Neurotoxicology and Teratology 36 (2013) 36–46

Contents lists available at SciVerse ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Disruption of social approach by MK-801, amphetamine, and fluoxetine in adolescent C57BL/6J mice

Sheryl S. Moy [a,b,*], Randal J. Nonneman [b], Geoffrey O. Shafer [a], Viktoriya D. Nikolova [a], Natallia V. Riddick [a], Kara L. Agster [a,b], Lorinda K. Baker [a,b], Darin J. Knapp [a,c]

[a] Department of Psychiatry, CB#7146, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA
[b] Carolina Institute for Developmental Disabilities, CB#7146, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA
[c] Bowles Center for Alcohol Studies, CB#7178, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form 28 July 2012
Accepted 30 July 2012
Available online 7 August 2012

Keywords:
Amphetamine
Autism
Ethanol withdrawal
Repetitive behavior
Social approach
Stereotypy

ABSTRACT

Autism is a severe neurodevelopmental disorder, diagnosed on the basis of core behavioral symptoms. Although the mechanistic basis for the disorder is not yet known, genetic analyses have suggested a role for abnormal excitatory/inhibitory signaling systems in brain, including dysregulation of glutamatergic neurotransmission. In mice, the constitutive knockdown of NMDA receptors leads to social deficits, repetitive behavior, and self-injurious responses that reflect aspects of the autism clinical profile. However, social phenotypes differ with age: mice with reduced NMDA-receptor function exhibit hypersociability in adolescence, but markedly deficient sociability in adulthood. The present studies determined whether acute disruption of NMDA neurotransmission leads to exaggerated social approach, similar to that observed with constitutive disruption, in adolescent C57BL/6J mice. The effects of MK-801, an NMDA receptor antagonist, were compared with amphetamine, a dopamine agonist, and fluoxetine, a selective serotonin reuptake inhibitor, on performance in a three-chamber choice task. Results showed that acute treatment with MK-801 led to social approach deficits at doses without effects on entry numbers. Amphetamine also decreased social preference, but increased number of entries at every dose. Fluoxetine (10 mg/kg) had selective effects on social novelty preference. Withdrawal from a chronic ethanol regimen decreased activity, but did not attenuate sociability. Low doses of MK-801 and amphetamine were also evaluated in a marble-burying assay for repetitive behavior. MK-801, at a dose that did not disrupt sociability or alter entries, led to a profound reduction in marble-burying. Overall, these findings demonstrate that moderate alteration of NMDA, dopamine, or serotonin function can attenuate social preference in wild type mice.

1. Introduction

Autism spectrum disorders (ASDs) are a set of etiologically diverse neurodevelopmental syndromes characterized by three core symptoms: social interaction deficits, impaired communication, and restricted repetitive behavior. A strong heritable component for susceptibility to ASD has been supported by high concordance rates between monozygotic twins (Bailey et al., 1995; Folstein and Rosen-Sheidley, 2001; Rosenberg et al., 2009). However, genetic and epidemiological studies have provided growing evidence for the importance of environmental factors in the development of the disorder (Hallmayer et al., 2011). For example, autism risk factors include maternal use of pharmaceutical agents with neurotoxic effects (Rasalam et al., 2005; Williams et al., 2001), maternal immune response to infection (Libbey et al., 2005), or exposure to environmental pollutants (Palmer et al., 2006; Shelton et al., 2012; Tian et al., 2011; Volk et al., 2011). A review by Herbert et al. (2006) identified 135 genes that have been shown to mediate responses to environmental challenge, and that are located within autism linkage regions. Overall, a complex combination of genetic predisposition and environmental contribution may underlie the broad range and differential severity of symptoms in autism.

