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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Received Nov 10, 2009; revised Jan 12, 2010; accepted Jan 22, 2010.

0006-3223/$36.00
doi:10.1016/j.biopsych.2010.01.022

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

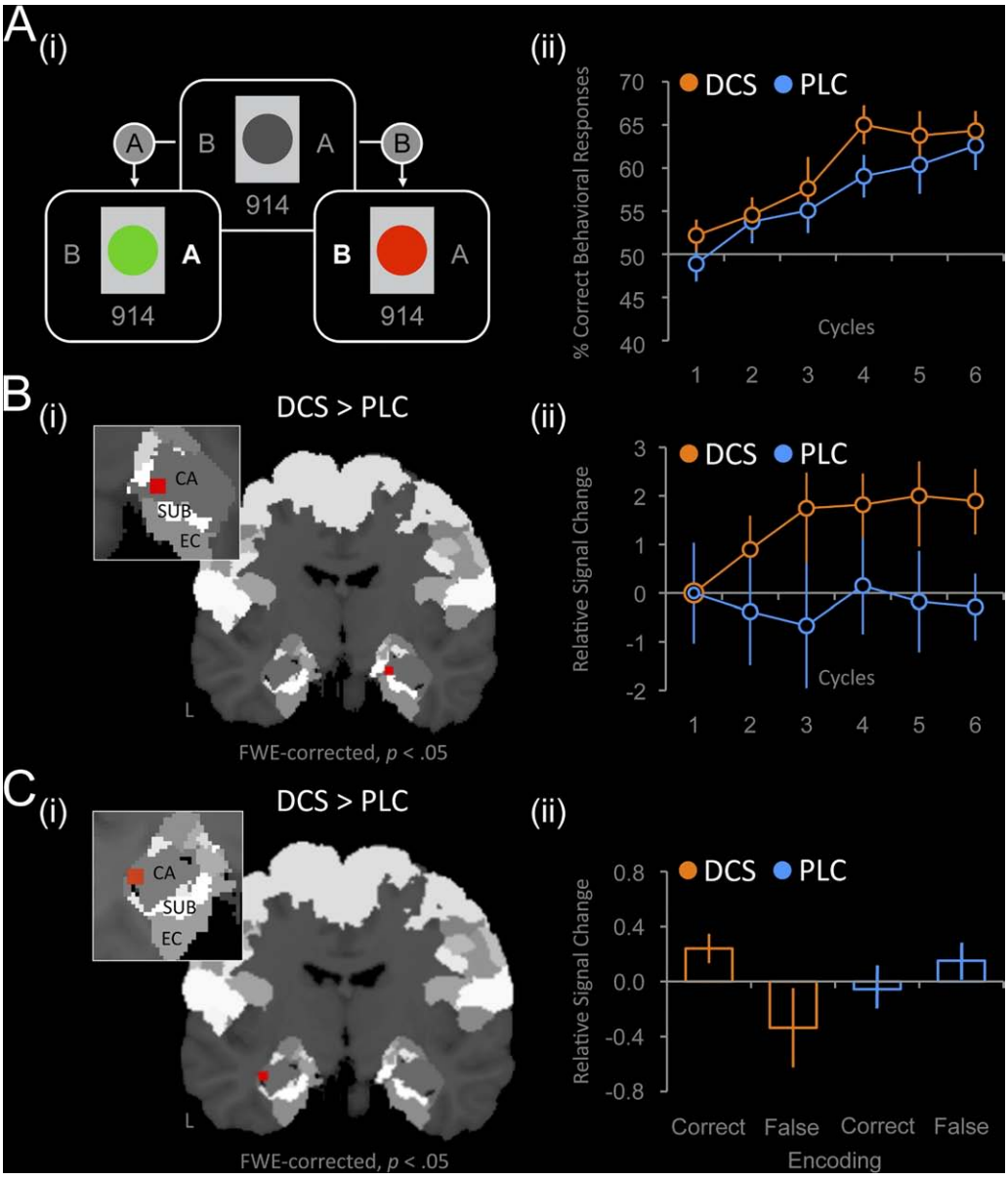

Figure 1. (A) Design of the feedback-guided item-category association task. (i) Subjects made push-button responses to judge the arbitrary category membership A or B of eight different three-digit numerical items presented repeatedly on-screen. In the first of six cycles, subjects had no knowledge of the correct category membership of each item and responded by guessing. Visual feedback (a gray circle changing to green for correct responses or to red for incorrect responses) immediately following each judgment informed subjects about the correct item-category association, thus enabling gradual increases in performance over subsequent cycles. (ii) A 250-mg single oral dose of D-cycloserine (DCS) decreased the number of cycles required to attain significantly improved performance compared with initial performance by two cycles (50%), thus resulting in a significant acceleration of learning although ultimate peak performance levels achieved were similar to the placebo (PLC) group. **(B)** Imaging data acquired with the item-category association task. (i) The probabilistic region-of-interest analysis revealed a differential effect of DCS over PLC treatment in the cornu ammonis (CA) region of the right hippocampus. (ii) Signal change courses demonstrated a trial repetition-related increase in CA responses under DCS treatment. **(C)** Imaging data acquired with the object-location association task. (i) Interaction contrasts revealed a differential effect of DCS over PLC treatment that probabilistically mapped to the CA region of the left hippocampus. (ii) The relative signal change profiles confirmed a DCS-induced enhancement of CA responses while encoding correct, but not incorrect, object-location associations. EC, encoding correct; FWE, family-wise error; SUB, subiculum.

were instructed to make a guess if they were uncertain about object locations.

Visual Stimulation Paradigm. We used a 10 × 10 checkerboard with a frequency of green-to-red switches of 8 per second over a block of 16-sec duration. Six blocks of checkerboard stimulation alternated with six blocks of rest, where a blank screen was presented.

Acquisition of Imaging Data

