State-Dependent Partial Occlusion of Cortical LTP-Like Plasticity in Major Depression

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The synaptic plasticity hypothesis of major depressive disorder (MDD) posits that alterations in synaptic plasticity represent a final common pathway underlying the clinical symptoms of the disorder. This study tested the hypotheses that patients with MDD show an attenuation of cortical synaptic long-term potentiation (LTP)-like plasticity in comparison with healthy controls, and that this attenuation recovers after remission. Cortical synaptic LTP-like plasticity was measured using a transcranial magnetic stimulation protocol, ie, paired associative stimulation (PAS), in 27 in-patients with MDD according to ICD-10 criteria and 27 sex- and age-matched healthy controls. The amplitude of motor-evoked potentials was measured before and after PAS. Patients were assessed during the acute episode and at follow-up to determine the state- or trait-character of LTP-like changes. LTP-like plasticity, the PAS-induced increase in motor-evoked potential amplitudes, was significantly attenuated in patients with an acute episode of MDD compared with healthy controls. Patients with remission showed a restoration of synaptic plasticity, whereas the deficits persisted in patients without remission, indicative for a state-character of impaired LTP-like plasticity. The results provide first evidence for a state-dependent partial occlusion of cortical LTP-like plasticity in MDD. This further identifies impaired LTP-like plasticity as a potential pathomechanism and treatment target of the disorder.

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INTRODUCTION

The World Health Organization lists major depressive disorder (MDD) as the leading cause for reduced quality of life due to illness worldwide (years of life lived with disability index) (WHO, 2012). Still, the pathophysiology of the disorder remains to be further elucidated. The *synaptic plasticity hypothesis* of MDD posits that alterations in synaptic plasticity represent a final common pathway underlying the biological and clinical characteristics of the disorder (Castrén, 2013). The current work was designed to further test the synaptic plasticity hypothesis of MDD.

Synaptic plasticity is a fundamental characteristic of animals and humans, which enables the brain to dynamically adapt to internal and external stimuli in a changing environment (Citri and Malenka, 2008). The main mechanism for the activity-dependent increase in synaptic efficacy is associative long-term potentiation (LTP) of glutamatergic synaptic transmission (Bliss and Collingridge, 1993), which is modulated by neurotrophic factors such as brain-derived neurotrophic factor (BDNF) (Cheeran *et al*, 2008; Kleim *et al*, 2006). Associativity of LTP is defined by concomitant

activation of two inputs to the same neuron. This form of LTP was first discovered in the hippocampus of mice (Barrionuevo and Brown, 1983). By now, associative LTP has also been demonstrated in a variety of neocortical networks.

Animal models of depression indicate an impairment of LTP in the hippocampus of rats exposed to chronic mild stress (Alfarez *et al*, 2003; Pavlides *et al*, 2002), a key factor in the precipitation of MDD in humans (Wurtman, 2005). In contrast, chronic mild stress in animals facilitates the induction of synaptic long-term depression (LTD) that decreases synaptic transmission (Holderbach *et al*, 2007; Xu *et al*, 1997). Diverse antidepressant treatments reverse these effects (Holderbach *et al*, 2007; Rocher *et al*, 2004).

Some studies have begun to translate this concept assessing non-invasive indices of synaptic plasticity in humans. Our group provided first evidence for reduced LTP-like plasticity in patients with MDD based on visually-evoked potentials (Normann *et al*, 2007; Teyler and Cavus, 2007). Further studies in humans were consistent with the concept of decreased synaptic plasticity in a dorsal executive network that comprises the hippocampus, as indexed by declarative memory consolidation (Nissen *et al*, 2010), and elevated synaptic plasticity in a ventral emotional network, as indexed by fear acquisition (Nissen *et al*, 2010) and fear extinction (Kuhn *et al*, 2014). However, these readouts comprise complex brain circuits and multi-synaptic pathways, making it difficult to determine the exact location and mechanisms of alterations.

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Figure 1 Study design. After screening, all participants (patients with major depressive disorder (MDD) and healthy controls) completed the paired associative stimulation (PAS) protocol (T1). Patients were reassessed at follow-up to determine the state- or trait-character of PAS-induced long-term potentiation (LTP)-like changes (T2).

