Nicotinic Acetylcholine Receptors Contribute to Learning-induced Metaplasticity in the Hippocampus

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Abstract

■ Hippocampal learning is thought to induce metaplasticity, which can facilitate subsequent learning. Administered at single low doses, the *N*-methyl-D-aspartate-type glutamate receptor antagonist memantine predominantly blocks α7 nicotinic acetylcholine receptors (α7 nAChRs). Placebo-controlled administration of a single low dose of memantine in a pharmaco-fMRI experiment may thus help characterize the role of α7 nAChRs in hippocampal metaplasticity. We hypothesized that if α7 nAChRs contribute to learning-induced metaplasticity in the hippocampus, blockade of these receptors with low-dose memantine would selectively interfere with a facilitation of subsequent learning without impairing hippocampal learning per se. To specifically test this hypothesis, we devised a randomized controlled trial in which healthy volunteers were administered a 20-mg single oral dose of memantine or placebo and scanned on three subsequent runs of a hippocampal learning task. Our results indicate no discrepancies in behavioral learning between low-dose memantine- and placebo-treated participants in the first and second run of this task. In the third run, however, only the placebo-treated group showed facilitated behavioral learning, an effect paralleled by decreased neural responses in the hippocampal cornu ammonis region. Our findings suggest that blockade of α7 nAChRs selectively interfered with a learning-induced facilitation of subsequent learning while leaving unimpaired hippocampal learning per se. Taken together, our results provide support for a relevant contribution of α7 nAChRs to learning-associated metaplasticity in the hippocampus. ■

INTRODUCTION

An important theme in the neurobiology of memory is the question of how current learning is influenced by prior learning. Emerging evidence suggests that learning-induced plasticity in the hippocampus is not a fixed uniform response, but instead plasticity is rapidly shaped by the trace left by prior neural activity. Such activity-dependent dynamic control over the onset and extent of hippocampal plasticity is known as metaplasticity (Hulme, Jones, Ireland, & Abraham, 2012; Abraham, 2008; Zelcer et al., 2006; Abraham & Bear, 1996; see also Byrne & Kandel, 1996).

One means of elucidating the molecular machinery underlying hippocampal metaplasticity is pharmaco-fMRI. In the present randomized placebo-controlled trial (RCT), we combined fMRI with probabilistic mapping of the hippocampus and its major subregions to study the behavioral and neural effects of memantine on hippocampal metaplasticity. Memantine is an antidementive agent licensed by the U.S. Food and Drug Administration and the European Medicines Agency to treat moderate-to-severe Alzheimer's disease (Howard et al., 2012). Owing to its fast off-rate kinetics and voltage-dependent affinity for *N*-methyl-D-aspartate-type glutamate receptors (NMDARs), memantine has been described as an atypical NMDAR antagonist; as such, it exerts neuroprotective and promnestic actions in the absence of neurobehavioral side effects by targeting tonic NMDAR overactivation and resultant excitotoxic neurodegeneration without compromising phasic NMDAR signaling and learning-related plasticity per se (Lipton, 2007; Parsons, Stoffler, & Danysz, 2007). Despite this unique profile, there is emerging evidence suggesting impaired rather than improved hippocampal plasticity following single-dose administration of memantine in healthy humans (Rammsayer, 2001; Schugens et al., 1997). Whereas NMDAR antagonism of low-dose memantine may be too weak to explain these findings, in vitro experiments have shown that the agent potently blocks α7 nicotinic acetylcholine receptors (α7 nAChRs; Aracava, Pereira, Maelicke, & Albuquerque, 2005; see also Pohanka, 2012). Densely expressed in the hippocampus, α7 nAChRs have both direct and indirect neuromodulatory roles by influencing procedural skill learning (Young, Meves, Tarantino, Caldwell, & Geyer, 2011) and attention/working

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memory operations (Young et al., 2007) and by controlling synaptic transmission and plasticity in the hippocampus per se (Taly, Corringer, Guedin, Lestage, & Changeux, 2009; Ge & Dani, 2005). Specifically, presynaptic α7 nAChRs enhance glutamate release in the hippocampal cornu ammonis (CA) region and postsynaptic α7 nAChRs promote the induction of NMDAR-dependent long-term potentiation in vitro, thereby augmenting synaptic efficacy (Dani & Bertrand, 2007; Levin & Simon, 1998).

In the light of evidence suggesting that α7 nAChRs exert a continual modulatory influence on hippocampal learning, we hypothesized that blockade of α7 nAChRs with low-dose memantine would not collapse learning in general but selectively impair a learning-induced metaplastic facilitation of subsequent learning (Hulme et al., 2012; Abraham, 2008; Dani & Bertrand, 2007; Levin & Simon, 1998; Abraham & Bear, 1996). To test this hypothesis, healthy adult volunteers were administered either a 20-mg single dose of memantine or placebo. They were then scanned on three subsequent runs of a task requiring gradual associative learning on new sets of item–category associations in each run. Similar tasks have previously been shown to heavily engage the hippocampus (Strange, Hurlemann, Duggins, Heinze, & Dolan, 2005; Strange, Fletcher, Henson, Friston, & Dolan, 1999), particularly the CA region (Onur et al., 2010), which is known as a key locus of learning-associated metaplasticity (Zelcer et al., 2006). To control for the possibility that potential intrahippocampal effects could be secondary to an extrahippocampal blockade of α7 nAChRs and/or reflect impaired attention/working memory operations rather than altered metaplasticity effects on learning, participants were additionally scanned on a face perception task and a numeric *n*-back task, which includes an attention (0-back) and a working memory (2-back) condition. We predicted that low-dose memantine would critically interfere with α7 nAChR-dependent metaplasticity in the hippocampal CA region, evident in the absence of a metaplastic facilitation of subsequent learning, while leaving unaffected learning per se.

