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Early Developmental Elevations of Brain Kynurenic Acid Impair Cognitive Flexibility in Adults: Reversal with Galantamine

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Abstract

Levels of kynurenic acid (KYNA), an endogenous $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) antagonist, are elevated in the brain of patients with schizophrenia (SZ) and might contribute to the pathophysiology and cognitive deficits seen in the disorder. As developmental vulnerabilities contribute to the etiology of SZ, we determined, in rats, the effects of perinatal increases in KYNA on brain chemistry and cognitive flexibility. KYNA's bioprecursor L-kynurenine (100 mg/day) was fed to dams from gestational day 15 (GD15) to postnatal day 21 (PD21). Offspring were then given regular chow until adulthood. Control rats received unadulterated mash. Brain tissue levels of KYNA were measured at PD2 and PD21, and extracellular levels of KYNA and glutamate were determined by microdialysis in the prefrontal cortex in adulthood (PD56-80). In other adult rats, the effects of perinatal L-kynurenine administration on cognitive flexibility were assessed using an attentional set-shifting task. L-Kynurenine treatment raised forebrain KYNA levels ~3-fold at PD2 and ~2.5-fold at PD21. At PD56-80, extracellular prefrontal KYNA levels were moderately but significantly elevated (+12%), whereas extracellular glutamate levels were not different from controls. Set-shifting was selectively impaired by perinatal exposure to L-kynurenine, as treated rats acquired the discrimination and intra-dimensional shift at the same rate as controls, yet exhibited marked deficits in the initial reversal and extra-dimensional shift. Acute administration of the $\alpha 7$ nAChR positive modulator galantamine (3.0 mg/kg, i.p.) restored performance to control levels. These results validate early developmental exposure to L-kynurenine as a novel, naturalistic animal model for studying cognitive deficits in SZ.

Keywords

schizophrenia; kynurenine; set-shifting; prefrontal cortex; glutamate; $\alpha 7$ nicotinic receptors

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Schizophrenia (SZ) is characterized by impairments in executive functions mediated by the prefrontal cortex (PFC), including working memory (Perlstein et al, 2001; Abi-Dargham et al, 2002), attention (Luck and Gold, 2008; Nuechterlein et al, 2009), and cognitive flexibility (Everett et al, 2001; Thoma et al, 2007). These deficits precede the onset of psychosis (Caspi et al, 2003), and their severity is predictive of functional outcome (Green et al, 2000). Despite this relationship, cognitive dysfunctions remain the most poorly treated of all symptoms in SZ (Bowie and Harvey, 2006). The development of valid animal models of “SZ-like” cognitive deficits is therefore critical for the rational discovery of more efficacious pharmacotherapeutics.

While pathological changes underlying the SZ phenotype are complex and numerous, two features have been well-established and should be incorporated into valid animal models of the disorder. First, impairments in neural development, reflecting interactions between genetic vulnerabilities and environmental risk factors, contribute to SZ (for review, see Tan et al, 2009). Second, these early developmental anomalies may lead to maturational dysregulations in several cortical transmitter systems that are critical for the expression of attention and cognitive flexibility, including acetylcholine (ACh; Sarter et al, 2011) and glutamate (Moghaddam and Javitt, 2012). Thus, the brain of persons with SZ shows cholinergic abnormalities, including decreased mRNA levels of the gene (CHRNA7) encoding the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR; Mexal et al, 2010) and reduced protein expression of the $\alpha 7$ nAChR in frontal cortex (Guan et al, 1999). These changes may play a role in the cognitive impairments seen in the disease (Leonard and Freedman, 2006). Moreover, in individuals with SZ, lower cortical glutamate transmission, assessed by *in vivo* imaging, correlates with impaired cognitive performance (Bustillo et al, 2006), and NMDA receptor mRNA expression correlates with the degree of antemortem cognitive deficits (Humphries et al, 1996). Finally, administration of NMDA receptor antagonists induces SZ-like cognitive deficits in both humans (for review, see Javitt, 2007) and animals (Stefani et al, 2003).

