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The Effect of Transient Increases in Kynurenic Acid and Quinolinic Acid Levels Early in Life on Behavior in Adulthood: Implications for Schizophrenia

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Abstract

Kynurenic acid is a tryptophan metabolite that is synthesized and released in the brain by astrocytes and acts as an antagonist of nicotinic acetylcholine receptors and N-methyl-D-aspartate glutamate receptors, both of which are critically involved in cognition as well as neural plasticity and brain development. The concentration of kynurenic acid is increased in the brains of persons with schizophrenia and this increase has been implicated in the cognitive and social impairments associated with the disease. In addition, growing evidence suggests that the increase in kynurenic acid may begin early in life. For example, exposure to influenza A virus during development results in a transient increase in kynurenic acid concentration that could disrupt normal brain development and lead to cognitive deficits later in life. Changes in kynurenic acid may thus provide a link between developmental exposure to viruses and the increased risk of subsequently developing schizophrenia. To test this, we mimicked the effects of influenza A exposure by treating rats with kynurenine, the precursor of kynurenic acid, on postnatal days 7-10. We observed a transient increase in both kynurenic acid and quinolinic acid during treatment. When rats were subsequently behaviorally tested as adults, those previously treated with kynurenine exhibited decreased social behavior and locomotor activity. In contrast, attentional function and fear conditioning were not affected. Together with other recent findings, these findings have several implications for understanding how viral-induced changes in tryptophan metabolism during development may contribute to schizophrenia-related symptoms later in life.

Keywords

schizophrenia; glia; attention; learning; memory; social

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1. Introduction

Kynurenic acid (KYNA), a final product of tryptophan metabolism, is synthesized and released in the brain by astrocytes (Schwarcz & Pellicciari, 2002) and acts as an endogenous antagonist of α7 nicotinic acetylcholine receptors (α7-nACh-Rs) and N-methyl-D-aspartate (NMDA) glutamate receptors (Hilmas et al., 2001; Parsons et al., 1997; Pereira et al., 2002; Stone, 1993). The concentrations of KYNA and its precursor kynurenine are significantly increased in the brains and cerebral spinal fluid of persons with schizophrenia (Erhardt et al., 2001; Linderholm et al., 2012; Schwarcz et al., 2001) and experimentally-induced elevations in KYNA concentration in adult rats reproduce the impairments in social behavior, attentional function, and contextual memory associated with this disease (Chess and Bucci, 2006; Chess et al., 2007, 2009; Erhardt et al., 2004; Geyer et al., 2001; Sams-Dodd, 1999; Shepard et al., 2003; Silver et al, 2003; Waters et al., 2004). Growing evidence now suggests that the increase in KYNA concentration in schizophrenia may begin early in life. For example, there is evidence that exposing mice to viruses during development can induce changes in KYNA levels in neonates. Indeed, exposure to influenza A virus on postnatal day (PND) 3 or 4 (which is comparable to late 2nd/early 3rd trimester in humans, Rice and Barone, 2000) produces a transient increase in KYNA concentration one week later (Asp et al., 2010; Holtze et al., 2008). In addition, genetic studies in humans have identified mutations associated with schizophrenia that result in increased KYNA levels, potentially at a very early age (Miller et al., 2004, 2006, 2008, 2009). These findings are significant because both α7-nACh-Rs and NMDA-Rs are critically involved in cognitive function and neural plasticity, as well as neural development (Bast et al., 2003; Broide and Leslie, 1999; Komuro and Rakic, 1993; Levin, 2002). Thus, early increases in KYNA concentration could disrupt normal brain development and lead to cognitive deficits later in life.

