Published in final edited form as:

Eur J Neurosci. 2012 May ; 35(10): 1605–1612. doi:10.1111/j.1460-9568.2012.08064.x.

PRE- AND POSTNATAL EXPOSURE TO KYNURENINE CAUSES COGNITIVE DEFICITS IN ADULTHOOD

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Abstract

Levels of kynurenic acid (KYNA), an endogenous product of tryptophan degradation, are elevated in the brain and cerebrospinal fluid of individuals with schizophrenia (SZ). This increase has been implicated in the cognitive dysfunctions seen in the disease since KYNA is an antagonist of the $\alpha 7$ nicotinic acetylcholine receptor and the NMDA receptor, both of which are critically involved in cognitive processes and in a defining neurodevelopmental period in the pathophysiology of SZ. We tested the hypothesis that early developmental increases in brain KYNA synthesis might cause biochemical and functional impairments in adulthood. To this end, we stimulated KYNA formation by adding the KYNA precursor kynurenine (100 mg/day) to the chow fed to rat dams from gestational day 15 to postnatal day 21 (PD 21). This treatment raised brain KYNA levels in the offspring by 341% on PD 2 and 210% on PD 21. Rats were then fed normal chow until adulthood (PD 56-PD 80). In the adult animals, basal levels of extracellular KYNA, measured in the hippocampus by *in vivo* microdialysis, were elevated (+12%), whereas extracellular glutamate levels were significantly reduced (–13%). In separate adult animals, early kynurenine treatment was shown to impair performance in two behavioral tasks linked to hippocampal function, the passive avoidance test and the Morris water maze test. Collectively, these studies introduce a novel, naturalistic rat model of SZ and also suggest that increases in brain KYNA during a vulnerable period in brain development may play a significant role in the pathophysiology of the disease.

Keywords

Rats; Cognition; Hippocampus; Kynurenic acid; Schizophrenia

INTRODUCTION

Impairments in neural development, caused by a convergence of genetic and environmental risk factors, play a critical role in the emergence of clinical symptoms in schizophrenia (SZ) (Weinberger, 1987; Lewis & Levitt, 2002). This, in turn, has led to the development of animal models based on presumed susceptibility genes (Ayhan *et al.*, 2009) or suspected perinatal hazards, such as maternal stress (Markham *et al.*, 2010), perinatal infection (Meyer & Feldon, 2010), and neonatal hippocampal damage (Tseng *et al.*, 2009).

Studies using cerebrospinal fluid or post-mortem brain tissue of SZ patients suggest that an excess of kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation, might play a causative role in the disease (Erhardt *et al.*, 2001; Schwarcz *et al.*, 2001; Miller *et al.*, 2006). More specifically, as KYNA is an endogenous antagonist of $\alpha 7$ nicotinic acetylcholine receptors ($\alpha 7$ nAChRs) and NMDA receptors, which are both critically involved in cognitive functions (Levin *et al.*, 2006; Robbins & Murphy, 2006), increased levels of the metabolite might be related to the cognitive deficits that are seen in individuals with SZ (Wonodi & Schwarcz, 2010). This suggested causality is directly supported by studies in rodents, which show cognitive dysfunctions reminiscent of SZ when brain KYNA levels are raised acutely (Shepard *et al.*, 2003; Erhardt *et al.*, 2004; Chess *et al.*, 2007; Chess *et al.*, 2009; Pocivavsek *et al.*, 2011; Alexander *et al.*, 2011). The association between excessive levels of brain KYNA and cognitive impairments is also bolstered by studies in humans, where a polymorphism in the gene of kynurenine 3-monooxygenase, a key kynurenine pathway enzyme that influences KYNA synthesis, has been correlated to cognitive endophenotypes (Wonodi *et al.*, 2011).