A TIM Trio MRI system (Siemens, Erlangen, Germany) operating at 3T was used to obtain T2*-weighted echo planar imaging (EPI) images with blood oxygenation level-dependent (BOLD) contrast. The following imaging parameters were applied for paradigm 1: repetition time, 2.24 sec; echo time, 30 msec; matrix size, 100 × 100; pixel size, 2 × 2 mm^2; slice thickness, 2.0 mm;

distance factor, 10%; field of view, 200 mm; flip angle, 90°; 36 axial slices (oriented centrally to the hippocampus); parallel acquisition technique (generalized autocalibrating partially parallel acquisitions); acquired volumes, 408. The following imaging parameters were applied for paradigms 2 and 3: repetition time, 2.20 sec; echo time, 30 msec; matrix size, 64 × 64; pixel size, 3.1 × 3.1 mm^2; slice thickness, 3.0 mm; distance factor, 10%; field of view, 200 mm; flip angle, 90°; 36 axial slices (oriented centrally to the hippocampus); acquired volumes, 464 (encoding, 172 volumes; retrieval, 292 volumes) (paradigm 2) and 100 (paradigm 3), respectively. The first four volumes were discarded to allow for T1 equilibration effects. In addition, we acquired high-resolution anatomical magnetic resonance images (T1-weighted three-dimensional magnetization-prepared rapid acquisition with gradient echo).

Analysis of Imaging Data

Image preprocessing was performed using Matlab7 (The MathWorks, Inc., Natick, Massachusetts) and Statistical Parametric Mapping 5 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm). The EPI images were corrected for head movements between scans by an affine registration (33). For realignment, a two-pass procedure was used by which images were initially realigned to the first image of the time series and subsequently realigned to the mean of all images. After completing the realignment, the mean EPI image for each subject was computed and spatially normalized to the Montreal Neurological Institute (MNI) template (34–36) using the unified segmentation function in Statistical Parametric Mapping 5. This algorithm is based on a probabilistic framework that enables the combination of image registration, tissue classification, and bias correction within the same generative model. The resulting parameters of a discrete cosine transform, which define the deformation field necessary to move the subjects' data into the space of the MNI tissue probability maps, were then combined with the deformation field transforming between the latter and the MNI single subject template. The ensuing deformation was subsequently applied to the individual EPI volumes. All images were hereby transformed into standard stereotaxic space and resampled at 2 × 2 × 2 mm^3 voxel size. The normalized images were spatially smoothed using an 8-mm full-width at half maximum Gaussian kernel.

