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Research Article

RESEARCH ARTICLE

BDNF-Val66Met-Polymorphism Impact on Cortical Plasticity in Schizophrenia Patients: A Proof-of-Concept Study

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) has been shown to be a moderator of neuroplasticity. A frequent BDNF-polymorphism (Val66Met) is associated with impairments of cortical plasticity. In patients with schizophrenia, reduced neuroplastic responses following non-invasive brain stimulation have been reported consistently. Various studies have indicated a relationship between the BDNF-Val66Met-polymorphism and motor-cortical plasticity in healthy individuals, but schizophrenia patients have yet to be investigated. The aim of this proof-of-concept study was, therefore, to test the impact of the BDNF-Val66Met-polymorphism on inhibitory and facilitatory cortical plasticity in schizophrenia patients.

Methods: Cortical plasticity was investigated in 22 schizophrenia patients and 35 healthy controls using anodal and cathodal transcranial direct-current stimulation (tDCS) applied to the left primary motor cortex. Animal and human research indicates that excitability shifts following anodal and cathodal tDCS are related to molecular long-term potentiation and long-term depression. To test motor-cortical excitability before and after tDCS, well-established single- and paired-pulse transcranial magnetic stimulation protocols were applied.

Results: Our analysis revealed increased glutamate-mediated intracortical facilitation in met-heterozygotes compared to val-homozygotes at baseline. Following cathodal tDCS, schizophrenia met-heterozygotes had reduced gamma-amino-

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butyric-acid-mediated short-interval intracortical inhibition, whereas healthy met-heterozygotes displayed the opposite effect. The BDNF-Val66Met-polymorphism did not influence single-pulse motor-evoked potential amplitudes after tDCS. **Conclusions:** These preliminary findings support the notion of an association of the BDNF-Val66Met-polymorphism with observable alterations in plasticity following cathodal tDCS in schizophrenia patients. This indicates a complex interaction between inhibitory intracortical interneuron-networks, cortical plasticity, and the BDNF-Val66Met-polymorphism. Further replication and validation need to be dedicated to this question to confirm this relationship.

Keywords: brain-derived neurotrophic factor, motor-cortical plasticity, schizophrenia, transcranial direct current stimulation, transcranial magnetic stimulation

Introduction

Brain-derived neurotrophic factor (BDNF) has been shown to be a moderator of neuroplasticity in the central nervous system through its activity-dependent release, and by balancing the intrasynaptic ratio with its precursor peptide pro-BDNF (Aicardi et al., 2004; Woo et al., 2005; Miyamoto, 2006). In healthy subjects, the BDNF G196A polymorphism, which results in a valine-to-methionine substitution at the amino-acid position 66 (Val66Met), has been consistently shown to reduce activity-dependent BDNF-secretion, to be involved in the regulation of activity- and stimulus-dependent plasticity and memory processes, and to influence episodic memory and learning processes (Egan et al., 2003; Pezawas et al., 2004; Woo et al., 2005; Miyamoto, 2006; Caroni et al., 2012). The impact of this BDNF-Val66Met-polymorphism on global and local (e.g., hippocampus) brain volumes is still subject to discussion (Molendijk et al., 2012). Further studies have addressed the influence of the BDNF-Val66Met-polymorphism on the pathophysiology of different neuropsychiatric disorders, including major depressive disorder, bipolar disorder, and schizophrenia (Autry and Monteggia, 2012). Post-mortem studies in schizophrenia patients have displayed evidence of reduced BDNF expression in the prefrontal cortex (Takahashi et al., 2000; Weickert et al., 2003; Hashimoto et al., 2005) and the hippocampus (Iritani et al., 2003; Durany and Thome, 2004). However, conflicting evidence has been reported on the impact of the BDNF-Valin-66-Methionine (Val66Met)-polymorphism on the age of onset, symptom severity, and MR-based brain volumes in schizophrenia (Pezawas et al., 2004; Numata et al., 2006; Naoe et al., 2007; Pillai, 2008; Dutt et al., 2009; Gruber et al., 2012).

Given the involvement of BDNF in neurotransmission and memory processes, previous studies using non-invasive brain stimulation have examined the relationship between the BDNF-Val66Met-polymorphism and motor-cortical plasticity (Chaieb et al., 2014). Studies using intermittent and continuous theta-burst stimulation showed conflicting findings for either decreased or absent responses in healthy met-allele carriers (Cheeran et al., 2008; Antal et al., 2010; Jayasekeran et al., 2011) or no differences between val-homozygotes and met-allele carriers (Li Voti et al., 2011; Mastroeni et al., 2013). Related results have been reported for plasticity induction by paired-associative stimulation (PAS), showing either reduced effects of PAS in met-allele carriers (Cheeran et al., 2008) or no group differences (Witte et al., 2012). With regards to the after-effects of transcranial direct current stimulation (tDCS), two studies using cathodal tDCS showed no differences between val-homozygotes and met carriers (Antal et al., 2010; Di Lazzaro et al., 2012) and a more pronounced increase of cortical excitability in met carriers following anodal tDCS (Antal et al., 2010). No significant differences were observed following transcranial random noise stimulation (Antal et al., 2010). A decreased response of met carriers was found in a homeostatic plasticity paradigm, pairing cathodal tDCS with 1Hz repetitve transcranial magnetic stimulation (repetitive TMS; Cheeran et al., 2008). The assessment of use-dependent plasticity showed reduced changes in motor cortical excitability in met carriers (Kleim et al., 2006). In one animal study, Fritsch et al. (2010) showed decreased BDNF secretion and long-term potentiation (LTP) following anodal tDCS in BDNF-mutant mouse primary motor cortex (M1) slices. Although the origin of these conflicting results has yet to be clarified, these studies suggest an association of the BDNF-Val66Met-polymorphism with the susceptibility to induce plasticity using non-invasive brain stimulation.