As demonstrated recently, the connection between KYNA and SZ may also have a developmental dimension (Akagbosu *et al.*, 2010). Thus, several of the above-mentioned risk factors, including perinatal stress and early infections (Müller & Schwarz, 2006; Meyer & Feldon, 2009) result in the activation of indoleamine-2,3-dioxygenase (IDO), a cytokine-responsive enzyme that catalyzes the degradation of tryptophan. In the blood, IDO stimulation leads to an increase in the formation of L-kynurenine (“kynurenine”) (Schröcksnadel *et al.*, 2006), a pivotal kynurenine pathway metabolite, which is readily transported across the blood-brain barrier (Fukui *et al.*, 1991). In the brain, kynurenine aminotransferases (KATs) subsequently convert kynurenine to KYNA. This irreversible process, preferentially catalyzed by the astrocytic enzyme KAT II (Guidetti *et al.*, 2007), results in the prompt release of newly formed KYNA into the extracellular milieu (Turski *et al.*, 1989) for interaction with $\alpha 7$ nAChRs and NMDA receptors.

The present study was designed to duplicate the effects of the presumed common feature of several perinatal SZ models, i.e. peripheral IDO activation, by administering the enzyme product, kynurenine, to pregnant and nursing dams during a sensitive period of pre- and postnatal brain development of their offspring. Pre- and early postnatal kynurenine treatment significantly raised KYNA levels in the pup brain, and this effect persisted into adulthood. In addition, the adult animals showed cognitive deficits reminiscent of those seen in SZ.

MATERIALS AND METHODS

Animals

Adult, pregnant Wistar rats (gestational age: 2 days) were obtained from Charles River Laboratories. Animals were housed in a temperature-controlled facility, which is fully accredited by the American Association for the Accreditation of Laboratory Animal Care, at the Maryland Psychiatric Research Center. The rats were kept on a 12h/12h light dark cycle and had access to food and water. Experimental protocols followed the ‘Principles of Laboratory Animal Care’ (NIH publication No. 86-23, 1996) and were approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine.

Each litter was culled to 10 pups on postnatal day (PD) 2 to normalize litter size and maximize the number of males. Special attention was paid to assure that kynurenine treatment did not disturb maternal behavior (i.e. nursing, nest building, and pup-retrieval) throughout the treatment period.

Male rats were tested experimentally on PD 2, PD 21, and PD 56–PD 80 (see Fig. 1). In all biochemical experiments, subjects were the progeny from 3–4 litters, with no more than 2–4 pups from any given litter. For the behavioral experiments, subjects were the progeny from 7 litters, with no more than 1–2 pups from any given litter. This design was implemented in order to minimize the contribution of individual litters.

Treatment

One week prior to the initiation of treatment, dams were acclimated to a wet mash diet. Rodent chow was ground finely in a food processor, and each dam ate approximately 30 grams per day. Beginning on gestational day (GD) 15, 100 mg of L-kynurenine sulfate (“kynurenine”) was thoroughly mixed into the food daily for kynurenine-treated dams, while controls continued to receive wet mash alone. Dams were fed the diet daily from GD 15 until their pups were PD 21. As the dams required greater caloric intake, additional wet mash food was provided in the course of the treatment. On PD 21, pups were weaned from the dams and pair-housed until at least PD 56. Upon weaning, all animals were given normal rodent chow pellets *ad libitum* (Fig. 1). All dams were euthanized on PD 21 for biochemical analyses.

Chemicals

KYNA, DL-3-hydroxykynurenine (3-HK) and glutamate were purchased from Sigma (St. Louis, MO, USA). L-Kynurenine sulfate (“kynurenine”; purity: 99.4%) was obtained from Sai Advantium (Hyderabad, India). Other chemicals were obtained from a variety of suppliers and were of the highest commercially available purity.

Microdialysis

To prepare for microdialysis experiments, rats were anesthetized with chloral hydrate (360 mg/kg, i.p.) and mounted in a David Kopf stereotaxic frame. A guide cannula (outer diameter: 0.65 mm) was positioned over the dorsal hippocampus (AP: −3.4 mm from bregma; L: 2.3 mm; V: 1.5 below dura) and secured to the skull with anchor screws and acrylic dental cement. On the next day, a microdialysis probe (CMA/10, membrane length: 2 mm, Carnegie Medicin AB, Stockholm, Sweden) was inserted through the guide, protruding vertically through the hippocampus. The probe inlet was connected to a microperfusion pump (CMA/100, Carnegie Medicin) set to a flow rate of 1 µl/min, and the freely moving animals were perfused with Ringer solution, pH 6.7, containing 144 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO₄ and 1.7 mM CaCl₂. The first 30-min fraction was discarded, and 8 consecutive 30-min fractions were collected during the following 4 h. Microdialysis data were not corrected for recovery from the dialysis probe.