Dysfunction of $\alpha 7$ nAChRs and NMDARs in SZ may be caused or exaggerated by disruptions in the kynurenine pathway of tryptophan degradation, which have been repeatedly documented in the disease (Erhardt et al, 2001; Schwarcz et al, 2001; Miller et al, 2006; Sathyaikumar et al, 2011). Specifically, abnormal kynurenine pathway metabolism leads to elevated levels of kynurenic acid (KYNA), an astrocyte-derived (Guillemin et al, 2001), endogenous $\alpha 7$ nAChR (Hilmas et al, 2001) and NMDAR (Alkondon et al, 2011) antagonist. In the cerebral cortex, as elsewhere in the brain, $\alpha 7$ nAChRs are often located presynaptically (Vizi and Lendvai, 1999), where they control the release of neurotransmitters, including glutamate (Konradsson-Geuken et al, 2010; Wu et al, 2010) and ACh (Zmarowski et al, 2009). Endogenous KYNA, functioning as a neuromodulator, regulates these processes bi-directionally (Zmarowski et al, 2009; Wu et al, 2010). Excessive blockade of $\alpha 7$ nAChRs by increased levels of KYNA may therefore be causally involved in the cognitive deficits seen in SZ. Supporting this concept, acute elevations of brain KYNA in adult rodents impair several SZ-like behaviors such as sensorimotor gating (Erhardt et al, 2004), working memory (Chess et al, 2007), contextual learning (Chess et al, 2009; Pocivavsek et al, 2011) and cognitive flexibility (Alexander et al, 2012).

As the gene expression of several kynurenine pathway enzymes is abnormal in SZ (Miller et al, 2006; Wonodi et al, 2011), elevations in brain KYNA levels in the disease may occur already during early development. Moreover, two known risk factors of SZ, i.e. perinatal immune activation (Müller and Schwarz, 2006; Asp et al, 2010) and stress during adolescence (Miura et al, 2011), activate indoleamine-2,3-dioxygenase (IDO), leading to increased formation of L-kynurenine (“kynurenine”), the direct bioprecursor of KYNA (Barry et al, 2009). Based on these findings, we designed the present study to test the

hypothesis that prolonged exposure to kynurenine during a sensitive period of early neurodevelopment would result in long-lasting impairments in cognitive flexibility in adulthood.

EXPERIMENTAL PROCEDURES

2.1 Animals

Wistar rats were maintained on a 12:12h light/dark cycle (lights on at 0600 h) in temperature- and humidity-controlled, AAALAC-approved animal facilities with *ad libitum* access to water. Animals used in behavioral experiments were food-deprived to 85% of their basal weight; all other animals received food *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committees of The Ohio State University and the University of Maryland School of Medicine in accordance with the NIH Guide for Care and Use of Laboratory Animals. Animals used for biochemical studies were obtained pregnant (gestational age: 2 days) from Charles River Laboratories. Animals used for the behavioral experiments were bred in the Ohio State colony. Based on a series of exploratory studies (results not shown here), gravid females received 100 mg/day of L-kynurenine sulfate (99.4% purity; Sai Advantium, Hyderabad, India) in 30 g of wet mash each day beginning on gestational day (GD) 15 and continuing through postnatal day (PD) 21, when pups were weaned. The dam's food intake was monitored each day. If the food presented on the previous day was completely consumed, 5 g of additional wet mash was provided on the following day. The day on which dams gave birth was denoted PD0. On PD2, litters were culled to 9-11 pups to standardize growth rates across all litters. Only male offspring were used in these studies. No obvious differences in maternal care (i.e. nest-building, nursing posture, pup retrieval) were noticed between the kynurenine-treated (devKYN) and control (devCTL) groups. Biochemical measures were taken on PD2 (KYNA levels in forebrain homogenate; devCTL: n = 6; devKYN: n = 6), PD21 (KYNA levels in PFC homogenate; devCTL: n = 7; devKYN: n = 7), and PD56-80 (extracellular KYNA and glutamate in PFC; devCTL: n = 6; devKYN: n = 7). For the biochemical analyses described below, subjects were taken from 4 litters of devCTL and devKYN rats, respectively. A maximum of 2 rats/litter were used in the analyses conducted at each age.

2.2 KYNA determination in tissue homogenate

Animals were euthanized (CO₂) at either PD2 or PD21, and the brain was rapidly removed, frozen on dry ice and stored at -80°C. In animals killed at PD21, the PFC was dissected out promptly after brain removal. On the day of the assay, tissues were thawed and sonicated in ultrapure water (1:5 w/v for PD2 tissue and 1:10, w/v for PD21 tissue). One hundred μ l of the homogenate were acidified with 25 μ l of 6% perchloric acid. After centrifugation (10 min; 12,000 $\times$ g), 20 μ l of the supernatant were applied to a 3- μ m C₁₈ reverse-phase column (80 mm $\times$ 4.6; ESA, Chelmsford, MA), and KYNA was isocratically eluted 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. KYNA was then detected fluorimetrically (excitation: 344 nm, emission: 398 nm; Shibata, 1988). The retention time of KYNA was approximately 7 min.