METHODS

Participants

The present RCT was approved by the Institutional Research Board (Identifier: 113/08) of the University of Bonn and by the German Federal Institute of Drugs and Medical Devices (Identifier: 4033608). Moreover, the study was registered in the Clinical Trials.gov database (Identifier: NCT00980408) provided by the U.S. National Institutes of Health. Volunteers gave informed consent before participation, and all investigation was conducted at the University of Bonn according to the principles expressed in the Declaration of Helsinki. Volunteers were free of current or past physical or psychiatric illness, as assessed by medical history and a Structured Clinical Interview for DSM-IV axis I and axis II disorders. Tobacco smokers and volunteers with known contraindications for MRI scanning were excluded from participation. To control for pretreatment differences in cognitive performance, all participants were administered a comprehensive neuropsychological test battery. Screening included the RAVLT (Rey Auditory Verbal Learning Test; Helmstaedter, Lendt, & Lux, 2001; Rey, 1941) to assess verbal learning skills; the DST (digit-span test) derived from the WAIS-R (revised version of the Wechsler Adult Intelligence Scale; Wechsler, 1997) to assess working memory; the LPS-4 (Leistungsprüfsystem Subtest 4; Horn, 1983) to assess nonverbal reasoning IQ; the MWT-B (Mehrfach-Wortschatz-Intelligenztest Teil B; Lehrl, 1978) to assess verbal IQ based on lexical decisions; the d2 test (Aufmerksamkeits- und Belastungstest d2; Brickenkamp, 1995) to assess visual attention and concentration; and the TMT (Trail-Making Test) part A and B (Raitan, 1958) to assess visual attention and task switching. Handedness was determined by the EHI (Edinburgh Handedness Inventory; Oldfield, 1971). Two-sample *t* tests confirmed no significant ($p < .05$) pretreatment differences in demographic and neuropsychological characteristics between the placebo- and memantine-treated samples (Table 1). All participants were instructed to maintain their regular sleeping and waking times and to abstain from caffeine and alcohol intake on the day before undergoing fMRI scanning. Compared with placebo (PLC), memantine (MEM) is generally well tolerated, and none of the participants reported side effects in interviews 4 hr after drug administration and immediately before the fMRI acquisition. From the 49 participants (MEM, $n = 24$; PLC, $n = 25$) initially enrolled in this study, 13 participants had to be excluded from further analysis. Six participants (MEM, $n = 4$; PLC, $n = 2$) had to be excluded either because of technical failures during data acquisition or because of excessive interscan motion (>2 mm translation, 1.5° rotation on one of the tasks). In postscan interviews, seven participants reported difficulties with task completion because of the scanner noise (MEM, $n = 4$; PLC, $n = 3$). Inspection of the behavioral data confirmed that response accuracy in the learning task was not greater than chance for these participants (see also Results: Item–Category Association Task), underlining the highly demanding nature of the applied learning task. In a previous RCT, testing the NMDAR coagonist D-cycloserine, this approach has been shown to reduce unsystematic variance, thus increasing statistical power to detect treatment-related effects (Onur et al., 2010). Consequently, subsequent analyses were performed on the behavioral and fMRI data acquired from 36 participants (MEM, $n = 17$; PLC, $n = 19$). Fischer's exact test ($p = .69$, two tailed) confirmed that there were no significant between-group differences in the proportion of participants excluded. To further control for confounding effects and a selection bias, significant treatment effects were recomputed for (1) a sample of all participants ($n = 43$; MEM, $n = 21$; PLC = 22) and (2) a subsample that included only participants who reached at least an a priori criterion of 55% mean correct responses for the three runs ($n = 34$; MEM, $n = 15$; PLC, $n = 19$; Fischer's exact test, two-tailed $p = .28$).

Experimental Protocol

The rationale for this RCT was to investigate the behavioral and neural effects of low-dose memantine relative to placebo on learning-induced metaplasticity in the hippocampus. A schematic synopsis of the experimental timeline is given in Figure 1A. This timeline accounts for the pharmacokinetic profile of memantine, that is, after ingestion of a 20-mg single oral dose, peak plasma levels are reached approximately 7 hr later with the elimination half-life ranging between 60 and 100 hr (Kornhuber & Quack, 1995). Whereas previous behavioral studies have administered higher doses to study functional impairments as a result of NMDAR blockade (Parsons et al., 2007; Rammsayer, 2001; Schugens et al., 1997), we administered a 20-mg dose because of our a priori experimental focus on α7 nAChRs and their hypothesized contribution to hippocampal metaplasticity (see also Results: Memantine Serum Levels).

Experimental Tasks

Item–Category Association Task

Hippocampal learning was probed using an item–category association task. In previous studies from our research team, this task showed a high sensitivity to detect pharmacological modulation of hippocampal learning (Hurlemann et al., 2010; Mihov et al., 2010; Onur et al., 2010; Figure 1B). Participants were required to make push-button responses to judge category membership "A" or "B" for three-digit numerical items (henceforth called stimuli) presented repeatedly. Participants were explicitly informed that category membership was arbitrary without any probabilistic rules and that they would have to guess the correct response for the first presentation. Once assigned, category membership remained constant over six presentations (cycles). For the first cycle, participants responded with a 50% probability of making the correct choice. A gray circle changed to green for correct and to red for incorrect responses. This feedback informed the participants about the correct item–category association and enabled gradually increased response accuracy over the subsequent cycles. To control for visuomotor learning, the response buttons for "A" or "B" changed, depending on the random lateralization of "A" and "B" on the screen. In total, participants completed three runs of the task with a new set of three-digit numerical items presented during each run. In each run, six different stimuli were presented over six cycles, leading to 36 trials (a trial is defined as the period composed of stimulus, subject's response, and feedback) per run and 108 trials for the entire experiment. Within each cycle, presentation of trials was randomized; trial duration was 3500 msec

Table 1. Demographic and Neuropsychological Sample Characteristics

Characteristic	*Placebo (n = 19)*	*Memantine (n = 17)*	*p*
Age (years)	25.7 (0.5)	24.7 (0.4)	.1
RAVLT Trials 1–5[a]	63.6 (1.8)	61.9 (1.1)	.3
RAVLT Trial 5[b]	14.1 (0.3)	13.7 (0.3)	.2
RAVLT Trial 6 Retention[c]	13.4 (0.4)	12.9 (0.4)	.3
RAVLT Trial 7 Delayed Recall[d]	13.1 (0.4)	13.2 (0.5)	.4
LPS-4[e]	29.7 (0.8)	30.1 (0.8)	.7
MWT-B[f]	31.4 (0.7)	31.3 (0.6)	.9
d2[g]	196.5 (10.6)	211.8 (11.0)	.3
TMT-A[h]	27.0 (1.8)	27.2 (1.8)	.9
TMT-B[h]	60.2 (4.5)	68.4 (4.7)	.2
Digit-span, forward[i]	8.4 (0.6)	9.1 (0.4)	.4
Digit-span, backwards[i]	9.0 (0.5)	8.3 (0.5)	.4
Latency (hr)[j]	8.9 (18.9)	9.8 (34.1)	.2

Two-sample *t* tests confirmed no significant ($p < .05$) pretreatment differences in demographic and neuropsychological characteristics between the placebo- and memantine-treated groups (displayed are mean and *SEM* for both groups). Verbal declarative memory performance was assessed using a German adaption of the RAVLT (Helmstaedter et al., 2001; Rey, 1941) and included [a]learning performance across five trials (maximum possible score 75), [b]performance trial 5 (maximum possible score 15), [c]susceptibility to interference (maximum possible score 15), and [d]delayed recall (maximum possible score 15). Nonverbal reasoning IQ was assessed by the [e]LPS (Leistungsprüfsystem) subtest 4 (maximum possible score, 40; Horn, 1983). Verbal IQ based on lexical decisions was assessed by the [f]MWT-B (Mehrfachwahl-Wortschatz-Intelligenz-Test Teil B; maximum possible score 37; Lehrl, 1978). Visual attention and concentration was assessed using the [g]d2 (Aufmerksamkeits- und Belastungstest d2; Brickenkamp, 1995). Visual attention and task-switching was assessed using the [h]TMT-A and [h]TMT-B (Trail-Making Test A, B; results displayed in seconds; Raitan, 1958). Working memory performance was assessed using the [i]DST (digit-span forward and backward test; maximum possible score 14) derived from the WAIS-R (revised version of the Wechsler Adult Intelligence Scale; Wechsler, 1997). [j]Latency refers to the interval between drug intake and start of the fMRI session (see also Figure 1A).