In patients with schizophrenia, impairments of neuroplastic responses following different forms of non-invasive brain stimulation have been consistently reported (Oxley et al., 2004; Hasan et al., 2011, 2013). Despite a likely impact of BDNF on the pathobiology of schizophrenia, the effects of the BDNF-Val66Met-polymorphism on cortical plasticity and on cortical excitability following non-invasive brain stimulation in patients with schizophrenia have not yet been investigated. Therefore, the aim of the present proof-of-concept study was to address this question for the first time by applying anodal and cathodal tDCS to the M1 of schizophrenia patients and healthy controls and by monitoring excitability changes before and after tDCS with single- and paired-pulse TMS paradigms. Paired-pulse TMS paradigms investigate the interaction of a first, sub-threshold conditioning stimulus followed by a second, suprathreshold test stimulus (Kujirai et al. 1993). Short intervals between both stimuli (2–5 ms) result in a subsequent inhibition of the test pulse (short-interval cortical inhibition [SICI]), whereas longer intervals (7–20 ms) lead to a facilitation (intracortical facilitation [ICF]; Kujirai et al. 1993). Pharmacological challenges in healthy subjects strongly indicate that SICI is mediated via $GABA_A$-neurotransmission, whereas ICF is related to glutamatergic neurontransmission (Ziemann, 2004). For tDCS, animal and human data indicate that the excitability changes following tDCS are long lasting, N-methyl-D-aspartate (NMDA)-dependent, and also polarity-dependent, and thus can be considered to be related to LTP (anodal) and long-term depression (LTD; cathodal). We first hypothesized that healthy and schizophrenia met-allele carriers would show reduced cortical inhibition and enhanced cortical facilitation compared to the respective val-homozygotes. Second, we hypothesized met-allele carriers would display less plasticity responses following tDCS compared to the val-homozygotes.

Methods

Subjects

In total, the data of 35 healthy controls and 22 schizophrenia patients from two foregoing studies (Hasan et al., 2011; Hasan, Nitsche, et al., 2012) were analyzed with regard to BDNF genotypes. As two different investigators (BR or MH) performed the

experiments for anodal and cathodal tDCS, each study group was considered to be independent. All schizophrenia patients, but not all healthy controls, received both stimulation polarities (see Table 1). After giving written informed consent, genotyping was conducted on 22 participants with schizophrenia (SZ; 8 female, 14 male, mean age = 30.1 years) and 35 healthy control (HC) participants (14 female, 21 male; anodal group: mean age = 27.1 years; cathodal group: mean age = 27.8 years). The local ethics committee of the University Medical Centre Goettingen approved the protocol, which was conducted in accordance with the Declaration of Helsinki. Participants with contraindication to TMS or tDCS, such as neurological illness, severe brain injury, dermatological disorders, brain tumors, or a history of dementia, and patients with concomitant benzodiazepine or mood stabilizer treatment were excluded. A clinical psychiatrist, blinded to the aims of the study, and a member of the study group (TW or AH) made an ICD-10 consensus diagnosis. All participants underwent a standardized test of hand preference (Annett, 1970) and all patients received an assessment of psychopathological symptoms (PANSS; Kay et al., 1987), disease severity (Guy and Bonato, 1976), and social functioning (Endicott et al., 1976). All schizophrenia patients (apart from three) were treated with antipsychotics (CPZ mean = 356.7 ± 393.5; 12 patients received monotherapy: of these, 5 patients were treated with risperidone, 6 with quetiapine, and 1 with olanzapine). In general, patients did not receive a concomitant medication (e.g., antidepressants, antihypertensive drugs, or benzodiazepines).

