Published in final edited form as:

Psychopharmacology (Berl). 2016 October ; 233(21-22): 3725–3735. doi:10.1007/s00213-016-4404-9.

Prenatal Kynurenine Exposure Results in Age-Dependent Changes in NMDA Receptor Expression and Conditioned Fear Responding

Michelle L. Pershing¹, David Phenix², Valentina Valentini³, Ana Pocivavsek⁴, Derick H. Lindquist^{1,2}, Robert Schwarcz⁴, and John P. Bruno^{1,2}

¹Department of Psychology, The Ohio State University, Columbus, OH, USA

²Department of Neuroscience, The Ohio State University, Columbus, OH, USA

³Department of Biomedical Sciences, University of Cagliari, Italy

⁴Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA

Abstract

Levels of kynurenic acid (KYNA), an endogenous negative modulator of alpha 7 nicotinic acetylcholine ($\pm 7nACh$) and antagonist at glutamatergic N-methyl-D-aspartate (NMDA) receptors, are elevated in the brain of patients with schizophrenia (SZ). In rats, dietary exposure to KYNA's immediate precursor kynurenine during the last week of gestation produces neurochemical and cognitive deficits in adulthood that resemble those seen in patients with SZ. The present experiments examined whether prenatal kynurenine exposure results in *age-dependent* changes in the kynurenine pathway (KP), expression of selected receptors, and cognitive function. Pregnant dams were fed unadulterated mash (progeny = ECON) or mash containing kynurenine (100 mg/day; progeny = EKYN) from embryonic day (ED) 15 to 22. Male offspring were assessed as juveniles, i.e. prior to puberty (postnatal day [PD] 32), or as adults (PD70) for brain KYNA levels, $\pm 7nACh$ and NMDA receptor gene expression, and performance on a trace fear conditioning (TFC) task. KYNA levels were comparable between juvenile ECON and EKYN rats whereas EKYN adults exhibited a ~3-fold increase in brain KYNA relative to ECONs. NR2A expression was persistently reduced (30–40%) in EKYN rats at both ages. Compared to ECON adults, there was a 50% reduction in NR1, and a trend toward decreased $\pm 7nACh$ expression, in adult EKYN rats. Surprisingly, juvenile EKYN rats performed significantly *better* in the TFC paradigm than controls, whereas adult EKYN animals showed the predicted deficits. Collectively, our results provide evidence that elevated KYNA in the fetal brain alters neuronal development and causes age-dependent effects on neurochemistry and cognitive performance.

FINANCIAL DISCLOSURES

The authors report no financial interests or potential conflicts of interest.

Keywords

alpha7 nicotinic receptors; glutamate; kynurenic acid; N-methyl-D-aspartate receptors; prefrontal cortex; schizophrenia; trace fear conditioning

INTRODUCTION

The levels of kynurenic acid (KYNA), a major metabolite of the kynurenine pathway (KP) of tryptophan degradation, are elevated in cerebrospinal fluid (CSF) and postmortem brain tissue of individuals with schizophrenia (SZ) and may be causally related to the pathophysiology of the disease (Erhardt et al., 2001; Linderholm et al., 2012; Schwarcz et al., 2001). Interest in this hypothesis is related to the fact that KYNA is a negative allosteric modulator of ± 7 nicotinic acetylcholine (± 7 nACh) and, at higher concentrations, a direct antagonist of N-methyl-D-aspartate (NMDA) receptors (Perkins and Stone, 1982, Albuquerque and Schwarcz, 2013), both of which play critical roles in brain development, a defining period in the etiology of SZ (Feinberg, 1982; Jansson and Akerman, 2014; Ohgi et al., 2015), and in several cognitive deficits seen in SZ patients (for reviews see Lewis, 1997; Millan et al., 2012). Moreover, SZ is associated with the genes of two key KP enzymes, tryptophan 2,3-dioxygenase (TDO) and kynurenine 3-monooxygenase (KMO), and functional abnormalities in these enzymes may be responsible for increased KYNA neosynthesis in the brain (Holtze et al., 2012; Miller et al., 2004; Sathyaikumar et al., 2011; Stone and Darlington, 2013; Wonodi et al., 2011).

As shown in the prefrontal cortex (PFC) of adult rats, even relatively modest KYNA increases, by negatively modulating ± 7 nACh and/or NMDA receptor function, reduce the local extracellular levels of several major neurotransmitters known to be critically involved in cognitive processes, including glutamate (Konradsson-Geuken et al., 2010; Pershing et al., 2015; Wu et al., 2010), acetylcholine (Zmarowski et al., 2009), dopamine (Pocivavsek et al., 2016) and GABA (Beggiato et al., 2014). Alone or jointly, and possibly also affected by maturational changes in ± 7 nACh and NMDA receptors (Lin et al., 2014a; Lin et al., 2014b; Stone and Darlington, 2013), these neurochemical effects may also partake in developmental insults that contribute to cognitive deficits seen in people with SZ. Notably, KYNA-induced neurotransmitter changes may also contribute to the structural abnormalities that are seen in the cerebral cortex of patients, including reduced volume (Rimol et al., 2010; Wright et al., 2000), irregular cellular organization (Akbarian et al., 1993; Benes et al., 2001), and diminished dendritic spine density (Glausier and Lewis, 2013).

Guided conceptually by the neurodevelopmental hypothesis of SZ, we demonstrated recently that the feeding of rat dams with KYNA's immediate bioprecursor kynurenine during the final week of gestation leads to elevated brain KYNA, diminished dendritic spine density, reduced expression of the metabotropic glutamate receptor mGluR2, reduced prefrontal glutamate levels following NMDA infusions into the nucleus accumbens, and impaired performance in the Morris water maze and the passive avoidance paradigm, in adult offspring (Pershing et al., 2015; Pocivavsek et al., 2014; do these two refs cover ALL listed effects?), Comparable results were obtained by elevating KYNA levels in the fetal brain

using a KMO inhibitor, which diverts KP metabolism toward increased KYNA formation (Forrest et al., 2013; Forrest et al., 2015). In all these studies, animals were left unaltered from birth until they were tested experimentally, raising questions about the nature and effects of *age-dependent* postnatal changes in animals that were exposed to elevated kynurenine prenatally. These questions are of obvious translational relevance given the typical post-pubertal onset of SZ symptoms in the clinical population and in several animal models (for review see Kinney et al., 2010).

The present study was designed to determine plasma kynurenine and brain KYNA levels, as well as $\pm 7nACh$ and NMDA receptor gene expression, in control (normal mash during embryonic development; “ECON”) and experimental rats (exposed to elevated KYNA as embryos; “EKYN”) at postnatal days (PD) 32 or 70. In separate ECON and EKYN rats at both ages, we studied impairment of executive function, which is thought to be predictive of SZ progression and functional outcome (Green, 1996; Green et al., 2004). To this end, we assessed trace fear conditioning (TFC), a cognitively challenging form of classical (Pavlovian) conditioning that can be readily studied in both juvenile and adult rodents (Barnet and Hunt, 2005; Goodfellow et al., 2016) and that depends upon the integrity of interactions between the hippocampus and the PFC (Raybuck and Gould, 2010).