Item-Category Association Task. An onset regressor was defined, indicating the onset times of all trials in which a correct behavioral response was recorded. An additional regressor indexing the number of repetitions of each stimulus as the parameter was included. The hemodynamic response to this event type was modeled using a canonical hemodynamic response function (HRF) and its first derivative, including the six head movement parameters as confounds. First-level linear baseline contrast was calculated comparing the regressors with the implicit baseline. This contrast was then taken to the second level, where it was subjected to an analysis of variance (ANOVA) with treatment (DCS vs. PLC treatment) as the between-subject factor. The *t* test analyses were used to constrain the direction of the observed effects. Unequal variances were compensated for by nonsphericity correction. In analogy to previous fMRI studies of gradual item-category learning that documented a linear adaptation of hippocampal responses as a function of trial repetitions (26,27), parametric modulation regressors were set to 1 to test for voxels with a repetition-dependent incline in BOLD signal amplitude and set to −1 to test for voxels with a repetition-dependent decline in BOLD signal amplitude. Bidirectional contrasts (i.e., DCS > PLC and PLC > DCS) were calculated. To report parameter estimates separately for each of the six cycles, a new model was estimated based on separate onset regressors for each cycle, again including only those trials in which a correct behavioral response was recorded. For a hypothesis-driven analysis, the left and right hippocampi were defined as ROIs (regions-of-interest) based on cytoarchitectonic probability maps derived from the histological analysis of 10 human postmortem brains (24,25). We applied corrections for multiple comparisons based on family-wise error (FWE; significance threshold $p < .05$). The feasibility of this probabilistic ROI approach has been confirmed by our previous work (37–41).

Object-Location Association Task. We defined four onset regressors specifying the onset times of encoding- and retrieval-related trials in which either correct or incorrect behavioral responses were recorded. Analogous to previous fMRI studies of object-location learning (28–30), our analysis focused on stimuli that were correctly recognized as "old." Depending on whether the object-location judgment for these stimuli succeeded or failed, encoding (E) and retrieval (R) trials were classified as either correct (EC, RC) or false (EF, RF). The hemodynamic response to each of these four different event types (subsequently referred to as accuracy) was modeled using a canonical HRF and its first derivative, including the six head movement parameters as confounds. First-level linear baseline contrasts were calculated comparing each onset regressor with the implicit baseline. These contrasts were then taken to the second level, where they were subjected to an ANOVA with accuracy as the within-subject factor and treatment as the between-subject factor. The *t* test analyses were used to constrain the direction of the observed effects. Unequal variances were compensated for by nonsphericity correction. Interactive contrasts were calculated separately for the encoding and retrieval phase, followed by an FWE-corrected probabilistic ROI analysis.

Visual Stimulation Paradigm. The visual stimulation was modeled by a boxcar function convolved with a canonical HRF. A design matrix comprising contrasts of alternating intervals of visual stimulation and rest, the time derivative, and the six head movement parameters as confounds was created. A first-level linear baseline contrast was calculated by comparing the boxcar function with the implicit baseline. This contrast was then taken to the second level, where it was subjected to an ANOVA with treatment as the between-subject factor. Unequal variances were compensated for by nonsphericity correction. Again, bidirectional contrasts were calculated using *t* test analyses.

Results

Behavior

Item-Category Association Task. In postscan interviews, 11 subjects reported that scanner noise had made the task too challenging for them. This was confirmed by near-floor behavioral performance, i.e., response accuracy was not greater than chance in all cases. Consequently, the behavioral and fMRI data acquired from these subjects had to be discarded from subsequent analyses of this task. For the remaining 29 subjects ($n = 15$ DCS; $n = 14$ PLC), a two-way repeated measures ANOVA with treatment group (PLC vs. DCS) as between-subject factor and cycle as within-subject factor revealed a main effect of group [$F(1,27) = 5.454$; $p = .027$] and a main effect of cycle [$F(5,135) = 8.696$; $p < .0001$] on performance but no group × cycle interaction effect [$F(5,135) = .233$; $p > .05$]. This indicates that treatment with a 250-mg single oral dose of DCS induced a