Consistent with this hypothesis, recent studies have shown that treating rats with kynurenine throughout adolescence (PND 27-53) increases KYNA levels during the treatment period, and produces memory deficits and decreased social behavior when rats are tested as adults, despite having normal KYNA levels at the time of behavioral testing (Akagbosu et al., 2012; Trecartin & Bucci, 2011). Similarly, treating rat dams with kynurenine-enriched food from gestational day 15 to PND 21 increased KYNA concentration in the offspring, which later exhibited impaired attention and memory as adults (Alexander et al., 2013; Pocivavsek et al., 2012). However, little research has focused on the long-term behavioral effects of increased KYNA concentration at earlier and more circumscribed time periods of development, as occurs following exposure to influenza A in mice. Indeed, it may be that the brain is particularly sensitive to increased KYNA levels at a specific time during development. Determining the effects of early exposure to KYNA is particularly important because developmental exposure to viruses has long been associated with an increased risk of subsequently developing schizophrenia (Karlsson, 2003; Yolken and Torrey, 2008), yet the underlying mechanism has remained unclear. Moreover, previous studies have revealed that a reduced immune response in persons with schizophrenia is associated with an increase in KYNA levels (Müller and Schwarz, 2010). An increase in KYNA levels at early ages may thus provide a significant link between viral exposure and subsequent cognitive dysfunction.

In the only published behavioral studies to date, Asp et al. (2009, 2010) exposed wild-type and immuno-compromised mice to influenza A on PND 3 or 4 and observed deficits in sensory gating and working memory in adulthood that were reminiscent of impairments observed in schizophrenia (Geyer et al., 2001; Silver et al., 2003), but only in the adult mice that were immuno-compromised. Although these data suggest that a viral-induced increase in KYNA concentration may not be sufficient to impact behavior in adults with normal

immune functioning, there are several unresolved questions. For example, the studies by Asp and colleagues (2009, 2010) were conducted in mice while most other studies of developmental KYNA exposure and behavior have been carried out in rats (Akagbosu et al., 2012; Trecartin & Bucci, 2011; Alexander et al., 2013; Pocivavsek et al., 2012), making it difficult to draw firm conclusions because of potential species differences as well as strain-specific differences in behavior in mice (e.g., Falls et al., 1997). In addition, not all of the cognitive and behavioral symptoms of schizophrenia are modulated by KYNA exposure, and the effects have also been shown to depend on age of treatment (Akagbosu et al., 2012; Chess et al., 2009; Trecartin and Bucci, 2011). Thus, it remains unclear whether neonatal KYNA exposure affects other types of behavior and cognitive function.

To address these issues, we treated neonatal rats with 100mg/kg of kynurenine (or vehicle solution) on PND 7-10 to mimic the effects of influenza exposure on KYNA levels. This dose of kynurenine results in a 2-3 fold increase in KYNA concentration in juvenile and adult rats (Akagbosu et al., 2012; Erhardt et al., 2004), mirroring the magnitude of the increase observed in schizophrenia (Erhardt et al., 2001; Schwarcz et al., 2001). Using kynurenine to manipulate KYNA concentration also has high translational value since an increase in kynurenine level is a primary factor leading to the increased KYNA concentration associated with schizophrenia (Miller et al., 2006; Linderholm et al., 2012). Upon reaching adulthood, rats were tested in a fear conditioning task and a social interaction paradigm previously shown to be sensitive to kynurenine treatment during adolescence (Chess et al., 2009; Trecartin and Bucci, 2011). Another set of rats was tested in an attentional orienting procedure that has features in common with the sensory gating paradigm used by Asp et al. (2010). We also measured the concentrations of KYNA and quinolinic acid (QUIN, another product of tryptophan/kynurenine metabolism) during kynurenine treatment as well as at the end of behavioral testing.

2. Materials and Methods

2.1. Subjects

Seven pregnant Long-Evans rats (~4 months old) were obtained from Harlan Laboratories (Indianapolis, IN) and maintained in individual cages on a 14:10 light-dark cycle with food (Purina standard rat chow; Nestle Purina, St. Louis, MO) and water available ad libitum throughout pregnancy and after giving birth. Pups were maintained on the same light-dark cycle and had free access to food and water throughout the experiment. At 25 days of age, the pups were weaned and group-housed (3-4 rats/cage) for the remainder of the study. All procedures were approved by the Dartmouth College Institutional Animal Care and Use Committee and carried out according to AAALAC guidelines.

