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Administration of Kynurenine during Adolescence, but not during Adulthood, Impairs Social Behavior in Rats

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Abstract

Kynurenic acid (KYNA) is a tryptophan metabolite that is present at high concentrations in the brains of persons with schizophrenia. This study tested the hypothesis that treatment with l-kynurenine (L-KYN), which increases KYNA concentration, would produce deficits in social behavior similar to those associated with schizophrenia. Rats treated with L-KYN throughout adolescence exhibited decreased social interaction when tested drug-free as adults. In contrast, neither acute nor chronic treatment during adulthood affected social behavior. These findings demonstrate that increases in KYNA concentration produce deficits in social behavior and that the adolescent brain is particularly susceptible to the effects of high KYNA concentration.

Keywords

l-kynurenine; schizophrenia; glutamate; acetylcholine; glia

1. Introduction

Although neuroleptic drugs are often successful in treating positive symptoms of schizophrenia (e.g., delusions, hallucinations), negative symptoms such as social withdrawal are less responsive to current drug therapies (Blin, 1999). Moreover, little is known about the neurobiological substrates of impaired social behavior. The present study tested the hypothesis that increased concentration of kynurenic acid (KYNA) contributes to deficits in social behavior. KYNA is a tryptophan metabolite that is increased in the brains of persons with schizophrenia (Schwarcz et al., 2001). Interestingly, KYNA is synthesized and released by astrocytes and acts as an endogenous antagonist of both NMDA glutamate receptors and $\alpha 7$ nicotinic acetylcholine receptors (Stone, 1993; Hilmas et al., 2001). Both receptors are critically involved in cognitive function (Bast et al., 2003; Gould and Higgins, 2003) and various studies have shown that pharmacological blockade of NMDA receptors induces social withdrawal (Sams-Dodd, 1999).

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Conflict of Interest. Both authors declare that they have no conflicts of interest.

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Rats were treated with 100mg/kg of l-kynurenine (L-KYN; the precursor of KYNA), which results in a 3–4 fold increase in KYNA concentration (Erhardt et al., 2004), consistent with the increase observed in persons with schizophrenia (Erhardt et al., 2001; Schwarcz et al., 2001). Importantly, rats were treated chronically throughout adolescence since genotypic alterations may underlie changes in KYNA concentration associated with schizophrenia (Miller et al. 2004; 2006; 2008; 2009) and thus expose the brain to high levels of KYNA during this critical stage of development. Indeed, there is substantial evidence that schizophrenia often develops during adolescence (Harrop and Trower, 2001). It is well established that NMDA receptors and $\alpha 7$ nicotinic acetylcholine receptors are involved in synaptic plasticity and neural development (Brodie and Leslie, 1999; Komuro and Rakic, 1993), and we have recently shown that increased KYNA concentration throughout adolescence has lasting impacts on cognitive function later in adulthood (Akagbosu et al., 2010). Thus, we expected that social behavior would be impaired in rats treated with L-KYN throughout adolescence.

2. Materials and methods

2.1. Subjects

Male Long Evans rats were obtained from Harlan Laboratories (Indianapolis, IN) and maintained in groups of 4 on a 12:12 light-dark cycle with food (Purina standard rat chow; Nestle Purina, St. Louis, MO) and water available ad libitum. All rats were allowed 6 days to acclimate to the vivarium before any treatment began and maintained according to AAALAC guidelines.

2.2. Drug preparation

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

2.3. Experimental design and treatment regimen

Experiment 1 (adolescent chronic group)—Sixteen rats were treated chronically with L-KYN or vehicle solution (0.1 M HEPES) beginning on postnatal day 27 and continuing through postnatal day 53, using a 3-day-on/3-day-off treatment regimen described previously (Akagbosu et al., 2010). This procedure reduces distress and also minimizes the potential for metabolic adaptations observed following daily L-KYN treatment (Vecsei et al., 1992). Using this procedure, KYNA concentration is increased 4-fold on days when rats are treated with L-KYN (Akagbosu et al., 2010). The social interaction task was carried out 8 days after the last injection (i.e., postnatal day 61), at which time KYNA concentration was no longer elevated (Akagbosu et al., 2010).

Experiment 2 (adult chronic group)—Sixteen adult rats were treated as described in Experiment 1 (8/group), beginning at 8 weeks of age. Thus, behavioral testing occurred at ~84 days of age.

Experiment 3 (acute treatment group)—Another set of 8 week old rats received a single injection of L-KYN (or vehicle) 2 hours before the social interaction task. This group of rats was tested at 61 days of age, like those in Experiment 1.

2.4. Behavioral Apparatus

The social interaction task was conducted in a white plastic tub measuring 119.4 cm x 59.7 cm x 59.7 cm and located in a small, dimly lit room. A clear Plexiglas cylinder (27.9 cm long x 7.6 cm diameter) was located in the center of the tub and used to separate the target

rat from the experimental rat during the test session. There were five holes (1.9cm) on each side of the cylinder, two holes on top, and one hole on either end.