The brain stimulation protocol *paired associative stimulation* (PAS) represents an important non-invasive tool to indirectly probe synaptic plasticity in humans (Stefan *et al*, 2000). In this protocol, electrical stimulation of a peripheral nerve (typically the median nerve) is repetitively paired with focal single-pulse transcranial magnetic stimulation (TMS) over the contralateral motor cortex. At an interstimulus interval of 25 ms, the electrical stimulus slightly precedes the TMS pulse in M1 leading to a LTP-like potentiation of synaptic transmission that can be measured as an increase in the TMS-evoked motor-evoked potential (MEP) amplitude in the corresponding hand muscle. This PAS-induced LTP-like plasticity shows similar properties to LTP in animal slice experiments, namely associativity, input specificity, and dependency on *N*-methyl-D-aspartate receptor (NMDAR) functioning (Stefan *et al*, 2002).

The PAS protocol has been used in a variety of neuropsychiatric disorders (Ziemann *et al*, 2008). For instance, in patients with schizophrenia (Frantseva *et al*, 2008) and high-functioning autism (Jung *et al*, 2013) a reduction in the PAS–LTP effect has been shown. Importantly, initial evidence indicates reduced LTP-like plasticity in patients with MDD relative to healthy controls (Player *et al*, 2013). However, this observation has not been replicated and it remains unclear whether reduced LTP-like plasticity recovers (state-marker) or persists after remission (trait-marker). Particularly, the characterization as a state-marker would identify reduced LTP-like plasticity as a potential pathomechanism of MDD and motivate further application of the broad preclinical knowledge on synaptic plasticity to treatment developments for MDD to overcome the current innovation crisis in psychopharmacology.

The aim of this study was to further characterize LTP-like plasticity in patients with MDD using PAS and to test the hypotheses that (1) cortical LTP-like plasticity is reduced in patients with an acute episode of MDD compared with healthy controls as indexed by a reduced increase in MEP amplitudes after PAS and (2) reduced LTP-like plasticity recovers after remission (state-character).

MATERIALS AND METHODS

Participants

A total of 27 patients (12 females, 15 males, aged 19–58 years) who met ICD-10 criteria for severe unipolar depression (F32.2 and F33.2) and 27 sex- and age-matched healthy controls (13 females, 14 males, aged 18–55 years)

participated in the study. Depressed patients were in-patients at the Department of Psychiatry and Psychotherapy, University Medical Center Freiburg, and were receiving psychotherapy and stable medication (>2 weeks) with one or more antidepressants according to standard guidelines for severe unipolar depression ($n=15$ venlafaxine, $n=5$ bupropion, $n=2$ tricyclic antidepressants, $n=1$ mirtazapine, $n=1$ lithium, $n=1$ lamotrigine, $n=1$ duloxetine, and $n=1$ no medication). All patients were free of benzodiazepines 2 weeks before and during the assessments (exclusion criterion). Depression severity was assessed using the 21-item Hamilton Depression Rating Scale (HAMD) (Hamilton, 1960) and the Beck Depression Inventory-Second Edition (BDI-II) (Beck *et al*, 1996). Criterion for inclusion was an HAMD score of ≥ 18 for MDD patients. Fifteen patients presented with a recurrent disorder. The total duration of illness at the time of assessment was 6.1 ± 7.3 years, the number of episodes was 3.2 ± 2.9 , and the duration of the current episode was 8.3 ± 7.5 months. According to the Massachusetts General Hospital Antidepressant Treatment Response Questionnaire (ATRQ) (Desseilles *et al*, 2011), eight patients were considered as resistant to antidepressant treatment during the current episode. Patients were excluded if they had another ICD-10 axis I disorder (lifetime history) or a comorbid personality disorder. Healthy controls were recruited in the community and were reimbursed for participation. They took part in an extensive diagnostic examination, including the Composite International Diagnostic Interview and standard clinical assessments, to rule out any mental or relevant physical disorders. All participants were right-handed according to the Edinburgh Handedness Inventory, did not take any CNS-active medication (other than antidepressants for patients), had no history of neurological illness, and did not meet any exclusion criteria for TMS. All participants reported a cigarette consumption of <10 cigarettes per day. All participants gave written informed consent before the study. The study was approved by the local Ethics Committee at the University Medical Center Freiburg (36/07) and was conducted in accordance with the Declaration of Helsinki.

Study Design

The study design is depicted in Figure 1. Patients with an acute episode of MDD and healthy controls underwent a PAS protocol (T1). After discharge from the hospital, patients were invited to participate in the same protocol to determine the state- or trait-character of PAS-induced

changes (follow-up, T2). To limit circadian effects, all measurements took place between 0830 and 1100 h in the morning.