2.2. Drug preparation

L-kynurenine (L-KYN; Sigma, St Louis, MO) was prepared fresh daily as described previously (Akagbosu et al., 2012; Chess et al., 2009).

2.3. Treatment regimen

On PND 7-10, an equal number of pups from each of the litters received daily intraperitoneal (i.p.) injections of either L-KYN (100 mg/kg; 2 ml/kg) or a comparable volume of 0.1 M HEPES buffer (vehicle, pH 7.0). In total, 22 pups were treated with L-KYN and another set 22 pups were treated with vehicle.

2.4. Experimental design

Two hours after the injection of L-KYN (or vehicle) on PND 10, four rats from each treatment condition were sacrificed to determine the concentrations of KYNA and QUIN

resulting from LKYN treatment. Rats were euthanized using isofluorane followed by rapid decapitation and brains were rapidly dissected and frozen on dry ice for analysis using high-performance liquid chromatography (HPLC; described below). The remaining 18 rats in each treatment condition were maintained until they reached adulthood and began behavioral testing at age 70 days.

One cohort of adult rats (10/group) was tested in the fear conditioning and social interaction tasks described below. A second cohort of adult rats (8/group) was tested in the attentional orienting procedure. After the last day of behavioral testing, 4 rats in each of the drug treatments conditions were sacrificed and brains processed to determine the concentrations of KYNA and QUIN at the time of behavioral testing as adults.

2.5. Behavioral apparatus, procedure, and observations

2.5.1. Social behavior and locomotor activity—Social behavior and locomotor activity were assessed as described previously (File, 1980; Hopkins et al., 2009; Trecartin and Bucci, 2011). Briefly, rats were individually placed in a white plastic tub containing an unfamiliar (target) rat in a restraint tube and allowed to explore for 10 minutes. The number of interactions with the target rat (placing the nose inside the restrainer) was recorded as measure of social behavior. In addition, the number of times the rat crossed perpendicular lines that were superimposed on the video image of the tub were counted and served as a measure of general locomotor activity.

2.5.2. Fear conditioning—Rats were trained in a standard fear conditioning task as described previously (Akagbosu et al., 2012; Keene & Bucci, 2008). Briefly, rats were placed in individual operant conditioning chambers and presented with 3 tone-shock pairings during the acquisition session. Twenty-four hours later, contextual fear memory was assessed by returning the rats to the chambers and measuring freezing behavior, which served as the indicator of conditioned fear (no shocks or tones were presented). Tone-specific fear memory was subsequently tested by placing the rats in a different chamber and replaying the tone (no shocks delivered).

2.5.3. Attentional orienting behavior—As described previously (Bucci and Burwell, 2004; Keene and Bucci, 2008), rats were placed in individual operant conditioning chambers and received 12 non-reinforced presentations of a light (10 sec in duration). Normal rats typically rear up on their hind legs and orient toward the visual stimulus (Holland, 1977, 1984), which is often-used indicator of attentional processing (Gallagher et al., 1990; Kaye and Pearce, 1984; Lang et al., 1997). Rearing behavior rapidly decreases (habituates) when the cue is not followed by reinforcement, reflecting a decrease in attention to a behaviorally irrelevant stimulus (Gallagher et al., 1990; Holland, 1997; Kaye & Pearce, 1984).

2.6. Behavioral Data Analyses

Analyses of the behavioral data were carried out as described previously (Keene and Bucci, 2008; Akagbosu et al., 2012; Trecartin and Bucci, 2011). Briefly, the number of social interactions and line crossings exhibited by rats previously treated with vehicle or L-KYN were analyzed using an independent samples t-test. For the fear conditioning task, freezing behavior during the training session, context test session, and tone test session was analyzed using a repeated measures analysis of variance (ANOVA) with Group (Vehicle, L-KYN) as the between subjects variable. For the acquisition session and tone test session, Trial was used as the within subjects variable. For the context test session data, freezing was measured in 64-second blocks and Block served as the within-subjects variable. In the attentional orienting task, the number of rears was analyzed using a repeated measures ANOVA with Group as the between subjects variable and Blocks of 4-trials as the within subjects variable.

