offspring on PD 21 (pre-puberty) and PD 35 (adolescence) (Table 1). Similarly, no significant group differences were seen in the levels of KYNA (Figure 4A–C) and 3-HK (Figure 4D–F) in the plasma, cortex, or hippocampus of either male or female mice in adulthood (PD 56–75). However, we noted a main effect of sex in 3-HK levels at PD 35 (Table 1) in both the cortex (two-way ANOVA, $F_{1,27} = 20.55$, $p = 0.0001$) and the hippocampus ($F_{1,27} = 7.068$, $p = 0.01$) at PD 56 (Figure 4E,F).

3.5 | E-4-Cl-Kyn: Biochemical Effects in the Fetal Brain, and no Impairment in Interhemispheric Signal Propagation in Adult Mice

The presence of newly formed 7-Cl-KYNA was verified in the fetal brain on ED 17/18. Treatment with 5 and 20 mg 4-Cl-KYN resulted in 16 ± 6.3 7-Cl-KYNA fmoles/mg tissue and 74.1 ± 15.0 7-Cl-KYNA fmoles/mg tissue, respectively (one-way ANOVA, $F_{2,6} = 17.30$, $p < 0.01$, $N = 3$) (Figure 5C). The higher dosing was selected for the subsequent electrophysiological study.

Offspring from dams treated daily with 20 mg 4-Cl-Kyn during the last week of gestation showed no impairment in adulthood due to diet alone ($F_{1,25} = 0.21$, $p = 0.651$) nor a diet by hemisphere interaction ($F_{1,25} = 3.74$, $p = 0.065$). As expected, contralateral latencies were significantly longer ($F_{1,25} = 36.91$, $p < 0.0001$) (Figure 5D).

4 | Discussion

In contrast to essentially identical experiments in rats, which consistently showed a significant elevation in brain KYNA levels in the adult offspring (Alexander et al. 2013; Pocivavsek et al. 2014; Pershing et al. 2015), kynurenine administration to pregnant mice during the final week of gestation did not affect the levels of KYNA in the brain of the offspring at the time of weaning (PD 21), during adolescence (PD 35), or in adulthood (PD 56). The prenatal kynurenine doses in mice (10 mg or 30 mg per day; i.e., approximately 300 mg/kg/day) were selected to elevate fetal KYNA concentrations to levels comparable to those observed in rats (Pocivavsek et al. 2014). Although fetal brain KYNA levels were increased to about half the levels seen in the rat (Beggiato et al. 2018), adult EKyn mice, like adult EKyn rats (Pocivavsek et al. 2014; Forrest et al. 2015; Buck et al. 2020), nonetheless exhibited distinct functional differences from ECon animals, as assessed in informative behavioral and electrophysiological experiments.

TABLE 1 | EKyn treatment does not affect plasma and forebrain levels of KYNA and 3-HK on PD 21 and PD 35.

		ECon	EKyn 30	ECon	EKyn 30	
Postnatal day (PD) 21		**Males**		**Females**		
KYNA	Plasma (nM)	46 ± 13	44 ± 8	51 ± 5	48 ± 6	
	Forebrain (fmoles/mg protein)	31 ± 3	21 ± 3	27 ± 5	29 ± 5	
3-HK	Plasma (nM)	10 ± 1	7 ± 1	6 ± 1	8 ± 2	
	Forebrain (fmoles/mg protein)	156 ± 27	201 ± 49	227 ± 43	208 ± 15	
Postnatal day (PD) 35						
KYNA	Plasma (nM)	48 ± 5	62 ± 8	56 ± 8	61 ± 11	
	Forebrain (fmoles/mg protein)	26 ± 2	18 ± 1	23 ± 4	22 ± 8	
3-HK	Plasma (nM)	8 ± 1	11 ± 7	9 ± 1	13 ± 3	
	Forebrain (fmoles/mg) protein)	225 ± 21	156 ± 18	138 ± 6	144 ± 25	* **Sex:** $F_{1,11} = 6.652$ 0.03

Note: Pregnant mice were treated with control mash (ECon) or kynurenine (30 mg per day; EKyn 30 mg) during the last week of gestation. See text for experimental details. Data are the mean ± SEM *$p < 0.05$.

