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Research report

Balb/c mice treated with D-cycloserine arouse increased social interest in conspecifics

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ABSTRACT

The genetically inbred Balb/cJ (Balb/c) mouse with functional alteration of its endogenous tone of NMDA receptor-mediated neurotransmission displays impaired sociability in a standard paradigm; this mouse strain has been proposed as a model of autism spectrum disorders (ASDs). Prior work showed that treatment of the Balb/c mouse with a centrally effective dose of D-cycloserine, a partial glycine$_B$ NMDA receptor agonist, improved several measures of its sociability. Additionally, D-cycloserine-treated Balb/c mice show greater preference for a social stimulus mouse than an inanimate object. We wondered if treatment with D-cycloserine also improved the social salience of the Balb/c mouse for "normally" sociable comparator strains. The current experiments explored whether C57Bl/6J (B6) and ICR mouse strains prefer D-cycloserine-treated to vehicle-treated Balb/c stimulus mice in a paradigm that evaluated social preference. The results showed that B6 mice prefer D-cycloserine-treated Balb/c mice to vehicle-treated Balb/c mice, suggesting that treatment could have resulted in normalization of "emitted" social cues.

1. Introduction

The endogenous tone of NMDA receptor-mediated neurotransmission is functionally altered in the genetically inbred Balb/cJ (Balb/c) mouse, relative to other inbred and outbred strains, as reflected in the heightened sensitivity of this strain to behavioral effects of MK-801 (dizocilpine), a noncompetitive NMDA receptor antagonist (Burket et al., 2010a; Deutsch et al., 1998, 1997a,b). For example, relative to other inbred and outbred comparator strains, Balb/c mice are more sensitive to elicitation of irregular episodes of intense jumping behavior, termed "popping," and circling behavior, as well as antagonism of electrically precipitated tonic hindlimb extension by MK-801 (Burket et al., 2010a; Deutsch et al., 1998, 1997a,b). Interestingly, this increased sensitivity to behavioral effects of MK-801 may not be due to changes in the relative expression of NMDA receptor subunits and splice variants within the frontal cortex and hippocampus, but may reflect changes within circuits that utilize NMDA receptors for synaptic transmission (Perera et al., 2008). Specifically, Balb/c mice and an outbred comparator strain did not differ in the immunoreactive protein content of two splice variants of the NR1 subunit, and the NR2A and NR2B subunits in hippocampus and frontal cortex (Perera et al., 2008). The Balb/c mouse also has impaired sociability, relative to the C57Bl/6J (B6) and Swiss Webster comparator strains, as reflected in its decreased locomotor activity in the presence of an enclosed and freely behaving salient social stimulus mouse; diminished time that it spends exploring and in the vicinity of an enclosed social stimulus mouse; and diminished number of discrete episodes of social approach toward, and anogenital sniffing of, a social stimulus mouse when the two mice are allowed to interact freely with each other, among other reliably rated behaviors in a standard mouse social procedure (Brodkin, 2007; Burket et al., 2010b; Jacome et al., 2011a; Sankoorikal et al., 2006).

The NMDA receptor was shown to be prominently involved in the regulation of normal sociability in mice (Halene et al., 2009; Labrie et al., 2008). For example, transgenic mice with diminished expression of the obligatory NR1 subunit of the NMDA receptor or expression of NMDA receptors with up to five-fold diminished affinity for the obligatory glycine co-agonist show impaired sociability in standard paradigms (Halene et al., 2009; Labrie et al., 2008). Because the Balb/c mouse with impaired sociability showed heightened sensitivity to MK-801, a compound whose major behavioral and pharmacological effects are attributed to interaction with NMDA receptors, and NMDA receptors are involved in the regulation of normal sociability, we tested D-cycloserine, a partial glycine$_B$ site agonist that binds to the strychnine-insensitive glycine binding site on the NMDA receptor, for its effects on the sociability of this strain (Burket et al., 2013; Deutsch et al., 2012, 2011a; Jacome et al., 2011b). Quite remarkably, D-cycloserine improved

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several measures of sociability in the Balb/c strain; however, it also improved several measures of sociability in the Swiss Webster comparator strain, raising the possibility that these prosocial effects are not strain-selective and an epiphenomenon of nonspecific effects of D-cycloserine on locomotor activity and anxiety. In the standard three-compartment sociability apparatus, several measures of sociability are dependent on the locomotor activity of the test mouse (Burket et al., 2010b; Deutsch et al., 2012, 2011a). The current experiment was undertaken to explore whether D-cycloserine improves the sociability of Balb/c mice in the absence of the potentially confounding nonspecific effect of increasing locomotor activity in general.

Thus, we wondered whether the social salience of enclosed D-cycloserine-treated Balb/c mice was greater than the social salience of enclosed vehicle-treated Balb/c mice. Specifically, we measured the amount of time 4-week old male B6 and 4-week old male ICR mice spent sniffing/exploring and in the compartment containing an enclosed D-cycloserine-treated 4-week old Balb/c mouse versus the time they spent sniffing/exploring and in the compartment containing an enclosed vehicle-treated 4-week old Balb/c mouse in a standard three-compartment apparatus.