MATERIALS AND METHODS

Animals

This study was conducted in male Wistar rats derived from in-house breeding. Animals were maintained on a 12:12h light/dark cycle in a temperature- and humidity-controlled, AAALAC-approved animal facility at The Ohio State University, and had *ad libitum* access to food and water. All procedures were approved by the Institutional Animal Care and Use Committee in accordance with the NIH Guide for Care and Use of Laboratory Animals. All necessary measures were taken to minimize pain and discomfort to the animals.

Kynurenine supplementation and treatment groups—Female breeders were habituated to wet rodent mash (Teklad Diets, Madison, WI, USA; 30 g per day) beginning on embryonic day (ED) 0. Dams received 100 mg/day of L-kynurenine sulfate (“kynurenine”, 99.4% purity; Sai Advantium, Hyderabad, India) in the wet mash each day from ED15 to ED22 (EKYN group). Control animals were fed unadulterated mash (ECON group). Standard rodent chow pellets were provided to all animals after birth. The day of birth was denoted postnatal day (PD) 0. On PD2, litters were culled to 9–11 pups to standardize growth rates across all litters and to maximize the number of males. Rats were weaned on PD21, and males were pair-housed by litter. All rats were handled approximately twice per week, until two males per litter were randomly selected for use in behavioral studies. Juvenile (pre-adolescent) rats ranged in age from PD31–34 (designated PD32 hereafter), and adult rats ranged in age from PD65–75 (designated PD70 hereafter).

One or two additional rats from each litter were selected for biochemical assays (plasma kynurenine and brain KYNA levels) and the determination of gene expression at each age. Due to the number of experimental outcome measures (i.e. both biochemical assessments and behavioral testing at two ages), the number of males per litter was not always sufficient

to fully balance littermates across all endpoints. Therefore, additional ECON or EKYN litters were added to biochemistry experiments to yield $n = 8$ litters per condition at PD32 and $n = 8$ –10 litters per condition at PD70, with each litter contributing one or two rats. Thus, biochemical assays were conducted on litters that contributed to behavioral measures (50% of samples) and those that did not (50% of samples), but which were treated identically in all other regards (prenatal treatment, culling, housing conditions, etc.).

Biochemical analyses

Rats (PD32: ECON: $n = 9$, EKYN: $n = 10$; PD70: ECON and EKYN: $n = 10$ each) were anesthetized (CO_2), and blood was collected by cardiac puncture into tubes containing disodium EDTA. After centrifugation ($1,200 \times g$, 10 min), the supernatant plasma was transferred to new Eppendorf tubes. Brains were rapidly removed from the skull, sectioned through the sagittal midline, and placed on ice. Both plasma and tissue samples were then promptly frozen on dry ice and stored at -80°C .

Plasma kynurenine determination—On the day of the assay, plasma samples were thawed and diluted 1:2 in ultrapure water. One hundred μL of diluted plasma were acidified with 25 μL of 6% perchloric acid, and samples were centrifuged ($12,000 \times g$, 10 min). In the resulting supernatant, kynurenine was analyzed by high performance liquid chromatography (HPLC) with fluorimetric detection, as previously described in Pocivavsek et al. (2014).

Kynurenic acid (KYNA) determination in tissue—Tissue was thawed, and the prefrontal cortex was dissected out and homogenized in ultrapure water (1:10 w/v). One hundred μL of homogenate were acidified with 25 μL of 6% perchloric acid, and samples were centrifuged ($12,000 \times g$, 10 min). In the resulting supernatant, KYNA was measured by HPLC with fluorimetric detection (Pocivavsek et al., 2014).

Quantitative polymerase chain reaction (qPCR)—Gene expression was determined for NMDA receptor subunits (NR1, NR2A, NR2B), $\pm 7\text{nACh}$ receptor, and GAPDH. To this end, total RNA was extracted from frontal cortices of PD32 (ECON: $n = 9$; EKYN: $n = 10$) and PD70 (ECON and EKYN: $n = 10$ each) rats using PureZol reagent (Bio-Rad, Hercules, CA, USA) and isolated using a NucleoSpin RNA II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Genomic DNA contamination was eliminated by performing DNase digestion using RNase-free DNase (Macherey-Nagel). cDNA was obtained from the extracted RNA (up to 1 μg /reaction) using iScript reverse transcription Supermix for RT-qPCR (Bio-Rad) under the following conditions: priming at 25°C for 5 min, reverse transcription for 30 min at 42°C , and inactivation of transcriptase by heating at 85°C for 5 min. The first-strand cDNA solution was diluted 5-fold with 0.5X Tris-EDTA buffer (Sigma-Aldrich, MO, USA) prior to qPCR.

qPCR was performed using the CFX96, C1000 Thermal Cycler (Bio-Rad) in a reaction mixture containing 10 μL Sso Advanced SYBR Green Supermix (Bio-Rad), 5 μL of each cDNA sample solution, 15 pmoles of forward and reverse gene-specific primer pairs (Integrated DNA Technologies, Coralville, IA, USA), and DNase-free water in a total volume of 20 μL . The thermal profile for PCR was as follows: initial denaturation at 95°C

for 30 sec followed by 39 temperature cycles of denaturation at 95°C for 5 sec, annealing/extension (62°C for 30 sec for $\pm 7nAChR$), and melting curves. Fluorescence was acquired at the end of each extension phase. Fold changes in mRNA expression levels were calculated according to the comparative cycle threshold (CT) method and normalized using the level of GAPDH mRNA (Schmittgen and Livak, 2008). The primers used for the five genes were: **GAPDH FW**: 5'-CAT CAA GAA GGT GGT GAA GCA-3', RV: 5'-CTG TTG AAG TCA CAG GAG ACA-3' (Rao et al., 2006); **NR1 FW**: 5'-GTT CTT CCG CTC AGG CTT TG-3', RV: 5'-AGG GAA ACG TCC TGC TTC CA-3' (Giza et al., 2006); **NR2A FW**: 5'-AGC CCC CTT CGT CAT CGT-3'; RV: 5'-GAC AGG GCA CCG TGT TCC T-3' (Giza et al., 2006); **NR2B FW**: 5'-AGC TGG TAG CCA TGA AC-3', RV: 5'-GAT CTT CCG GTC AGA CAT-3' (Giza et al., 2006); **Alpha7 FW**: 5'-TGC ACG TGT CCC TGC AAG GC-3, RV: 5'-GTA CAC GGT GAG CGG CTG CG-3' (Thomsen and Mikkelsen, 2012). Primer quality was evaluated by standard curve, melting curve and the presence of a single PCR product after gel electrophoresis.

Behavioral analyses

Conditioning apparatus—All training and testing occurred in standard operant boxes (Coulbourn Instruments, Allentown, PA), enclosed in sound-attenuating chambers. Each operant box had two stainless steel walls, two Plexiglas walls, and a grid floor composed of 0.5 cm stainless steel bars placed approximately 1.5 cm apart. A small animal shock generator (model H13–15; Coulbourn Instruments) and neon grid scrambler connected to the grid floor provided the unconditioned stimulus (US), foot shock.