Biochemical analyses

Kynurenine determination in serum—Animals were euthanized using CO₂, and whole blood was collected in tubes containing 25 µl K₃-EDTA (0.15 %) as an anticoagulant. The blood was gently mixed and centrifuged (300 × g, 15 min) to separate serum and blood cells. The supernatant serum was transferred to Eppendorf tubes, frozen on dry ice and stored at −80°C. On the day of the assays, the samples were thawed, and 100 µl of serum were acidified with 25 µl of 6% perchloric acid. After centrifugation (12 000 × g, 10 min), 20 µl of the supernatant were subjected to high performance liquid chromatography (HPLC). Kynurenine was isocratically eluted from a 3-µm C₁₈ reverse-phase column (80 mm × 4.6; ESA, Chelmsford, MA, USA), using a mobile phase containing 250 mM zinc acetate, 50 mM sodium acetate, and 3% acetonitrile (pH adjusted to 6.2 with glacial acetic acid), using a flow rate of 1.0 ml/min. In the eluate, kynurenine was detected fluorimetrically (excitation:

365 nm, emission: 480 nm; Perkin Elmer Series 200 fluorescence detector, Waltham, MA). The retention time of kynurenine was approximately 6 min.

KYNA and 3-HK determination in tissue—Animals were euthanized using CO₂, and the brain was rapidly removed. The forebrain (PD 2) or the hippocampus (PD 21) was frozen on dry ice and stored at −80°C. On the day of the assays, tissues were thawed and sonicated in ultrapure water (1:5, w/v, for PD 21 brains, 1:10, w/v for adult brains). One hundred µl of the homogenate were acidified with 25 µl of 6% perchloric acid. After centrifugation (12 000 × g, 10 min), 20 µl of the supernatant were analyzed by HPLC to detect either KYNA or 3-HK.

KYNA was eluted as described above for kynurenine and was then detected fluorimetrically (excitation: 344 nm, emission: 398 nm). The retention time of KYNA was approximately 7 min.

3-HK was eluted from a 3 µm HPLC column (HR-80; 80 mm × 4.6 mm; ESA), using a mobile phase consisting of 1.5 % acetonitrile, 0.9 % triethylamine, 0.59 % phosphoric acid, 0.27 mM EDTA, and 8.9 mM sodium heptane sulfonic acid, and a flow rate of 0.5 ml/min. In the eluate, 3-HK was detected electrochemically using an HTEC 500 detector (Eicom Corp., San Diego, CA, USA; oxidation potential: + 0.5 V). The retention time of 3-HK was approximately 11 min.

KYNA and glutamate determination in microdialysate—The extracellular concentrations of both KYNA and glutamate in microdialysates were determined fluorimetrically using 15 µl of the same sample for each assay. Thus, KYNA was quantitated as described for tissue above, and glutamate was determined after o-phthalaldehyde/2-mercaptoethanol derivatization and gradient elution (excitation: 390 nm; emission: 460 nm) (Wu *et al.*, 2010). The electrochemical methodology described above proved insufficient for determining 3-HK levels in microdialysis samples.

Behavioral testing

Contextual memory—The passive avoidance apparatus had two compartments of equal size (22 cm high, 18 cm wide and 16 cm deep), one illuminated and the other in darkness, separated by a guillotine door. On day 1, the acquisition trial, the animal was first placed in the illuminated compartment. The door was then opened, prompting the rat to move rapidly into the preferred dark compartment. The latency to enter the dark compartment was recorded and defined as the “approach latency”. After the door was closed, an inescapable foot shock (0.56 mA for 1 sec; Seliger, 1977; Gschanes *et al.*, 1998) was delivered through metal rods of the floor. Twenty-four h later, in the retention trial, the rat was again placed in the light compartment, and the guillotine door was opened. The “avoidance latency”, i.e. the time from opening the guillotine door to the time of entering the dark compartment, was recorded.