Transcranial Magnetic Stimulation

The PAS protocol closely followed standard procedures (Stefan *et al*, 2000; Ziemann *et al*, 2004). TMS was applied using a figure-of-eight coil with an outer diameter of 90 mm that was connected to a Magstim 200 stimulator (The Magstim Company, Whitland, UK). The coil was held tangentially to the skull over the right primary motor cortex (M1) with the handle pointing in a posterior direction and laterally at an angle of 45° away from the midline. By moving the coil over M1 while administering stimuli of suprathreshold intensity at 0.25 Hz, the optimal coil position for eliciting MEPs of maximal amplitude of the abductor pollicis brevis (APB) muscle was identified ('hotspot'). This coil position was recorded using a stereotaxic, optically tracked navigation system, consisting of a camera (Polaris Vicra P6, NDI, Waterloo, ON, Canada), custom-made software (BrainView, Fraunhofer Institute (IPA), Stuttgart, Germany), and passive sphere markers (Jung *et al*, 2010), and kept constant throughout the measurements. Resting motor threshold (RMT) was determined using a maximum-likelihood threshold-hunting paradigm (Awiszus, 2003) that consisted of 16 TMS stimuli at 0.25 Hz. The stimulation intensity for MEP measurements was adjusted to elicit MEPs with peak-to-peak amplitudes of on average 600–1400 μ V (SI_{1mV}), and was kept constant throughout all measurements. The stimulation intensity was expressed as the percentage of the maximal stimulator output (% MSO). Twenty TMS pulses were administered at a frequency of 0.1 Hz before and 2, 30, and 60 min after the end of PAS. The mean peak-to-peak amplitude was calculated by averaging the individual peak-to-peak amplitudes of the 20 TMS pulses via Signal Software (CED, UK). Participants were instructed to relax the target muscle during all measurements. Relaxation was monitored visually via an electromyogram (EMG).

Electromyographic Recording

MEPs were recorded from the left APB muscle at rest using silver/silver chloride electrodes (AMBU, Ballerup, Denmark) in a belly-tendon montage. EMG signals were band-pass filtered (20–2000 Hz), amplified using an Ekida DC universal amplifier (EKIDA GmbH, Helmstadt, Germany), digitized at a 5 kHz sampling rate using a MICRO1401mkII data acquisition unit (CED), and stored on a computer for offline analysis. MEPs with preceding muscle activity were excluded from the analysis.

Paired Associative Stimulation

The PAS protocol comprised 200 pairs of peripheral and cortical stimuli, given at a frequency of 0.25 Hz (total duration ~13 min). The peripheral pulse was delivered to the median nerve of the left wrist at an intensity of 300% of the sensory perceptual threshold by a Digitimer DS7 electrical stimulator (Digitimer, Welwyn Garden City, Hertfordshire, UK). Stimuli were constant current square

wave pulses with a duration of 1000 μ s. TMS intensity was set at SI_{1mV} as determined before PAS. The interstimulus interval (ISI) between the peripheral and cortical stimulation was 25 ms. As the level of attention influences PAS effectiveness (Stefan *et al*, 2004), participants were instructed to direct their attention to the stimulated hand during the PAS intervention. They received intermittent electrical stimuli to the thumb of the stimulated hand via a bipolar electrode (200% perceptual threshold, cathode proximal, constant current square wave pulses, duration 200 μ s). Participants were informed that up to 10 electrical stimuli would be administered and instructed to silently count the number during the protocol. In fact, a total of four electrical stimuli were applied at the midpoint of the interval between successive paired stimuli. After the PAS intervention, participants were asked to report the number of stimuli delivered to the thumb.

Experimental TMS Design and Data Analysis

Mean MEP amplitudes and RMTs were assessed before the PAS intervention (pre-PAS) and at three time points after PAS (post 1: 2 min, post 2: 30 min, and post 3: 60 min). The number of TMS stimuli before PAS was limited to 200 to prevent an interference with the subsequent induction of plasticity (Delvendahl *et al*, 2010). Figure 2 shows the timeline of measurements.

For each participant, an individual mean of the 20 MEP amplitudes was calculated for each time point of the investigation (pre-PAS, post 1, post 2, post 3). In line with Player *et al* (2013), the post-PAS measures (post 1, post 2, post 3) were normalized by dividing them by the baseline measure (pre-PAS) to account for interindividual variance in TMS responses. All post-PAS analyses were performed with normalized data.

Neuropsychological Tests

To control for general neuropsychological effects, we conducted the Digit Span test of the Hamburg–Wechsler Intelligence Scale for Adults (Tewes, 1991) and the subtest Alertness of the Test for Attentional Performance (TAP) (Zimmermann and Fimm, 1992). The Digit Span test measures short-term memory capacity and requires participants to immediately recall spoken strings of digits in a forward and backward order. The subtest Alertness is a simple reaction time test. Participants have to respond to a visual stimulus with and without an auditory cue (tonic alertness and phasic alertness, respectively) by pressing a response button as fast as possible.