Footshock reactivity—To ensure that TFC results were not unduly influenced by group differences in sensory perception, foot shock reactivity was assessed in juvenile (PD32) and adult (PD70) ECON and EKYN rats ($n = 3$ per condition per age). Each rat was placed into the conditioning chamber and allowed to explore for 120 sec. An ascending series of 10 foot shocks (0.1 to 1.0 mA) was then administered in 0.1 mA increments with a 30 ± 5 sec intertrial interval. Rat behavior was recorded using a Panasonic HC-V720 high definition video camera, and responses were scored off-line by observers blind to the treatment condition. The following criteria, adapted from Nielsen and Crnic (2002), were used: 0 = No Response (no reaction to shock); 1 = Flinch/Rear (jerky movement; shift in body posture; attentional orienting; but with two paws remaining on floor); 2 = Hop/Run (forward or backward movement, less than half the length of the testing chamber or small vertical movement less than body height); and 3 = Jump (horizontal motion greater than half of the chamber or vertical motion greater than body height with all four paws leaving the floor in a springing motion). The shock intensity at which rat vocalizations occurred was also noted for each subject. Shock reactivity was determined by calculating the mean shock intensity at which each response occurred.

Trace fear conditioning—TFC and retention testing were conducted over 3 sessions, separated by ~24 h, beginning at PD32 or PD70 (ECON: $n = 10$; EKYN: $n = 12$, at each age). The conditioned stimulus (CS) was a 15 sec, 2.8 kHz, 80 dB tone presented through a speaker located at the top of each chamber. The US was a 1.0 sec, 1.0 mA scrambled foot shock. TFC consisted of 10 CS-US trials. For each trial, the 15 sec tone CS was followed 10

sec later (the trace interval) by the 1.0 sec footshock US, resulting in a 25 sec interstimulus interval (ISI) from CS onset to US onset. The intertrial interval (ITI) was 240 ± 30 sec. Prior to placing each subject inside, each chamber was wiped with a water/vinegar (5:1) solution. Approximately 4 min after the last trial, rats were removed from the chamber and transported to their home cages.

Approximately 24 h later, rats were returned to the conditioning chamber to measure conditioned fear (i.e. freezing behavior) in the absence of the CS and the US. After a 120 sec baseline, freezing was measured for 10 min (in 2 min bins). Forty-eight hours after conditioning, CS-evoked freezing was assessed independent of the conditioning context—i.e., rats were placed in a novel context located in a dark room lit with one red overhead light. The chamber was scented with Windex®, the grid bars were covered with gray opaque Plexiglas, a pink geometric shape was attached to the front door, a small magnet was placed on one inside metal wall, and a small fan was turned on, providing 60 dB white ambient noise. Unconditioned (generalized) fear to the novel context (i.e., freezing) was measured during the 1 min immediately preceding the first CS-alone trial. Rats were presented a total of 10 CS-alone trials (180 ± 30 sec ITI) and freezing was measured during the 15 sec tone CS and the 10 sec trace interval.

Freezing analysis—Freezing was defined as cessation of all movement except that required for respiration (Fanselow, 1980) and was expressed as the percentage of the recording interval that rats were engaged in freezing. Freezing behavior was recorded with a video camera (Model WDSR-2005SC; Circuit Specialists, Inc., Mesa, AZ) mounted to the top (ceiling) of the conditioning chamber. The interior was illuminated by an infrared light source to observe freezing behavior. The video signal was ported to FreezeScan (CleverSys, Inc., Reston, VA), a video-based tool that detects and quantifies freezing behavior within experimenter-defined time bins.

Statistical analyses—With the exception of body weight, which was expressed as the litter average, all data were calculated using individual subjects as the experimental unit. Body weights, plasma kynurenine, brain KYNA, gene expression, and freezing data were analyzed using single-factor, multi-factorial or repeated-measures ANOVAs. The Huynh-Feldt correction was utilized to reduce Type II errors associated with repeated measures ANOVAs (Vasey and Thayer, 1987). Significant main effects or interactions were followed by a minimum number of t-tests to determine between-group effects. Data analyses were performed using the SPSS statistics program (V22, IBM corporation, Armonk, NY), and significance was defined as $P < 0.05$.

RESULTS

Growth Rate of ECON and EKYN Pups

There were no apparent differences in maternal care (nest-building, nursing posture, pup retrieval) between ECONs and EKYNs (data not shown). Offspring were weighed weekly from PD7 through PD65. Average litter weights were calculated separately for ECONs ($n = 16$ litters) and EKYNs ($n = 17$ litters). Prenatal treatment with kynurenine did not affect body weight disproportionately in the offspring at any age tested, i.e. there were no

significant differences in growth rate between ECON and EKYN rats during the pre-weaning (PD7-21) or post-weaning (PD28-65) periods ($P = 0.242$; repeated measures analysis) (data not shown).

Plasma kynurenine and brain KYNA

The long-term effects of prenatal exposure to elevated kynurenine on subsequent KP metabolism was assessed by measuring plasma kynurenine and brain KYNA levels in juvenile (PD32) and adult (PD70) offspring (Figure 1). Two-way ANOVA (age $\times$ prenatal condition) indicated that plasma kynurenine levels were similar between ECON and EKYN juvenile rats ($2.6 \pm 0.2 \mu\text{M}$ and $2.5 \pm 0.2 \mu\text{M}$, respectively, $P = 0.70$) and ECON and EKYN adult rats ($1.7 \pm 0.2 \mu\text{M}$ and $1.9 \pm 0.2 \mu\text{M}$, respectively; $P = 0.5$). In contrast, there was a highly significant effect of age on brain KYNA levels in ECON and EKYN rats ($F_{1,35} = 8.143$, $P = 0.007$). Brain KYNA levels were similar in juvenile ECON and EKYN rats (181.4 ± 51.0 and $149.8 \pm 35.3 \text{ fmol/mg protein}$, respectively; $t_{17} = 0.536$, $P = 0.599$). In contrast, brain KYNA levels in adult EKYN rats ($443.2 \pm 108.7 \text{ fmol/mg protein}$) were 293% higher than in adult ECON rats ($112.8 \pm 17.3 \text{ fmol/mg protein}$), resulting in a significant group difference ($t_{18} = -3.003$, $P = 0.008$).

Gene expression: NMDA and $\pm 7\text{nACh}$ receptors

Several genes encoding receptors which are implicated in the etiology of SZ, are antagonized by KYNA and play crucial roles in the expression of TFC, were analyzed by qPCR at PD32 and PD70 (NR1, NR2A, NR2B and $\pm 7\text{nAChR}$) (Figure 2A). Compared to age-matched ECONs, there was a 29% reduction in NR2A in PD32 EKYN rats ($t_{17} = 2.216$, $P = 0.041$). This decrease in EKYNs persisted through adulthood (-38% compared to ECONs; $t_{18} = 2.314$, $P = 0.033$). At PD70, but not at PD32, there was also a significant 50% reduction in NR1 expression in EKYN rats compared to ECONs ($t_{18} = 2.550$, $P = 0.020$). No group differences were noted for the NR2B subunit or for $\pm 7\text{nAChRs}$ in either juvenile (PD32) rats or adult (PD70) rats. Of possible interest, and in line with our previous observation (Persing et al., 2015), adult EKYN rats showed a modest, yet non-significant trend toward reduced expression of $\pm 7\text{nAChRs}$ compared to adult ECONs (-17% ; $t_{18} = 1.153$, $P = 0.264$). Moreover, as illustrated in Figure 2B, the ratio of NR2A/NR2B gene expression, which was comparable at PD32 and PD70 in control rats ($P = 0.76$), was slightly decreased (reflecting an increase in NR2B relative to NR2A) in EKYN rats compared to ECONs at both ages by 29% and 39%, respectively ($P = 0.06$ and $P = 0.07$, respectively).