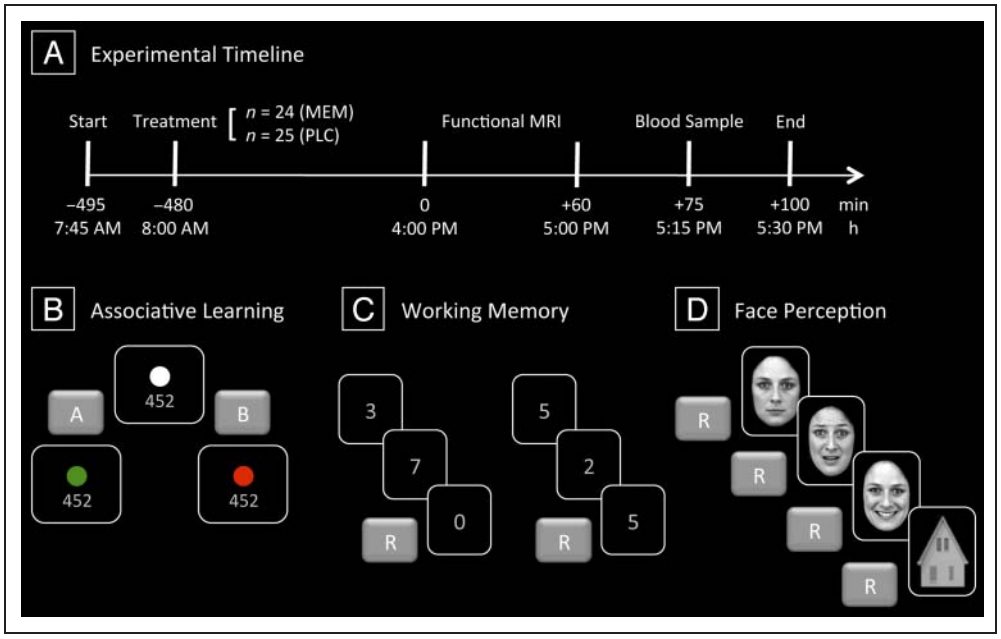

Figure 1. Experimental timeline and schematic synopsis of fMRI tasks. (A) Participants were administered either placebo (PLC) or memantine (MEM) 8 hr (t = −480 min) before fMRI scanning. Four participants were scanned per day, starting at 4:00 p.m. and finishing at 8:00 p.m. Total scanning time for each participant was approximately 60 min (t = +60 min); after fMRI scanning a venous blood sample was drawn (t = +75) for memantine serum level analysis. This timeline accounts for the specific pharmacokinetic profile of memantine. (B) An item–category association task was administered to probe hippocampus-dependent learning. Participants had to gradually learn associations between three-digit numerical items and category membership "A" or "B." Category membership was arbitrary, but once assigned, remained constant over six presentations. After each judgment, visual feedback (a gray circle changing to green for correct responses or to red for incorrect responses) was given to inform participants about the correct item–category association, thus enabling gradually increased response accuracy over the subsequent cycles. Participants were scanned on three subsequent runs of the task with different sets of three-digit numerical items. (C) Working memory performance and associated neural activity was assessed using a numerical n-back task in a blocked design with an attention (0-back) and a working memory condition. During the 0-back condition, the target item was designated (zero), whereas during the 2-back condition participants had to press the button each time the presented item was identical to the item presented two trials before. (D) A passive viewing face perception task was incorporated as a noncognitively demanding control task. Participants were shown fearful, neutral, and happy facial expressions. Additionally, pictures of houses were presented, which served as a control condition. Participants had to press a button in response to each stimulus, thus assuring attentive stimulus processing. Abbreviations: MEM = memantine-treated individuals; PLC = placebo-treated individuals.

(stimulus–response duration = 2500 msec, feedback duration = 1000 msec) and the duration of the jittered intertrial interval was 2250 msec (1500–3000 msec). Each run had a mean duration of approximately 3.5 min, at which 72 scans were recorded. The total scanning time was 10.5 min, resulting in 216 scans for the entire task.

Numerical n-Back Task

Attention/working memory performance and associated neural activity were assessed using a numeric n-back task (Figure 1C) in a blocked design with two conditions: in the 2-back working memory condition, participants had to respond by button press when the presented number was identical to the number presented two trials before; in the 0-back attention condition, participants had to respond by button press each time the number zero was presented on-screen. Numerical items (henceforth called stimuli) were presented for 300 msec with an ISI of 1700 msec. Each block comprised 25 stimuli (including six targets) resulting in a block length of 50 sec. In total, five blocks of both conditions were presented. The sequence of blocks was randomized, and blocks were separated by a low-level baseline during which a fixation cross was presented in the center of the screen. The specific type of condition was defined by an instruction slide displayed for 1500 msec before the start of each block. In total, 195 scans were recorded; the task lasted approximately 10 min.

Face Perception Task

To stimulate the face-processing network, we used a face perception task that reliably evoked robust activity in face-processing regions in previous studies (Onur et al., 2012; Goossens et al., 2009; see also Patin & Hurlemann, 2011; Figure 1D). Specifically, participants were exposed to photographs depicting 40 individuals showing fearful, neutral, and happy facial expressions selected from the validated "The Karolinska Directed Emotional Faces" database (Oosterhof & Todorov, 2008; Lundqvist, Flykt, & Ohman, 1998) and pictures of houses, which served as a non-face control condition. All stimuli were grayscaled and equated for size and luminance. Stimuli were presented block-wise. Each stimulus was presented for 2625 msec, with an ISI varying between 250 and 1500 msec, hence resulting in a mean block length of 14.5 sec. Each block comprised four stimuli of the same emotional category (fearful, happy, neutral) or four houses. In total, 10 blocks of fearful, happy, and neutral faces were presented. The sequence of blocks was randomized, and blocks were

separated by a low-level baseline during which a fixation cross was presented in the center of the screen. Participants had to press a button in response to each face occuring on-screen to ensure that stimuli were attended to. In total, 296 scans were recorded, and the task lasted approximately 20 min.