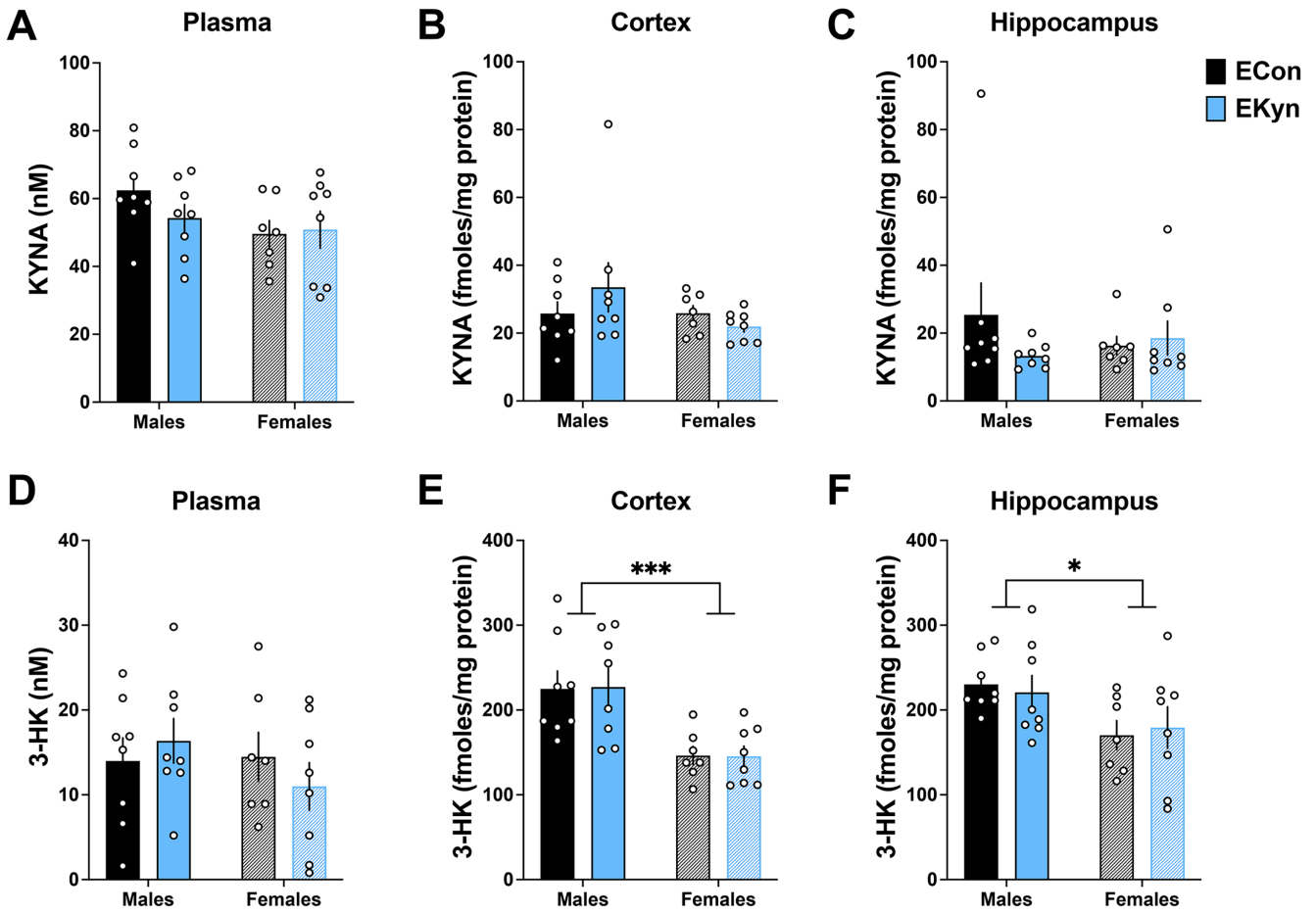

FIGURE 4 | EKyn treatment does not affect the levels of KYNA and 3-HK in plasma, cortex, and hippocampus of adult mice (PD 56–85). Pregnant mice were fed control mash (ECon) or kynurenine (30 mg per day; EKyn) during the last week of gestation. KP metabolites were measured as detailed in the text. Data are the mean ± SEM; $^{*}p < 0.05$; $^{***}p < 0.001$.

The compromised performance in the Barnes maze, a spatial navigation task that evaluates cognitive flexibility by engaging the frontal cortex during reversal learning (Sunyer et al. 2007), was observed preferentially in male EKyn mice. This is reminiscent of the sex-specific impairments in maze navigation (Buck et al. 2020), as well as perturbations in sleep and arousal (Rentschler et al. 2021), seen in adult male EKyn rats. Notably, the resilience of adult female EKyn mice in the behavioral paradigm assessed in the present study is also similar to the long-lasting effects of other perinatal insults in rodents (Andersen and Pouzet 2004; Bath and Pimentel 2017). These findings parallel the well-established sex differences in the onset and severity of SZ (Tamminga 1997; Abel et al. 2010) and, specifically, trends in sex-dependent cognitive dysfunctions in individuals with the disease (Leger and Neill 2016; Mendrek and Mancini-Marïe 2016; Xia et al. 2021). Regarding underlying molecular mechanism(s), all these results are in line with, and support, a significant role of both KP metabolism and gonadal hormones in neurodevelopment and in the (patho)physiology of behavioral features (Arnold 1985; Bale and Epperson 2015; Becker and Koob 2016; Rubinow and Schmidt 2018). However, the greater vulnerability