2.7 Biochemistry

Whole brain tissue (minus the cerebellum) was used to determine the levels of KYNA and QUIN following L-KYN treatment.

2.7.1. Determination of KYNA concentration—The concentration of KYNA was determined using HPLC as described previously (Akagbosu et al., 2012) and group differences analyzed using an independent samples t-test.

2.7.2. Determination of QUIN concentration—QUIN levels in brain homogenate were quantified by electron- capture negative chemical ionization gas chromatography using a minor modification of the procedure described by Heyes and Markey (1988). The modifications included a different internal standard ($^{13}C_7$)-QUIN and precipitation with perchloric acid followed by liquid /liquid extraction using prepurified ethyl acetate. This extract was evaporated in a vacuum centrifuge and the QUIN and ($^{13}C_7$)-QUIN esterified to their dihexafluoroisopropanol esters. After extraction into heptane, the samples were quantified using the molecular ions at m/z 467 from QUIN and m/z 474 from ($^{13}C_7$)-QUIN. Intra- and inter-assay variations at plasma concentrations of 240, 600, and 1200 pmol/mL were <6% and <7%, respectively. The lower limit of quantification was ~20 pmol/mL (CV = 10.4%, n=5). Group differences in QUIN concentration were analyzed using an independent samples t-test.

3. Results

3.1. KYNA and QUIN Concentration

The concentrations of KYNA and QUIN measured after the L-KYN injection on PND 10 and after behavioral testing on PND 75 are shown in Table 1. On PND 10, there was significant increase in the level of KYNA [$t(6)=4.3$, $p<0.005$)] and in the level of QUIN [$t(6)=2.6$, $p<0.04$] in the brains of rats treated with L-KYN compared to vehicle-treated controls. After behavioral testing, the concentrations of KYNA and QUIN did not differ significantly between L-KYN-treated and vehicle-treated rats ($p=0.28$, $p=0.09$, respectively).

3.2. Social interaction and locomotor activity

The number of social interactions exhibited by adult rats in each group is shown in Figure 1A. Compared to rats that were previously treated with vehicle on PND 7-10, those treated with L-KYN displayed significantly fewer social interactions with an unfamiliar rat [$t(17)=2.3$, $p<0.04$]. In addition, rats treated L-KYN exhibited a marginally significant decrease in the number of lines crossed while exploring the test chamber [$t(17)=2.2$, $p=0.05$], as shown in Figure 1B. There was no significant correlation between the number of social interactions and the line crossings in the data set as a whole ($r^2=0.1$, $p=0.2$) or in the individual groups (Vehicle: $r^2=0.003$, $p=0.9$; L-KYN, $r^2=0.05$, $p=0.4$).

3.3. Fear conditioning

As summarized in Figure 2, freezing behavior was comparable in adult rats that had been previously treated with vehicle or L-KYN as neonates. There were no significant group differences in post-shock freezing during the acquisition session ($p=0.8$) and no significant differences during the context ($p=0.9$) or tone test sessions ($t=0.4$).

3.4. Attentional orienting behavior

Orienting behavior during presentations of a non-reinforced visual cue is shown in Figure 3. A repeated measures ANOVA revealed a significant main effect of Block [$F(2,28)=9.9$,

p<0.001], indicating that the amount of rearing behavior decreased across trials. There was no significant main effect of Group (p=0.3) and no significant Group X Block interaction (p=0.5), indicating that rats previously treated with vehicle or L-KYN exhibited comparable patterns of orienting behavior and habituation.

4. Discussion

The present study was designed to examine how a neonatal increase in KYNA concentration, similar to that which occurs following an influenza A infection (Holtze et al., 2008), effects cognition and social behavior later in adulthood in rats. Administering L-KYN to rats on PND 7-10 mimicked the effects of neonatal exposure to influenza A by transiently elevating the concentration of KYNA in whole-brain tissue (Asp et al., 2010; Holtze et al., 2008). Indeed, the concentration of KYNA measured on PND 10 was ~3X higher in rats that were treated with L-KYN compared to vehicle-treated control rats, mirroring the magnitude of the increase observed following viral exposure (Asp et al., 2010). When rats were subsequently tested as adults, a number of behavioral measures were impacted in rats that had previously been treated with L-KYN. These findings have several implications for understanding how viral-induced changes in tryptophan metabolism during development may contribute to schizophrenia-related symptoms later in life.