Foot shock sensitivity

Table 1 summarizes a descriptive analysis of the mean foot shock intensity (mA) at which various behaviors (flinch, hop/run, jump, and/or vocalizing) emerged in ECON and EKYN rats at both ages. With the exception of one ECON and one EKYN rat at PD70, rats were insensitive to the lowest shock intensity. While the sample size was relatively small ($n = 3/\text{group}$), the results were extremely similar across the two treatment groups and two ages. Thus, shock reactivity scores increased across groups in almost an identical fashion as a function of shock intensity. Vocalizations were noted at shock intensities of 0.4 mA and above. These similarities suggest that differences in freezing behavior, described below, cannot be attributed to differences in the salience of the foot shock stimulus.

Trace fear conditioning

TFC was performed to examine the effects of prenatal exposure to kynurenine on the acquisition and expression of conditioned fear in adolescence and adulthood. At either age, freezing behavior during TFC acquisition did not differ between treatment groups (data not shown). Juveniles and adults of both groups also showed similar levels of freezing throughout the 10 min context test (ranging from $34 \pm 3\%$ to $41 \pm 3\%$ freezing across ages and conditions) (Figure 3A). There were no significant effects of prenatal condition ($F_{1,40} = 0.742$, $P = 0.394$), age ($F_{1,40} = 0.009$, $P = 0.926$), or age $\times$ condition interaction ($F_{1,40} = 0.941$, $P = 0.338$). Despite the absence of a significant interaction, we noticed an apparent difference in the context test between PD70 ECON and EKYN rats. A post-hoc comparison revealed modest but significant deficits in PD70 EKYN rats ($t_4 = 3.897$, $P = 0.017$), and this effect is consistent with previously reported findings (see Discussion).

All animals were subsequently presented with 10 CS-alone test trials in a novel context. Due to equipment malfunction, CS-alone freezing data were lost for two PD32 EKYN rats. As illustrated in Figure 3B, 2×2 ANOVAs showed that prenatal kynurenine treatment produced age-dependent alterations in freezing to the tone CS ($F_{1,38} = 13.664$, $P = 0.001$) and trace interval ($F_{1,38} = 9.908$, $P = 0.003$). ECON and EKYN rats were subsequently compared within each age, revealing that PD32 EKYN rats froze significantly more than ECON rats during the CS ($66 \pm 4\%$ vs $54 \pm 3\%$; $t_{18} = -2.872$, $P = 0.010$) and trace interval ($69 \pm 4\%$ vs $56\% \pm 4\%$; $t_{18} = -2.349$, $P = 0.030$). In contrast, PD70 EKYN rats froze significantly less than ECONs during the CS ($58 \pm 3\%$ vs. $72 \pm 5\%$; $t_{20} = 6.259$, $P = 0.021$) and trace interval ($57 \pm 4\%$ vs $69 \pm 4\%$; $t_{20} = 4.414$, $P = 0.049$). This effect was not due to differences in baseline freezing in the novel context, as all rats froze at roughly the same rate during the pre-tone period ($P = 0.228$).

DISCUSSION

We demonstrated previously that the administration of KYNA's bioprecursor, kynurenine, to pregnant Wistar rats during the last week of gestation results in elevated brain KYNA and deficits in prefrontal- and hippocampal-mediated tasks in adulthood (Persching et al., 2015; Pocivavsek et al., 2014). The present study was designed to expand on this work, and that of others (Forrest et al., 2015; Stone and Darlington, 2013), by investigating whether prenatal exposure to kynurenine (from ED15-22) causes (i) *age-related* long-term changes in the gene expression of selected glutamatergic and nicotinic receptors, and/or (ii) cognitive dysfunction. To that end, male ECON and EKYN rats were assessed as juveniles (PD32) or adults (PD70). We made several new observations. First, while we confirmed that prenatal kynurenine exposure causes a significant elevation in cerebral KYNA levels in adult rats (Persching et al., 2015), no such effect was seen in juvenile animals. This indicates post-pubertal changes in cerebral kynurenine pathway metabolism in EKYNs. Second, NR1 mRNA expression was substantially reduced (~50%) in adult but not in juvenile EKYN rats. Third, NR2A mRNA expression was reduced by ~30% in the brain of both juvenile and adult EKYN rats, leading to a lower NR2A/NR2B ratio at both ages. Finally, TFC test performance was diminished in adult EKYN rats but unexpectedly improved in juvenile EKYNs. The relevance of these findings, including the utility of prenatal kynurenine

exposure as a translationally valid experimental approach for studying cognitive deficits in SZ and other major psychiatric diseases, is discussed below.

KYNA, an astrocyte-derived metabolite of the KP of tryptophan degradation, has been repeatedly postulated to contribute to the pathophysiology of SZ (for reviews see Erhardt et al., 2009; Hashimoto, 2014; Schwarcz et al., 2012). This hypothesis is not only based on the observation that KYNA levels are increased in the brain and the CSF of patients with SZ (Erhardt et al., 2001; Linderholm et al., 2012; Schwarcz et al., 2001) but also on the fact that KYNA is an antagonist of NMDA and $\pm 7nACh$ receptors, which are both critically involved in brain development and cognitive processes (see Introduction). Notably, the pathogenesis of SZ is related to genetics as well as environmental influences early in life. Genes encoding the KP enzymes tryptophan 2,3-dioxygenase (TDO) (Holtze et al., 2012; Miller et al., 2004) and kynurenine 3-monooxygenase (KMO) (Aoyama et al., 2006; Wonodi et al., 2011) are abnormal in SZ patients, KMO activity is reduced in the brain of people with SZ (Sathyaikumar et al., 2011; Wonodi et al., 2011), and known risk factors such as maternal infections and stress acutely stimulate cerebral KP metabolism in the fetus (reviewed in Notarangelo and Pocivavsek, 2016). Experimentally induced elevations of KYNA in the fetal brain may therefore duplicate the effects of one or more of these etiological factors.

Using an identical prenatal kynurenine treatment paradigm as in the present study, we demonstrated previously that brain KYNA levels were substantially elevated on ED22 yet returned to normal levels in pups at PD2, i.e. shortly after kynurenine was removed from the dam's diet (Pershing et al., 2015; Pocivavsek et al., 2014). However, although all experimental animals received a regular diet after birth, adult EKYN rats had significantly higher cerebral KYNA levels than age-matched ECONs. We confirmed here that brain levels of KYNA were increased in adult EKYNs but did not find differences between EKYNs and ECONs at PD32. The fact that kynurenine levels in the blood, which can readily influence cerebral KYNA formation (Gál and Sherman, 1978), was unchanged in EKYN rats at PD70 suggests that the enhanced production of KYNA in adult EKYNs was caused by slowly developing alterations in KP metabolism *within the brain*, which do not become effective until after puberty. Underlying mechanisms may involve hormonal changes, which are known to affect KP dynamics (McGinty and Rose, 1969), perhaps leading to a secondary reduction in KMO activity and thus shunting the pathway towards enhanced KYNA formation (Abdel-Tawab et al., 1975; Danesch et al., 1987; Danesch et al., 1983; Gibney et al., 2014).