TMS Procedure

The complete protocol has been described previously (Hasan et al., 2011). Briefly summarized, subjects were examined in a comfortable sitting position with arms supported passively. Electromyographic activity was recorded by surface electrodes placed over the right first-dorsal interosseus muscle (FDI). Raw signals were amplified, bandpass-filtered (2–10 kHz) and digitized using a standard amplifier (Keypoint Portable, Medtronic Co.). TMS was performed over the left M1 with a standard 70 mm TMS figure-of-eight magnetic coil and a MagPro X 100 magnetic stimulator (Medtronic Co.). Throughout all experiments, the coil was held tangentially to the head, with the handle pointing backwards and in a 45° angle lateral to the midline. The stimulation site that produced the largest motor-evoked potential (MEP) at moderately suprathreshold stimulation intensities was defined and marked as the optimal coil position.

tDCS Procedure

Transcranial direct current stimulation was applied after baseline TMS examination. The tonic electrical field with a stimulation intensity of 1 mA was induced using a CE-certified stimulator (DC-Stimulator-Plus, NeuroConn GmbH) through saline-soaked rectangular-surface sponge-electrodes (7 x 5 cm) for a duration time of 9 minutes in the cathodal and 13 minutes in the anodal condition (Nitsche et al. 2000, 2001). These standard stimulation durations have been consistently shown to be optimal for the induction of cortical excitability alterations lasting for about 1 h after tDCS (Nitsche et al., 2008). The stimulation electrode was placed over the left M1 on the spot of the optimal coil position, and the second electrode was placed over the contralateral supraorbital forehead.

Cortical Excitability

Resting motor threshold (RMT), expressed as a percentage of maximum stimulator output, was defined as the lowest intensity that produced a minimum MEP of 50 μV in the relaxed FDI in at least 5 of 10 trials. Single-pulse MEPs were recorded from the motor-cortical representation of the right FDI (MEP-size) to monitor the global effects of tDCS on cortico-spinal excitability. TMS intensity was adjusted before tDCS to evoke MEPs of 1 mV size on average (S1mV) and was kept unchanged for the after-effect assessment (Hasan et al., 2011). Forty MEPs were recorded before tDCS and 5 min after the stimulation. Follow-up measurements of cortical excitability parameters were started immediately. The cortical silent period (CSP), a measure of cortical inhibition, was obtained by recording from the FDI muscle under voluntary contraction while stimulating the contralateral M1 with 120% RMT. The mean CSP duration was calculated from 10 trials. Short-latency intracortical inhibition and intracortical facilitation (SICI, ICF) were recorded with a standardized

Table 1. Demographic and clinical characteristics of the participants. CGI: clinical global impression; CPZ: chlorpromazine equivalent dose; GAF: global assessment of functioning; PANSS: positive and negative syndrome scale. Data are presented as mean ± standard deviation. [a]Chi2-test; [b]one-way-ANOVA; [#]all schizophrenia patients participated in both anodal and cathodal tDCS within one week, resulting in the same psychopathological scores, CPZ-equivalents, and duration of illness. HC either received only cathodal (n = 15) or only anodal (n = 13) tDCS or both stimulation paradigms (n = 7). All analyses were conducted for the anodal and cathodal samples as independent groups.

Variable	SZ-val	SZ-met	HC-val	HC-met	Statistics
n_{ges} = 57[#]	n = 14	n = 8	n = 17	n=18	
Gender	6 F, 8 M	2 F, 6 M	8 F, 9 M	6 F, 12 M	p=0.693 [a]
Age (years)	36.79 ± 7.47	27.75 ± 7.34	30.29 ± 9.12	33.67 ± 9.73	p=0.080 [b]
Handedness	13 R, 1 L	8 R, 0 L	17 R, 0 L	16 R, 2 L	p=0.436 [a]
BDNF					
val/val	14	-	17	-	
val/met	-	8	-	18	p=0.438 [a]
PANSS Scores					
Total	58.50 ± 12.6	52.14 ± 10.4	-	-	p=0.264 [b]
Positive	14.29 ± 5.2	11.71 ± 4.9	-	-	p=0.292 [b]
Negative	16.21 ± 4.4	16.14 ± 3.6	-	-	p=0.971 [b]
General	28.00 ± 5.8	24.29 ± 5.4	-	-	p=0.175 [b]
GAF	56.00 ± 11.0	64.43 ± 8.4	-	-	p=0.093 [b]
CGI	4.57 ± 0.65	3.86 ± 1.1	-	-	p=0.070 [b]
CPZ (daily)	382.55 ± 484.9	30.4.93 ± 122.3	-	-	p=0.685 [b]
Duration of illness (years)	7.73 ± 4.6	2.04 ± 2.7	-	-	p=0.024 [b]

paired-pulse protocol (Kujirai et al., 1993; conditioning stimulus: 80% RMT; test stimulus: intensity that produced resting MEPs averaging 0.7–1.3 mV [S1mV]; ISIs: 3 and 12). A minimum of 20 trials with each ISI and 40 trials with the test stimulus alone were performed. RMT and S1mV were adjusted for the paired-pulse protocols and for CSP after tDCS (Chen, 2004).

BDNF Genotyping

Genomic DNA was extracted from ethylenediaminetetraacetic acid anti-coagulated venous blood samples using the chemagic MSM I system (PerkinElmer Chemagen Technologie GmbH). BDNF Val66Met (rs6265) was genotyped on a 7900HT Fast Real-Time PCR System (Life Technologies) using TaqMan®SNP Genotyping Assay C-11592758_10 (Life Technologies) and the standard protocol for allelic discrimination. Accuracy was assessed by duplicating 15% of the original sample, and reproducibility was 100%.