of males to adverse events very early in life (Mueller and Bale 2007, 2008; Brunton and Russell 2010; Carney 2019; Hunter et al. 2019), and especially the apparent species-specific role of KP metabolism in this context (Jayawickrama et al. 2017; Baratta et al. 2018; Bjorke-Monsen et al. 2023), clearly require further detailed investigation.

Our electrophysiological experiments revealed differences in signal transmission across the corpus callosum, a major interhemispheric white matter tract, in both male and female EKyn mice in adulthood. Specifically, we observed a longer latency for the manifestation of evoked potentials contralateral to the stimulation site in these animals, indicating impaired conduction velocity. Since acute, direct exposure of tissue slices to KYNA did not affect eLFP latencies, prenatal stimulation of KP metabolism apparently impaired the development of brain circuits that are involved in signal propagation in the EKyn animals. The most obvious underlying cause are abnormalities in the extent and integrity of myelination, which influence signal speed (Salzer and Zalc 2016). For example, EKyn treatment may delay myelination of the corpus callosum, which normally occurs prior to adulthood in mice (Sturrock 1980). Of interest in view of the hypothetical translational relevance of the EKyn model, persons with SZ show reduced white matter integrity (Kochunov and Hong 2014; Chiappelli et al. 2016), which may be causally related to impaired reaction times in cognitive tasks (Roalf et al. 2015), and increased KYNA levels have been repeatedly documented in brain tissue and CSF of patients diagnosed with SZ (Schwarcz et al. 2001; Nilsson et al. 2005; Miller et al. 2006;