Although the mechanistic basis for the abnormal behavioral profiles in ASD is not yet known, genetic analyses in human populations have implicated several genes important for synaptic function, including *SHANK3*, which encodes a member of the post-signaling complex of glutamatergic synapses, and *GRIN1*, which encodes the obligatory NMDAR1 subunit of the NMDA (*N*-methyl-D-aspartate) receptor (Abrahams and Geschwind, 2008; Geschwind, 2011; Voineagu et al., 2011). Dysregulation of excitatory and inhibitory signaling in brain has been implicated in ASD (Rubenstein, 2010), suggesting that abnormal neurotransmission induced by environmental factors could exacerbate genetic predisposition for core symptoms. In line with this premise,

* Corresponding author at: Carolina Institute for Developmental Disabilities, CB#7146, University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA. Tel.: +1 919 966 3082; fax: +1 919 966 5657.
E-mail addresses: ssmoy@med.unc.edu (S.S. Moy), nonneman@unc.edu (R.J. Nonneman), gos@case.edu (G.O. Shafer), Viktoriya_Nikolova@med.unc.edu (V.D. Nikolova), natallia_riddick@med.unc.edu (N.V. Riddick), agster@email.unc.edu (K.L. Agster), lorinda@med.unc.edu (L.K. Baker), darin_knapp@med.unc.edu (D.J. Knapp).

0892-0362/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ntt.2012.07.007

work in rodent models has demonstrated that developmental exposure to neurotoxins can have long-term effects on glutamate, dopamine, and serotonin function, including altered sensitivity to the behavioral effects of NMDA receptor antagonists (Basta-Kaim et al., 2011; Fortier et al., 2004; Graham et al., 2012; Moy and Breese, 2002; Ozawa et al., 2006; Shi et al., 2003; Vorhees et al., 2012; Zuckerman et al., 2003).

The acute administration of NMDA receptor antagonists can decrease rodent social interaction, as well as induce stereotyped, perseverative responses (Corbett et al., 1995; De Moura Linck et al., 2008; Gao et al., 2009; Nilsson et al., 2001, 2006; Steinpreis et al., 1994). Mice with reduced function of *Grin1* recapitulate many ASD features, including overt social deficits, abnormal repetitive behavior, self-injurious responses, sensory hyper-reactivity, and impaired sensorimotor gating (Duncan et al., 2004; Halene et al., 2009; Mohn et al., 1999; Moy et al., 2008a, 2012). However, most of these changes have been reported in adult mice. We have recently found that, in contrast to a marked lack of sociability in adulthood, *Grin1* knockdown mice have significant hypersociability during adolescence (Moy et al., 2012). Further, there is preliminary evidence that memantine, an NMDA antagonist, can increase social interaction in subjects with autism (Doyle and McDougle, 2012). The following studies examined whether acute disruption of NMDA receptor function in adolescent C57BL/6J mice (ages 5 to 7 weeks) leads to exaggerated social preference, similar to that observed with constitutive reduction of NMDA receptors, in a three-chamber choice task.

Significant impairment of social interaction has also been reported following dysregulation of dopaminergic (Gendreau et al., 1998, 2000; Rodriguiz et al., 2004; Steinpreis et al., 1994) and serotonergic (Homberg et al., 2007; Zhuang et al., 1999; for reviews, see Gingrich and Hen, 2001; Insel and Winslow, 1998) neurotransmission in animal models. Therefore, the effects of MK-801 (Dizocilpine), an NMDA receptor antagonist, on social approach were compared to those of amphetamine, a dopamine agonist, and fluoxetine, a selective serotonin reuptake inhibitor. In addition, the role of anxiety in social deficits was investigated by testing mice following withdrawal from chronic ethanol, which has been shown to induce anxiety-like behavior and social interaction deficits in rats (File et al., 1993, Knapp et al., 2004; Moy et al., 1997; Wills et al., 2010).

The marble-burying assay has been proposed as a measure of perseverative motor responses, relevant to abnormal repetitive behavior in autism, obsessive-compulsive disorder, and other neuropsychiatric conditions (Jimenez-Gomez et al., 2011; Londei et al., 1998; Takeuchi et al., 2002; Thomas et al., 2009). Although *Grin1* knockdown mice exhibit hyperactivity and increased stereotypy, including over-grooming to the point of self-injury, they have profound deficits in marble-burying (Riddick et al., 2011). Similarly, MK-801 treatment in ICR mice has been shown to attenuate marble-burying, but at a dose with significant stimulant effects on locomotor activity (Egashira et al., 2008). The present studies determined the effects of MK-801 and amphetamine on marble-burying at low doses that did not alter sociability or, in the case of MK-801, change entries in the social approach task.