Statistics

For statistical analysis, SPSS 22 for Windows (IBM) was used. Level of significance was set at $\alpha = 0.05$ for gender, hand preference, and BDNF genotype. Chi²-tests were computed to test for different distributions between the study groups. One-way analysis of variance (ANOVA) was used to compare mean ages and baseline excitability between the groups. MEP size was calculated as the mean MEP amplitude both individually and then interindividually, both before and after stimulation. As the normality assumption was violated (Kolmogorov–Smirnov-test, p between <0.001 and 0.007) for 1 mV-MEP, RMT, SICI, and ICF, square-root transformations were applied to meet the requirements for repeated-measures-ANOVA (RM-ANOVA). RM-ANOVAs were computed with time (baseline and post-tDCS) as a within-subject factor and stimulation type (anodal, cathodal), BDNF genotype (val/val [val-group] and val/met and met/met [met-group]), and study group (SZ, HC) as between-subject factors. Dependent variables were RMT, S1mV, MEP size, CSP, SICI, and ICF, at baseline and after tDCS stimulation (between-factor time [pre, post]). In cases of significant interaction effects, independent-sample t-tests for inter-group comparisons and paired-sample t-tests for intra-group pre-to-post comparisons (all two-tailed; $p < 0.05$; not adjusted for multiple comparisons; adjusted values are presented in Supplementary Table 1) were conducted to detect different distributions between the groups. In cases of lacking interactions, no further t-tests were conducted. In the linear models, sphericity was tested with the Mauchly's test and, if necessary (Mauchly's test <0.05), the Greenhouse-Geisser correction was used. Effect sizes for the between-group comparisons were calculated. Data in tables are presented as mean ± standard deviation; in all figures, error bars refer to the standard error and graphs show untransformed data.

Results

Sociodemographic and Clinical Characteristics

The study groups did not differ significantly with respect to gender distribution ($p = 0.693$), handedness ($p = 0.436$), or age ($p = 0.080$). According to PANSS measures, patients suffered from moderate to severe positive and negative symptoms, likely accompanied by directed degrees of illness and impairments of social functioning (Table 1).

Distribution of Genetic Polymorphisms

No significant group differences were detected for the BDNF-Val66Met-polymorphism ($p = 0.438$; schizophrenia patients (SZ)-val: n = 14, 64,6%; SZ-met: n = 8, 36,4%; healthy-controls (HC)-val: n = 17, 48,6%; HC-met: n = 18, 51,4%; Table 1) and genotyping results did not deviate from the Hardy-Weinberg equilibrium ($p = 0.11$).

Cortical Excitability at Baseline

Overall ANOVA showed a significant difference between the val-homozygotes and met-allele carriers for ICF ($F_{1,56} = 11.187$, $p = 0.002$, $\eta^2 = 0.169$). For all other baseline parameters, no differences were observed. Further group comparisons of cortical excitability parameters at baseline were conducted between schizophrenia and healthy control val-homozygotes and met-allele carriers (SZ-val, SZ-met, HC-val, and HC-met) by applying ANOVAs to both the anodal and the cathodal sample. Analysis of the anodal sample revealed a significant difference for ICF ($F_{1,41} = 3.380$, $p = 0.028$, $\eta^2 = 0.211$). Further independent-sample t-test analyses showed significantly higher baseline ICF in HC met-allele carriers compared to the HC val-group ($t_{(18)} = 2.444$, $p = 0.025$, d = 1.105). A non-significant similar pattern was found when comparing the SZ met-group to the SZ val-group ($t_{(20)} = 1.746$, $p = 0.096$, d = 0.766; Table 2 and Figure 1). For the cathodal sample, the ANOVA analysis revealed no significant group differences ($F_{1,43} = 1.078$, $p = 0.369$, $\eta^2 = 0.075$), though the data of the cathodal sample showed the same numeric distribution. It is possible,that, in contrast to our findings in the anodal sample, significant differences failed to be observed for the cathodal sample due to the higher standard deviations and due to a different BDNF-Val66Met-polymorphism distribution. No other variables showed differences across groups (Table 2).

In summary, we observed higher baseline ICF in healthy and schizophrenia met-allele carriers compared to the respective val-homozygotes. ICF represents the activity of facilitatory and glutamate-mediated cortical networks (Chen. 2004; Ziemann, 2004) and the presented results indicate that the met-allele is associated with a cortical disinhibition.

Impact of tDCS on Cortical Excitability

RMT, S1mV, 1mV MEP. and aMEP

A RM-ANOVA for 1mV-MEP revealed significant effects of time × BDNF and time × stimulation, but no further interactions or main effects (all $p \geq 0.069$; Table 3 and Figure 2). Since analyses did not show a significant time × BDNF × group × stimulation interaction, no further subgroup RM-ANOVAs were conducted.