general improvement of performance. In addition, one-way repeated measures ANOVAs showed that both the PLC [$F(5,70) = 4.413$; $p = .001$] and DCS treatment groups [$F(5,70) = 5.412$; $p < .001$] significantly improved performance across cycles. Post hoc multiple comparisons between performance on cycle 1 and subsequent cycles using the Bonferroni paired *t* test revealed that for the DCS-treatment group performance on cycles 4 [$t(14) = 3.812$; $p = .004$], 5 [$t(14) = 3.442$; $p = .015$], and 6 [$t(14) = 3.607$; $p = .009$] was significantly improved but that there were no significant differences between performance on these last three cycles (all p values $> .05$). By contrast, in the PLC group, only performance on cycle 6 was significantly improved compared with cycle 1 [$t(13) = 3.386$; $p = .018$], although cycle 5 nearly achieved this [$t(13) = 2.944$; $p = .067$]. These results imply that learning speed was considerably faster in the DCS-treated group. Results are shown in Figure 1A(ii). Neither reaction times nor response misses differed between the PLC and DCS treatment groups (Figure S1 in Supplement 1). Furthermore, treatment had no differential effect on performance in the control condition, with all 29 subjects achieving a response accuracy of nearly 100% (all p values $> .05$).

Object-Location Association Task. In contrast to the item-category association task, no subjects reported any difficulty in performing the object-location association task in postscan interviews and this was confirmed by their performance scores. Two-sample *t* tests confirmed that both groups ($n = 20$ DCS; $n = 20$ PLC) performed almost identically on the retrieval of object-location associations [PLC group, 48.1 ± 13.8%; DCS group, 46.3 ± 15.1%; $t(38) = -.48$; $p > .05$]. Further, recognition of new objects did not differ between treatment groups [PLC group, 82.7 ± 14.7%; DCS group, 81.9 ± 13.7%; $t(38) = -1.11$; $p > .05$]. Neither reaction times nor response misses differed between treatment groups (all p values $> .05$).

Imaging

Item-Category Association Task. The probabilistic ROI analysis demonstrated a parametric effect of DCS over PLC treatment in the CA region of the right hippocampus (MNI coordinates xyz = 20, −11, −20; $p < .05$, FWE-corrected) (Figure 1B[i]). In the DCS group, but not in the PLC group, this CA response increased across cycles (Figure 1B[ii]). Thus, the faster learning seen in the item-category association task following DCS administration was paralleled by increased CA activity.

Object-Location Association Task. We found an interaction effect with the within-subject factor accuracy (EC vs. EF) and the between-subject factor treatment, which was restricted to the encoding phase of the task and probabilistically mapped to the left hippocampal CA region (MNI coordinates xyz = −36, −10, −21; $p < .05$, FWE-corrected) (Figure 1C[i–ii]). An across-group main effect of accuracy (RC vs. RF) was restricted to the retrieval phase of the task, evident in robust bilateral hippocampal activation (Table S2 in Supplement 1). No further suprathreshold effects occurred in this analysis.

Visual Stimulation Paradigm. Within-group analyses showed robust neural responses to checkerboard stimulation in bilateral visual cortex. However, between-group comparisons revealed no differential activations (Figure S2 in Supplement 1). This argues against nonspecific DCS effects resulting from a global potentiation of NMDAR activity or homogeneous changes in cerebral hemodynamics.

Discussion

In the present study, we combined conventional fMRI with cytoarchitectonic probabilistic mapping to make an initial attempt at a subdivision-level investigation of the effects of a 250-mg single oral dose of DCS on hippocampal function probed with two declarative learning tasks. Parametric analysis of fMRI data acquired with the item-category association task revealed that DCS enhanced hippocampal activity, an effect that probabilistically mapped to the CA region. Specifically, the corresponding signal change courses illustrate that DCS elevated CA activity across trial repetitions, whereas CA activity remained at baseline in the PLC group. This profile supports the hypothesis that DCS may increase the efficiency of learning by indirectly upregulating glutamate signaling via NMDAR to above-threshold levels, thereby recruiting previously silent CA synapses for the benefit of faster learning (12,42). The latter is evident at the behavioral level, where DCS reduced the number of trial repetitions required to attain significant improvement compared with initial performance by two cycles (50%), hence resulting in an overall significant enhancement of declarative learning, although ultimate peak performance levels achieved were similar to the control group and reaction times were unaffected. Our results are thus compatible with a report of a 50% reduction of trial repetitions in DCS-treated rabbits tested on a hippocampus-dependent gradual associative learning task (10).

