D-Cycloserine Facilitates Socially Reinforced Learning in an Animal Model Relevant to Autism Spectrum Disorders

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Background: There are no drugs that specifically target the social deficits of autism spectrum disorders (ASD). This may be due to a lack of behavioral paradigms in animal models relevant to ASD. Partner preference formation in the prairie vole represents a social cognitive process involving socially reinforced learning. D-cycloserine (DCS) is a cognitive enhancer that acts at the *N*-methyl-D-aspartate receptor to promote learning. If DCS enhances socially reinforced learning in the partner preference paradigm, it may be useful in combination with behavioral therapies for enhancing social functioning in ASD.

Methods: Female prairie and meadow voles were given DCS either peripherally or directly into one of three brain regions: nucleus accumbens, amygdala, or caudate putamen. Subjects were then cohabited with a male vole under conditions that do not typically yield a partner preference. The development of a preference for that stimulus male vole over a novel male vole was assessed using a partner preference test.

Results: A low dose of DCS administered peripherally enhanced preference formation in prairie voles but not meadow voles under conditions in which it would not otherwise occur. These effects were replicated in prairie voles by microinfusions of DCS into the nucleus accumbens, which is involved in reinforcement learning, and the amygdala, which is involved in social information processing.

Conclusions: Partner preference in the prairie vole may provide a behavioral paradigm with face, construct, and predictive validity for identifying prosocial pharmacotherapeutics. D-cycloserine may be a viable treatment strategy for social deficits of ASD when paired with social behavioral therapy.

Key Words: Animal model, glutamate, NMDA receptor, prairie vole, social cognition, social impairment

Despite the growing public health concern over autism spectrum disorders (ASD), there have been few advances in the development of pharmacotherapeutic treatment options for these neurodevelopmental disorders. Most existing pharmacotherapies for individuals with ASD are simply relabeled drugs commonly used for the treatment of other neuropsychiatric disorders, which, in ASD, target only peripheral comorbid symptoms rather than key features like social impairment (1). Consequently, there is a significant need for the use of animal models and behavioral paradigms relevant to ASD to gain understanding of the fundamental neurobiology of the core endophenotypes of ASD so that informed pharmacotherapies based on biology can be developed. Given the heterogeneous nature of ASD, targeting individual endophenotypes may be a more viable approach for drug development than targeting the global etiology. For this reason, we have focused on behavioral paradigms that may be useful in screening drugs that enhance social cognitive function in animal models, with the presumption that similar pharmacotherapeutic approaches may enhance social cognition in patients with ASD.

Cognitive enhancers such as D-cycloserine (DCS) have gained considerable attention in recent years for their potential in facilitating selective cognitive processes in the treatment of psychiatric disorders such as phobias, social anxiety, obsessive-compulsive disorder, and posttraumatic stress disorder (2–5). D-cycloserine is a partial agonist of the *N*-methyl-D-aspartate (NMDA) glutamate receptor that binds to the glycine site of the NMDA receptor, enhancing receptor activation only in the presence of glutamate (6). The NMDA receptor plays a pivotal role in long-term plasticity, the neuronal correlate of memory (7). D-cycloserine enhances many different forms of learning and memory (8–12), which suggests the drug may also be effective in improving social memory and cognition.

Currently, there are two rodent behavioral paradigms that are particularly well suited to the investigation of the neurobiological mechanisms underlying social cognition and for screening compounds that may enhance social cognition: social recognition in the mouse (*Mus musculus*) and partner preference formation in the socially monogamous prairie vole (*Microtus ochrogaster*) (13,14).

Social recognition paradigms in mice have revealed an important role for the amygdala (Amyg) in social information processing. Mice discriminate novel from familiar mice using olfactory cues and habituate to a familiar mouse following repeated exposures (15). Mice genetically deficient in oxytocin (OT) fail to habituate to a conspecific after repeated exposure and therefore fail to discriminate familiar from novel conspecifics (16). Silencing OT receptor expression or infusion of OT receptor antagonist into the Amyg disrupts social recognition in wild-type mice, while microinjections of OT directly into the medial Amyg rescues social recognition (17,18). D-serine, a compound related to DCS, increases social recognition in rats at high doses (19). Correspondingly, antagonists of the NMDA receptor prevent the expression of social memory (20).

Social bonding in the monogamous prairie vole, which is assessed in the laboratory using a partner preference paradigm, is a higher order and multidimensional social cognitive process that involves functional circuits for social recognition, social reward and reinforcement, and associative social learning (21). In this paradigm, the social learning phase (e.g., the initial cohabitation) can be manipulated pharmacologically to either accelerate or inhibit the

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Received Oct 14, 2010; revised Jan 7, 2011; accepted Jan 24, 2011.

0006-3223/$36.00
doi:10.1016/j.biopsych.2011.01.026

formation of the social bond, consequently enabling the identification of compounds and neural circuits that affect social learning. In female prairie voles, OT and dopamine interact in the nucleus accumbens (NAcc) to promote partner preference formation (22–24). Direct infusion of OT into the brain during the social learning phase accelerates partner preference formation in the absence of mating (25). Infusion of an OT antagonist or a D2 dopamine antagonist directly into the NAcc prevents mating-induced partner preference formation (23). An alternative animal model relevant to ASD is the meadow vole (*Microtus pennsylvanicus*), which, despite being closely related to the prairie vole, is relatively asocial and does not typically display partner preferences, in part because of a lack of OT receptors in the NAcc (26).

