The *N*-Methyl-D-Aspartate Receptor Co-agonist D-Cycloserine Facilitates Declarative Learning and Hippocampal Activity in Humans

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Background: The *N*-methyl-D-aspartate receptor (NMDAR) is critical for learning-related synaptic plasticity in amygdala and hippocampus. As a consequence, there is considerable interest in drugs targeting this receptor to help enhance amygdala- and hippocampus-dependent learning. A promising candidate in this respect is the NMDAR glycine-binding site partial agonist D-cycloserine (DCS). Accumulating clinical evidence indicates the efficacy of DCS in the facilitation of amygdala-dependent fear extinction learning in patients with phobic, social anxiety, panic, and obsessive-compulsive disorder. An important unresolved question though is whether the use of DCS can also facilitate hippocampus-dependent declarative learning in healthy people as opposed to being restricted to the fear memory domain.

Methods: In the present study, we investigated whether or not DCS can facilitate hippocampus-dependent declarative learning. We have therefore combined functional magnetic resonance imaging with two different declarative learning tasks and cytoarchitectonic probabilistic mapping of the hippocampus and its major subdivisions in 40 healthy volunteers administered either a 250 mg single oral dose of DCS or a placebo.

Results: We found that DCS facilitates declarative learning as well as blood-oxygen level dependent activity levels in the probabilistically defined cornu ammonis region of the hippocampus. The absence of activity changes in visual control areas underscores the specific action of DCS in the hippocampal cornu ammonis region.

Conclusions: Our findings highlight NMDAR glycine-binding site partial agonism as a promising pharmacological mechanism for facilitating declarative learning in healthy people.

Key Words: Cognitive enhancement, D-cycloserine, declarative learning, fMRI, hippocampus, memory, NMDA receptor

Since the discovery of neuroenhancement by low-dose strychnine (1), ample evidence has accrued to show that drugs can augment learning. A crucial target of current neuroenhancement strategies is the *N*-methyl-D-aspartate receptor (NMDAR), which is the predominant molecular device for triggering learning-related synaptic plasticity in amygdala and hippocampus (2,3). Among the regulatory binding sites on the NMDAR is the glycine-binding site, which is distinct from the glutamate/aspartate-binding site and must be co-activated for NMDAR-mediated signaling (4,5). Whereas direct pharmacological stimulation via the glutamate/aspartate-binding site bears the risk of NMDAR overactivity and excitotoxicity (6), indirect stimulation via the co-agonist glycine-binding site offers a relatively safe and feasible pharmacological mechanism for facilitating NMDAR function (7). One important candidate agent in this respect is the cyclic glycine analogue and high-affinity glycine-binding site partial agonist D-cycloserine (DCS). Studies in rodents indicate that DCS augments both amygdala- and hippocampus-dependent learning (8–10), and accumulating evidence from preclinical and clinical studies in humans suggests that DCS promotes both the consolidation (11) and extinction (12) of conditioned fear. Specifically, augmentation with DCS enhances responses to exposure-based cognitive-behavioral therapy (CBT) in patients with phobic (13), social anxiety (14,15), panic (16), and obsessive-compulsive disorder (17,18), most likely by potentiating amygdalar NMDAR activity related to fear extinction learning (19). While these human studies implicate the efficacy of DCS as a cognitive enhancer in the non-declarative domain of fear memory, no such evidence has yet emerged for declarative (episodic and semantic) learning (20), despite its critical dependence on NMDAR activity in the hippocampus, and in particular, the cornu ammonis (CA) region ([21]; see also [22,23]). Against this background, we devised a randomized controlled trial including 40 adult healthy volunteers, which combined functional magnetic resonance imaging (fMRI) with cytoarchitectonic probabilistic mapping of the hippocampus and its major subdivisions (24,25) to explore both the behavioral correlates and intrahippocampal location of putative DCS effects on declarative learning. Given evidence in rodents that DCS increased the rate of gradual learning in a hippocampus-dependent task (10), we used an fMRI paradigm that required gradual learning of item-category associations from visual trial-by-trial feedback (see also [26,27]), thereby enabling us to assess a DCS-induced modulation of task-related hippocampal responses on both the behavioral and neural level. The item-

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category association task was complemented by an object-location association task specifically addressing the spatial-contextual component of declarative learning (28–30), which has also been shown to be enhanced by DCS in rodents (22,23). Thus, the priority for the choice of these particular declarative learning tasks was their potential to evoke robust hippocampal responses and their susceptibility to the facilitative influence of DCS, as suggested by analogous experiments in rodents. In addition, all subjects were scanned on a checkerboard visual stimulation task, with the aim to control for nonspecific DCS effects possibly resulting from a global potentiation of NMDAR activity or homogeneous changes in cerebral hemodynamics.