2.3 Microdialysis

Rats (PD56) were anesthetized with chloral hydrate (360 mg/kg, i.p.) and mounted in a stereotaxic frame. A guide cannula (outer diameter: 0.65 mm) was positioned unilaterally over the PFC (AP: 3.2 mm anterior to bregma, L: $\pm$ 0.6 mm from the midline, DV: 2.0 mm below dura; hemispheres counterbalanced) and secured to the skull with anchor screws and acrylic dental cement. On the next day, a microdialysis probe (CMA/10, membrane length 3.0 mm, Carnegie Medicin, Stockholm, Sweden) was inserted and connected to a

microperfusion pump. The freely moving animals were perfused (1 μ l/min) 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 sample was discarded, and 60-min fractions were collected during the next 4 h (4 fractions). Basal release data were expressed in absolute concentrations and not corrected for recovery from the dialysis probe.

2.4 KYNA and glutamate determination in microdialysate

The concentrations of basal levels of KYNA and glutamate in microdialysate were determined fluorimetrically as described previously (Rassoulpour et al, 2005), using 15 μ l of the sample for each assay when both analytes were measured. Thus, KYNA was quantitated as described for tissue above, and glutamate was determined fluorimetrically after o-phthalaldehyde/2-mercaptoethanol derivatization and gradient elution (excitation 390 nm; emission: 460 nm). The basal efflux data for both KYNA and glutamate were consistent across the collection intervals, not varying by more than 10-12% from interval to interval.

2.5 Attentional set shifting task

2.5.1 Apparatus—Rats were trained to dig in terra cotta pots (4" diameter) for a cereal reward. The outer surface of the pots was covered with a textured cloth, the inside filled with a distinct digging medium, and each pot was scented with a specific odor. The testing chamber consisted of a wooden box (91 cm $\times$ 40.5 cm $\times$ 25.4 cm; L $\times$ W $\times$ H) covered in self-adhesive contact paper, in which one third was divided lengthwise to create two choice chambers.

2.5.2 Training—Habituation for behavioral studies began on PD56, when single-housed rats were started on food restriction. Training began once rats reached a body weight of 85% of pre-habituation values. All training and testing procedures were modeled after those used by Birrell and Brown (2000). For approximately one week prior to testing, rats were habituated to the testing chamber for 20 min/day, with no discriminatory stimuli or rewards available. Starting 3 days prior to testing (i.e. Day 0), the animals were placed in the testing environment for 20 min with a pot filled with pine shavings and baited with several pieces of cereal reward. The next day (Day 1), the rats were trained to dig in the pot to retrieve the reward until they successfully retrieved a reward on 10 consecutive trials. During the inter-trial interval, the rat was separated from the choice chambers by a moveable barrier. The onset of each trial was signaled by the removal of the barrier to allow access to the pots. On Day 2, rats were trained to discriminate between 2 pots that differed across one of the possible stimulus dimensions (odor, texture, and digging medium), until they successfully chose the 'correct' exemplar for 10 consecutive trials under each stimulus dimension.

2.5.3 Testing—On Day 3, rats were tested consecutively in 7 separate discriminations (stages), in which they had to choose the 'correct' pot by discriminating between two exemplars of the relevant dimension. The list of exemplars is presented in Table 1. For each stage of the task, testing continued until the rat reached a criterion of 6 consecutive correct choices. In a simple discrimination (SD), the pots differed in only one of the three stimulus dimensions. In the compound discrimination (CD), the pots differed across two dimensions, one relevant (i.e. odor) and one irrelevant (i.e. texture), but the correct (e.g. jasmine) and incorrect (e.g. gardenia) exemplars within those dimensions were the same as those in the SD. For all reversal stages (REV), the rats had to learn that the previously correct exemplar was now incorrect and vice versa. For the intra-dimensional shift (ID) stage, all new exemplars were introduced, but the same stimulus dimension (i.e. odor) was relevant. For the extra-dimensional shift (ED) stage, all new exemplars were introduced again, but this time the previously irrelevant dimension was now relevant (e.g. texture). The order of stages was the same for all animals, but the stimulus dimensions and pairs of exemplars used for

each rat were counterbalanced across animals such that, within a treatment group, each dimension was represented at each stage of the task. For the first 4 trials of every stage, subjects were allowed to explore the alternate pot following an incorrect choice. These were termed exploration trials. The criterion for advancing from one stage to the next was 6 consecutive correct trials (exploration trials were included in this criterion). In addition to correct choices, the number of errors to criterion was also recorded. An error occurred when the rat digs in the incorrect pot or fails to make a choice in the allotted time (90 sec during exploration trials; 60 sec thereafter). The average time to complete the task was 1.25 hr, with a range of 45 min – 3 hr, depending upon the acquisition rate of the rats for the various stages.