First, the results indicate that cognition and social behavior in adulthood are not uniformly affected by exposure to increased KYNA during PND 7-10. For example, attentional orienting behavior was unaffected in L-KYN-treated rats, a finding that is consistent with the observation of normal sensory gating in wild-type mice exposed to influenza A as neonates (Asp et al. 2010). In contrast, we observed fewer social interactions in rats previously exposed to L-KYN. Together with our previous finding that social behavior was decreased following treatment with L-KYN during adolescence, but not by L-KYN treatment during adulthood (Trecartin and Bucci, 2011), these data suggest that social behavior is particularly sensitive to changes in the level of KYNA during development. Still other types of behavior, such as conditioned freezing (i.e., fear learning/memory), were unaffected by neonatal treatment (present study) but were instead found to be sensitive to L-KYN treatment during adolescence (Akagbosu et al., 2012) and adulthood (Chess et al., 2009). Collectively, as illustrated in Table 2, these findings indicate that the effects of L-KYN treatment on behavior in adult rats depends on the age at which L-KYN is administered as well as the behavioral domain that is being assessed by a particular task. Although the basis of these age and task-dependent effects is currently unknown, they may reflect differences in the maturation of the brain systems that underlie specific functions (e.g., hippocampus and contextual memory; prefrontal cortex and attention) and/or region-specific differences in the distribution of α7-nACh-Rs and NMDA receptors during development.

We also found that locomotor behavior in adults was impacted by treatment with L-KYN on PND 7-10, as evidenced by a decrease in line crossings in the social interaction chamber. Although it is possible that the decrease in locomotor behavior contributed to the reduction in social interactions exhibited by rats previously treated with L-KYN in the present study, several observations suggest that is unlikely. For instance, the correlation between the number of social interactions and line crossings was not statistically significant. In addition, freezing behavior during the fear conditioning task, which could also be affected by a basal change in locomotor activity, was not different between the vehicle-treated rats and L-KYN-treated rats. Regardless, the decrease in locomotor activity observed here provides additional evidence that behavioral changes following L-KYN treatment depend on the time of intervention. Indeed, there was no change in locomotor behavior in adult rats that had been treated with L-KYN during adolescence or adulthood (Akagbosu et al., 2012).

Another explanation for the observed decreases in social interaction and locomotor behavior is that they were due to changes in tryptophan metabolites other than KYNA. An additional new finding in the current study was that the QUIN concentration was also increased by L-KYN administration on PND 10. This is consistent with the observation that levels of the transcripts of the biosynthetic enzymes leading to QUIN production are increased in mice treated with influenza A (Holtze et al., 2008). Like KYNA, QUIN is also a downstream metabolic product of kynurenine in the tryptophan degradation pathway. QUIN acts as an NMDA agonist (Stone and Perkins, 1981) and it has been previously shown that intracerebroventricular infusions of QUIN reduce social interaction and locomotor behavior (Lapin et al., 1996). Interestingly, L-KYN during adulthood does not have a significant effect on QUIN levels (Shepard et al., 2003) nor did it affect locomotor behavior (Akagbosu et al., 2012), further suggesting that the changes in locomotor behavior observed following neonatal exposure to L-KYN may be due to changes in QUIN concentration.