BDNF Genotyping

To further explore the potential effect of the BDNF on synaptic plasticity, we determined the BDNF genotype. Genomic DNA was purified from 3 ml of whole blood. In all, 50 ng of genomic DNA was used to amplify a 281-bp PCR product surrounding the site of the Val66Met polymorphism for subsequent direct sequencing by GATC Biotech in Konstanz. Direct sequencing was performed with 3.2 pmol of the reverse primer used for initial PCR amplification. Genotypes of each subject were determined

Figure 2 Timeline of the measurements. Motor-evoked potential (MEP) amplitudes and resting motor threshold (RMT) were measured before and at three points in time after paired associative stimulation (PAS). PAS was performed with an interstimulus interval of 25 ms between electrical stimulation of the median nerve and single-pulse transcranial magnetic stimulation of the contralateral motor cortex to induce long-term potentiation (LTP)-like plasticity.

Table 1 Demographic and Clinical Characteristics

	Patients (n = 27)	Healthy controls (n = 27)	t/χ^2	p
<i>Demographic parameters</i>				
Age (years)	38.7 ± 11.2	37.3 ± 10.3	0.5	0.652
Sex (m/f)	15/12	14/13	$\chi^2 = 0.1$	0.785
Years in school	11.8 ± 2.0	12.5 ± 1.1	−1.6	0.125
<i>Clinical characteristics</i>				
BDI-II	23.6 ± 11.3	2.1 ± 3.0	9.6	<0.001
HAMD-21	29.6 ± 6.3	—	—	—
PSQ	65.9 ± 13.0	27.0 ± 15.9	9.8	<0.001
<i>Neuropsychological tests</i>				
TAP (tonic alertness)	255.6 ± 48.6	258.5 ± 40.7	−0.2	0.810
TAP (phasic alertness)	254.7 ± 56.8	257.1 ± 33.7	−0.2	0.849
Memory span	14.8 ± 4.3	15.5 ± 4.3	−0.6	0.549

Abbreviations: BDI-II, Beck Depression Inventory-Second Edition; HAMD-21, 21-item Hamilton Depression Rating Scale; PSQ, Perceived Stress Questionnaire; TAP, attention test battery.

Data represent means ± standard deviations. Student's *t*-tests for independent samples were used, where applicable. Significant results are given in bold.

following two independent rounds of direct sequencing (Fritsch *et al*, 2010). The following primers were used: 5'-CAGGTGAGAAGAGTGATGACCA-3' (forward) and 5'-GCATCACCTGGACGTGTAC-3' (reversed).

Data Analysis

We used IBM SPSS 21 for statistical analysis. Data are reported as means ± standard deviations. Student's *t*-tests for independent samples were used to compare demographic/clinical characteristics and baseline (pre-PAS) TMS parameters between the groups (MDD and controls).

All post-PAS analyses were performed with normalized data. To verify that the PAS intervention in our study caused a comparable effect size in healthy controls as shown in previous studies, we calculated Cohen's d_z (small: <0.5; medium: ≥0.5; large: ≥0.8) for the difference in MEP amplitudes before (pre-PAS) and after PAS (averaged across post 1, post 2 and post 3) in healthy controls.

A 2 × 3 repeated measures analysis of variance (rm-ANOVA) with the between-subject factor Group (MDD vs controls) and the within-subject factor Time (post 1, post 2, post 3) was conducted in normalized MEP amplitudes at the first TMS measurement (T1). To further determine the state- or trait-character of signal changes, patients were classified as remitters (BDI-II ≤ 12) or non-remitters (BDI-II > 12) according to standard definitions of their BDI-II score at follow-up (T2). A 2 × 3 rm-ANOVA with the between-subject factor Group (remitters vs non-remitters) and the within-subject factor Time (post 1, post 2, post 3) was conducted in normalized MEP amplitudes at the follow-up TMS measurement (T2). Partial eta square values (η_p^2) were calculated as effect sizes for ANOVAs (low: <0.06; medium: ≥0.06; large: ≥0.14). *Post hoc t*-tests were performed for significant results of the ANOVAs. Pearson correlations were employed for exploratory correlation analyses. The level of statistical significance was set at $p < 0.05$ (two-tailed).

RESULTS

Demographic and Clinical Characteristics

Demographic and clinical characteristics are listed in Table 1. Patients with MDD and healthy controls did not differ in the distribution of age, sex, and years in school. Depressed patients reported significantly higher levels of depression and stress than healthy controls. Parameters of alertness and short-term memory did not differ between the groups.