2.5. Behavioral Procedures

Social interaction was assessed using a procedure adapted from File and colleagues (File, 1980; File and Seth, 2003) as described previously (Hopkins et al., 2009). Briefly, an unfamiliar 'target' rat was placed in the cylindrical apparatus inside the center of the tub and then an 'experimental rat' that had previously been treated with vehicle or L-KYN was placed in the right-hand corner of the tub. The experimenter left the room and the rat was allowed 10 min to explore the tub, after which the rats were removed and all surfaces cleaned with disinfectant.

2.6. Behavioral observations

2.6.1. Social behavior—The primary measure of social interaction was the number of times the experimental rat approached and sniffed inside a hole in the cylinder that contained the target rat. Walking around and continuously sniffing the cylinder was also counted as interaction. Behavioral observations were conducted by an observer who was blind to the identity of the experimental rats using videotapes of the session.

2.6.2. Locomotor behavior—The videotape of the social interaction session was scored a second time to assess locomotor activity by drawing two lines perpendicular to the long sides of tub on the video screen, dividing it into 3 equal areas. An observer blind to condition counted the number of times each rat crossed one of the lines. Assessing locomotor activity was important because any group differences that emerged in social behavior could merely be due to treatment-related changes in locomotor activity. Moreover, measuring locomotor behavior during the social interaction test provided a direct task-relevant measure of activity.

2.7. Data analysis

The number of interactions or line crossings was analyzed using independent measures *t*-tests. All statistical analyses were conducted using an alpha level of 0.05.

3. Results

The amount of time rats engaged in social interaction is illustrated in Figure 1. Rats treated with L-KYN during adolescence exhibited significantly less social interaction compared to vehicle-treated rats when they were tested as adults [$t(14)=2.5$, $p < 0.03$]. In contrast, neither chronic nor acute treatment with L-KYN during adulthood had an effect on social interaction ($ps > 0.9$). The amount of locomotor activity exhibited during the social interaction procedure is shown in Table 1. Importantly, locomotor activity did not differ between the L-KYN and vehicle groups in any of the experiments ($ps > 0.1$).

4. Discussion

Here we demonstrate that treatment with L-KYN, which has been shown to increase KYNA levels, decreases social behavior in rats in consistent with the observed social dysfunction in persons with schizophrenia (Sams-Dodd, 1999). Moreover, the adolescent brain may be particularly sensitive to the effects of increased KYNA concentration. Indeed, rats that were treated chronically as adults did not exhibit any impairment in social behavior. Together these findings indicate that prolonged antagonism of NMDA and/or $\alpha 7$ nicotinic acetylcholine receptors during development can lead to social impairments later in adulthood.

It is unlikely that the observed deficits in social interaction were merely due to drug-induced changes in locomotor behavior, since activity levels were comparable in rats treated with L-KYN and control rats in each experiment (consistent with prior data; Akagbosu et al., 2010). Likewise, the decrease in social interaction in rats treated during adolescence was not due to high levels of KYNA at the time of testing since rats were tested 8 days after the final injection and we have previously shown that KYNA levels are no longer elevated at that time (Akagbosu et al., 2010). Moreover, at the time of behavioral testing, the rats treated during adolescence were the same age as rats in the adult-acute treatment group, which did not exhibit any changes in social behavior. These findings further support the notion that the adolescent brain may be hypersensitive to L-KYN treatment.

It is possible that the effects of L-KYN administration could be due to changes in the concentration of kynurenine metabolites other than KYNA. However, previous data indicate that a higher dose of L-KYN (150mg/kg compared to 100mg/kg in our study) failed to significantly increase levels of quinolinic acid, for example (Shepard et al., 2003). Nevertheless, this is a question that should be addressed further in future studies. Similarly, it is of interest that the three groups of rats used in this study had different baseline levels of social interaction. Although this may simply be due to age-related differences in exploratory activity (Stansfield and Kirstein, 2006) or the effects of acute versus repeated injection stress, it would be of interest to further investigate interactions between variable such as age and gender with regard to the effects of L-KYN administration on social behavior.

In summary, the present findings add to a growing literature indicating that increased concentration of KYNA leads to cognitive deficits commonly associated with schizophrenia. Deficits in spatial working memory, sensory gating, contextual memory, and attention have been observed after acute treatment with L-KYN (Chess and Bucci, 2006; Chess et al., 2007, 2009; Erhardt et al. 2004; Shepard et al., 2003). Other cognitive domains, such as object memory (Akagbosu et al., 2010) and social behavior (present report), are insensitive to acute elevations in KYNA, but are impaired in drug-free adults that had been previously exposed to KYNA during development. This differential pattern of effects following exposure to KYNA at different ages and for different durations may provide new insight into the neural substrates underlying cognitive and social dysfunction in schizophrenia.

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Figure 1.

Chronic treatment with L-KYN during adolescence decreased the amount of time rats interacted with an unfamiliar rats when they were later tested drug-free as adults. In contrast, neither chronic nor acute exposure during adulthood impaired social behavior. Data are means $\pm$ SEM.

Table 1

Number of line crossings during the social interaction task

	Vehicle	L-KYN
Adolescent chronic	34 ± 4.4	41.8 ± 1.4
Adult Chronic	41.9 ± 1.5	40.9 ± 2.7
Adult Acute	25.3 ± 3.2	31.1 ± 4.8