Our findings raise the crucial question as to whether DCS modulates item-category associative learning by facilitating encoding- and/or retrieval-related operations in the hippocampal CA region. To determine whether one or both of these operations are susceptible to DCS action, subjects also completed an object-location association task, which tested encoding and retrieval of object-location associations separately from each other (28–30). In rodents, object-location learning engages the hippocampal area CA1 and is enhanced by DCS treatment (23). However, consistent with the observed lack of a DCS behavioral effect on peak performance in item-category associative learning, no overall beneficial effect was found in the object-location association task either. Nevertheless, interaction contrasts revealed a differential effect of DCS over PLC that again projected to the hippocampal CA region and was exclusively restricted to the encoding phase of the task. From these results, it appears that DCS enhanced CA responses during encoding of object-location associations but did not affect retrieval of these associations.

One important consideration in this context is the differentiation between a specific facilitation of learning-related NMDAR activity in the hippocampal CA region as opposed to nonspecific DCS effects resulting from a global potentiation of NMDAR activity or homogeneous changes in cerebral hemodynamics. To control for potential nonspecific DCS effects, all subjects were scanned on a checkerboard visual stimulation paradigm. Although the visual cortex is clearly an important site of NMDAR-mediated synaptic plasticity (43), between-group comparisons failed to find a differential effect of DCS on visual cortical responses to checkerboard stimulation. This absence of an effect supports the notion of a specific facilitative influence of DCS on learning-related CA activity. Thus, the present pharmacological fMRI study is the first to suggest that a 250-mg single oral dose of DCS improves declarative learning in healthy people through selective enhancement of hippocampal CA activity.

Despite substantial evidence from behavioral studies in rodents that DCS facilitates performance in hippocampus-dependent learning tasks (10,22,23), behavioral studies in humans have

failed so far to demonstrate beneficial effects of DCS in tests of immediate and delayed recall of verbal and nonverbal items (7,44), suggesting that the facilitative influence of DCS on hippocampal function is limited (20). However, we note that these negative studies used a substantially lower dose of DCS (50 mg) than the one we administered in our study (250 mg). Support for a dose-dependent variation in the efficacy of DCS comes from two fear extinction studies: Ledgerwood *et al.* (45) found effect sizes of .54, .80, and 1.43 for DCS versus PLC in rodents treated with 2.5, 5, and 10 mg, respectively, whereas Ressler *et al.* (13) found effect sizes of .36 and .86 for DCS versus PLC in phobic patients treated with 50 mg and 500 mg, respectively.

Given this empirical background, dosing, frequency, and chronicity of DCS treatment appear to be critical as to whether the use of DCS can be expanded to promote hippocampus-dependent declarative learning, aside from its experimental application in exposure-based CBT. Current hypotheses regarding the facilitation of declarative learning with DCS emphasize its potential to alleviate age-associated cognitive decline and to augment clinical response to CBT in patients, where a greater emphasis is placed on cognitive restructuring techniques, informational strategies, and skill acquisition interventions (44). However, if DCS indeed facilitates declarative learning by activating previously silent CA synapses, as is suggested by our results, it may be ineffective in conditions characterized by progressive hippocampal degeneration and synaptic loss (46,47).

RH was supported by a German Research Foundation Grant (HU1302/2-2) and by a Starting Independent Researcher Grant jointly provided by the Ministry of Innovation, Science, Research and Technology of the State of North Rhine-Westphalia and the University of Bonn. KMK was supported by the Biotechnology and Biological Sciences Research Council.

We thank M.X. Cohen for helpful comments on a previous version of the manuscript and K. Linkert and A. Kirsch for research assistance.

The authors report no biomedical financial interests or potential conflicts of interest.

ClinicalTrials.gov: The influence of glutamate on memory in humans; http://clinicaltrials.gov; NCT00980408.

Supplementary material cited in this article is available online.

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