Acquisition of fMRI Data

fMRI employing BOLD contrast was performed on a 1.5-T Siemens Avanto MRI system (Siemens, Erlangen, Germany) using a T2*-weighted echoplanar EPI sequence (imaging parameters: repetition time = 3000 msec, echo time = 50 msec, matrix size = 64 × 64, pixel size = 3.3 × 3.3 mm^2, slice thickness = 3.0 mm, distance factor = 10%, field of view = 210, flip angle = 90°, 36 axial slices). In addition, high-resolution anatomical images were acquired on the same scanner using a T1-weighted 3D MPRAGE sequence (imaging parameters: repetition time = 1660 msec, echo time = 3.09 msec, matrix size = 256 × 256, pixel size = 1 × 1 mm^2, slice thickness = 1.0 mm, field of view = 256, flip angle = 15°, 160 sagittal slices). All stimuli were presented by means of liquid crystal display video goggles (Nordic NeuroLab, Bergen, Norway) connected to a PC running Presentation 14 (Neurobehavioral Systems, Inc., Albany, CA). Before fMRI scanning, participants underwent a training session including different sets of stimuli to allow familiarization with the experimental tasks.

Analysis of fMRI Data

Preprocessing

The first five volumes of each functional time series were discarded to allow for T1 equilibration. Images were corrected for head movement between scans by an affine registration (Ashburner & Friston, 2003). For realignment, a two-pass procedure was used where images were initially realigned to the first image of the time series and subsequently re-realigned to the mean of all images. Next the mean functional image was coregistered to the T1 image of each participant. Normalization parameters were determined by segmenting the T1 images using the default tissue probability maps as priors. These normalization parameters were subsequently used to spatially normalize all functional images to the standard anatomical Montreal Neurological Institute (MNI) space. The normalized images were spatially smoothed using an 8-mm FWHM Gaussian kernel and raw time series were detrended by the application of a high-pass filter (cutoff period, 128 sec). MRI data were analyzed using a random-effect approach within the framework of the general linear model as implemented in SPM8 (Wellcome Trust Centre for Neuroimaging, London, U.K.; www.fil.ion.ucl.ac.uk/spm).

Item–Category Association Task

On the basis of our initial hypothesis, two separate models were computed to test for treatment effects (A) on the general ability to learn associations and (B) on the metaplastic facilitation of subsequent learning. Possible effects of treatment on associative learning were addressed by analyzing drug effects within the three runs of the task. Therefore, an onset regressor for the three runs was defined, indicating the onset times of all trials in which a correct behavioral response was recorded. In line with previous studies (Onur et al., 2010; Strange et al., 1999, 2005), learning effects within the runs of the task were modeled by adding a regressor indexing the number of repetitions of each stimulus as the parameter. The hemodynamic response was modeled using a canonical hemodynamic response function (HRF), including the six head movement parameters as confounds. Parametric regressors were pooled across the three runs of the task and set to 1 to test for voxels with a repetition-dependent incline and to −1 for voxels with a repetition-dependent decline in BOLD signal amplitude. These individual first-level contrasts ("repetition incline," "repetition decline") were taken to the second level and subjected to an ANOVA with treatment (memantine [MEM] vs. placebo [PLC]) as between-subject factor. Possible effects of treatment on metaplastic facilitation of subsequent learning were addressed by analyzing drug effects across the three runs of the task. Therefore, separate onset regressors were defined for each run, indicating the onset times of all trials at which a correct behavioral response was recorded. The hemodynamic response was modeled using an HRF including the six head movement parameters as confounds. The individual first-level contrasts ("Correct Trials Run 1," "Correct Trials Run 2," "Correct Trials Run 3") were passed to a second-level repeated-measures ANOVA. A flexible factorial design was used to model the between-subject factor Treatment (MEM vs. PLC) and the within-subject factor run (Runs 1–3). The main effect of the factor Group was assessed by aggregating individual activity maps for the three runs on the first level, which were then entered in a second-level two-sample *t* test.

Numerical Working Memory Task

Conditions (2-back, 0-back) were modeled by a boxcar function convolved with an HRF (Friston et al., 1995). A design matrix including the contrasts of the different blocks and the six head movement parameters was created. Specific effects were assessed by applying appropriate linear contrasts to the parameter estimates of the experimental trials resulting in *t* statistics for each voxel. Effects of treatment on working memory were analyzed using *t* tests for the contrast "2-back > 0-back." Effects on attention were analyzed using separate *t* tests for the attentional 0-back condition compared with the implicit baseline.

Face Perception Task

Conditions (fearful faces, happy faces, neutral faces, and houses) were modeled by a boxcar function convolved with an HRF (Friston et al., 1995). A design matrix comprising

contrasts of alternating intervals of the different blocks and the six head movement parameters was created. Specific effects were assessed by applying appropriate linear contrasts to the parameter estimates of the experimental trials resulting in t statistics for each voxel. To control for treatment-induced homogenous changes in cerebral hemodynamics between-group differences in the visual network ("all stimuli > implicit baseline") were analyzed. To further control for nonspecific treatment effects on brain functions that are independent from hippocampal learning between-group differences in the face-processing network ("all faces > houses") were analyzed.

Statistical Thresholding and Definition of ROIs

All results are reported at a threshold of $p < .05$ corrected for multiple comparisons based on family-wise error (FWE) within ROIs appropriately chosen according to the task (bilateral hippocampus ROI encompassing the CA, dentate gyrus and entorhinal cortex subregions for the learning task, and bilateral pFC ROI encompassing BA 46 and BA 47 for the working memory task). The ROI encompassing the bilateral hippocampus was defined by means of the SPM Anatomy toolbox, Version 1.8 (Eickhoff et al., 2005, 2007), which provides cytoarchitectonic probability maps derived from the histological analysis of 10 human postmortem brains; the ROI encompassing BA 46 and BA 47 were defined using the WFU Pickatlas toolbox, Version 3.0 (Maldjian, Laurienti, & Burdette, 2004; Maldjian, Laurienti, Kraft, & Burdette, 2003; Tzourio-Mazoyer et al., 2002).

RESULTS

Behavioral Data

Item–Category Association Task

In the first step, learning performance for the participants who were included and excluded from further analysis was inspected (see also Methods: Participants). Participants had to guess the correct responses for the first presentation (cycle) of each stimulus within a run and performed at chance (50%). With increasing stimulus presentations, participants improved performance as demonstrated by (A) repeated-measures ANOVA testing for Cycle × Performance (percent correct responses) effects collapsed across the three runs, $F(5, 31) = 19.34$, $p < .001$, and (B) responses 20.69% (*SEM*, 2.61) above chance level after six cycles. Separate repeated-measures ANOVAs for the three runs confirmed that participants improved during each run [Run 1, $F(5, 31) = 3.91$, $p = .002$; Run 2, $F(5, 31) = 5.56$, $p < .001$; Run 3, $F(5, 31) = 17.01$, $p < .001$]. In contrast, participants who reported having difficulties due to scanner noise failed to improve with increasing stimulus presentations (<1%, *SEM*, 3.91, above chance after six cycles collapsed across the three runs; Figure 2).