Spatial navigation and reference memory—The Morris water maze (MWM)(Morris, 1984) was used to study spatial navigation and reference memory. The maze pool (180 cm in diameter) was surrounded by distinct extra-maze spatial cues. To habituate the rat to the procedure and environment, each rat was allowed to navigate the pool for 120 sec one day prior to training the rat to locate a hidden platform. On the first day of training, the hidden platform (10 cm in diameter, approximately 2 cm below water level) was placed in the NW quadrant and remained there across training trials and days. Rats were placed into the maze at different locations (directions NW, NE, SW, and SE) in a counterbalanced order across trials and animals. Each animal was given up to 120 sec to locate the hidden platform. Upon

finding the platform, the animal was required to stay on it for 15 sec. If an animal did not find the platform, it was gently guided to the platform location and made to stay on the platform for 15 sec. The inter-trial interval was 120 sec, and each animal was tested on 4 trials on each of 6 consecutive days. On the seventh day, the hidden platform was removed. The animal was then given 120 sec to navigate the pool, allowing us to test retention and spatial navigation strategy ("probe trial"). Immediately following the probe trial, each animal was tested again, with the platform made visible by a red flag and raised slightly above water level ("visible trial"). This trial was used to monitor visual acuity and general sensorimotor capabilities. Behavior during all trials was recorded by a Noldus EthoVision tracking system (Leesburg, VA, USA).

Statistical analysis

For all biochemical analysis, comparisons were made using an unpaired Student's t-test. Results from the passive avoidance paradigm were also analyzed using an unpaired Student's t-test. MWM escape latency across days was analyzed using a two-way repeated measures Analysis of Variance (ANOVA) with treatment group as a between-subject factor and days as a within-subject factor. A Huynh-Feldt correction was applied to the repeated measures ANOVA as a precaution against non-homogeneity of variance and Type 1 errors (Vasey & Thayer, 1987). Post-hoc analyses were performed using a Bonferroni t-test correction with an adjusted $\alpha = P < 0.008$ per comparison. Swim speed, probe trial and visual platform trial comparisons were made using an unpaired Student's t-test. Statistical significance in Student's t-test was defined as $P < 0.05$. All statistical analyses were performed using SPSS 12.0 software.

RESULTS

Kynurenine feeding from GD 15 until weaning increases serum kynurenine levels

To corroborate the efficacy of our treatment regimen, we first determined the effect of the kynurenine diet on serum kynurenine levels in the treated dams. All mothers were euthanized on PD 21, when the pups were weaned. At that time, kynurenine-treated mothers had significantly higher levels of serum kynurenine compared to mothers that were fed the control diet (control: $1.1 \pm 0.2 \mu\text{M}$, kynurenine-treated: $24.3 \pm 5.9 \mu\text{M}$; $F_{1,18} = 23.936$, $P = 0.001$) (Fig. 2A)

Kynurenine levels in the serum of pups on PD 2 were below the detection limit (i.e. $< 0.3 \mu\text{M}$). However, on PD 21, serum kynurenine levels were significantly elevated in the offspring of kynurenine-treated dams compared to control pups (control: $1.9 \pm 0.2 \mu\text{M}$, kynurenine-treated: $16.9 \pm 0.7 \mu\text{M}$; $F_{1,14} = 3.909$, $P < 0.001$) (Fig. 2B).

Brain KYNA levels are elevated with kynurenine treatment from GD 15 until weaning

Pups of kynurenine-treated dams also showed substantial increases in KYNA levels in the brain. Thus, on PD 2, the KYNA content in the forebrain of treated pups was significantly higher than in controls (control: 70.0 ± 13.2 fmoles/mg protein, kynurenine-treated: 257.1 ± 55.5 fmoles/mg protein; $F_{1,10} = 3.442$, $P = 0.008$). 3-HK levels in the forebrain on PD 2 were below the detection limit (i.e. < 100 fmoles/mg protein). On PD 21, KYNA levels were determined specifically in the hippocampus and found to be elevated in perinatally treated pups (control: 73.4 ± 15.0 fmoles/mg protein, kynurenine-treated: 154.3 ± 11.5 fmoles/mg protein; $F_{1,14} = 0.436$, $P = 0.001$) (Fig. 3A). In the same hippocampal tissues, 3-HK levels were also significantly elevated in pups from kynurenine-treated dams (control: 450.8 ± 58.9 fmoles/mg protein, kynurenine treated: 2165.0 ± 454.7 fmoles/mg protein; $F_{1,13} = 68.325$, $P = 0.002$) (Fig. 3B).