TMS Measurements

Comparable TMS parameters before PAS between the groups (T1). As listed in Table 2, TMS parameters before the PAS intervention (mean MEP amplitudes, RMTs, and stimulation intensities) did not differ between patients in an acute episode of MDD and healthy controls.

Validity of the PAS intervention. Importantly, an analysis in healthy controls demonstrated the validity of the PAS intervention. Specifically, the effect size (Cohen's d_z) for the difference in MEP amplitudes before (pre-PAS) and after PAS (averaged across post 1, post 2 and post 3) in healthy controls was $d_z = 0.71$. This effect size is comparable to the prior study by Player *et al* (2013), reporting an LTP-like increase in MEP amplitudes after PAS with an effect size of $d_z = 0.67$ in healthy controls. As visualized in Figure 3a,

Table 2 Pre-PAS TMS Parameters at the First TMS Measurement (T1)

	Patients (n = 27)	Healthy controls (n = 27)	t	p
MEP amplitudes (mV)	0.9 ± 0.2	0.9 ± 0.3	-0.6	0.537
Resting motor threshold (% MSO)	35.9 ± 5.2	37.8 ± 5.6	-1.3	0.208
Stimulation intensity TMS (% MSO)	47.5 ± 8.8	48.7 ± 7.4	-0.6	0.583

Abbreviations: MEP, motor-evoked potential; MSO, maximum stimulator output; mV, millivolt; TMS, transcranial magnetic stimulation.

Data represent means ± standard deviations. Student's *t*-tests for independent samples were used.

healthy controls showed a significant increase in MEP amplitudes at post 2 (30 min) and post 3 (60 min) after the PAS intervention.

Partial occlusion of LTP-like plasticity in patients with an acute episode of MDD compared with healthy controls (T1). As the first main finding (Figure 3a), the 2 × 3 rm-ANOVA with the between-subject factor Group (MDD vs controls) and the within-subject factor Time (post 1, post 2, post 3) showed a significant main effect for the factor Group ($F_{1,52} = 4.48$, $p = 0.039$, $\eta_p^2 = 0.079$) and a significant main effect for the factor Time ($F_{1,52} = 7.36$, $p = 0.001$, $\eta_p^2 = 0.124$). The Group × Time interaction was not significant ($F_{1,52} = 0.27$, $p = 0.765$, $\eta_p^2 = 0.005$). *Post hoc* group comparisons revealed a significantly lower amplitude of MEPs at post 2 for MDD patients (1.08 ± 0.30) compared with controls (1.35 ± 0.52) ($t_{52} = -2.41$, $p = 0.020$); the group comparisons at post 1 (1.00 ± 0.27 vs 1.17 ± 0.51 , $t_{52} = -1.47$, $p = 0.148$) and post 3 (1.28 ± 0.45 vs 1.49 ± 0.78 , $t_{52} = -1.21$, $p = 0.231$) were not significant. To further determine the characteristics of the main effect of Time, we conducted *post hoc* tests in both groups. These tests showed a highly significant increase in MEP amplitudes 30 min (post 2; $t_{26} = 3.6$, $p = 0.001$) and 60 min (post 3; $t_{26} = 3.3$, $p = 0.003$) after PAS relative to pre-PAS values in healthy controls, indicative for a valid LTP-like effect after PAS in controls. MDD patients did not demonstrate a significant increase in MEP amplitudes compared with baseline (post 1: $t_{26} = 0.8$, $p = 0.939$; post 2: $t_{26} = 1.4$, $p = 0.180$) until post 3 ($t_{26} = 3.2$, $p = 0.004$). Together, this pattern of results indicates a valid induction of LTP-like plasticity in both groups with a partial occlusion of LTP inducibility in patients with MDD in the acute episode.

Figure 3 Long-term potentiation (LTP)-like plasticity in patients with major depressive disorder (MDD) and healthy controls. (a) First paired associative stimulation (PAS) assessment (T1). Patients in the acute episode of MDD demonstrated a partial occlusion of LTP-like plasticity in comparison with controls. (b) Follow-up PAS assessment (T2). At follow-up, patients with remission showed an intact inducibility of LTP-like plasticity at post 2, whereas the deficits persisted in patients without remission. Means ± SEM are shown. Filled symbols indicate significant increases ($p < 0.05$) in post-PAS MEP amplitudes referred to baseline (with a value of 1 indicating no change in the motor-evoked potential (MEP) amplitude after the PAS intervention). Asterisks indicate significant group differences (Student's *t*-test, $p < 0.05$).