In the next step, treatment effects within and across the runs of the task were analyzed in two separate analyses. In a first analysis, possible effects of treatment on associative learning were addressed by analyzing drug effects within the runs of the task. A two-way repeated-measures ANOVA with the Percent Correct Responses as

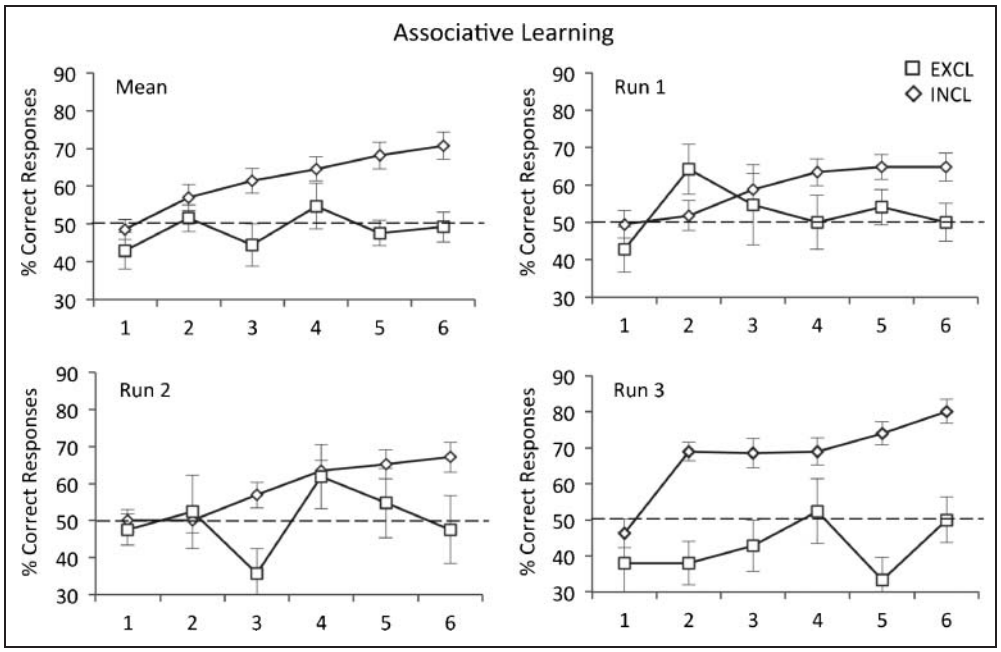

Figure 2. Learning performance profiles of the subjects that were included or excluded based on self-reported difficulties because of the noisy scanning environment. Diagrams show means and standard errors of the mean (*SEM*). With increasing stimulus presentations, participants ($n = 36$) improved mean (collapsed across the three runs) and run-specific performance (all $p < .05$). In contrast, participants who reported difficulties with task completion because of scanner noise ($n = 7$) failed to improve with increasing stimulus presentations (mean response accuracy < 1%, *SEM*, 3.91, above chance after six cycles). Abbreviations: EXCL = participants who reported concentration difficulties because of scanner noise; INCL = participants who did not report difficulties because of scanner noise.

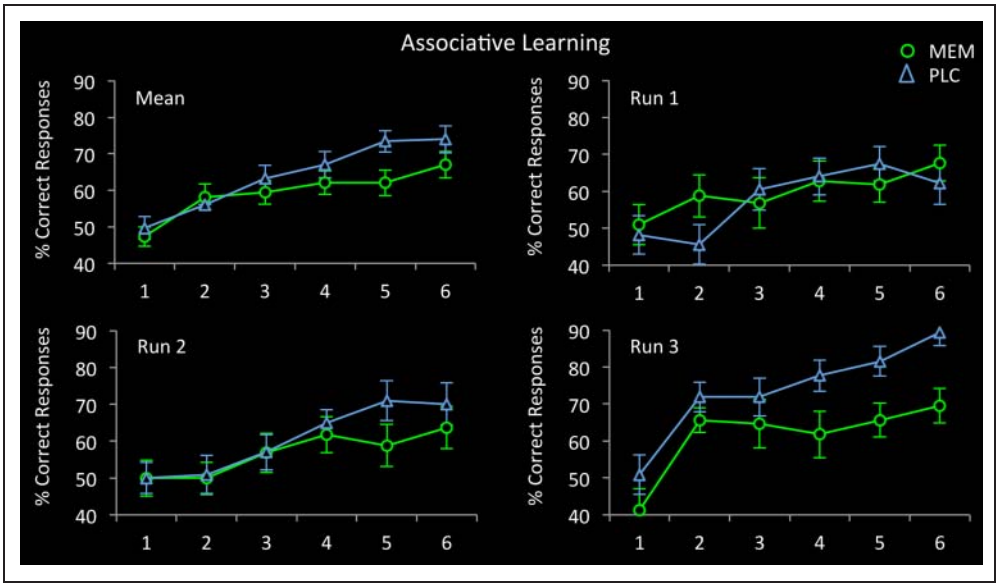

Figure 3. Learning performance profiles of the memantine- and placebo-treated participants within the runs of the associative memory task. Diagrams show means and standard errors of the mean (*SEM*). Memantine- ($n = 17$) and placebo-treated ($n = 19$) participants improved mean performance across cycles ($p < .05$) but did not differ in performance within the runs of the task. Further exploratory analyses for the separate runs revealed that both groups showed performance improvements within each run ($p < .05$); however, placebo-treated participants demonstrated better learning performance in the third run ($p < .05$). Abbreviations: MEM = memantine-treated individuals; PLC = placebo-treated individuals.

dependent variable, Treatment (PLC vs. MEM) as between-subject factor, and Cycle (Cycles 1–6) as within-subject factor was used. Results revealed a main effect of Cycle, $F(5, 30) = 19.16, p < .001$, but no main effect of Group, $F(1, 34) = 1.84, p = .18$, or Group × Cycle interaction, $F(5, 30) = 1.53, p = .18$, indicating that the placebo- and memantine-treated groups both improved across cycles but did not differ in performance within the runs of the task (learning performance profiles of the treatment groups are plotted in Figure 3). These effects remained stable for the entire sample along with the subsample, which only included participants who reached the a priori defined performance criterion (Table 2). Additional two-way repeated-measures ANOVAs were used to explore treatment effects on learning performance within the separate runs of the task. Findings confirmed that within each run both groups improved performance across cycles [main effect of Cycle, Run 1, $F(5, 30) = 3.79$; Run 2, $F(5, 30) = 5.33$; Run 3, $F(5, 30) = 16.68$; all $p > .01$]. A Group × Cycle interaction effect did not appear in the separate runs [Run 1, $F(5, 30) = 3.79$; Run 2, $F(5, 30) = 5.33$; Run 3, $F(5, 30) = 16.68$; all $p > .01$] nor did a main effect of Group in the first two runs. However, the PLC group showed better learning performance in the third run [main effect of Group, Run 1, ($F(1, 34) = 0.16$; Run 2, $F(1, 34) = 0.88$; Run 3, $F(1, 34) = 5.85, p = .02$; Figure 3].