However, to test whether tDCS induced a plasticity response at all, we performed paired-sample t-tests for MEP-amplitudes in all study groups. This showed an increase in MEP-amplitudes in all subgroups following anodal tDCS (SZ-val $t_{(13)} = 3.348$, $p = 0.005$; SZ-met $t_{(7)} = 4.685$, $p = 0.002$; HC-val $t_{(11)} = 4.020$, $p = 0.002$; HC-met $t_{(7)}$=3.374, p=0.012). In the cathodal condition, a plasticity response was observed in healthy subjects (HC-val $t(9) = 2.682$, $p = 0.025$; HC-met $t(11) = 1.860$, $p = 0.090$), but not in schizophrenia patients (SZ-val $t_{(13)} = 0.816$, $p = 0.429$; SZ-met $t_{(7)} = 0.665$, $p = 0.527$) in the cathodal group (Figure 2). One previous study with healthy subjects showed significant enhanced plasticity response (MEP amplitudes) following anodal tDCS and a trend towards a more pronounced decrease in excitability after cathodal tDCS in met-allele carriers (Antal et al., 2010; Chaieb et al., 2014). To compare our results to this analysis, we conducted additional RM-ANOVAs and one-way ANOVAs in our healthy sample. We found an MEP

Table 2. Cortical excitability at baseline. aMEP: active MEP; CSP: cortical silent period; HC: healthy controls; ICF: intracortical facilitation; MEP: motor-evoked potential; RMT: resting motor threshold; S1mV: stimulation intensity to generate 1mV MEP; SICI: short-interval intracortical inhibition; SZ: schizophrenia patients;. -met: met-allele carriers; -val: val-homozygotes. Data are presented as mean ± standard deviation and bold letters plus asteriks (*) signifying *p*-values ≤0.05.

Variable	n	SZ-val	n	SZ-met	N	HC-val	n	HC-met	ANOVA	
Anodal sample										
RMT (%)	14	53.7 ± 8.5	8	53.8 ± 5.6	12	48.8 ± 8.0	8	51.4 ± 3.7	F = 1.466	*p* = 0.239
S1mV (%)	14	61.5 ± 10.5		64.9 ± 9.9		58.5 ± 10.9		63.8 ± 8.2	F = 0.996	*p* = 0.405
1mV MEP (mV)	14	938.3 ± 219.3		953.3 ± 172.6		1049.3 ± 195.8		941.3 ± 210.1	F = 0.651	*p* = 0.587
aMEP (mV)	14	8458.9 ± 3268.2		10943.6 ± 3928.5		7709.9 ± 2244.0		9735.8 ± 3252.6	F = 1.017	*p* = 0.396
CSP (ms)	14	137.3 ± 40.9		165.5 ± 37.5		122.3 ± 42.8		132.3 ± 44.7	F = 1.819	*p* = 0.160
SICI 3ms (%)	14	31.9 ± 20.8		36.3 ± 18.1		27.1 ± 29.1		17.2 ± 11.9	F = 1.835	*p* = 0.157
ICF 12ms (%)	14	170.2 ± 86.5		240.9 ± 136.3		132.9 ± 49.0		202.8 ± 74.2	F = 3.380	***p* = 0.028***
Cathodal sample										
RMT (%)	14	60.6 ± 8.0	8	58.6 ± 9.4	10	55.3 ± 7.1	12	56.2 ± 8.3	F = 0.930	*p* = 0.435
S1mV (%)	14	70.1 ± 10.4		66.8 ± 11.2		61.9 ± 9.6		62.3 ± 9.8	F = 1.588	*p* = 0.207
1mV MEP (mV)	14	969.9 ± 250.3		820.9 ± 233.9		958.9 ± 438.5		1020.3 ± 227.0	F = 1.033	*p* = 0.388
aMEP (mV)	14	6772.6 ± 3600.9		5792.3 ± 1384.2		6731.9 ± 2783.1		7629.9 ± 3050.5	F = 0.457	*p* = 0.714
CSP (ms)	14	146.7 ± 33.0		143.1 ± 41.2		115.5 ± 31.8		129.4 ± 39.0	F = 1.480	*p* = 0.235
SICI 3ms (%)	14	34.6 ± 32.2		35.6 ± 40.6		26.4 ± 28.1		48.1 ± 56.9	F = 1.078	*p* = 0.370
ICF 12ms (%)	14	177.3 ± 164.8		239.5 ± 200.0		134.2 ± 57.6		211.2 ± 106.4	F = 1.369	*p* = 0.267

increase in both HC val-homozygotes and HC met-allele carriers following anodal tDCS, demonstrated by a significant main effect for time ($F_{1,18}$ = 27.827, $p < 0.0001$). However, the analysis of the time × BDNF interaction remained non-significant ($F_{1,18}$ = 3.416, p = 0.081). Subsequent independent-sample *t*-tests of the post-tp pre-ratios showed a numeric difference for an augmented increase in excitability in the case of met-allele carriers compared to val-homozygotes ($t_{(18)}$ = 1.912, p = 0.072, d = 0.799), confirming one foregoing study (Antal et al., 2010).