Moreover, the quantity of NMDA-Rs in the brain has been shown to reach adult levels by embryonic day 19 (Sanchez et al., 2010), whereas the development of nACh-Rs is more protracted and peak levels are not observed until the 3rd postnatal week (Adams, 2003). Thus, the behavioral effects of increased KYNA and/or QUIN concentration following flu exposure (Holtze et al., 2008; Asp et al., 2009, 2010), or following treatment with L-KYN on PND 7-10, are likely mediated more by NMDA-Rs than nACh-Rs. In contrast, previous studies have shown that L-KYN administration during adulthood impairs sensory gating (Erhardt et al., 2004; Shepard et al., 2003), an effect that was not mediated by NMDA-Rs (Shepard et al., 2003) and likely involves the antagonism of nACh-Rs instead. Thus, it is possible that the specific behavioral effects observed following L-KYN treatment depend in part on the respective expression levels of NMDA-Rs and nACh-Rs. This is underscored by the finding that KYNA potently inhibits α7-nAChR activation at an IC_{50} of 7μM in cultured hippocampal neurons, while the IC_{50} for KYNA-induced blockade of NMDA-Rs is 235μM (Hilmas et al., 2001). Future studies could investigate this notion further by comparing the present findings to those obtained when L-KYN is administered around PND 21, when nACh-Rs are more numerous.

It is possible that effects on fear learning/memory or attentional function would have been observed in the present study if the L-KYN treatment extended beyond PND 10. However, we specifically chose to limit the L-KYN treatment to PND 7-10 for several reasons. First, a primary goal of the study was to mimic the effects of neonatal influenza exposure on KYNA levels. In the influenza studies (Asp et al., 2010; Holtze et al., 2008), rats were exposed to the virus on PND 3 or 4 and KYNA was found to be elevated on PND 13, but not PND 7 or 24 (no other days were tested). Thus, we chose to begin injections late in the day on PND 7 so that we could elevate KYNA levels as early as possible during development (in an attempt to maximize the influence of KYNA on the developing brain), while retaining translational relevance to the influenza studies. Secondly, continuing beyond day PND 10 was impractical since systemic L-KYN administration no longer increases KYNA concentration after 3-4 days of treatment because of metabolic changes (Vescei et al., 1992). Future studies are therefore needed to more precisely characterize the time course of changes in KYNA concentration following influenza A exposure and to assess corresponding behavioral effects. If the metabolic changes associated with repeated treatment can be surmounted, using L-KYN to mimic the effects of viral exposure on KYNA and QUIN remains an attractive method since it eliminates the potential confounding effects of viral exposure on other organ systems.

Importantly, the behavioral changes we observed in adults that had been treated with L-KYN as neonates could not be attributed to an increase in KYNA at the time of testing. Indeed, the concentration of KYNA was not significantly different in L-KYN-treated rats

and vehicle-treated rats when they were reached adulthood, as was also the case following neonatal influenza A exposure (Asp et al., 2010). From an experimental perspective, this is ideal in that it allows us to distinguish the behavioral effects of early exposure to increased levels of KYNA from the effects of high KYNA levels at the time of testing. This is significant since we have shown previously that an acute increase in KYNA concentration on the day of behavioral training impairs contextual fear memory (Chess et al., 2009). On the other hand, the transient nature of the increase in KYNA after neonatal viral exposure (Asp et al. 2009, 2010) or neonatal treatment with L-KYN (present study) indicates that these early changes in KYNA may not contribute to the increases in KYNA levels observed in adults with schizophrenia (Schwarcz et al., 2001).

In summary, the present findings provide new evidence that exposure to increased levels of tryptophan metabolites, such as KYNA and QUIN, during development can affect behavior in adulthood (Akagbosu et al., 2012; Alexander et al., 2013; Pocivavsek et al., 2012; Trecartin and Bucci, 2011). Both of these substances act on neurotransmitter systems (glutamatergic and cholinergic) that are critically involved in cognitive function as well as normal brain development and neural plasticity, and may thus contribute to cognitive and behavioral deficits associated with various neuropsychiatric disorders, such as schizophrenia. Moreover, changes in KYNA concentration can modulate dopamine levels, and vice versa (Rassoulpour et al., 2005; Wu et al., 2000, 2007). Thus, alterations in tryptophan metabolites can influence the levels of several of the major neurotransmitters that have been implicated in schizophrenia. However, the current findings together with those of Asp et al. (2009, 2010) indicate that early exposure to KYNA and QUIN may only affect certain types of behavior. Moreover, the resulting behavioral consequences may be more apparent in immuno-compromised animals than in animals with normal immune systems (Asp et al., 2009, 2010). This may be particularly important to consider for the development and eventual use of new ‘kynurenergic’ therapies for schizophrenia (Erhardt et al., 2009; Schwarcz et al., 2010, 2012; Thevandavakkam et al., 2010; Wonodi & Schwarcz, 2010) since growing evidence suggests that schizophrenia may be associated with immune dysfunction that could results in elevated levels of kynurenine and KYNA (Müller and Schwarz, 2010).