Kynurenine treatment from GD 15 until weaning: neurochemical effects in the hippocampus of adult animals

After treatment was terminated on PD 21, the male pups not used for the initial biochemical analyses were weaned and group-housed until the completion of the study. When the animals reached adulthood (PD 56–80), the levels of KYNA and 3-HK were assessed in hippocampal tissue homogenates. KYNA levels (control: 130.7 ± 13.3 fmoles/mg protein, kynurenine-treated: 150.1 ± 12.4 fmoles/mg protein; $F_{1,16} = 0.008$, $P = 0.302$) and 3-HK levels (control: 253.2 ± 20.4 fmoles/mg protein, kynurenine-treated: 219.3 ± 17.7 fmoles/mg protein; $F_{1,16} = 0.115$, $P = 0.229$) were not significantly different between the two groups (Fig. 4A,B).

In contrast to tissue levels, hippocampal microdialysis in rats treated perinatally with kynurenine revealed that *extracellular* KYNA levels were increased by 12% compared to control animals ($F_{1,21} = 2.142$, $P = 0.023$) (Fig. 5A). The absolute concentration of KYNA recovered from the microdialysis probe in control rats was 2.5 ± 0.1 nM. 3-HK levels in hippocampal microdialysates were found to be below the detection limit (i.e. < 2 nM). In the same samples, extracellular glutamate levels in adult rats receiving kynurenine during early development were decreased by 13% ($F_{1,21} = 1.444$, $P = 0.003$) (Fig. 5B). The absolute concentration of extracellular glutamate in the hippocampus of control rats was 2.1 ± 0.1 μ M.

Cognitive deficits in animals receiving kynurenine from GD 15 until weaning

Next, we investigated whether pre- and postnatal kynurenine treatment affected performance in two behaviors known to be hippocampally-mediated, i.e. passive avoidance and spatial learning (MWM). Animals were examined on both tasks in a counterbalanced order, with at least 4 days between tasks. In addition, the age range (PD 56–80) of adult animals was balanced across behavioral experiments. On the first day of passive avoidance testing, the acquisition trial, adult rats from the control group and the kynurenine-treated group did not differ significantly in approach latencies (control: 23.9 ± 5.9 sec; kynurenine-treated: 30.0 ± 6.0 sec; $F_{1,22} = 0.254$, $P = 0.479$) (Fig. 6A). However, when tested 24 h later (retention trial), the avoidance latencies of the animals treated with kynurenine from GD 15 until weaning were significantly shorter than those of control animals (control: 161.2 ± 28.3 sec; kynurenine-treated: 50.3 ± 9.6 sec; $F_{1,22} = 13.715$, $P = 0.001$) (Fig. 6B).

Compared to controls, adult rats treated pre- and postnatally with kynurenine showed an overall significant effect on escape latency across days in the MWM ($F_{1,22} = 3.832$, $P = 0.005$). Post-hoc analysis revealed a significant increase in escape latency on days 2 ($P = 0.001$), 4 ($P = 0.003$) and 5 ($P = 0.001$) (Fig. 7A). These data demonstrated that kynurenine treatment during a sensitive period of development slows the rate of acquisition in the spatial learning task in adulthood.