For cathodal tDCS, we found an MEP decrease in both healthy met carriers and val-homozygotes, as shown by a significant effect of time ($F_{1,20}$ = 11.491, p = 0.003), but no further time × BDNF interaction ($F_{1,20}$ = 2.135, p = 0.159). Subsequent independent-sample *t*-tests of the post- to pre-ratios revealed no significant differences between met-allele carriers and val-homozygotes ($t_{(20)}$ = 1.355, p = 0.191, d = 0.565), also confirming the findings of Antal et al. (2010). Neither analysis for the SZ subjects showed a significant interaction or subgroup difference. RM-ANOVAs revealed no significant or trend-level effects for all other dependent variables (RMT, S1mV, and aMEP).

In summary, we were able to confirm previous findings (Antal et al., 2010; Chaieb et al., 2014) of an enhanced facilitatory plasticity response in terms of increased MEP amplitudes following anodal tDCS and of no differences following cathodal tDCS in healthy met-allele carriers compared to val-homozygotes.

SICI, ICF, and CSP

For SICI, the RM-ANOVA revealed significant effects of time × BDNF × group, time × group × stimulation, and time × BDNF × group × stimulation, but no further interactions or main effects. Subsequent RM-ANOVAs of the anodal and cathodal samples showed significant effects of time × BDNF × group in the cathodal condition, but not in the anodal condition (Table 3). Hence, no further analyses were conducted regarding the anodal-tDCS subgroup.

For cathodal tDCS, two separate subsequent RM-ANOVAs were conducted, and revealed a significant time × group effect for the met-allele carriers and no effect for the val-homozygotes.

To further investigate the impact of BDNF-Val66Met-polymorphism in the cathodal subgroup, paired-sample *t*-tests were computed, and showed significantly lower SICI following cathodal tDCS in the SZ met-allele carriers ($t_{(7)}$ = 2.662, p = 0.0324), but not in the SZ val-homozygotes ($t_{(13)}$ = 0.448, p = 0.6617; Figure 3). The HC met-allele carriers showed a converse effect with significantly-pronounced SICI ($t_{(11)}$ = 2.246, p = 0.0462), and the HC val-homozygotes showed reduced SICI ($t_{(9)}$ = 2.400, p = 0.0399) following cathodal tDCS (Figure 3).

Independent-sample *t*-tests not corrected for multiple comparisons showed significantly lower SICI after cathodal tDCS for SZ patients compared to HC participants in the case of met-allele carriers ($t_{(18)}$ = 2.5999, p = 0,0181, d = 1.123), but not for val-homozygotes ($t_{(22)}$ = 0.3538, p = 0.7268, d = 0.144; Figure 3).

Neither baseline nor post-tDCS SICI values differed significantly between val-homozygotes and met-allele carriers in either SZ (baseline: $t_{(20)}$ = 0.056, p = 0.956, d = 0.025; post-tDCS: $t_{(20)}$ = 1.390, p = 0.180, d = 0.583) or HC (baseline: $t_{(20)}$ = 1.191, p = 0.247, d = 0.513; post-tDCS: $t_{(20)}$ = 1.018, p = 0.321, d = 0.428). No significant effects were found by RM-ANOVAs run on CSP duration and ICF data.

In summary, we observed reduced SICI following cathodal tDCS in the schizophrenia met-allele carriers compared to the schizophrenia val-homozygotes. The healthy control met-allele carriers displayed a converse effect, showing significantly-pronounced SICI after cathodal tDCS compared to the healthy val-homozygotes. As SICI involves GABA-mediated intracortical inhibitory networks, this result indicates that the met-allele influences inhibitory networks following cathodal tDCS in dependence of the diagnosis.

Correction for Multiple Testing

The presented uncorrected results of this proof-of-concept study do not provide broad evidence that can be generalized, and thus need to be considered as preliminary and confirmed in subsequent larger studies. However, to test the generalizability of findings, Bonferroni-adjustment for multiple comparisons was performed for independent-sample *t*-tests (n = 6). Adjusting the results for these six subgroup comparisons results in the situation that the significant group differences presented in the paragraphs before did not survive correction for multiple testing. Please see Supplementary Table 1 for a detailed presentation of corrected and uncorrected independent-sample *t*-tests.