Both juvenile and adult rats underwent TFC, in which a 15 sec tone CS was followed by an aversive footshock US 10 sec later (the trace interval). Compared to age-matched ECONs, TFC retention deficits were seen in adult, but not in juvenile, EKYN rats. Freezing behavior after re-exposure to the training context was significantly impaired in adult EKYN rats, in line with prior studies that used early-life kynurenine treatment. For example, Bucci and colleagues showed that acute KYNA elevations during early postnatal development resulted in impaired context fear conditioning in adult rats (Iaccarino et al., 2013). Notably, these results are generally consistent with the human SZ literature, which describes a variety of context-mediated impairments, including context fear conditioning (Brebion et al., 2007; Hemsley, 2005; Waters et al., 2004).

Based on freezing behavior in response to the tone CS and trace interval, treatment group differences were also seen during CS-alone test trials. Relative to ECON controls, EKYN rats froze significantly less as adults and significantly more as juveniles, i.e. treatment interacted with age at the time of testing. This age-treatment interaction, which may be informative with respect to the etiology of neuropsychiatric diseases, is probably caused by abnormal maturational changes in EKYN rats. Specifically, TFC deficits in *adult* EKYNs could be causally related to the substantial, approximately 3-fold, elevation in brain KYNA levels in these animals compared to ECONs. This increase may be sufficient to reduce the release of both glutamate (Konradsson-Geuken et al., 2009; Konradsson-Geuken et al., 2010; Wu et al., 2010) and acetylcholine (Zmarowski et al., 2009) in the PFC, especially in response to mesolimbic stimulation (Persching et al., 2015), and thus to antagonize NMDAR and ± 7 nAChR function (Erhardt et al., 2009). Adult EKYN rats also showed an approximately 50% reduction in the cortical expression of the NR1 subunit, which is ubiquitously expressed in all NMDA receptors and contains the glycine B site that recognizes KYNA as a ligand (Kessler et al., 1989), and a trend (–17%) towards a reduction in the expression of ± 7 nAChRs, another physiological target of KYNA (Albuquerque and Schwarcz, 2013). Adult EKYN rats also display a significant reduction in dendritic spines on prefrontal pyramidal neurons, which mediate excitatory cortical input (Persching et al., 2015). Collectively, these effects indicate that glutamatergic and cholinergic neurotransmission in the PFC is markedly compromised in adult EKYN animals. TFC depends on the prefrontal cortex and working memory in order to sustain the CS neural signal across the trace interval and become associated with the US (Gilmartin et al., 2014; Gilmartin and McEchron, 2005). It is plausible CS-evoked neuronal spiking cannot be effectively maintained during the trace interval in adult EKYN rats, resulting in a weaker CS-US fear memory and significantly diminished freezing during the CS-alone test trials.

In contrast to adult EKYNs, *juvenile* EKYN rats did not show elevated brain KYNA levels or a reduction in NR1 gene expression compared to age-matched controls. This may account for the lack of impairment in the TFC test. In fact, the juvenile EKYN rats unexpectedly performed *better* than juvenile ECON rats, i.e. they froze more when tested during the tone CS and trace interval (cf. Figure 3). Improved TFC test performance by juvenile EKYN rats may be causally related to an altered NR2A/NR2B expression ratio, independent of changes in KYNA and NR1 expression. Synaptic plasticity is influenced by many factors, including the NR2A/NR2B ratio (Shouval et al., 2002; Yashiro and Philpot, 2008), such that a decrease in the ratio enhances the induction and maintenance of long-term potentiation (Cui et al., 2011; Xu et al., 2009). Relative to NR2A, NR2B-containing NMDA receptors gate more calcium due to longer channel open times (Erreger et al., 2005), prolonging the slow reverberating neural dynamics that are required for sustained spiking (following stimulus termination) and working memory (Wang et al., 2008; Wang et al., 2013). Infusion of a NR2B antagonist (Ro25-6981) into the medial PFC of rats blocks TFC but not, importantly, context fear conditioning or delay fear conditioning, in which the CS and US overlap in time (Gilmartin et al., 2013). Inasmuch, the decrease in NR2A expression seen in juvenile EKYN rats – producing a proportional increase in NR2B – could, during TFC, maintain NR2B-dependent spiking following CS offset more reliably than in juvenile ECONs. This would result in stronger CS-US fear memory and, at the time of testing, significantly enhanced

freezing throughout the tone and trace interval. Provisional support for this idea comes from Forrest et al. (2013), who observed enhanced long-term potentiation on PD21 in the offspring of dams that were repeatedly treated with a KMO inhibitor during the last week of gestation.

Ongoing studies in our laboratories are designed to further define the irregular maturational changes, which account for the emergence of cognitive deficits in adult EKYN animals following an adolescent period of PFC remodeling, i.e. from about PD35 to PD45 (Flores-Barrera et al., 2014). Elaboration of these abnormal developmental mechanisms will be critical for understanding – and eventually interfering with – enhanced feed-forward inhibition of PFC synapses in adult EKYN rats, which is proposed to impede CS-evoked spiking across the trace interval, yielding a weakened CS-US associative memory and impaired TFC test performance.

In summary, the current study revealed that prenatal kynurenine exposure results in distinct pre- and post-pubertal changes in KYNA levels, NMDAR gene expression, and cognitive function. In light of the crucial role of developmental processes in the etiology of SZ and other major neuropsychiatric disorders (Davis et al., 2016; O'Donnell, 2011; Selemon and Zecevic, 2015), the present demonstration of distinct age-dependent differences in TFC following prenatal kynurenine exposure is of substantial translational interest. Together with complementary recent studies from other laboratories (Chess et al., 2009; DeAngeli et al., 2014; Erhardt et al., 2009; Iaccarino et al., 2013; Kegel et al., 2014; Stone and Darlington, 2013), our findings provide further stimulus for examining KP impairments before and during adolescence, and for studying their role in the emergence of cognitive dysfunctions in adulthood. Investigations currently in progress utilize a variety of pharmacological approaches to prevent cognitive deterioration in EKYN rats by appropriately timed interventions with agents that specifically interfere with KYNA neosynthesis or function (Notarangelo and Pocivavsek, 2016; Wu et al., 2014).

Acknowledgments

This study was supported by NIMH grants MH083729 (to JPB and RS) and P50103222 (to RS). AP is supported by NIH grant K12 HD43489-14.