Four treatment groups were trained and tested for performance in the set shifting task. Initially, two groups were tested in order to reveal cognitive deficits as a result of the developmental exposure to elevated KYNA; devCTL + saline (as a control injection for galantamine, see below; $n = 6$) and devKYN + saline ($n = 8$). Another two groups were tested in order to determine the potential pro-cognitive effects of the $\alpha 7$ nAChR positive modulator galantamine (GAL; Sigma, St. Louis, MO; 3.0 mg/kg, i.p., 45 min prior to task onset) (Samochocki et al., 2003; Lopes et al., 2007); devCTL + GAL ($n = 6$) and devKYN + GAL ($n = 8$). As with the biochemistry experiments, the treatment groups in the behavioral studies were designed to distribute the sample variance over multiple litters. Subjects were the progeny from 6 different litters. Each litter contributed to each of the four treatment conditions described above, with no more than 2-3 pups from any litter represented in a single treatment condition.

2.6 Statistical analyses

All data are expressed as the mean $\pm$ S.E.M.

2.6.1 Biochemical experiments—One-way ANOVAs were performed to compare group means of devCTL and devKYN animals at PD2 and PD21. Separate two-way ANOVAs were used to analyze the microdialysis KYNA and glutamate data from adult rats (PD56-80).

2.6.2 Behavioral experiments—An overall three-way ANOVA (STAGE of task as a within-subject factor; GROUP (CTL vs KYN) and DRUG (sal vs GAL) as between-subject factors) was conducted on the number of trials necessary to reach criterion. A parallel three-way ANOVA was conducted on the number of errors committed as animals reached criteria although the stages were limited to REV1 and ED as these were the stages that revealed group differences. In both the correct choice and error analyses, targeted one-way ANOVAs were performed to determine between-drug group effects on a particular stage of the task. In all ANOVAs, the Huynh-Feldt correction was utilized to reduce Type I errors associated with repeated measures ANOVAs (Vasey and Thayer, 1987). In addition, paired samples *t*-tests were performed to compare CD vs. REV1 and ID vs. ED within certain treatment conditions.

RESULTS

3.1 Biochemical measures

Exposure to kynurenine during early development resulted in a significant three-fold increase in forebrain KYNA levels, relative to controls, at PD2 ($F_{1,11} = 14.132$, $P = 0.004$) (Figure 1). This increase was maintained throughout the kynurenine treatment regimen, as KYNA remained elevated (2.5-fold increase) in homogenates of PFC at the onset of weaning at PD21 ($F_{1,13} = 11.648$, $P = 0.005$) (Figure 2).

Microdialysis was used to determine extracellular levels of KYNA (Figure 3, top) and glutamate (Figure 3, bottom) in the PFC in adult animals (PD56-80), well after the termination of the perinatal kynurenine treatment regimen, but at a period within the range devoted to behavioral testing. Absolute levels of basal KYNA and glutamate were analyzed by two-way, repeated measures ANOVA. With regard to KYNA levels, devKYN rats differed from devCTL rats ($F_{1,11} = 27.97$, $P < 0.001$), and the magnitude of this difference varied with the collection interval ($F_{3,33} = 2.95$, $P = 0.047$). A series of one-way ANOVAs comparing devCTL vs devKYN at each hour revealed that the two groups differed at each collection interval (all P 's < 0.05). As for glutamate levels, there were no significant differences between treatment groups (P 's > 0.45). The mean control levels of extracellular KYNA and glutamate were 2.43 ± 0.03 nM and 1.99 ± 0.07 μ M, respectively.

3.2 Performance in an attentional set-shifting task

The reliability of the set-shifting task within the devCTL and devKYN groups can be illustrated by comparing performance in those animals that received saline vehicle injections as controls (Figure 4) vs two groups of animals that did not receive saline injections and were tested 6 months earlier (not used in the main analysis). A focus on the 3 most critical stages (REV1; ID; and ED) of the task revealed very similar performances in the respective groups. The mean ($\pm$ S.E.M.) trials to criterion for devCTL vs devCTL-SAL were; 13 ± 3.2 vs 12 ± 1.7 ; 7 ± 0.8 vs 8 ± 0.8 ; and 9 ± 2.2 vs 13 ± 2.0 in REV1, ID, and ED, respectively (all P 's > 0.05). Likewise, performance was also reliable in the devKYN group. A focus on stages REV1; ID; and ED of the task revealed equivalent performances in the respective groups. The mean ($\pm$ S.E.M.) trials to criterion for devKYN vs devKYN-SAL were; 23 ± 2.3 vs 22 ± 2.1 ; 8 ± 1.0 vs 12 ± 2.2 ; and 20 ± 2.3 vs 23 ± 2.8 in REV1, ID, and ED, respectively (all P 's > 0.05).