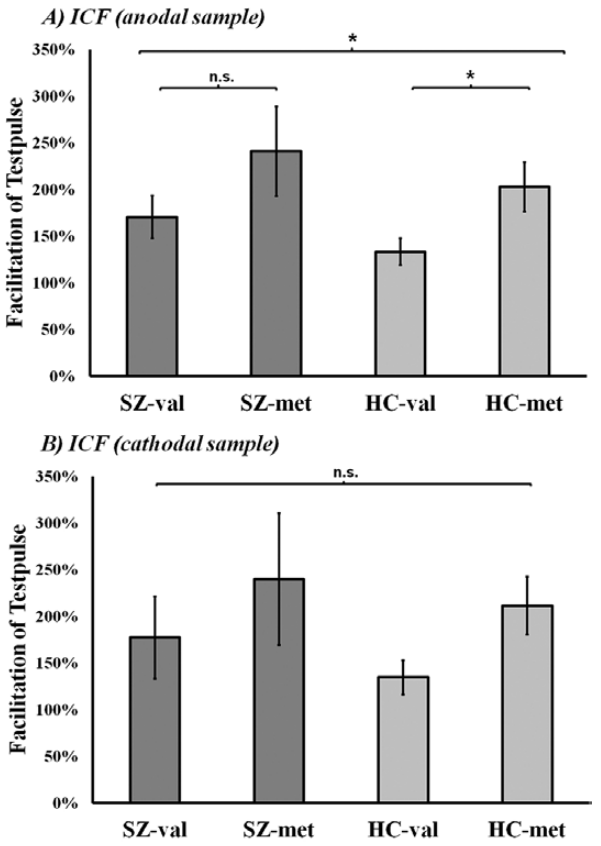

Figure 1. Intracortical facilitation at baseline. (A) ICF (anodal sample) shows significant differences regarding baseline ICF across all groups and between HC-val compared to HC-met, as well as a trend-level difference between SZ-val and SZ-met. (B) ICF (cathodal sample) shows no significant differences regarding baseline ICF across all groups (*$p \leq 0.05$). Data are presented as mean ± standard error of the mean.

Table 3. Results of RM-ANOVAs for SICI values. RM-ANOVAs show significant time × BDNF × group, time × group × stimulation, and time × BDNF × group × stimulation interactions for SICI values in the whole sample and significant time × BDNF × group and time × group interactions for SICI values in the cathodal subsample and bold letters plus asteriks (*) signifying *p*-values ≤0.05.

	Hypothesis *df*, *error df*	*F* value	*p* value
1mV-MEP values (whole sample)			
Time	1, 78	**11.128**	**0.001***
Time x BDNF		**4.267**	**0.042***
Time x Group		1.360	0.247
Time x Stimulation		**38.399**	**<0.001***
Time x BDNF x Group		0.978	0.326
Time x BDNF x Stimulation		0.162	0.688
Time x Group x Stimulation		3.404	0.069
Time x BDNF x Group x Stimulation		0.858	0.357
SICI values (whole sample)			
Time	1, 78	0.361	0.550
Time x BDNF		2.342	0.130
Time x Group		0.574	0.451
Time x Stimulation		2.497	0.118
Time x BDNF x Group		0.284	0.596
Time x BDNF x Stimulation		**4.125**	**0.046***
Time x Group x Stimulation		**4.595**	**0.035***
SICI values (cathodal sample)			
Time	1, 40	0.875	0.355
Time x BDNF		0.471	0.496
Time x Group		**6.568**	**0.014***
Time x BDNF x Group		**12.672**	**0.001***
SICI values (anodal sample)			
Time	1, 38	0.013	0.909
Time x BDNF		2.279	0.139
Time x Group		0.170	0.682
Time x BDNF x Group		0.660	0.422

Discussion

The results of this proof-of-concept study provide the first preliminary evidence for BDNF-associated alterations in inhibitory cortical plasticity following tDCS in schizophrenia patients and for an impact of the BDNF met-allele on cortical facilitatory networks. More specifically, schizophrenia met-allele carriers showed reduced SICI following LTD-inducing cathodal tDCS, whereas healthy met-allele carriers displayed the opposite effects. These effects could be observed neither in val-homozygotes nor following LTP-inducing anodal tDCS. The baseline increase in ICF can be considered to be hyperexcitable, and indicates impaired inhibitory regulatory processes in met-allele carriers independent from disease state. Our preliminary findings enable a novel view of the inhibitory and plasticity deficits in schizophrenia patients and extend prior findings of BDNF-induced alterations of cortical plasticity in healthy subjects.

BDNF Effects on Cortical Excitability

Met-allele carriers had more ICF before tDCS compared to the val-homozygotes, indicating motor-cortical hyperexcitability. It has been suggested that ICF is generated in excitatory neural circuits of the motor cortex, which are at least partially distinct from the SICI-generating circuits (Chen, 2004; Ziemann, 2004). For ICF generation, the excitation balance is shifted from inhibition towards facilitation, whereas the inhibitory processes are controlled by GABA-mediated inhibitory postsynaptic potentials (Connors et al., 1988; Ziemann, 2004). Pharmacological challenges in healthy subjects further indicate that ICF critically depends on the activity of NMDA and GABAergic receptors, as both NMDA-receptor antagonists and GABA agonists decrease ICF (Ziemann et al., 1996, 1998; Schwenkreis et al., 1999). Therefore, one could speculate that the met-allele, largely independent from disease state, leads to motor-cortical hyperexcitability as the result of an increased facilitation mediated by NMDA and GABA receptors. However, SICI, a marker of GABAergic intracortical inhibition (Ziemann, 2004), did not differ significantly at baseline between val-homozygotes and met carriers in both groups. This could indicate the observed increase in ICF in the met-allele carriers is mainly mediated by a glutamatergic pathway with less influence of GABAergic neurotransmission.