Abbreviations

±7nACh	alpha 7 nicotinic acetylcholine
CS	conditioned stimulus
CSF	cerebrospinal fluid
ECON	embryonic control treatment
ED	embryonic day
EKYN	embryonic kynurenine treatment
KP	kynurenine pathway

KYNA	kynurenic acid
NMDA	N-methyl-D-aspartate
PD	postnatal day
PFC	prefrontal cortex
SZ	schizophrenia
TFC	trace fear conditioning
UCS	unconditioned stimulus

References

- Abdel-Tawab GA, El-Zoghby SM, Saad AA. Relationship between pyridoxal phosphate and some synthetic oestrogens, gonadotropin and thyroxine in their effects on kynurenine hydrolase and kynurenine aminotransferase enzymes of normal mouse liver. *Acta Vitaminologica et Enzymologica*. 1975; 29:326–331. [PubMed: 1244117]
- Akbadian S, Bunney WE Jr, Potkin SG, Wigal SB, Hagman JO, Sandman CA, Jones EG. Altered distribution of nicotinamide-adenine dinucleotide phosphate-diaphorase cells in frontal lobe of schizophrenics implies disturbances of cortical development. *Archives of General Psychiatry*. 1993; 50:169–177. [PubMed: 7679891]
- Albuquerque EX, Schwarcz R. Kynurenic acid as an antagonist of alpha7 nicotinic acetylcholine receptors in the brain: Facts and challenges. *Biochemical Pharmacology*. 2013; 85:1027–1032. [PubMed: 23270993]
- Aoyama N, Takahashi N, Saito S, Maeno N, Ishihara R, Ji X, Miura H, Ikeda M, Suzuki T, Kitajima T, et al. Association study between kynurenine 3-monooxygenase gene and schizophrenia in the Japanese population. *Genes Brain Behavior*. 2006; 5:364–368.
- Barnet RC, Hunt PS. Trace and long-delay fear conditioning in the developing rat. *Learning and Behavior*. 2005; 33:437–443. [PubMed: 16573214]
- Beggiato S, Tanganelli S, Fuxe K, Antonelli T, Schwarcz R, Ferraro L. Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. *Neuropharmacology*. 2014; 82:11–18. [PubMed: 24607890]
- Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. *Biological Psychiatry*. 2001; 50:395–406. [PubMed: 11566156]
- Brebion G, David AS, Jones HM, Ohlsen R, Pilowsky LS. Temporal context discrimination in patients with schizophrenia: associations with auditory hallucinations and negative symptoms. *Neuropsychologia*. 2007; 45:817–823. [PubMed: 16996090]
- Chess AC, Landers AM, Bucci DJ. L-kynurenine treatment alters contextual fear conditioning and context discrimination but not cue-specific fear conditioning. *Behavioural Brain Research*. 2009; 201:325–331. [PubMed: 19428652]
- Cui Y, Jin J, Zhang X, Xu H, Yang L, Du D, Zeng Q, Tsien JZ, Yu H, Cao X. Forebrain NR2B overexpression facilitating the prefrontal cortex long-term potentiation and enhancing working memory function in mice. *PLoS One*. 2011:e20312. [PubMed: 21655294]
- Danesch U, Gloss B, Schmid W, Schutz G, Schule R, Renkawitz R. Glucocorticoid induction of the rat tryptophan oxygenase gene is mediated by two widely separated glucocorticoid-responsive elements. *EMBO Journal*. 1987; 6:625–630. [PubMed: 3582368]
- Danesch U, Hashimoto S, Renkawitz R, Schutz G. Transcriptional regulation of the tryptophan oxygenase gene in rat liver by glucocorticoids. *The Journal of Biological Chemistry*. 1983; 258:4750–4753. [PubMed: 6187742]

- Davis J, Eyre H, Jacka FN, Dodd S, Dean O, McEwen S, Debnath M, McGrath J, Maes M, Amminger P, et al. A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis. *Neuroscience and Biobehavioral Reviews*. 2016; 65:185–194. [PubMed: 27073049]
- DeAngeli NE, Todd TP, Chang SE, Yeh HH, Yeh PW, Bucci DJ. Exposure to Kynurenic Acid during Adolescence Increases Sign-Tracking and Impairs Long-Term Potentiation in Adulthood. *Frontiers in Behavioral Neuroscience*. 2014; 8:451. [PubMed: 25610382]
- Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. *Neuroscience Letters*. 2001; 313:96–98. [PubMed: 11684348]
- Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenic acid: potential in the treatment of psychiatric disorders. *CNS Drugs*. 2009; 23:91–101. [PubMed: 19173370]
- Erreger K, Dravid SM, Banke TG, Wyllie DJ, Traynelis SF. Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. *Journal of Physiology*. 2005; 563:345–358. [PubMed: 15649985]
- Fanselow MS. Conditional and unconditional components of post-shock freezing. *Pavlovian Journal of Biological Science*. 1980:177–182. [PubMed: 7208128]
- Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research*. 1982; 17:319–334. [PubMed: 7187776]
- Flores-Barrera E, Thomases DR, Heng LJ, Cass DK, Caballero A, Tseng KY. Late adolescent expression of GluN2B transmission in the prefrontal cortex is input-specific and requires postsynaptic protein kinase A and D1 dopamine receptor signaling. *Biological Psychiatry*. 2014; 75:508–516. [PubMed: 24041503]
- Forrest CM, Khalil OS, Pisar M, Darlington LG, Stone TW. Prenatal inhibition of the tryptophan-kynurenine pathway alters synaptic plasticity and protein expression in the rat hippocampus. *Brain Research*. 2013; 1504:1–15. [PubMed: 23353758]
- Forrest CM, McNair K, Pisar M, Khalil OS, Darlington LG, Stone TW. Altered hippocampal plasticity by prenatal kynurenine administration, kynurenine-3-monooxygenase (KMO) deletion or galantamine. *Neuroscience*. 2015; 310:91–105. [PubMed: 26365611]
- Gál EM, Sherman AD. Synthesis and metabolism of L-kynurenine in rat brain. *Journal of Neurochemistry*. 1978; 30:607–613. [PubMed: 681935]
- Gibney SM, Fagan EM, Waldron AM, O'Byrne J, Connor TJ, Harkin A. Inhibition of stress-induced hepatic tryptophan 2,3-dioxygenase exhibits antidepressant activity in an animal model of depressive behaviour. *The International Journal of Neuropsychopharmacology*. 2014; 17:917–928. [PubMed: 24472498]
- Gilmartin MR, Balderston NL, Helmstetter FJ. Prefrontal cortical regulation of fear learning. *Trends in Neuroscience*. 2014; 37:455–464.
- Gilmartin MR, Kwapis JL, Helmstetter FJ. NR2A- and NR2B-containing NMDA receptors in the prelimbic medial prefrontal cortex differentially mediate trace, delay, and contextual fear conditioning. *Learning & Memory*. 2013; 20:290–294. [PubMed: 23676200]
- Gilmartin MR, McEchron MD. Single neurons in the medial prefrontal cortex of the rat exhibit tonic and phasic coding during trace fear conditioning. *Behavioral Neuroscience*. 2005; 119:1496–1510. [PubMed: 16420154]
- Giza CC, Maria NS, Hovda DA. N-methyl-D-aspartate receptor subunit changes after traumatic injury to the developing brain. *Journal of Neurotrauma*. 2006; 23:950–961. [PubMed: 16774479]
- Glausier JR, Lewis DA. Dendritic spine pathology in schizophrenia. *Neuroscience*. 2013; 251:90–107. [PubMed: 22546337]
- Goodfellow MJ, Abdulla KA, Lindquist DH. Neonatal ethanol exposure impairs trace fear conditioning and alters NMDA receptor subunit expression in adult male and female rats. *Alcoholism, Clinical and Experimental Research*. 2016; 40:309–318.
- Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? *The American Journal of Psychiatry*. 1996; 153:321–330. [PubMed: 8610818]
- Green MF, Kern RS, Heaton RK. Longitudinal studies of cognition and functional outcome in schizophrenia: implications for MATRICS. *Schizophrenia Research*. 2004; 72:41–51.