Chronic exposure to kynurenine during early development significantly impaired performance in the set-shifting task ($F_{1,24} = 9.959$, $P < 0.001$), but the deficits were isolated to certain stages of the paradigm ($F_{6,144} = 2.692$, $P = 0.017$; Figure 4). The source of this impairment was revealed by subsequent one-way ANOVAs, comparing each stage of the task between the two experimental groups (treated with the saline vehicle as adults). Compared with devCTL + saline rats, devKYN + saline animals showed the same rule acquisition (SD or CD) and intra-dimensional shifting (ID) (all P values > 0.05), indicating that perinatal exposure to kynurenine did not cause generalized deficits in motivation, discriminatory abilities or rule learning. As anticipated, both groups required more trials to complete REV1 than CD, and this difference reached significance in the devKYN animals ($t_7 = -5.345$, $P = 0.001$). Furthermore, compared to controls, devKYN animals showed a significant impairment in the initial reversal learning (REV1, $F_{1,13} = 13.966$, $P = 0.003$), but not in subsequent reversal stages (REV2, REV3; both P 's > 0.3). Compared to controls, early developmental kynurenine administration also caused impairments in the ability to learn an ED ($F_{1,13} = 6.225$, $P = 0.028$).

Acute, systemic galantamine (GAL) administration (3.0 mg/kg, i.p.) completely restored performance in devKYN + GAL animals to levels observed in the devCTL + GAL group (Figure 5). Thus, devKYN + GAL animals were not impaired in rule acquisition (SD, CD) or ID shifting (all P values > 0.05) when compared to devCTL + GAL rats. However, unlike the devKYN group (see Figure 4), reversal learning in devKYN + GAL animals was similar to that seen in the devCTL + GAL group in REV1 ($F_{1,13} = 0.448$, $P = 0.516$). Also, GAL administration restored performance in the ED stage to levels seen in the devCTL + GAL group ($F_{1,13} = 0.022$, $P = 0.885$). Finally, GAL administration had no facilitative effect on task performance in control animals. Visual comparison of Figures 4 and 5 indicates that devCTL + GAL animals performed similar to animals in the devCTL + SAL group in all stages of the task.

The mean ($\pm$ S.E.M.) number of errors committed by each group of rats during the REV1 and ED stages of the task is depicted in Figure 6. These two stages were selected as the performance analyses above revealed significant group differences in trials to acquisition. The number of errors differed as a function of stage of test ($F_{1,25} = 16.145$, $P < 0.001$) and kynurenine-treatment ($F_{1,25} = 15.741$, $P < 0.001$). As indicated in Figure 6, and consistent with the correct choice data shown in Figure 4, the devKYN + SAL group produced more errors than the devCTL + SAL group in the REV1 ($F_{1,13} = 10.078$, $P = 0.008$) and ED ($F_{1,13} = 5.477$, $P = 0.037$) stages of the task.

DISCUSSION

The present experiments determined the effects of early developmental exposure to kynurenine on brain KYNA levels and on performance in an attentional set-shifting task. Several novel findings can be reported. First, early treatment with the bioprecursor kynurenine (from GD15 – PD21) resulted in elevations of prefrontal KYNA as early as PD2, and these increases persisted into adulthood, long after kynurenine exposure had ceased. Second, as adults, rats that were exposed perinatally to kynurenine exhibited deficits in both initial reversal learning (REV1) and ED shifting, leaving rule acquisition and ID shifting intact. Finally, acute systemic administration of GAL, prior to task onset, normalized performance of kynurenine-treated rats during the REV1 and ED stages of the task. The discussion that follows focuses on several issues related to the interpretation and implications of these results, including: a) potential neuronal mechanisms underlying the enduring impairments in cognitive flexibility, b) the degree to which the cognitive deficits following early exposure to kynurenine are regionally or behaviorally selective, as well as age-dependent, c) the ability of acute GAL to reverse deficits in set-shifting behavior, and d) the validity of early exposure to kynurenine as a novel, naturalistic model of cognitive deficits in SZ.