Table 3 Clinical Characteristics and Pre-PAS TMS Parameters of Patients Who Participated in the Follow-Up Assessment (T2)

	Remitters (n = 8)	Non-remitters (n = 8)	t/ χ^2	p
<i>Demographic parameters</i>				
Sex (m/f)	6/2	4/4	$\chi^2 = 1.1$	0.608
Age (years)	39.0 ± 10.8	44.5 ± 11.9	1.0	0.350
<i>Clinical characteristics</i>				
BDI-II	6.6 ± 3.7	29.4 ± 11.7	5.2	<0.001
PSQ	37.7 ± 16.2	70.6 ± 16.2	4.1	0.001
<i>Pre-PAS TMS parameters</i>				
MEP amplitudes (mV)	0.8 ± 0.2	0.9 ± 0.3	0.8	0.418
Resting motor threshold (% MSO)	35.8 ± 4.0	39.3 ± 6.3	1.3	0.202
Stimulation intensity TMS (% MSO)	46.4 ± 6.7	46.4 ± 9.1	<0.01	>0.999

Abbreviations: BDI-II, Beck Depression Inventory-Second Edition; MEP, motor-evoked potential; MSO, maximum stimulator output; mV, millivolt; PSQ, Perceived Stress Questionnaire; TMS, transcranial magnetic stimulation. Data represent means ± standard deviations. Student's *t*-tests for independent samples were used, where applicable. Significant results are given in bold.

State-like character of reduced LTP-like plasticity in MDD (T2). To further determine the state- or trait-character of signal changes, patients were classified as remitters (BDI-II ≤ 12) or non-remitters (BDI-II > 12) according to standard definitions of their BDI-II score at follow-up (T2). In all, 16 of the 27 patients were reassessed, 10 patients declined their participation and 1 patient was excluded due to the intake of a benzodiazepine. The interval between the first TMS measurement (T1) and the follow-up TMS measurement (T2) was 246 ± 84 days. The medication remained stable between T1 and T2. Eight patients were remitted as defined by BDI-II ≤ 12, and eight patients were not remitted. As listed in Table 3, the distribution of sex and age did not differ between remitted and non-remitted patients. By selection, non-remitted patients reported significantly higher levels of depression and stress than remitted patients. There were no differences in pre-PAS TMS parameters at T2 between the two groups.

As the second main finding (Figure 3b), the 2 × 3 rm-ANOVA with the between-subject factor Group (remitters vs non-remitters) and the within-subject factor Time (post 1, post 2, post 3) showed a significant Group × Time interaction ($F_{2,28} = 3.7$, $p = 0.038$, $\eta_p^2 = 0.208$), no significant effect of Group ($F_{1,14} = 0.3$, $p = 0.601$, $\eta_p^2 = 0.020$), and no significant effect of Time ($F_{2,28} = 1.7$, $p = 0.198$, $\eta_p^2 = 0.109$). To further determine the direction of the significant interaction, *post hoc t*-tests were conducted. These tests demonstrated that patients in remission showed a restoration of LTP-like plasticity as indexed by a significant increase in MEP amplitudes at post 2 ($t_7 = 2.7$, $p = 0.032$). In contrast, the MEP amplitudes of non-remitted patients did not significantly increase at any point in time (for all $p > 0.1$), indicating a persistent occlusion of LTP-like plasticity in

non-remitters. *Post hoc* group comparisons (*t*-tests for independent samples) revealed a significantly higher MEP amplitude in remitters (1.46 ± 0.49) than in non-remitters (1.0 ± 0.34) at post 2 ($t_{14} = -2.2$, $p = 0.047$); there were no significant group differences at post 1 (1.13 ± 0.38 vs 0.94 ± 0.32 , $t_{14} = 1.1$, $p = 0.299$) and post 3 (1.24 ± 0.53 vs 1.23 ± 0.45 , $t_{15} = 0.04$, $p = 0.966$). Together, these results indicate that the reduced inducibility of LTP-like plasticity is, at least partially, normalized in patients after remission (state-character).

To control that the subgroups of patients classified as remitters or non-remitters at follow-up (T2) did not already differ at T1, we calculated a 2 × 3 rm-ANOVA with the between-subject factor Group (remitters vs non-remitters) and the within-subject factor Time (post 1, post 2, post 3) for the first TMS measurement (T1). This rm-ANOVA showed no significant Group × Time interaction ($F_{2,28} = 0.5$, $p = 0.598$, $\eta_p^2 = 0.036$) and no significant effects for the factor Group ($F_{1,14} = 0.001$, $p = 0.972$, $\eta_p^2 = 0.0001$) or Time ($F_{2,28} = 2.9$, $p = 0.074$, $\eta_p^2 = 0.169$), demonstrating that the induction of LTP-like plasticity was impaired in both groups (remitters and non-remitters) in the acute episode of MDD.