In a second analysis, possible effects of treatment on metaplastic facilitation of subsequent learning were addressed by analyzing drug effects across the runs of the task. A two-way repeated-measures ANOVA with the number of Correct Responses per Run (Σ correct responses across Cycles 2–6) defined as dependent variable, Treatment (PLC

Table 2. Behavioral Performance Criteria Underlying Analysis 1

Selection Criterion	*ME Cycle F(df)*	*ME Treatment F(df)*	*IE Cycle × Treatment F(df)*
None, $n = 43$ (MEM, $n = 21$; PLC, $n = 22$)	17.21 (5, 37)*	3.10 (1, 41)	2.12 (1, 41)
Self-report, $n = 36$ (MEM, $n = 17$; PLC, $n = 19$)	19.16 (5, 30)*	1.84 (1, 34)	1.53 (1, 34)
50% correct responses, $n = 34$ (MEM, $n = 15$; PLC, $n = 19$)	24.92 (5, 28)*	1.53 (1, 32)	1.70 (1, 32)

To control for potential confounding effects of participant selection the two-way repeated-measures ANOVA with the Percent Correct Responses as dependent variable, Treatment (PLC vs. MEM) as between-subject factor, and Cycle (Cycles 1–6) as within-subject factor was recomputed for the entire sample ($n = 43$) and a subsample including only participants who reached an a priori defined performance criterion. Results from the initial analysis ($n = 36$) remained stable, arguing against strong confounding effects of the selection criteria applied. Shown are *F* values and corresponding degrees of freedom. *p* Values of <.05 were considered significant. Abbreviations: MEM = memantine-treated individuals; PLC = placebo-treated individuals; ME = main effect; IE = interaction effect.

*Significant at $p < .05$.

vs. MEM) as between-subject factor, and Run (Runs 1-3) as within-subject factor revealed a main effect of Run, $F(2, 33) = 11.24, p < .001$, a Group × Run interaction effect, $F(2, 33) = 3.29, p = .04$, but no main effect of Group, $F(1, 34) = 1.81$, $p = .19$. Again, effects remained stable for the entire sample and the subsample which included only participants who reached the a priori defined performance criterion (Table 3). Separate one-way repeated-measures ANOVAs for each treatment group, with run as within-subject factor, showed only the PLC group improved performance across runs, $F(2, 17) = 13.49, p < .001$, whereas the MEM group failed to improve across runs, $F(2, 15) = 1.79, p = .18$. Post hoc multiple comparisons for analysis of within-group effects using Bonferroni-corrected paired t tests revealed that the PLC group showed improved overall performance in the third run (77% correct responses; *SEM* = 3.30) compared with the first (60% correct responses; *SEM* = 3.54), $t(18) = -5.67, p < .001$, and second run (63% correct responses; *SEM* = 3.63), $t(18) = -3.83, p = .001$. In contrast there was no such difference in the MEM group (all ps > .071). A between-group comparison restricted to the third run revealed that the PLC group (77% correct responses; *SEM* = 3.30) learned better in this run than the MEM group (66% correct responses; *SEM* = 3.61), $t(34) = 2.43, p = .02$ (Figure 4A).

In addition to treatment effects on response accuracy, effects on response latencies for the correct trials were analyzed using two-way repeated-measures ANOVA with treatment (PLC vs. MEM) as between-subject factor and cycle (Cycles 1–6) as within-subject factor. This analysis revealed a main effect of cycle, $F(5, 30) = 10.68, p < .001$, but no main effect of group, $F(1, 34) = 1.65, p = .20$, or Group × Cycle interaction effect, $F(5, 30) = 1.46$, $p = .20$, indicating that both groups responded faster across cycles. Effects of treatment across the runs of the task were analyzed using a two-way repeated-measures ANOVA with Mean Response Latencies per run as dependent variable, Treatment (PLC vs. MEM) as between-subject factor, and Run (Runs 1-3) as within-subject factor. This analysis revealed a main effect of Run, $F(2, 33) = 24.64, p < .001$, but no main effect of Group, $F(1, 34) = 1.65, p = .21$, and no Group × Cycle interaction effect, $F(2, 33) = 0.60$, $p = .55$.

Numerical Working Memory Task

For the working memory task the correct response rates, false alarm rates, the number of missed items, and the RTs for correct responses were analyzed using separate two-way repeated-measures ANOVAs with Treatment (PLC vs. MEM) as the between-subject factor and Condition (0-back vs. 2-back) as the within-subject factor. Analyses revealed a main effect of Condition for the correct responses, $F(1, 34) = 15.28, p < .001$, false alarms, $F(1, 34) = 41.36, p < .001$, missed items, $F(1, 34) = 11.15, p = .002$, and RTs, $F(1, 34) = 33.94, p < .001$, confirming the higher working memory load of the 2-back condition compared with the 0-back condition. Neither a main effect of Group (all ps > .423) nor Group × Run interaction effects (all ps > .45) reached statistical significance, arguing against substantial treatment effects on attention or working memory performance.

fMRI Data

Item–Category Association Task

As with the behavioral data, separate analyses were computed to analyze treatment effects within and across the runs of the task. First, analysis of treatment effects within the runs of the task revealed both a lack in significant main effects for repetition within runs ("repetition incline," "repetition decline") and treatment group, and a lack of significant interaction effect. Second, analysis of treatment effects across the runs of the task revealed a significant main effect in the right thalamus [MNI coordinates $x = 10, y = -10, z = 16$; $F(1, 68) = 32.47, p < .05$, FWE corrected], left anterior cingulate [MNI coordinates $x = -2, y = 22, z = -4$; $F(1, 68) = 32.15, p < .05$, FWE corrected], and the right superior frontal gyrus [MNI coordinates $x = -62, y = -50,$

Table 3. Behavioral Performance Criteria Underlying Analysis 2

Selection Criterion	*ME Run F(df)*	*ME Treatment F(df)*	*IE Run × Treatment F(df)*
None, $n = 43$ (MEM, $n = 21$; PLC, $n = 22$)	6.45 (2, 40)*	2.70 (1, 41)	4.38 (1, 41)*
Self-report, $n = 36$ (MEM, $n = 17$; PLC, $n = 19$)	11.24 (2, 33)*	1.81 (1, 34)	3.29 (2, 33)*
50% correct responses, $n = 34$ (MEM, $n = 15$; PLC, $n = 19$)	4.66 (2, 31)*	4.27 (1, 32)	1.19 (1, 32)*

To control for potential confounding effects of participant selection the two-way repeated-measures ANOVA with the percent correct responses as dependent variable, treatment (PLC vs. MEM) as between-subject factor, and run (Runs 1–3) as within-subject factor was recomputed for the entire sample ($n = 43$) and a subsample including only participants who reached an a priori defined performance criterion. Results from the initial analysis ($n = 36$) remained stable, arguing against strong confounding effects of the selection criteria applied. Shown are F values and corresponding degrees of freedom. p Values of <.05 were considered significant. Abbreviations: MEM = memantine-treated individuals; PLC = placebo-treated individuals; ME = main effect; IE = interaction effect.