4.1 Neural mechanisms mediating reversal learning and extra-dimensional shifts

Insights into the mechanisms contributing to the enduring performance deficits in rats exposed to elevated KYNA during development may emerge from consideration of the neural processes that mediate reversal and ED deficits in intact rats or in animals acutely treated with neuroactive drugs. The literature suggests a dissociation between the ventromedial regions of the PFC (mPFC) and the orbitofrontal cortex (OFC) in mediating the two task components (REV, ED) that were affected by prolonged elevations in KYNA. Thus, the OFC is necessary for the expression of reversal learning, whereas the mPFC is critical for ED shifting (McAlonan and Brown, 2003; Ghods-Sharifi et al, 2008; Rygula et al, 2010). Decreases in cortical cholinergic transmission impair serial reversal learning (Cabrera et al, 2006) and may be central to the deficits described here. In this regard, we recently reported that intra-mPFC infusions of the cholinergic receptor antagonists mecamylamine or scopolamine led to deficits in the REV1, but not the ED stages of the task (Brooks et al, 2010). Whether this mediation of reversal learning by cholinergic transmission is due to the mPFC or reflects connections between the mPFC and the OFC remains to be resolved. Notably, acute elevations of cortical KYNA are associated with a trend toward decreased basal levels of prefrontal ACh and a marked attenuation of evoked increases in ACh levels (Zmarowski et al, 2009). We are currently determining the effect of prolonged elevations of KYNA on basal and stimulated levels of ACh in the PFC.

Available evidence also suggests an important role for prefrontal glutamatergic transmission in the expression of ED. Collectively, the results indicate that NMDA receptor activation is obligatory for such performance (Stefani et al, 2003; Pedersen et al, 2009; Brooks et al, 2010; Dalton et al, 2011). In the present study, developmental exposure to kynurenine produced enduring, modest increases in cortical KYNA but did not affect basal levels of

glutamate in the PFC in adulthood (Figure 3). However, we recently reported that adult animals that had been treated with kynurenine perinatally are markedly impaired in their capacity to evoke cortical glutamate release following mesolimbic stimulation (Alexander et al, 2011). This attenuation of stimulated prefrontal glutamate release and the subsequent reduction in NMDA receptor activity may contribute to the set-shifting deficits observed in the present study.

Finally, perinatal elevations of KYNA, due to the subsequent reduction in $\alpha 7$ nAChR-mediated transmitter release during critical periods of development, may disrupt several more general neurodevelopmental processes, including: 1) activity-dependent competition for synaptic space (Hua et al, 2005), 2) spine density in PFC (Drakew et al, 1999), 3) the extent of pruning of synaptic contacts in PFC/OFC (Teicher et al, 2003), and 4) loss of synchrony of neuronal oscillations in areas critical to the performance of the set-shifting task (Uhlhaas and Singer, 2011). Alone or collectively, these neurodevelopmental changes may result in significant dysregulations in the maturation of distributed neural subcortical-cortical circuits that may ultimately contribute to deficits in cognitive flexibility.

4.2 Regional, behavioral, and age-dependent selectivity of the effects of elevated KYNA

The long-lasting neurochemical and behavioral effects of developmentally elevated brain KYNA levels are not limited to prefrontal transmission or cognitive flexibility. Using the same protocol as described here, we recently demonstrated that perinatal exposure to kynurenine produced elevations in extracellular levels of KYNA in the hippocampus at PD56-80 (Pocivavsek et al, 2012). In contrast to the present results from the PFC, however, these rats also showed a significant reduction in extracellular hippocampal glutamate levels as adults. As in the present study, perinatal elevations of KYNA were associated with persistent cognitive deficits. Thus, a group of devKYN-treated rats, tested as part of the same set of experiments, exhibited deficits in two hippocampally-mediated behaviors, i.e. passive avoidance and acquisition of a spatial memory (Pocivavsek et al, 2012).

The timing of kynurenine treatment used here was designed to span a rather broad pre- and postnatal period, comprising developmental stages where perturbations are known to significantly affect cognitive functions in adults (Peterschmitt et al, 2007; Marquis et al, 2008; Alexander et al, 2009; Brady et al, 2010; Brooks et al, 2011; Pocivavsek et al, 2012). In follow-up studies based on the present results, we are currently evaluating whether exposure to kynurenine, and hence elevation of brain KYNA levels, during more restricted periods of early development, or extended treatments during adulthood, would have similarly enduring, adverse effects on cognitive processes. Notably, intermittent systemic kynurenine injections in adolescent rats cause persistent impairments in contextual fear memory and novel object recognition (Akagbosu et al, 2010) as well as deficits in social behavior (Trecartin and Bucci, 2011).