- Hashimoto K. Targeting of NMDA receptors in new treatments for schizophrenia. *Expert Opinion on Therapeutic Targets*. 2014; 18:1049–1063. [PubMed: 24965576]
- Hemsley DR. The schizophrenic experience: taken out of context? *Schizophrenia Bulletin*. 2005; 31:43–53. [PubMed: 15888424]
- Holtze M, Saetre P, Engberg G, Schwieler L, Werge T, Andreassen OA, Hall H, Terenius L, Agartz I, Jönsson EG, Schalling M, Erhardt S. Kynurenine 3-monooxygenase polymorphisms: relevance for kynurenic acid synthesis in patients with schizophrenia and healthy controls. *J Psychiatry Neurosci*. 2012 Jan; 37(1):53–7. [PubMed: 21693093]
- Iaccarino HF, Suckow RF, Xie S, Bucci DJ. The effect of transient increases in kynurenic acid and quinolinic acid levels early in life on behavior in adulthood: Implications for schizophrenia. *Schizophrenia Research*. 2013; 150:392–397.
- Jansson LC, Akerman KE. The role of glutamate and its receptors in the proliferation, migration, differentiation and survival of neural progenitor cells. *Journal of Neural Transmission*. 2014; 121:819–836. [PubMed: 24562403]
- Kegel ME, Bhat M, Skogh E, Samuelsson M, Lundberg K, Dahl ML, Sellgren C, Schwieler L, Engberg G, Schuppe-Koistinen I, et al. Imbalanced kynurenine pathway in schizophrenia. *International Journal of Tryptophan Research*. 2014; 7:15–22. [PubMed: 25288889]
- Kessler M, Terramani T, Lynch G, Baudry M. A glycine site associated with N-methyl-D-aspartic acid receptors: Characterization and identification of a new class of antagonists. *Journal of Neurochemistry*. 1989; 52:1319–1328. [PubMed: 2538568]
- Kinney DK, Hintz K, Shearer EM, Barch DH, Riffin C, Whitley K, Butler R. A unifying hypothesis of schizophrenia: Abnormal immune system development may help explain roles of prenatal hazards, post-pubertal onset, stress, genes, climate, infections, and brain dysfunction. *Medical Hypotheses*. 2010; 74:555–563. [PubMed: 19836903]
- Konradsson-Geuken A, Gash CR, Alexander K, Pomerleau F, Huettl P, Gerhardt GA, Bruno JP. Second-by-second analysis of alpha 7 nicotine receptor regulation of glutamate release in the prefrontal cortex of awake rats. *Synapse*. 2009; 63:1069–1082. [PubMed: 19637277]
- Konradsson-Geuken A, Wu HQ, Gash CR, Alexander KS, Campbell A, Sozeri Y, Pellicciari R, Schwarcz R, Bruno JP. Cortical kynurenic acid bi-directionally modulates prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. *Neuroscience*. 2010; 169:1848–1859. [PubMed: 20600676]
- Lewis DA. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology*. 1997; 16:385–398. [PubMed: 9165494]
- Lin H, Hsu FC, Baumann BH, Coulter DA, Anderson SA, Lynch DR. Cortical parvalbumin GABAergic deficits with alpha7 nicotinic acetylcholine receptor deletion: Implications for schizophrenia. *Molecular and Cellular Neurosciences*. 2014a; 61:163–175. [PubMed: 24983521]
- Lin H, Hsu FC, Baumann BH, Coulter DA, Lynch DR. Cortical synaptic NMDA receptor deficits in alpha7 nicotinic acetylcholine receptor gene deletion models: Implications for neuropsychiatric diseases. *Neurobiology of Disease*. 2014b; 63:129–140. [PubMed: 24326163]
- Linderholm KR, Skogh E, Olsson SK, Dahl ML, Holtze M, Engberg G, Samuelsson M, Erhardt S. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophrenia Bulletin*. 2012; 38:426–432. [PubMed: 20729465]
- McGinty F, Rose DP. Influence of androgens upon tryptophan metabolism in man. *Life Sciences*. 1969; 8:1193–1199. [PubMed: 5358665]
- Millan MJ, Agid Y, Brune M, Bullmore ET, Carter CS, Clayton NS, Connor R, Davis S, Deakin B, DeRubeis RJ, et al. Cognitive dysfunction in psychiatric disorders: characteristics, causes and the quest for improved therapy. *Nature Reviews: Drug Discovery*. 2012; 11:141–168. [PubMed: 22293568]
- Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiology of Disease*. 2004; 15:618–629. [PubMed: 15056470]
- Nielsen DM, Cnric LS. Automated analysis of foot-shock sensitivity and concurrent freezing behavior in mice. *Journal of neuroscience methods*. 2002; 115:199–209. [PubMed: 11992671]

- Notarangelo FM, Pocivavsek A. Elevated kynurenine pathway metabolism during neurodevelopment: Implications for brain and behavior. *Neuropharmacology*. 2016; doi: 10.1016/j.neuropharm.2016.03.001
- O'Donnell P. Adolescent onset of cortical disinhibition in schizophrenia: insights from animal models. *Schizophrenia Bulletin*. 2011; 37:484–492. [PubMed: 21505115]
- Ohgi Y, Futamura T, Hashimoto K. Glutamate signaling in synaptogenesis and NMDA receptors as potential therapeutic targets for psychiatric disorders. *Current Molecular Medicine*. 2015; 15:206–221. [PubMed: 25817855]
- Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res*. 1982 Sep 9; 247(1):184–7. [PubMed: 6215086]
- Pershing ML, Bortz DM, Pocivavsek A, Fredericks PJ, Jorgensen CV, Vunck SA, Leuner B, Schwarcz R, Bruno JP. Elevated levels of kynurenic acid during gestation produce neurochemical, morphological, and cognitive deficits in adulthood: Implications for schizophrenia. *Neuropharmacology*. 2015; 90:33–41. [PubMed: 25446576]
- Pocivavsek, A., Notarangelo, FM., Wu, HQ., Bruno, JP., Schwarcz, R. Astrocytes as pharmacological targets in the treatment of schizophrenia: Focus on kynurenic acid. In: Pletnikov, M., Waddington, J., editors. *Modeling the psychopathological dimensions of schizophrenia*. New York: Academic Press; 2016. p. 423–443.
- Pocivavsek A, Thomas MA, Elmer GI, Bruno JP, Schwarcz R. Continuous kynurenine administration during the prenatal period, but not during adolescence, causes learning and memory deficits in adult rats. *Psychopharmacology*. 2014; 231:2799–2809. [PubMed: 24590052]
- Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proceedings of the National Academy of Sciences of the USA*. 2006; 103:8721–8726. [PubMed: 16731620]
- Raybuck JD, Gould TJ. The role of nicotinic acetylcholine receptors in the medial prefrontal cortex and hippocampus in trace fear conditioning. *Neurobiology of Learning and Memory*. 2010; 94:353–363. [PubMed: 20727979]
- Rimol LM, Hartberg CB, Nesvag R, Fennema-Notestine C, Hagler DJ Jr, Pung CJ, Jennings RG, Haukvik UK, Lange E, Nakstad PH, et al. Cortical thickness and subcortical volumes in schizophrenia and bipolar disorder. *Biological Psychiatry*. 2010; 68:41–50. [PubMed: 20609836]
- Sathyasaikumar KV, Stachowski EK, Wonodi I, Roberts RC, Rassoulpour A, McMahon RP, Schwarcz R. Impaired kynurenine pathway metabolism in the prefrontal cortex of individuals with schizophrenia. *Schizophrenia Bulletin*. 2011; 37:1147–1156. [PubMed: 21036897]
- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols*. 2008; 3:1101–1108. [PubMed: 18546601]
- Schwarcz R, Bruno JP, Muchowski PJ, Wu HQ. Kynurenines in the mammalian brain: When physiology meets pathology. *Nature Reviews Neuroscience*. 2012; 13:465–477. [PubMed: 22678511]
- Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biological Psychiatry*. 2001; 50:521–530. [PubMed: 11600105]
- Selemon LD, Zecevic N. Schizophrenia: a tale of two critical periods for prefrontal cortical development. *Translational Psychiatry*. 2015; 5:e623. [PubMed: 26285133]
- Shouval HZ, Bear MF, Cooper LN. A unified model of NMDA receptor-dependent bidirectional synaptic plasticity. *Proceedings of the National Academy of Sciences of the USA*. 2002; 99:10831–10836. [PubMed: 12136127]
- Stone TW, Darlington LG. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. *British Journal of Pharmacology*. 2013; 169:1211–1227. [PubMed: 23647169]
- Thomsen MS, Mikkelsen JD. The alpha7 nicotinic acetylcholine receptor complex: one, two or multiple drug targets? *Current Drug Targets*. 2012; 13:707–720. [PubMed: 22300038]