*Significant at $p < .05$.

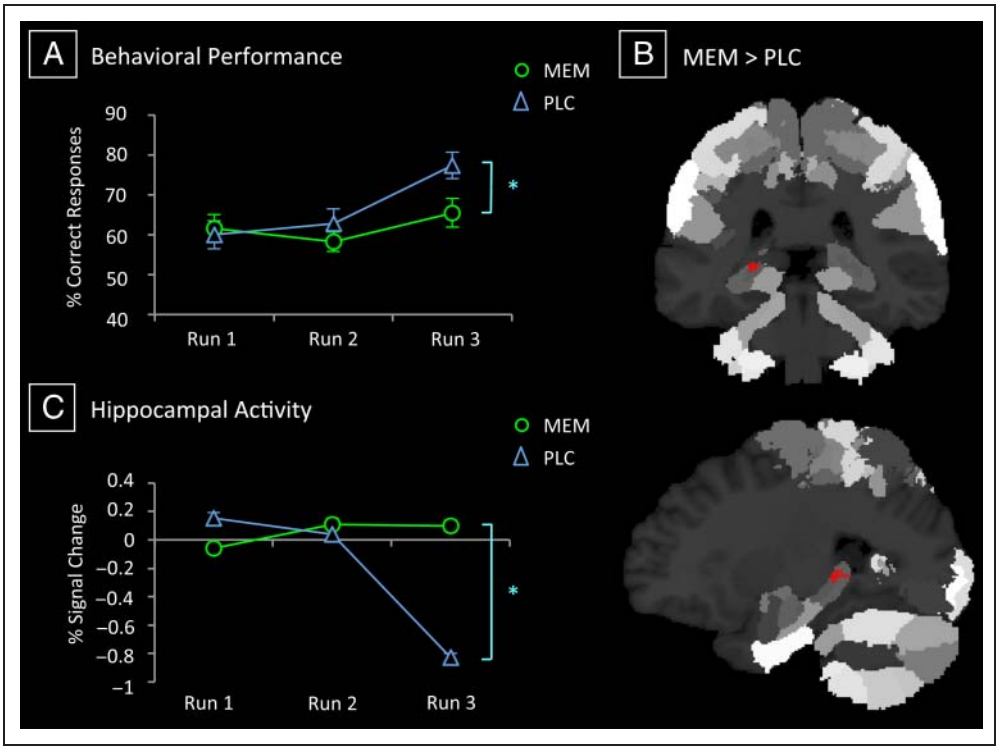

Figure 4. Learning performance and associated neural activity of the memantine- and placebo-treated participants between the runs of the associative memory task. Diagrams show means and standard errors of the mean (*SEM*). (A) Post hoc Bonferroni-corrected paired *t* tests revealed that the placebo-treated ($n = 19$) individuals showed improved performance in the third run compared with performance in the first ($t18 = -5.67$, $p < .001$) and the second ($t18 = -3.83$, $p = .001$) run, whereas the memantine-treated ($n = 17$) participants failed to improve in the third run (all ps $> .071$). Between-group analyses revealed a significantly better learning performance for the placebo-treated individuals in the third run ($t34 = 2.43$, $p = .02$). (B) The probabilistic ROI analysis revealed a significant effect of placebo over memantine treatment in the CA region of the left hippocampus. For illustrational purpose only, findings are displayed at an uncorrected ($p < .001$) significance level. (C) Signal change profiles as extracted from the left hippocampal CA region. Post hoc Bonferroni-corrected paired *t* tests revealed a significant decrease in CA activity from Run 1 to Run 3 ($t18 = 5.26$, $p < .001$) for the placebo-treated group, whereas the memantine-treated group showed a significant increase in CA activity from Run 1 to Run 3 ($t16 = -2.93$, $p = .010$) and from Run 1 to Run 2 ($t16 = -3.15$, $p = .006$). Between-group analyses revealed significantly larger neural responses in participants treated with memantine ($t34 = -3.71$, $p = .001$). Abbreviations: MEM = memantine-treated individuals; PLC = placebo-treated individuals.

$z = 16$; $F(1, 68) = 32.03$, $p < .05$, FWE corrected] but no significant main effect for treatment group. Hypotheses-driven ROI analysis revealed a significant Group × Run interaction effect located in the CA region of the left hippocampus [MNI coordinates $x = -26$, $y = -34$, $z = 0$; $F(1, 68) = 20.23$; $p < .05$, FWE-corrected; Figure 4B]. Effects remained stable for the entire sample [MNI coordinates $x = -24$, $y = -38$, $z = 4$; $F(1, 82) = 19.20$; $p < .05$, FWE-corrected] and the subsample that included only participants who reached the a priori defined performance criterion [MNI coordinates $x = -25$, $y = -35$, $z = 1$; $F(1, 64) = 21.53$; $p < .05$, FWE-corrected]. For further analysis, individual percent signal change values for suprathreshold voxels were extracted from an 8-mm radius sphere centered at the coordinates of the Group × Run interaction (rfxplot toolbox for SPM; Glascher, 2009). A two-way repeated-measures ANOVA with Treatment Group (PLC vs. MEM) as between-subject factor and Run as within-subject factor revealed no main effect of Group, $F(1, 34) = 0.35$, $p = .56$, or run, $F(2, 33) = 1.47$, $p = .24$, but a significant Group × Run interaction effect $F(2, 33) = 13.28$, $p < .001$. Separate one-way repeated-measures ANOVAs for the treatment groups with Run as within-subject factor showed that both the PLC group, $F(2, 17) = 9.44$, $p < .001$, and the MEM group, $F(2, 15) = 5.69$, $p = .008$, changed in CA activity across runs. Post hoc multiple comparisons for the within-group effects using Bonferroni-corrected paired *t* tests revealed that CA activity significantly decreased from Run 1 to Run 3 in the PLC group, $t(18) = 5.26$, $p < .001$, whereas the MEM group showed a significant increase in CA activity from Run 1 to Run 3, $t(16) = -2.93$, $p = .010$ [and also from Run 1 to Run 2; $t(16) = -3.15$, $p = .006$; Figure 4C]. A between-group comparison restricted to the third run revealed that the MEM group had a larger neural response in the CA region, $t(34) = -3.71$, $p = .001$. To explore extrahippocampal treatment effects an exploratory whole-brain analysis was computed ($p < .001$, uncorrected, cluster size $>$ 20 voxels). The unrestricted analysis only revealed a significant effect in the left hippocampus [MNI coordinates $x = -24$, $y = -34$, $z = 2$; $F(1, 68) = 24.24$, $p < .001$, uncorrected; cluster size = 41], indicating hippocampus-specific effects.