2. Material and methods

2.1. Animals

Sets of male C57BL/6J mice (n = 12–24 mice) were purchased from the Jackson Laboratory (JAX; Bar Harbor, ME, USA), or obtained from C57BL/6J breeding pairs from JAX. Mice received from JAX were 3 to 4 weeks of age upon arrival, and were given one week to acclimate to the new housing conditions. Mice were group-housed in ventilated cages, and given free access to water and Prolab RMH 3000 chow, or water and liquid diet for the ethanol study (described below). The housing room had a 12-hour light/dark cycle (lights off at 7:00 p.m.). All procedures were conducted in strict compliance with the policies on animal welfare of the National Institutes of Health (stated in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, 1996 edition) and the University of North Carolina.

2.2. Study design

2.2.1. Effects of altered NMDA, dopamine, or serotonin function on social approach

4–5 separate sets of mice (JAX) were used to test each compound: MK-801 (0.2, 0.3, or 0.5 mg/kg), amphetamine (1.0, 2.0, or 3.0 mg/kg), and fluoxetine (5.0, 10.0, and 20.0 mg/kg), with at least half of the subjects from each separate set treated with vehicle. At 4–5 weeks in age, mice were given a one-hour open-field session, without any drug treatment. This procedure served to familiarize mice with the laboratory setting and with exploration of a novel environment. At 5–7 weeks of age, each mouse was given a single drug or vehicle treatment, and then tested in the 3-chamber choice task for social approach. A separate set of mice (n = 25; JAX) was tested with vehicle and fluoxetine (10 mg/kg), to confirm selective drug effects observed in the first group. For each test, experimenters were blinded to drug treatment condition.

2.2.2. Effects of ethanol withdrawal on social approach

At 5–6 weeks of age, mice (n = 8; JAX) were weighed, housed 2 per cage, and placed for 14 days on a modified Lieber-DeCarli liquid diet (PMI Micro-Stabilized Alcohol Rodent Liquid Diet; Test Diet, Richmond, IN, USA). The diet included 5% sucrose to increase palatability. Mice continued to have free access to water throughout the liquid diet regimen. For the first 2 days of the regimen, all mice were given regular liquid diet. The control group (n = 4) remained on this diet for the entire regimen. For the ethanol-withdrawal group (n = 4), mice were introduced to liquid diet containing 2.4% ethanol for 2 days, and then received 4.8% ethanol for the following 10-day period. The amount of diet given to the control group was matched to the mean amount of diet consumed by the ethanol-treatment group on the previous day, to insure both groups were drinking the same overall amount. On the morning of the test day, the ethanol liquid diet was replaced with regular liquid diet to induce withdrawal in the ethanol-exposed mice. 5–6 h after replacement of the ethanol diet, mice were evaluated in the 3-chamber test for sociability, but not the test for social novelty (due to time constraints of the withdrawal period).

2.2.3. Effects of reduced NMDA-receptor function on marble-burying

Two sets of mice were evaluated for MK-801 effects on marble-burying. Subjects in the first set were experimentally-naïve, male C57BL/6J mice (n = 24), bred in the UNC animal facility. Each mouse received one test with either MK-801 (0.2 or 0.3 mg/kg) or vehicle (n = 8 per treatment group). Subjects in the second set were 25 C57BL/6J mice (JAX) first used to confirm effects of fluoxetine (10 mg/kg) in the social approach test. One-two weeks following the social test, mice were re-assigned to 3 treatment groups (0.05 or 0.1 mg/kg MK-801, or vehicle, with 8–9 mice per group), balanced for initial fluoxetine or vehicle exposure, and assessed for marble-burying.

2.2.4. Effects of enhanced dopamine function on marble-burying

Subjects were experimentally-naïve, male C57BL/6J mice (n = 21), bred in the UNC animal facility. Each mouse first received one marble-bury test with either amphetamine (1.0 or 2.0 mg/kg) or vehicle (n = 7 per treatment group). One week later, all subjects were re-assigned to 2 new treatment groups (balanced for initial drug exposure), and re-tested in the marble-bury assay. Treatments for this second test were 1.5 mg/kg amphetamine (n = 10 mice), and vehicle (n = 11 mice).

2.3. Testing procedures

2.3.1. Three-chamber social choice test

Mice were tested in an automated 3-chamber box, using our published methods (Moy et al., 2004, 2007; Nadler et al., 2004). Dividing