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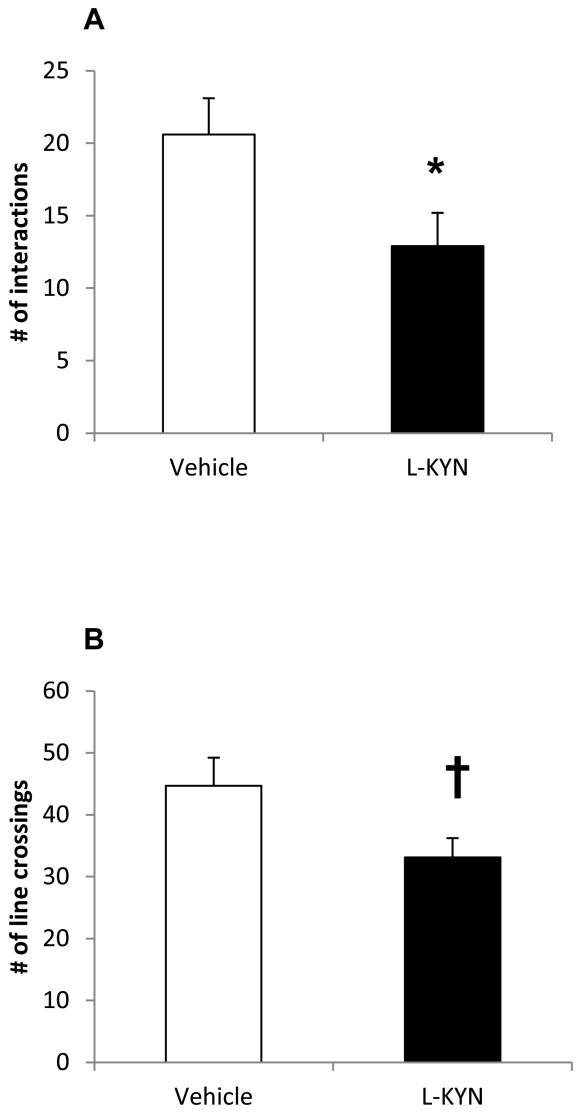

Figure 1.

(A) The number of social interactions displayed by adult rats that had been treated with either vehicle or L-KYN on PND 7-10 (n=10/group). The number of social interactions reflects the number of contacts made by an experimental rat with holes in the cylinder containing the target rat during a 10-min session. The number of social interactions was significantly decreased in rats that had been treated with L-KYN on PND 7-10. **(B)** Locomotor activity measured during the social interaction task. Data reflect the number of times rats in each group crossed a line that separated the arena into thirds. There was a marginally significant decrease in line crossings in adult rats that had received L-KYN on PND 7-10. Data are means ± SEM. *$p<0.05$, $^{\dagger}p=0.05$.

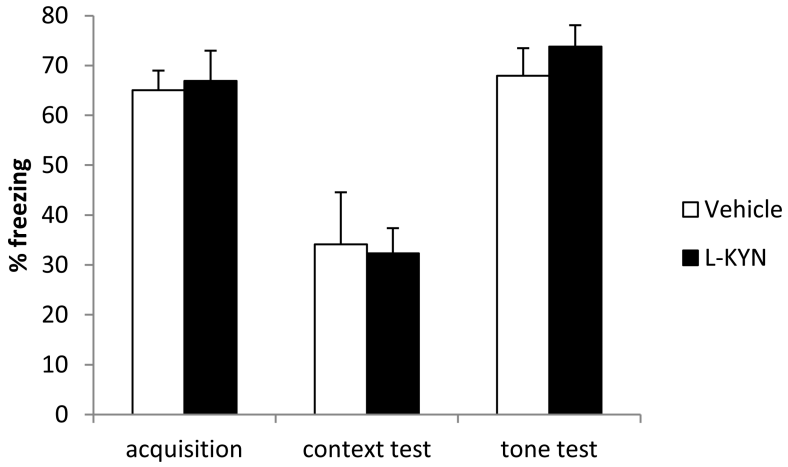

Figure 2.