4.3 Reinstatement of performance by galantamine: the role of $\alpha 7$ nicotinic receptors

In our perinatally kynurenine-treated animals, deficits in REV1 and ED performance were eliminated (Figure 4) following an acute systemic injection of GAL (Lopes et al, 2007). Notably, the same acute GAL treatment also eliminates set-shifting deficits that are elicited by a single systemic kynurenine injection in adult rats (Alexander et al, 2012). Although GAL also inhibits the activity of acetylcholinesterase (Lillienfeld, 2002), pharmacokinetic considerations (Geerts et al., 2005) and *in vitro* studies (Lopes et al., 2007) suggest that these effects probably reflect the drug's ability to positively modulate $\alpha 7$ nAChR function at an allosteric potentiating site of the receptor. In line with this interpretation, the adverse effects of an acute systemic kynurenine application on cognition are also readily neutralized by the $\alpha 7$ nAChR partial agonist SSR180711 (Pershing et al., 2011). Taken together with

other reports (Hilmas et al., 2001; Wu et al., 2010), these studies confirm the relevance of $\alpha 7$ nAChRs as a preferred target of KYNA *in vivo*. As KYNA levels remain elevated long after the cessation of perinatal kynurenine exposure (Pocivavsek et al, 2012 and Figure 3), this treatment may cause ongoing abnormalities not only in the PFC but throughout the neuronal circuits that are involved in the modulation of set-shifting behavior (see Section 4.1 above). In addition to neutralizing the effects of abnormally high KYNA levels within the PFC, acute GAL treatment, as used in the present study, might therefore also normalize chemical disparities in a distributed neural system that had been dysregulated by prolonged, negative modulation of $\alpha 7$ nAChRs. Consequently, current studies in our laboratories are designed to test, for example, whether perinatal kynurenine exposure causes long-lasting reductions in extracellular glutamate, ACh and dopamine in various components of the neural circuit involved. We would then examine if these persistent neurochemical changes, too, are reversible by an acute administration of GAL.

4.4 Validity of developmental elevations of KYNA as a model of cognitive deficits in SZ

Perinatal elevation of brain KYNA provides an animal model for the cognitive deficits seen in individuals with SZ. This model has significant face, construct and predictive validity. Leading developmental hypotheses of SZ suggest that genetic polymorphisms (for review see Weinberger, 2005), together with immunological and other environmental risk factors (Meyer et al., 2009; Brown and Patterson, 2011), alter the functional maturation of the brain. These factors may also contribute toward increased KYNA levels (Erhardt et al, 2001; Schwarcz et al, 2001; Wonodi et al, 2011; Holtze et al, 2012). Elevations in KYNA and the ensuing antagonism of $\alpha 7$ nAChRs can then be readily envisioned to exacerbate dysregulations in cholinergic (Guan et al, 1999; Crook et al, 2001), glutamatergic (Bustillo et al, 2011; Corti et al, 2011) and dopaminergic (Laruelle et al, 1999; Abi-Dargham et al, 2002) transmission and thus aggravate pathology. The resulting deficits in reversal learning and ED performance closely resemble impairments seen in patients with SZ (Tyson et al, 2004; Pantelis et al, 2009). Finally, the beneficial effects of GAL in this animal model parallel recent clinical findings that $\alpha 7$ nAChR agonists exhibit modest pro-cognitive effects in patients with SZ (Schubert et al, 2006; Buchanan et al, 2008). Collectively, these observations support the heuristic value of experimental elevations in brain KYNA levels before weaning as a novel and valid animal model of the cognitive deficits seen in SZ.

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ABBREVIATIONS

7nAChR	alpha7 nicotinic acetylcholine receptor
ACh	acetylcholine
CD	compound discrimination
devCTL	developmental control treatment
devKYN	developmental kynurenine treatment
ED	extra-dimensional shift
GAL	galantamine treatment

GD	gestational day
ID	intra-dimensional shift
IDO	indoleamine-2,3-dioxygenase
KYNA	kynurenic acid
NMDA	N-methyl-D-aspartic acid
NMDAR	NMDA receptor
OFC	orbitofrontal cortex
PD	postnatal day
PFC	prefrontal cortex
REV	reversal
SD	simple discrimination
SZ	schizophrenia

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HIGHLIGHTS FROM THIS MANUSCRIPT

- A novel neurodevelopmental animal model of schizophrenia is presented
- Developmental kynurenine treatment results in elevated KYNA levels into adulthood
- Adults with elevated KYNA levels during development exhibit deficits in cognitive flexibility
- These cognitive deficits are ameliorated by acute treatment with galantamine

Figure 1.

Effects of pre- and postnatal exposure to kynurenine on forebrain tissue levels of KYNA at PD2. Dams were exposed to kynurenine (100 mg/day) as a component of a wet chow mash from GD15 to weaning on PD21 (devKYN). Control animals were fed a standard wet mash without kynurenine (devCTL). Male progeny ($n = 6$ per group) were euthanized at PD2. KYNA levels are expressed as the mean $\pm$ S.E.M. ^a = $P < 0.05$ vs. devCTL.

Figure 2.