Retention of the newly learned task and spatial navigation strategy were assessed in one single probe trial 24 h after the last training session. Animals treated pre- and postnatally with kynurenine crossed into the area formerly occupied by the platform less frequently than control animals (control: 5.1 ± 1.2 crosses; kynurenine-treated: 1.8 ± 0.6 crosses; $F_{1,22} = 0.973$, $P = 0.017$) (Fig. 7B). Furthermore, on average, animals exposed to kynurenine perinatally also spent less time in the quadrant formerly containing the platform (NW) than controls (Fig. 7C). Swim speeds did not differ significantly between the two groups (control: 34.0 ± 1.0 cm/sec; kynurenine-treated: 31.6 ± 1.4 cm/sec; $F_{1,22} = 7.111$, $P = 0.001$). Finally, the escape latencies of the two experimental groups were found not to differ significantly on the visible trial immediately after the probe trial (data not shown), indicating that early kynurenine treatment did not cause gross visual deficits later in life.

DISCUSSION

In the present study, we explored long-term biochemical and behavioral consequences of prolonged kynurenine treatment during prenatal and early postnatal development in rats. After confirming that oral administration of kynurenine to the dam had the desired effect, i.e. that precursor application stimulated cerebral kynurenine pathway metabolism in the pups, we observed remarkably enduring effects once the offspring reached adulthood. Using hippocampal *in vivo* microdialysis, we first found, unexpectedly, that adult rats which had been treated with kynurenine during early development showed a modest but statistically significant increase in the extracellular levels of KYNA in the hippocampus, even though the animals were fed normal rat chow after weaning. This elevation was accompanied by a reduction in basal levels of extracellular glutamate in the same microdialysis samples. In separate adult animals, we observed impaired performance in two behavioral tasks, namely passive avoidance and the MWM test. Since both the neurochemical changes and the behavioral abnormalities in the developmentally treated animals signify dysfunction of the hippocampus, a brain region with conspicuous anatomical and functional changes in SZ (Tamminga *et al.*, 2010), the present study introduces a novel animal model with apparent construct, and, conceivably, predictive validity for SZ research.

The selection of orally administered kynurenine to generate the new paradigm was based on several considerations. First, as kynurenine readily crosses the blood-brain barrier (Fukui *et al.*, 1991) and is then rapidly transaminated by KATs, we could reasonably anticipate that its acute or sub-chronic systemic administration would cause the desired increases in brain KYNA (Swartz *et al.*, 1990; Olsson *et al.*, 2009). Second, we had conducted preliminary experiments (not shown) in which we optimized the preparation of kynurenine-laced chow and ascertained that continuous intake of 100 mg of kynurenine per day for 4 weeks, as described here, is well tolerated and does not result in weight loss of the dam or gross phenotypic abnormalities in the pups when compared to respective controls. In the present study, we verified the efficacy of this treatment regimen by demonstrating increased serum kynurenine levels in both the dams and the pups at PD 21, and by showing elevated levels of KYNA in the pup brain at PD 2 and PD 21. Not unexpectedly (Gál & Sherman, 1980), kynurenine treatment also raised hippocampal 3-HK levels in the pups at the time of weaning, and the possible long-term effects of this increase will need to be evaluated in separate studies. Finally, we intended to create a developmental model that would reproduce the peripheral IDO activation that is common to animals exposed to stress or a variety of other challenges to the immune system, i.e. established risk factors for SZ (cf. Introduction), without, however, introducing stressful circumstances or immune activators *per se*. In other words, we applied kynurenine, the product of IDO, to avoid direct enzyme manipulations or other upstream interventions.

Similar to the recent study by Akagbosu *et al.* (2010), who reported no lasting effect on the brain tissue levels of KYNA in adulthood after rats received intermittent kynurenine injections (each 100 mg/kg, i.p.) between PD 27 and PD 53, we did not observe significant changes in hippocampal KYNA or 3-HK tissue content in our perinatally kynurenine-treated animals in adulthood. However, a trend towards elevated KYNA levels (Fig. 5A) prompted a re-examination using *in vivo* microdialysis in separate adult animals. These studies revealed that the basal *extracellular* levels of KYNA in the hippocampus of rats that had received kynurenine until weaning were indeed modestly but significantly increased compared to controls (basal extracellular 3-HK was below our detectability limit.) As in conditions of acute elevations of hippocampal KYNA (Pocivavsek *et al.*, 2011), this KYNA increase was accompanied by a significant reduction in basal extracellular glutamate levels. The cause(s), temporal development and regional distribution of these chronic neurochemical changes are currently being studied in our laboratories. Notably, we recently

observed that our pre- and postnatal kynurenine regimen also produces long-lasting elevations in extracellular KYNA levels in the prefrontal cortex. This increase in KYNA was associated with marked deficits in cognitive flexibility, a prefrontally-mediated impairment evocative of the cognitive endophenotype seen in SZ (Alexander et al., manuscript in preparation).