Episode duration and severity of depression. There were no significant correlations between the normalized post-PAS MEP amplitudes (post 1, post 2, and post 3) and the duration of the current episode ($p > 0.1$ for all time points), the total duration of illness ($p > 0.6$), the HAMD score ($p > 0.3$), or the BDI-II score ($p > 0.5$) for patients with MDD at the first TMS measurement (T1).

BDNF genotype. The BDNF genotype was determined in 50 of 54 participants (26 patients and 24 healthy controls). The distribution of the BDNF genotypes (patients: 13 Val-Val vs 13 Met carriers; controls: 17 Val-Val vs 7 Met carriers) did not significantly differ between the groups ($\chi^2_1 = 2.3$, $p = 0.133$). At both TMS measurements (T1 and T2), a 2 × 2 × 3 rm-ANOVA with the between-subject factors Group (T1: patients and healthy controls; T2: remitters and non-remitters, respectively) and Genotype (Val-Val and Met carriers) and the within-subject factor Time (post 1, post 2, post 3) showed no effect for any factor or interaction ($p > 0.1$), indicating that the genotype did not significantly influence the results. These findings are limited by power deficits of the subgroup analysis.

DISCUSSION

The results of this study provide further evidence for the synaptic plasticity hypothesis of MDD. Our findings corroborate previous reports on reduced LTP-like plasticity in patients with MDD (Nissen *et al*, 2010; Normann *et al*, 2007; Player *et al*, 2013) and complement preclinical findings of LTP deficits in animal models of depression (Alfarez *et al*, 2003; Pavlides *et al*, 2002). Our results extend prior research by providing first evidence for a state-character of impaired LTP-like plasticity, ie, a restoration after remission. The state-dependent partial occlusion of synaptic plasticity might therefore represent a pathomechanism for the clinical manifestation and a promising treatment target of the disorder.

As a first main finding, we demonstrated a significant impairment of LTP-like plasticity in patients with an acute episode of MDD compared with healthy controls using PAS and confirmed a prior report (Player *et al*, 2013). As in this report, we showed a robust LTP-like effect after PAS in healthy controls. In contrast to Player *et al*, who detected no significant LTP-like effect in patients at any point in time (0–60 min after PAS), we observed a significant increase in MEP amplitude 60 min after PAS, indicative of a rather partial than complete occlusion of LTP inducibility in MDD. This divergence might, at least partly, be explained by the higher proportion of patients with antidepressant medication in our study (26/27 vs 18/23 patients), which has been shown to enhance synaptic plasticity (Castrén, 2004). Furthermore, the duration of the current MDD episode was shorter in our study (8.3 ± 7.5 months vs 27.9 ± 20.9 months), which might point to a stronger impairment in patients with a longer episode. In the current study, we did not observe a significant correlation between the duration of the episode and LTP-like plasticity. Generally, it appears likely that MDD is characterized by a partial, rather than a complete occlusion of LTP-like plasticity, thought to reflect a vital brain function.

As a second main finding, we provided first evidence for a restoration of LTP-like plasticity after remission. This identified the impairment of LTP-like plasticity as a state-dependent biomarker and a possible pathomechanism of the clinical manifestation of the disorder. Up to now, there has been no demonstration of normalized indices of synaptic plasticity after recovery from a depressive episode in humans. Player *et al* (2014) demonstrated an increase in PAS-induced LTP-like plasticity in MDD patients after a treatment course of anodal transcranial direct current stimulation (tDCS) to the left prefrontal cortex. However, the design of the study did not allow disentangling whether the increase in plasticity resulted from a clinical improvement or from independent effects of tDCS on the motor cortex.