Methods and Materials

Subjects

Forty healthy volunteers (20 female volunteers, 20 male volunteers; mean age, 24.7 years; age range, 18.9–34.6 years) were recruited by advertisement and provided written informed consent before the study, which was approved by the University of Bonn Institutional Research Ethics Board (Identifier: 113/08) and the German Federal Institute of Drugs and Medical Devices (Identifier: 4033608). The study period commenced in June 2008 and was completed by March 2009. The study was registered as a randomized controlled trial in the European Clinical Trials database (Identifier: 2007-005215-26) as well as in the Clinical Trials.gov database (Identifier: NCT00980408) provided by the US National Institutes of Health. All subjects were determined to be free of current or past physical (including daltonism) or psychiatric illness by medical history and diagnoses according to the Structured Clinical Interview for DSM-IV axis I disorders (SCID-I) and axis II disorders (SCID-II). Moreover, subjects were assessed with a comprehensive neuropsychological test battery (Table S1 in Supplement 1). Furthermore, subjects were briefed on magnetic resonance imaging (MRI) safety and instructed to maintain their regular bed and wake times and to abstain from caffeine and alcohol intake on the day before the fMRI scan.

Experimental Protocol

The rationale of this randomized, double-blind, placebo (PLC)-controlled, parallel-group study was to prove whether a 250 mg single oral dose of DCS facilitates declarative learning in healthy subjects. According to the product information (King Pharmaceuticals, Ltd., Ballybofey, Co. Donegal, Ireland), DCS (D-4-amino-3-isoxazolidone) is an antibiotic effective against *Mycobacterium tuberculosis*. Following capsule ingestion, plasma concentrations are detectable within 1 hour, whereas peak plasma levels of approximately 10 mg/L are achieved 3 to 4 hours after dosage administration. Data from the antibiotic use of DCS at doses of >1 g daily indicate that the drug has excellent central bioavailability (31), with peak cerebrospinal fluid levels corresponding to 80% to 100% of peak plasma concentrations ([32]; see also [7]). The elimination half-life of DCS is in the range of 8 to 12 hours. In view of this pharmacokinetic profile, subjects received a single capsule containing either verum or a lactose PLC 4 hours before the fMRI scan. Drug allocation was gender-balanced. A 250-mg dose of DCS was administered, as cognitive-enhancing effects of the agent have been documented for a dose range of 50 to 500 mg daily (8,12). According to the scan protocol, we scanned 4 subjects per day, starting at 2:00 PM and finishing at 6:00 PM; until they were scanned, subjects were placed in a quiet room with reading materials. Before the fMRI scan, subjects performed training versions of the experimental tasks. Inside the scan room, a mirror system was used for stimulus presentation (viewing distance, 254 cm). Stimuli subtended a visual angle of 8.2° horizontally and 6.5° vertically. Stimulus delivery and response recording in the experimental tasks were carried out with Presentation12 (Neurobehavioral Systems, Inc, Albany, California).

Imaging Paradigms

Item-Category Association Task. This fMRI paradigm required subjects to make push-button responses to judge the category membership A or B of three-digit numerical items presented repeatedly on screen. Subjects were informed that there was no underlying rule defining which item belonged to category A or B and that category membership of each item was based on an arbitrary and randomized algorithm before the start of the task. Once assigned, category membership remained constant over six presentations (cycles). For the first cycle, subjects had no knowledge of the correct category membership and thus responded by guessing. Visual feedback immediately followed each category judgment, in which a gray circle changed to green for correct responses or to red for incorrect responses. The feedback informing subjects about the correct item-category association thereby enabled them to gradually improve response accuracy greater than chance over subsequent cycles. To avoid simple visuomotor learning, the response buttons for A and B changed depending on the random lateralization of A and B on screen. In the control condition of the task, subjects were instructed to dichotomically categorize numerical items smaller than 500 as A and items larger than 500 as B. In total, subjects completed three runs of the learning condition and one run of the control condition, with eight trials (four items in each category) presented over six cycles during each of these runs. Within each cycle, trials were presented in a random order. Hence, the number of trials per run was 48, leading to 192 trials over the entire paradigm. The trial duration was 3500 msec (stimulus-response duration 2500 msec; feedback duration 1000 msec) and the jittered intertrial interval 2250 msec (1500–3000 msec) (Figure 1A[i]). In contrast to previous studies (26,27), numerical items instead of symbols, objects, or scenes were presented to increment task difficulty and counteract near ceiling behavioral performance, which would render the paradigm insensitive to further DCS-induced improvement in performance.

Object-Location Association Task. This fMRI paradigm was composed of an encoding phase separated from a retrieval phase (28–30). Colored photographs of natural and artificial objects served as stimuli. The baseline display consisted of a green cross, which divided the screen into four quadrants. For encoding, 64 stimuli were randomly selected from a pool of 96 stimuli. The selected stimuli randomly occurred with a duration of 2000 msec in one of the four screen quadrants and were each followed by an interstimulus interval of 1450 msec. Subjects were instructed to memorize each item and its on-screen location. To ensure sufficient attentive processing, subjects engaged in a dichotomous push-button artificial-versus-natural judgment task. There was a 5-min break between the encoding (duration 6.1 min) and retrieval (duration 10.4 min) phases, during which subjects maintained their position in the MRI scanner. During the retrieval phase, the complete set of 96 stimuli was presented in a random order. Stimuli were presented for 1500 msec followed by an interstimulus interval of 2650 msec. Subjects performed a push-button old-versus-new recognition judgment, combined with an object-location judgment for objects classified as old. Subjects