Our behavioral results, too, complement and expand the study by Akagbosu et al. (2010), who showed detrimental effects of intermittent kynurenine administration to adolescent rats on contextual fear memory and novel object recognition in adulthood. Thus, long-term cognitive impairments, here illustrated by dysfunction in two classic hippocampus-dependent tests – passive avoidance and MWM –, are also seen when animals are exposed to an excess of kynurenine at a much younger age. This extends the susceptible period to the very early stages of brain development, i.e. a time when animals exhibit heightened vulnerability to a variety of environmental factors. Moreover, the demonstration of increased extracellular KYNA levels in the adult hippocampus of perinatally kynurenine-treated rats suggests a scenario in which persistent blockade of γ -AChRs, initiated very early in development, might underlie the emergence of cognitive deficits later in life. In adult animals *in vivo*, even modest elevations in brain KYNA cause inhibition of γ -AChRs, KYNA's primary target, and this effect reliably results in a rapid, secondary reduction in extracellular glutamate levels (Wu et al., 2010; Pocivavsek et al., 2011; Alexander et al., 2011). A reduction in glutamate, as well as direct inhibition of γ -AChRs and NMDARs, impairs performance in passive avoidance (Roesler et al., 1998; Marubio & Paylor, 2004) and MWM (Curzon et al., 2006; Boess et al., 2007). Therefore, as both receptors are critically involved in mediating hippocampal processes, the long-lasting increase in KYNA and the associated reduction in glutamate (Fig. 3) may jointly underlie the cognitive deficits seen in adult animals in our study. Notably, the hippocampus is richly endowed with these receptors from an early age, though the ontogenetic characteristics of their interplay and the changing effect of KYNA on these receptors during development are complex and not yet fully understood (Ben-Ari et al., 1997; Dwyer et al., 2009; Alkondon et al., 2011).

The present results are in line with a large number of reports on long-term cognitive impairments following a wide variety of pre- and early postnatal experimental interventions (cf. Introduction). Collectively, these studies have provided an impressive number of heuristically valuable models, which are widely used to investigate brain changes leading to the cognitive impairments seen in SZ. Interestingly, several of the perinatal insults used to generate these models, including stress and infection, are known to cause both acute and long-lasting elevations in brain kynurenine and KYNA, probably as a consequence of an initial activation of IDO (Kennett & Joseph, 1981; Asp et al., 2010; Raison et al., 2010; Miura et al., 2011). Conceptually similar to the conclusion reached by Akagbosu et al. (2010), we propose that supplying the very young, developing brain with greater-than-physiological amounts of KYNA duplicates this mechanism and thus provides an attractive naturalistic animal model for studying the pathophysiology of SZ. The appeal of this model for SZ research is further enhanced by the fact that KYNA levels are elevated in brain and cerebrospinal fluid of individuals with SZ (Erhardt et al., 2001; Schwarcz et al., 2001), and that a polymorphism in the gene for kynurenine 3-monooxygenase, an enzyme involved in the regulation of KYNA biosynthesis (Russi et al., 1992; Holtze et al., 2011), is associated with cognitive SZ endophenotypes (Wonodi et al., 2011).