Effects of pre- and postnatal exposure to kynurenine on KYNA levels in the prefrontal cortex at PD21. Dams were exposed to kynurenine (100 mg/day) as a component of a wet chow mash from GD15 to weaning on PD21 (devKYN). Control animals were fed a standard wet mash without kynurenine (devCTL). Male progeny ($n = 7$ per group) were euthanized at PD21. KYNA levels are expressed as the mean $\pm$ S.E.M. ^a = $P < 0.05$ vs. devCTL.

Figure 3.

Effects of pre- and postnatal exposure to kynurenine on basal extracellular levels (mean $\pm$ S.E.M.) of KYNA (top) and glutamate (bottom) in the prefrontal cortex in adult rats (PD56-80). Dams were exposed to kynurenine (100 mg/day) as a component of a wet chow mash from GD15 to weaning on PD21. Control animals were fed a standard wet mash without kynurenine. All rats were fed standard laboratory chow pellets from the time of weaning until the microdialysis studies were conducted between PD56 and PD80. See text for absolute basal KYNA and glutamate levels in devCTL ($n=6$) and devKYN animals ($n=7$) of the levels seen in devCTL rats ($n=6$). **a** = $P < 0.05$ vs. devCTL.

Figure 4.

Mean trials to criterion ($\pm$ S.E.M.) for adult rats (PD56-80) in various stages of the attentional set-shifting task. Animals were derived from two treatment conditions: devCTL + SAL (saline; $n = 6$) and devKYN + SAL ($n = 8$). Saline was administered as a vehicle solution for subsequent groups that received galantamine as a potential cognition-enhancer. Both groups of rats readily acquired the single (SD) and compound (CD) discriminations but required more trials to learn the initial reversal (REV1). The devKYN + SAL group required more trials than the devCTL + SAL group to acquire REV1. Each group demonstrated comparable abilities to form an attentional set, as evidenced by the rapid acquisition of an intra-dimensional shift (ID) to a novel stimulus. DevKYN rats exhibited a marked deficit in the ability to make an extra-dimensional shift (ED). ^a = significantly different, within treatment group, from the trials to criterion for the CD stage; ^b = significantly different, within the REV1 stage, from the devCTL + SAL group; ^c = significantly different, within treatment group, from the trials to criterion for the ID stage; ^d = significantly different, within the ED stage, from the devCTL + SAL group (all P values < 0.05).

Figure 5.

Mean trials to criterion ($\pm$ S.E.M.) for adult rats (PD56-80) in various stages of the attentional set-shifting task. The pro-cognitive effects of galantamine (GAL) were assessed in two treatment groups: devCTL+ GAL ($n = 6$) and devKYN + GAL ($n = 8$). GAL (3 mg/kg, i.p.) was injected 45 min prior to the onset of the task. Both groups of rats readily acquired the single (SD) and compound (CD) discriminations but required more trials to learn the initial reversal (REV1). The devKYN + SAL group required more trials than the devCTL + SAL group to acquire REV1 (Fig. 4). This difference was eliminated following the administration of GAL (Fig. 5). ^a = significantly different, within treatment group, from the respective trials to criterion for the CD stage; ^b = significantly different, within treatment group, from the trials to criterion for the ID stage (all P values < 0.05).

Figure 6.

Mean errors to criterion ($\pm$ S.E.M.) for adult rats (PD56-80) in select stages of the attentional set-shifting task. The focus was on the REV1 and ED stages as these were the stages that revealed significant group differences in Figure 4. The error analysis paralleled the effects shown in Figure 4; namely that the number of errors made in the run to criterion was significantly higher in the devKYN + SAL group, during REV1 and ED, than any of the remaining 3 groups. ^a = devKYN + SAL different from any other group in that particular stage (both P values < 0.05).

Table 1

Perceptual dimensions (odor, digging medium, texture of pot) and specific stimuli used in the attentional set-shifting task. Different stimuli were used for training and testing. Each testing session utilized three pairs of digging pots that were used for specific clusters of stages in the task. One pair was used for the single discrimination (SD), compound discrimination (CD), and the first reversal (REV1). Another pair was used for the intra-dimensional shift (ID) and second reversal (REV2). A third pair was used for the extra-dimensional shift (ED) and the third reversal (REV3). The assignment of these pairs of stimuli to stage clusters was randomized among the treatment groups.

Dimension	Training Set	Pair 1	Pair 2	Pair 3
Odor	Lavender Raspberry	Cinnamon Patchouli	Gardenia Jasmine	Rose Lilac
Medium	White paper Green paper	Light foam Dark foam	Plastic beads Metallic beads	Plastic buttons Gold buttons
Texture	Paper Parafilm	Velour fabric Cotton fabric	Felt fabric Cotton fabric	Yarn Cotton fabric