2. Methods

2.1. Animals

Experimentally naïve, 4-week old male, outbred ICR (Charles River Laboratories, Wilmington, MA) and genetically inbred B6 test mice (Jackson Laboratories, Bar Harbor, ME) were housed 2 per cage, in hanging clear Plexiglas cages with free access to food and water, and maintained on a 12 h light/dark cycle. The stimulus mice were 4-week old male, inbred Balb/mice (Jackson Laboratories, Bar Harbor, ME), housed 4 per cage. Housing conditions were adopted from prior literature (Sankoorikal et al., 2006). Mice were individually weighed prior to drug administration. All animal procedures were approved by the Eastern Virginia Medical School Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

D-Cycloserine (Sigma–Aldrich Co., St. Louis, MO) was dissolved in 0.9% saline and prepared each day of the experiment. D-Cycloserine (320 mg/kg or saline) was injected intraperitoneally in a volume of 0.01 ml/g of body weight 20 min prior to testing sociability. As we previously reported, the dose of D-cycloserine (320 mg/kg, ip) was selected based on earlier dose–response data, its reliable demonstration of prosocial effects in 4- and 8-week old male Balb/c mice on a large variety of sociability outcome measures, and literature pertaining to its half-life, clearance from plasma and uptake into brain (Deutsch et al., 2012, 2011a; Wlaź et al., 1994).

2.3. Apparatus

The three-compartment testing apparatus consisted of a black Plexiglas rectangular box (52.07 cm × 25.40 cm × 22.86 cm), without a top or bottom. The center compartment was slightly smaller (12.07 cm × 25.40 cm) than the two end compartments that were of equal size (19.05 cm × 25.40 cm). Inverted wire cups (Galaxy Cup, Kitchen Plus, http://www.kitchen-plus.com) were placed in each side of the end compartments during the acclimation and test sessions (discussed below); D-cycloserine-treated and vehicle-treated Balb/c mice were enclosed in these inverted wire cups during the test sessions. 500 ml glass bottles were placed on top of the inverted wire cups to prevent climbing during testing. After each test mouse was studied, the apparatus and wire cups were thoroughly cleaned with Quatricide PV solution, as required by Eastern Virginia Medical School's Institutional Animal Care and Use Committee.

2.4. Social preference procedure

We adapted our current sociability procedure to explore the social "choice" preferences of the B6 and ICR test mice (i.e., whether they "preferred" the D-cycloserine-treated or vehicle-treated Balb/c stimulus mouse) (Burket et al., 2010b; Deutsch et al., 2012, 2011a; Shah et al., 2013). After a 10-min period of acclimation during which neither D-cycloserine-treated nor vehicle-treated Balb/c mice were enclosed in the inverted wire cups in the two lateral compartments of the sociability apparatus, test mice were observed and videotaped for 30 min while they were allowed to explore the apparatus, which contained the enclosed D-cycloserine-treated and vehicle-treated Balb/c "stimulus" mice in the lateral compartments. The amount of time the test mice spent sniffing/exploring in the vicinity of and in the compartment containing the enclosed D-cycloserine- and vehicle-treated Balb/c mice was measured. In prior studies, we showed that the time test mice spend sniffing/exploring and in the vicinity of the enclosed stimulus mouse can be measured reliably (Burket et al., 2013; Deutsch et al., 2011a, 2012; Jacome et al., 2011a). All sessions were conducted in dim lighting and videotaped using a Sony HDR-CX560V HD Video Camera (Sony Corp., Tokyo, Japan) for future viewing and data collection.

2.5. Statistics

Paired *t*-tests were used to determine effects of D-cycloserine on the social salience of the enclosed Balb/c stimulus mouse for B6 and ICR test mice. Specifically, for both the vehicle and D-cycloserine treatment conditions, within strain comparisons were made with respect to time spent in the compartments containing the D-cycloserine and vehicle-treated Balb/c mice and time spent exploring (i.e., sniffing) the inverted cups containing D-cycloserine- and vehicle-treated Balb/c mice. A two sample independent *t*-test was used to compare locomotor activity (i.e., number of transitions between compartments) between the ICR and B6 test mice.

3. Results

The locomotor activity of the 4-week old male ICR test mice (i.e., number of transitions between compartments) was significantly greater than that of 4-week old male B6 test mice ($t = -3.41$, $p < 0.01$) during the 10-min period of acclimation or habituation to the apparatus (see Fig. 1). For purposes of analysis, the time test mice spent sniffing/exploring and in the compartments of the enclosed D-cycloserine- and vehicle-treated Balb/c mice were broken into three 10-min epochs (see Fig. 2). The data show that during the first two 10-min epochs, the B6 strain of mouse spent significantly more time in the compartment containing the enclosed D-cycloserine-treated Balb/c mouse than in the compartment containing the vehicle-treated Balb/c mouse ($t = -3.24$, $p < 0.01$ and $t = -4.78$, $p < 0.001$ for both the first and second 10-min epochs, respectively) (Panel 2A). The B6 strain also spent significantly more time sniffing/exploring the inverted cup containing the enclosed D-cycloserine-treated Balb/c mouse compared to the time spent sniffing/exploring the inverted cup containing the enclosed vehicle-treated Balb/c mouse ($t = -3.47$, $p < 0.01$ and $t = -2.84$, $p < 0.01$ for both the first and second 10 min epochs) (Panel 2B). Interestingly, the ICR strain did not show any preference for sniffing/exploring or spending time in the compartment containing the D-cycloserine-treated Balb/c mouse (data not shown).

When the data were summed over the entire 30-min period of observation (see Fig. 3), only the B6 mouse strain spent