Although a role for glutamate in partner preference formation has not yet been demonstrated, we hypothesize that DCS, acting in the Amyg and NAcc, will facilitate social learning during the initial cohabitation, accelerating partner preference formation. G-protein coupled receptors, like dopamine and OT receptors, can potentiate the action of NMDA receptors in the encoding of long-term behavioral changes (27). The effect of DCS on the enhancement of social learning should therefore be most profound in brain areas that mediate OT-dependent social functions, like the Amyg and NAcc. It is likely, though, that DCS will not facilitate partner preferences in meadow voles because they lack some of the neural substrates essential for socially reinforced learning of olfactory cues.

We propose that DCS will accelerate bonding in female prairie voles by enhancing socially reinforced learning through modulation of NMDA receptors in socially relevant brain regions. If our assertion is correct and DCS accelerates partner preference formation during the learning phase of the paradigm, then DCS may be a useful adjunct to behavioral based therapies currently used to enhance social function in ASD.

Methods and Materials

Subjects

Subjects were adult (60–120 days of age), sexually naive female prairie voles or meadow voles, and stimulus male voles were adult (90–180 days of age), sexually experienced prairie voles or sexually naive meadow voles. All prairie voles were generated from an in-house breeding colony originally derived from wild-caught populations in Illinois (prairie voles) or Pennsylvania (meadow voles). After weaning at 21 days of age, subjects were housed in same sex pairs or trios with water and Purina rabbit chow provided ad libitum. All experiments were done in accordance to the Institutional Animal Care and Use Committee at Emory University.

Peripheral Effects of DCS on Partner Preference in Prairie Voles

Adult, gonadally intact female prairie voles were injected intraperitoneally with either saline or D-cycloserine dissolved in saline (Sigma-Aldrich, St. Louis, Missouri; C6880; 0 mg/kg $n = 6$; 10 mg/kg $n = 7$; or 20 mg/kg $n = 7$). The doses used were based on the functional doses of DCS for appetitive learning in other rodent models (28). Immediately after the injection, female voles were placed into the cage of a novel, sexually experienced stimulus male vole for a 6-hour cohabitation period. As female prairie voles are induced ovulators, the female voles were nonreceptive and should not have mated. Following the cohabitation period, the subjects were tested for partner preference. Partner preference was tested in a three-cage apparatus. The familiar partner male vole and the novel stranger male vole were tethered, one in each of the two end cages. The female vole was then placed in the center nonsocial cage and allowed to freely move through the three cages. The amount of time the female vole spent in social proximity with each male vole was recorded using the VoleTracker (Wang Laboratory, Florida State University) beam-break infrared monitoring system (29). The infrared beams were placed just beyond the reach of each tethered animal, such that a beam break indicated that the female vole entered the social proximity zone in which social contact was possible. The amount of time the female vole spent in the social proximity zone for each stimulus animal was used as a measure of time spent with the partner and the stranger for the determination of a partner preference.

Central Effects of DCS on Partner Preference in Prairie Voles

In an effort to further specify the area of action of DCS, we made some refinements in the partner preference paradigm. All experimental prairie vole female subjects were ovariectomized to ensure nonreceptivity throughout the testing. The pairs were video recorded during the social learning period to verify that they did not mate. The method of quantifying social interaction between the female and male voles was also refined through a move to automated computerized scoring, using SocialScan 2.0 (CleverSys Incorporated, Reston, Virginia) (30). The validity of the automated computerized scoring was previously assessed. SocialScan 2.0 correlated highly with manual scoring of partner preference ($R = .904$) (30).

Adult, ovariectomized female prairie voles were bilaterally cannulated into the nucleus accumbens, the amygdala, or the caudate-putamen (CP) using stereotaxic methods. Ovariectomy was performed at approximately 60 days and animals were allowed to recover for 14 days before beginning the study. Subjects were anesthetized using isoflurane and 26-gauge bilateral guide cannulae (Plastics One, Roanoke, Virginia) aimed at the NAcc (anterior 1.7 mm, bilateral $\pm$1 mm, ventral $-$3.5 mm to bregma), Amyg (anterior $-$1.3 mm, bilateral $\pm$2.7 mm, ventral $-$6.1 mm), or CP (anterior 1.7 mm, bilateral $\pm$1 mm, ventral $-$2.5 mm to bregma) were implanted. Location of the cannulae was verified postexperimentally in Nissl-stained brain sections. The coordinates used for the Amyg group targeted specifically the medial amygdala; however, any cannulae that hit within the amygdala were included in the analysis. After 2 to 3 days of recovery, subjects received microinjections with a 33-gauge internal cannula (Plastics One) that extended 1 mm below the guide cannula into the target area. The needle was connected to a Hamilton syringe (Hamilton, Reno, Nevada) via polyethylene-20 tubing (Plastics One), through which the solution was injected slowly over the course of 1 minute. The internal needle was left in place for 2 minutes after the injection to prevent backflow.

The effect of DCS on partner preference was tested in each brain location independently. Animals received either a bilateral control injection of Ringer's solution or 10 μg of DCS dissolved in 500 nL of Ringer's solution (Fisher Scientific, Pittsburg, Pennsylvania) per side into the NAcc ($n = 11$–12/treatment), Amyg ($n = 11$/treatment), or CP ($n = 12$/treatment). The 10 μg dose is based on the effective dose needed for intra-amygdalar infusion in studies examining fear learning in rats (31). Immediately after the injection, the female voles were placed into the cage of a novel sexually experienced male vole for a 6-hour cohabitation period. Subjects were video recorded during the cohabitation to ensure no mating occurred. Mating was not observed in any of these animals. Following the cohabitation period, the subjects were tested for partner preference (Figure 1).

Partner preference testing was conducted in a three-chamber arena in which a novel male stranger was tethered at one end and