The new model can be expected to provide fresh insights into the role of a malfunctioning kynurenine pathway in the pathophysiology of SZ. In particular, our paradigm introduces a tool to examine how an increase in brain KYNA during sensitive stages of brain development adversely affects the maturation of neuronal networks, leading to cognitive and possibly other functional impairments and structural changes reminiscent of SZ. Studies

currently in progress in our laboratories are designed to identify the critical period of vulnerability to excessive KYNA in greater detail, and to evaluate the role of astrocytes, which harbor essentially all KAT II in the *adult* mammalian brain (Guidetti *et al.*, 2007), in the progression of pathology. As selective inhibition of KAT II, the major KYNA-synthesizing enzyme in rat and human brain, leads to increases in extracellular glutamate and cognitive improvements (Potter *et al.*, 2010; Pocivavsek *et al.*, 2011), we are also exploring possible beneficial effects of co-administering a KAT II inhibitor with kynurenine during the vulnerable period of brain development. These studies, intended to prevent early, excessive KYNA accumulation in the brain, might not only speak to mechanisms of pathogenesis but might also have translational value for the treatment of SZ and, possibly, other psychiatric diseases (Wonodi & Schwarcz, 2010).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by USPHS grants MH83729 and NS25296.

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Figure 1. Experimental design and kynurenine treatment regimen

Kynurenine-treated dams were fed a diet of wet rodent chow mash supplemented with 100 mg kynurenine per animal per day from GD 15 until PD 21. On PD 21, offspring were weaned from their mothers and pair-housed until adulthood. Biochemical and behavioral studies were performed in adulthood, as indicated.

Figure 2. Pre- and postnatal kynurenine treatment increases serum kynurenine levels in dams and their offspring

Exposure to kynurenine (100 mg/day) from GD 15 until weaning raised kynurenine levels in the serum of both mothers (A) and pups (B) on PD 21. Data are the mean $\pm$ SEM ($n = 10$ per group for dams; $n = 8$ per group for offspring). *** $P < 0.001$ vs. control.

Figure 3. Pre- and postnatal kynurenine treatment increases KYNA and 3-HK levels in the hippocampus

Rats were exposed to kynurenine (100 mg/day) from GD 15 until weaning. Kynurenine pathway metabolites were measured in hippocampal tissue of pups that were euthanized on PD 21. Levels of KYNA (**A**) and 3-HK (**B**) were increased in offspring fed kynurenine. Data are the mean $\pm$ SEM ($n = 8$ per group). ** $P < 0.01$ and *** $P < 0.001$ vs. control.

Figure 4. Pre- and postnatal kynurenine treatment does not affect tissue levels of KYNA and 3-HK in the hippocampus in adulthood

Rats were exposed to kynurenine (100 mg/day) from GD 15 until weaning, and kynurenine pathway metabolites were determined in hippocampal tissue of animals that were euthanized during adulthood (PD 56-80). Levels of KYNA (**A**) and 3-HK (**B**) did not differ significantly between controls and kynurenine-treated rats ($P > 0.05$). Data are the mean $\pm$ SEM ($n = 9$ per group).

Figure 5. Effects of pre- and postnatal kynurenine treatment on extracellular KYNA and glutamate levels in the hippocampus of adult animals

Rats were exposed to kynurenine (100 mg/day) from GD 15 until weaning. In adulthood (PD 56-80), 30-min hippocampal microdialysate samples were collected consecutively for 4 h. The averaged data are expressed as a percentage of control for extracellular KYNA (**A**) and glutamate (**B**) (mean $\pm$ SEM; $n = 12$ for controls; $n = 11$ for kynurenine-treated rats). See Results for absolute analyte concentrations in control rats. * $P < 0.05$ and ** $P < 0.01$ vs. control.

Figure 6. Animals exposed to kynurenine during pre- and early postnatal development show contextual memory impairment in adulthood

Rats were exposed to kynurenine (100 mg/day) from GD 15 until weaning and were tested in the passive avoidance paradigm during adulthood (PD 56-80; $n = 12$ per group). No differences in approach latency were observed on the training day (**A**), but avoidance latency differed significantly between the two groups 24 h later (**B**). All data are the mean $\pm$ SEM. *** $P < 0.001$ vs. control.

Figure 7. Spatial learning and reference memory is impaired in animals receiving kynurenine during pre- and early postnatal development

Rats were exposed to kynurenine (100 mg/day) from GD 15 until weaning and were tested in the MWM during adulthood (PD 56- 80; $n = 12$ per group). **A**) Test across 6 days of training; **B**) Number of platform crossings during the probe trial (video tracking analysis); **C**) Percent of time spent in the target quadrant during the probe trial (video tracking analysis). All data are the mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ vs. control.