- Vasey MW, Thayer JF. The continuing problem of false positives in repeated measures ANOVA in psychophysiology: a multivariate solution. *Psychophysiology*. 1987; 24:479–486. [PubMed: 3615759]
- Wang H, Stradtman GG, Wang XJ, Gao WJ. A specialized NMDA receptor function in layer 5 recurrent microcircuitry of the adult rat prefrontal cortex. *Proceedings of the National Academy of Sciences of the USA*. 2008; 105:16791–16796. [PubMed: 18922773]
- Wang M, Yang Y, Wang CJ, Gamo NJ, Jin LE, Mazer JA, Morrison JH, Wang XJ, Arnsten AF. NMDA receptors subserve persistent neuronal firing during working memory in dorsolateral prefrontal cortex. *Neuron*. 2013; 77:736–749. [PubMed: 23439125]
- Waters FA, Maybery MT, Badcock JC, Michie PT. Context memory and binding in schizophrenia. *Schizophrenia Research*. 2004; 68:119–125. [PubMed: 15099596]
- Wonodi I, Stine OC, Sathyaikumar KV, Roberts RC, Mitchell BD, Hong LE, Kajii Y, Thaker GK, Schwarcz R. Downregulated kynurenine 3-monooxygenase gene expression and enzyme activity in schizophrenia and genetic association with schizophrenia endophenotypes. *Archives of General Psychiatry*. 2011; 68:665–674. [PubMed: 21727251]
- Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET. Meta-analysis of regional brain volumes in schizophrenia. *The American Journal of Psychiatry*. 2000; 157:16–25. [PubMed: 10618008]
- Wu HQ, Okuyama M, Kajii Y, Pocivavsek A, Bruno JP, Schwarcz R. Targeting kynurenine aminotransferase II in psychiatric diseases: promising effects of an orally active enzyme inhibitor. *Schizophrenia Bulletin* 40 Suppl. 2014; 2:S152–158.
- Wu HQ, Pereira EF, Bruno JP, Pellicciari R, Albuquerque EX, Schwarcz R. The astrocyte-derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. *Journal of Molecular Neuroscience*. 2010; 40:204–210. [PubMed: 19690987]
- Xu Z, Chen RQ, Gu QH, Yan JZ, Wang SH, Liu SY, Lu W. Metaplastic regulation of long-term potentiation/long-term depression threshold by activity dependent changes of NR2A/NR2B ratio. *The Journal of Neuroscience*. 2009; 29:10.
- Yashiro K, Philpot BD. Regulation of NMDA receptor subunit expression and its implications for LTD, LTP, and metaplasticity. *Neuropharmacology*. 2008; 55:14.
- Zmarowski A, Wu HQ, Brooks JM, Potter MC, Pellicciari R, Schwarcz R, Bruno JP. Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *The European Journal of Neuroscience*. 2009; 29:529–538. [PubMed: 19187269]

Figure 1.

Effects of prenatal exposure to kynurenine (KYN) on levels of kynurenine in the plasma and kynurenic acid (KYNA) in the PFC in juveniles (PD32) and adults (PD70). Data are the mean $\pm$ SEM. PD32: ECON: $n = 9$; EKYN: $n = 10$; PD70: $n = 10$ per group. Two-way ANOVA (age $\times$ prenatal condition) revealed no differences in plasma kynurenine; however, there was a significant interaction of age and prenatal condition for brain KYNA levels. EKYN vs ECON at PD70: ** $P < 0.01$.

Figure 2.

(A) Relative expression of mRNA levels of NMDAR subunits and $\pm 7nAChR$ at PD32 and PD70. Data (mean $\pm$ SEM) for EKYNs are normalized, for their respective age group, to ECONs, which were set to a value of 1.0 ($n = 11$ each/age group) * $P < 0.05$, ** $P < 0.01$. (B) Bar graphs showing a strong trend ($P < 0.06$; $P < 0.07$ at PD32, PD70, respectively) toward decreases in the NR2A/NR2B ratio in EKYN rats at both ages.

Figure 3.

Percent time freezing in the training chamber during A) context test (absence of CS or US on Day 2) and B) cue test (CS in a novel context on Day 3). (ECON: $n = 10$, EKYN: $n = 12$ per age). Contextual fear appeared to be similar in juvenile rats but modestly impaired in the adult rats ($P = 0.17$). On Day 3 during the critical cue test, EKYNs froze more than controls as juveniles, but less than controls as adults. * $P < 0.05$; ** $P < 0.01$.

Table 1

Shock Sensitivities as Function of Age and Treatment Conditions

Shock score	PD32		PD70	
	ECON	EKYN	ECON	EKYN
	Mean $\pm$ SEM (mA)	Mean $\pm$ SEM (mA)	Mean $\pm$ SEM (mA)	Mean $\pm$ SEM (mA)
1	0.32 $\pm$ 0.06	0.34 $\pm$ 0.05	0.34 $\pm$ 0.07	0.25 $\pm$ 0.06
2	0.65 $\pm$ 0.05	0.68 $\pm$ 0.05	0.63 $\pm$ 0.05	0.55 $\pm$ 0.06
3	0.92 $\pm$ 0.04	0.88 $\pm$ 0.06	0.84 $\pm$ 0.09	0.80 $\pm$ 0.06
Vocalize	0.68 $\pm$ 0.07	0.77 $\pm$ 0.05	0.77 $\pm$ 0.05	0.77 $\pm$ 0.05