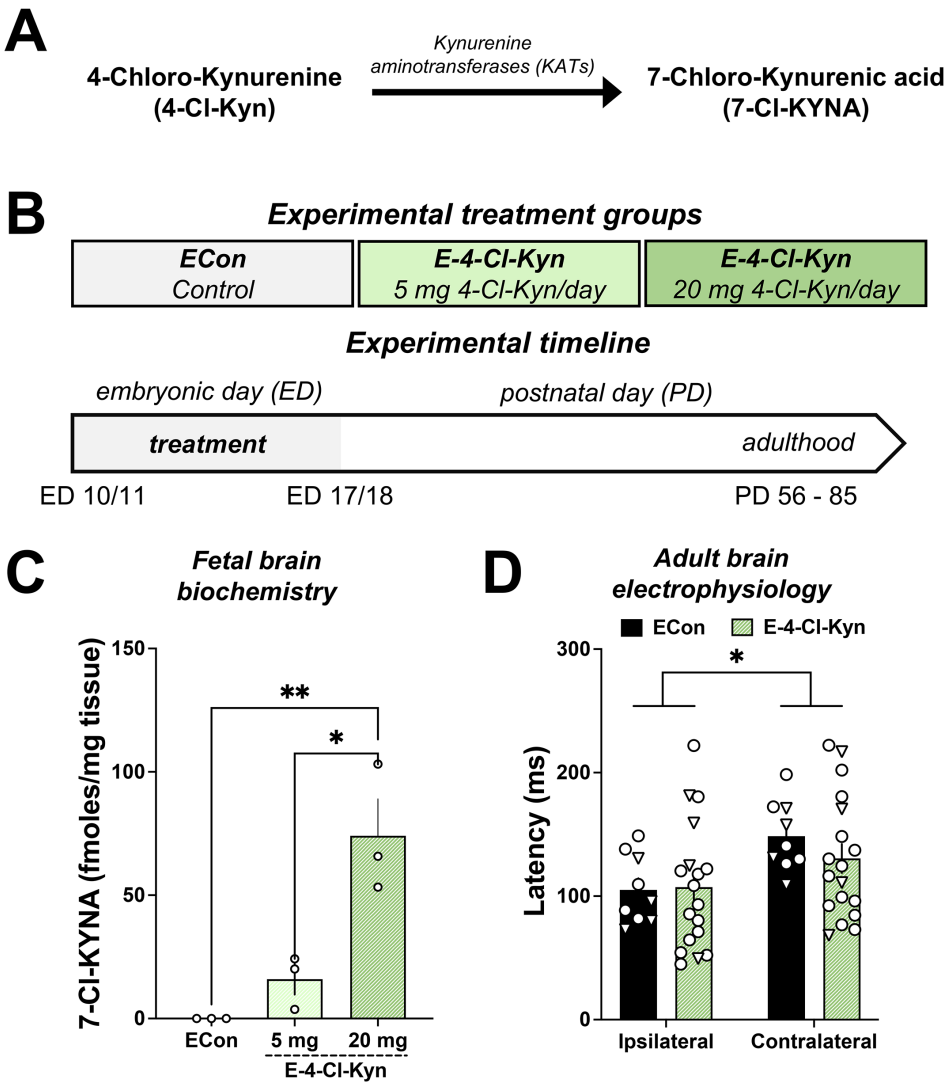

FIGURE 5 | Embryonic 4-chloro-kynurenine (E-4-Cl-Kyn) treatment produces 7-Cl-KYNA in the fetal brain but does not impair interhemispheric signal propagation in adult mice (PD 56–85). (A) Schematic representation of the enzymatic conversion of 4-Cl-Kyn to 7-Cl-KYNA. (B) Experimental design; 4-Cl-Kyn was fed to pregnant dams during the last week of gestation, i.e., ED 10/11 to ED 17/18. (C) 7-Cl-KYNA levels in the brain of E-4-Cl-Kyn mice on ED 17/18. (D) eLFP latency in male (circles) and female (triangles) E-4-Cl-Kyn mice in adulthood (PD 56–85). Data are the mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$.

Linderholm et al. 2010; Sathyasaikumar et al. 2010; Kindler et al. 2020). Abnormal elevation of brain KYNA during the prenatal period may therefore lead to reduced myelination (Maas et al. 2017) in EKyn mice (and rats). Although we found no evidence of reduced corpus callosum myelination (Figure S3), this conclusion is tempered by the limitations of the current study. To perform this histological assay, we made use of the preserved slices used for electrophysiology, which were too old to be cut into the ultra-thin sections normally used for such analysis. In addition, myelin density measures just one aspect of axonal integrity, and it is possible that other alterations in the corpus callosum (e.g., axon diameter, orientation, or density) may be present in the EKyn mice and account for the impaired transmission seen here.