The synaptic plasticity hypothesis has the potential to refine major limitations of the classic monoamine hypothesis (Heninger *et al*, 1996) and might unravel novel pathways to treatment. Specifically, the time course of the proposed recovery of synaptic transmission parallels the delay of the clinical effect of antidepressants, while the neurochemical effects occur fast after drug administration (Nestler *et al*, 2002). Of particular note, accumulating evidence suggests that the glutamatergic system that regulates synaptic plasticity is central to the pathomechanism and treatment of MDD (Sanacora *et al*, 2008), whereas the monoaminergic system seems to exert a rather modulatory influence. Abnormal activity of the glutamatergic system is likely to contribute to the impairments observed in MDD patients. Glutamate is the major excitatory neurotransmitter in the brain and regulates synaptic transmission and plasticity by activating ionotropic α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and NMDA receptors, and metabotropic glutamate receptors (mGluR1–8). It has been demonstrated that the induction of LTP involves postsynaptic NMDA receptor activation (Stefan *et al*, 2002) and postsynaptic modifications of AMPA receptor function and localization (Malenka and Nicoll, 1999). Hence, the observed partial occlusion of LTP-like plasticity in patients with MDD

might be driven by alterations in glutamatergic synaptic transmission.

Several therapeutic agents targeting the glutamatergic system are currently being explored in an attempt to remedy the presumed impairment of glutamate-mediated synaptic plasticity (Duman and Aghajanian, 2012). For instance, the rapid-acting agent ketamine, which has been shown to have fast and relatively sustained antidepressant effects in animal models of depression (Koike *et al*, 2011) and patients with MDD (Berman *et al*, 2000; Zarate *et al*, 2006), produces profound effects on fast excitatory glutamate transmission and rapidly induces synaptogenesis in rodents (Li *et al*, 2010, 2011), similar to LTP. Ketamine increases glutamate transmission in the prefrontal cortex, presumably by eliciting a disinhibition of GABAergic input that results in an enhancement of the firing rate of glutamatergic neurons thought to underlie ketamine induction of synaptogenesis (Li *et al*, 2010, 2011). Interestingly, the antidepressant effect of ketamine is attenuated in both animals and humans with the Met allele of the BDNF gene (Laje *et al*, 2012; Liu *et al*, 2012), an observation that may render BDNF genotyping an important biomarker for the clinical response to ketamine in humans. However, in our study, we did not observe a significant effect of the BDNF polymorphism.

The synaptic plasticity hypothesis provides a conceptual framework for the beneficial effect of a combined treatment of antidepressants and psychotherapy. Thus, antidepressant treatment might open a window for plasticity and recovery, particularly when combined with beneficial input fostered by psychotherapy, which can be considered as a form of learning and thus requires LTP on the molecular level. Interestingly, D-cycloserine (4-amino-isoxazolidin-3), a partial NMDA receptor agonist, has been found to facilitate fear extinction in rats (Walker *et al*, 2002) and to enhance the efficacy of exposure-based therapies for anxiety disorders (for an overview, please refer to Norberg *et al*, 2008). Further trials are warranted to determine whether D-cycloserine can be considered to be an effective enhancer of other types of psychotherapy, particularly in MDD.

The PAS protocol represents an important method to assess associative synaptic LTP-like plasticity in humans. In comparison with previously used indices, such as visually-evoked potentials or memory studies, the neural pathways of the TMS-PAS protocol are well defined and the protocol is relatively independent of higher cognitive functions that might bias results in MDD studies. The replication of a prior report (Player *et al*, 2013) strengthens the concept of impaired LTP-like plasticity in the acute episode of MDD. Critically, our study extends prior research by identifying partial occlusion of LTP-like plasticity as state dependent. The distinction between state- and trait-markers is important to inform future work on potential pathomechanisms, treatment targets, and treatment monitoring.

Still, several limitations need to be addressed. First, 26 out of 27 patients were on antidepressant medication shown to influence neuroplasticity. However, antidepressants rather enhance synaptic plasticity (Batsikadze *et al*, 2013; Normann *et al*, 2007), suggesting that, in line with Player *et al* (2013), the observed deficits might be even stronger in patients without medication. Second, the sample size in the follow-up TMS measurement was relatively small and the findings require replication. Third, we assume that our results in the

motor cortex are representative for other brain regions, including the prefrontal cortex and the hippocampus. However, synaptic plasticity in other networks is difficult to characterize electrophysiologically in humans. Of particular note, current models of emotion regulation propose inverse changes, ie, enhanced synaptic plasticity, in a ventral emotional system, comprising eg the amygdala (Phillips *et al*, 2003a, b). Interestingly, Rajji *et al* (2013) demonstrated PAS-induced changes in the dorsolateral prefrontal cortex of healthy participants by combining TMS and electroencephalography (EEG). This technique may allow for future testing in MDD. Further studies in animals and humans using a variety of direct assessments and proxies are needed to fully characterize potential alterations of synaptic plasticity in MDD.

In conclusion, the demonstration of a state-dependent partial occlusion of LTP-like plasticity in patients with MDD supports the synaptic plasticity hypothesis of MDD and might contribute to the development of novel treatments.

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