Summary of the amount of freezing behavior observed during the acquisition session, context test session, and tone test session of the fear conditioning task. There were no group differences during any phase of training (n=10/group). Data are means ± standard error.

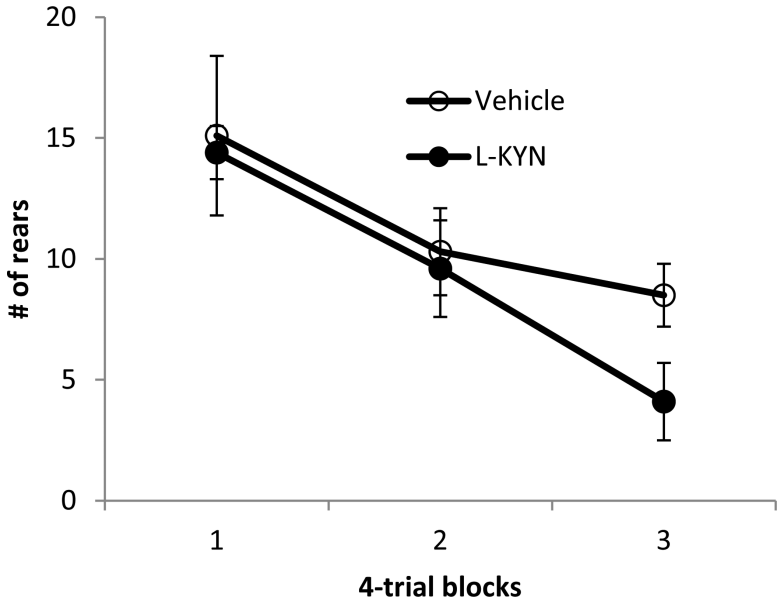

Figure 3.

Unconditioned orienting (rearing behavior) observed during repeated presentation of a non-reinforced visual stimulus. The number of breaks in the photobeams used to detect rearing behavior is shown on the y-axis. Blocks of trials (first 4, middle 4, and last 4 trials) are shown on the x-axis. Rearing behavior habituated across trials and there were no significant group differences (n=8/group). Data are means ± standard error.

Table 1

Concentrations (pmol/gm) of KYNA and QUIN in brain tissue 2 hours after L-KYN on PND 10, and immediately after behavioral testing in adults.

	PND 10		Adult	
Treatment group	**KYNA (n=4)**	**QUIN (n=4)**	**KYNA (n=4)**	**QUIN (n=4)**
Vehicle	11.2 ± 0.9	----- †	19.2 ± 5.3	58.7 ± 15.3
L-KYN	32.3 ± 4.8 *	64.6 ± 12.9 *	35.4 ± 12.8	95.5 ± 9.0

Data are means ± SEM.

* $p<0.05$ compared to Vehicle group

† levels below limit of detection

Table 2

Behavioral effects in adult rats previously treated with L-KYN at different ages

	Age when L-KYN was administered		
	Adult	**Adolescent**	**PND 7-10**
Contextual fear memory	Decreased[1]	Decreased[2]	No effect
Social behavior	No effect[3]	Decreased[3]	Decreased
Locomotor behavior	No effect[3]	No effect[3]	Decreased
Attentional set shifting	Decreased[4]	Decreased[5*]	Decreased[5*]

[1] Chess et al., 2009

[2] Akagbosu et al., 2012

[3] Trecartin & Bucci, 2011

[4] Alexander et al, 2012

[5] Alexander et al., 2013

* rats were exposed to L-KYN from gestational day 15 through PND 21 (Alexander et al., 2013; Alexander et al., 2012)