Notably, and of special relevance in light of the established role of NMDA receptors in the fetal brain (Bhutta and Anand 2002; Herlenius and Lagercrantz 2004), our study of E-4-Cl-Kyn mice did not recapitulate the results of the present EKyn experiment, refuting the possibility that the impairments seen in the electrophysiological and behavioral experiments were due solely to prenatal NMDA antagonism. Rather, these results suggest that other molecular targets of KYNA—or possibly of other KP metabolites—account for the initiation of abnormal features that are seen in EKyn animals postnatally (Alves et al. 2024; Stone et al. 2024). It is important to note that, at this time, there is no evidence to indicate that 7-Cl-KYNA (formed in E-4-Cl-KYN mice) might also act on other known KYNA targets (Wang et al. 2006; DiNatale et al. 2010; Pocivavsek et al. 2024). Therefore, additional interpretations ought to be explored in this context.

The present findings should also be considered in the broader context of KP modulation. Previous work has shown that increased conversion of peripheral kynurenine to KYNA in adulthood may confer resilience to depressive-like symptoms

(Agudelo et al. 2014). In contrast, our results emphasize that elevated central KYNA during critical stages of neurodevelopment may entail risks, as reflected in persistent cognitive and electrophysiological abnormalities. This contrast underscores the importance of both the site (peripheral vs. central) and timing (developmental vs. adult) of KP metabolism in determining its effects on brain function. Careful evaluation of these factors will be essential for understanding the balance between neuroprotective and potentially deleterious consequences of KP modulation. Studies currently in progress in our laboratory are therefore designed to assess the effects of high KYNA levels in the prenatal brain of EKyn mice by biochemical and pharmacological methods and to examine subsequent white matter abnormalities in these animals using an array of informative histological approaches.

5 | Conclusion

Taken together, the behavioral and physiological abnormalities in adult EKyn mice, which were observed in the apparent absence of changes in brain KYNA—and also of 3-HK levels, arguing against a shift toward the "neurotoxic" branch of the kynurenine pathway in EKyn animals (see Figure 1)—indicate a critical role of as yet unidentified neurodevelopmental malfunctions following prenatal exposure to high kynurenine levels. In particular, the possible contribution of altered NMDA receptor function to these abnormalities warrants further investigation, given the receptor's known sensitivity to KP metabolites (Pocivavsek et al. 2024). Notably, cerebral KP metabolism in mice and rats may react differently to abnormal perinatal events (Fujigaki et al. 1998; Allegri et al. 2003; Murakami and Saito 2013). Our laboratories are now in the process of elaborating both pre- and postnatal mechanism(s) that are responsible for EKyn-induced biochemical, functional—and possibly structural—irregularities in mice using animals with a genetically-induced impairment in KP metabolism (Giorgini et al. 2013). In view of the remarkable translational relevance of these animals (Giorgini et al. 2013; Tufvesson-Alm et al. 2018), these studies can be expected to provide useful information regarding the role of cerebral KP metabolism in the etiology and pathophysiology of neuropsychiatric illnesses.

Author Contributions

S.B., P.L.B., K.V.S., F.M.N., R.S., and A.P. conceptualized the project. S.B., P.L.B., S.M., M.A.R.T., M.V.P., and K.V.S. performed experiments. R.S. and A.P. obtained funding for the project. All authors participated in data analysis, interpretation, and manuscript preparation.

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Conflicts of Interest

Dr. Schwarcz is a co-founder of Kynexis BV, which develops a KYNA synthesis inhibitor for the treatment of cognitive deficits in persons with schizophrenia. Other authors report no conflicts.

Data Availability Statement

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

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ejn.70185.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.