Numerical Working Memory Task

A whole-brain one-sample *t* test for the contrast "2-back > 0-back" confirmed that the working memory task evoked widespread responses in the frontoparietal working memory networks (Wager & Smith, 2003) spanning frontoparietal regions (results not shown). An anatomically defined ROI analysis of these regions, however, yielded no

significant between-group differences for the 0-back attentional condition or the 2-back working memory condition, which argues against substantial treatment effects in the domains of attention or working memory.

Face Perception Task

In accordance with our previous studies (Becker et al., 2013; Onur et al., 2012; Patin & Hurlemann, 2011), the administered task evoked robust activity in widespread visual networks ("all stimuli > implicit baseline") and the face processing network ("all faces > houses"; Fusar-Poli et al., 2009) including the right fusiform gyrus (MNI coordinates $x = 39, y = -43, z = -17$; $t(35) = 5.61$; $p < .05$, FWE-corrected; results not shown). However, there were no significant between-group differences, arguing against treatment-induced homogenous changes in cerebral hemodynamics and non-specific treatment effects on brain function.

Memantine Serum Levels

Analysis of venous blood samples collected after fMRI data acquisition revealed memantine serum levels in the expected range of 19–33 μg/L (mean = 23.5 μg/L, $SD = 3.4$ μg/L) following administration of a 20-mg single oral dose (Kornhuber & Quack, 1995). Given a cerebrospinal fluid/serum ratio of 0.52 for memantine (Kornhuber & Quack, 1995), resultant brain concentrations can be expected in a range of 50–80 nM, which is too low to reach the K_I value of 650 nM for NMDARs, but exceeds the K_I value of 30 nM for α7 nAChRs (Aracava et al., 2005; see also Pohanka, 2012). Thus, the observed behavioral and neural effects of memantine may be almost exclusively related to α7 nAChR antagonism. Our analyses revealed no dose–response associations between memantine serum levels and behavioral performance indices or extracted percent signal changes (all ps > .05).

DISCUSSION

In the present RCT, we investigated the behavioral and neural effects of low-dose memantine across three independent runs of a hippocampus-dependent associative learning task. Although memantine- and placebo-treated participants showed no discrepancies regarding behavioral learning and associated neural activity in the first and second run of this task, only the placebo-treated group exhibited a significant improvement of behavioral learning in the third run. This performance gain was paralleled by declining hippocampal responses on the neural level, an effect that was probabilistically mapped to the left CA. Importantly, this facilitation was absent in volunteers treated with low-dose memantine, although their general ability to rapidly learn associations was preserved. This result appears unlikely if the agent had significantly interfered with NMDAR-dependent synaptic plasticity during learning per se.

Given that participants had to learn a new set of item–category associations in each run of the task, we interpret the converging behavioral and neural effects observed in the placebo-treated group as the result of activity-dependent increases in synaptic efficacy because of α7 nAChR-mediated upregulation of plasticity in the hippocampal CA region (Dani & Bertrand, 2007; Ge & Dani, 2005; Levin & Simon, 1998; see also Taly et al., 2009). In this context, we need to take into account, though based on the characteristic behavioral deficits occurring in knockout mice, α7 nAChRs have also been implicated in procedural skill learning (e.g., acquisition of rules in probabilistic learning tasks; Young et al., 2011) and attention/working memory operations (Young et al., 2007) in addition to their hypothesized role in the regulatory control of hippocampal synaptic transmission and plasticity (Dani & Bertrand, 2007; Ge & Dani, 2005; Levin & Simon, 1998). Deficient procedural skill learning because of blockade of extrahippocampal α7 nAChRs would typically result in a relatively delayed execution of highly practiced cognitive tasks. Given the absence of between-group differences in response latencies, however, this interpretation is not supported by our data. Moreover, behavioral performance in the numerical *n*-back task and associated neural activity patterns remained unchanged, suggesting that blockade of α7 nAChRs with low-dose memantine was insufficient to significantly compromise attention/working memory operations. Furthermore, administration of memantine had no modulatory impact on the neural substrates of face perception, such that confounding effects of α7 nAChR activity changes elsewhere in the brain or nonspecific modulatory effects on cerebral hemodynamics also appear unlikely. In this context, we acknowledge that both control tasks were applied only once, such that we cannot exclude that adaptive effects of treatment might have become obvious across repeated runs of the control tasks. Taken together, our results argue against the interpretation that the observed effects of memantine are predominantly driven by or indirectly reflective of reduced extrahippocampal α7 nAChR activity.

In concert with mounting evidence that low-dose memantine interferes with hippocampal plasticity in healthy humans (Rammsayer, 2001; Schugens et al., 1997), we believe that our data primarily reflect intrahippocampal drug action. However, in contrast to previous studies (Rammsayer, 2001; Schugens et al., 1997), we had an a priori experimental focus on challenging α7 nAChR-dependent processes and thus administered a low oral dose of memantine. As a consequence, memantine-treated participants performed slightly less efficiently and had to afford more hippocampal resources in the third run of the task than placebo-treated participants. This specific profile is consistent with our a priori hypothesis that pharmacological blockade of α7 nAChRs would impair a learning-induced facilitation of subsequent learning in the hippocampal CA region rather than altering learning

per se (Young et al., 2007; Ge & Dani, 2005). In conclusion, our findings provide converging behavioral and neural evidence for a critical role of α7 nAChRs in learning-associated metaplasticity in the hippocampus.

A key implication of learning-associated metaplasticity is that the underlying molecular control mechanisms facilitating subsequent learning are already operative during current learning (Abraham, 2008; Zelcer et al., 2006). In this study, for instance, a possible α7 nAChR-mediated metaplastic upregulation of presynaptic glutamate release in the first and/or second run of the task may have facilitated subsequent plasticity induction in the third run of the task, thereby promoting performance in the placebo-treated group but not in the memantine-treated group. Thus, metaplasticity control mechanisms may commence to function rapidly after their initial engagement to put hippocampal synapses and networks into a learning-sensitive state (Abraham, 2008; Zelcer et al., 2006). Harnessing these mechanisms may prove to have important clinical usefulness, particularly as the more tempting direct pharmacological manipulations of hippocampal plasticity are likely to be limited by severe side effects (Abraham, 2008; see also Taly et al., 2009).

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