BDNF Effects on Neuroplasticity

Following LTD-inducing cathodal tDCS, SICI was reduced in schizophrenia met-allele carriers, but pronounced in healthy met-allele carriers. Healthy controls showed a numeric MEP increase in met-allele carriers following anodal tDCS. Genotype had no influence on the decrease of MEPs following cathodal tDCS. In schizophrenia patients, genotype did not influence the tDCS-mediated MEP modulation in both stimulation polarities.

Previous research addressing the influence of the BDNF-Val66Met-polymorphism on different measures of neural

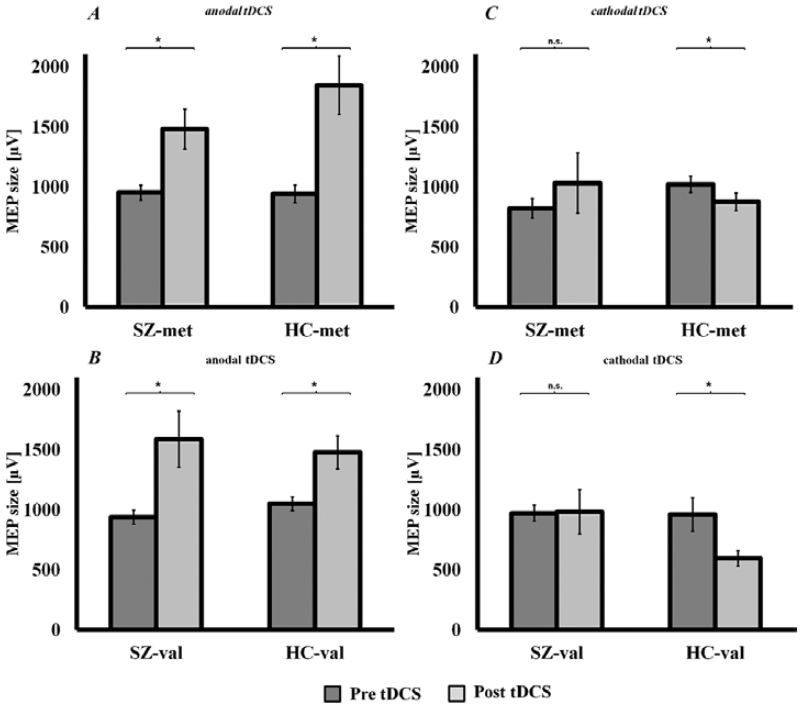

Figure 2. MEP size before and after anodal and cathodal tDCS. Panels A and B display MEP sizes before and after anodal tDCS in healthy and schizophrenia val-homozygotes and met-allele carriers. Panels C and D display MEP sizes before and after cathodal tDCS in healthy and schizophrenia val-homozygotes and met-allele carriers. Following anodal tDCS, increased MEP amplitudes were observed in all subgroups (SZ-val $t_{(13)} = 3.348$, $p = 0.005$; SZ-met $t_{(7)} = 4.685$, $p = 0.002$; HC-val $t_{(11)} = 0.002$; HC-met $t_{(7)} = 0.012$). Following cathodal tDCS, decreased MEP-amplitudes were observed in healthy subjects [HC-val t(9) = 2.682, $p = 0.025$; HC-met t(11) = 1.860, $p = 0.090$], but not in schizophrenia patients (SZ-val $t_{(13)} = 0.816$, $p = 0.429$; SZ-met $t_{(7)} = 0.665$, $p = 0.527$). Data are presented as mean ± standard error of the mean.

plasticity has shown heterogeneous and partly contradictory results that point to a possible reduction in hippocampal volume (Egan et al., 2003; Pezawas et al., 2004; Molendijk et al., 2012), reduced hippocampal activation (Hariri et al., 2003), and lower performance in episodic memory tasks (Hariri et al., 2003; Dempster et al., 2005; Goldberg et al., 2008; Kambeitz et al., 2012) in healthy met-allele carriers. Evidence from non-invasive brain stimulation studies indicates an association of the BDNF-Val66Met-polymorphism with altered neuroplasticity in healthy controls. Three previous studies have used tDCS to examine this relationship (Cheeran et al., 2008; Antal et al., 2010; Di Lazzaro et al., 2012). Di Lazarro et al. (2012) reported no significant differences between val-homozygotes and met-allele carriers with respect to MEP size, SICI, or ICF following cathodal tDCS. In contrast, Antal et al. (2010) found more pronounced after-effects of both anodal and cathodal tDCS in addition to no response to iTBS in the case of met-allele carriers. No significant differences were observed following tRNS. The third study, by Cheeran et al. (2008), showed reduced or absent after-effects following iTBS and cTBS in met carriers, a decreased response to PAS, and decreased reversal of cathodal tDCS-induced inhibition through 1Hz-rTMS (homeostatic plasticity). Our results in healthy subjects principally confirm the findings of Antal et al., showing a trend towards a more pronounced enhancement of MEPs after anodal tDCS, and also extend this previous study by showing increased SICI following cathodal tDCS in healthy met-allele carriers. Taken together, these findings are indicative of a complex interaction between the BDNF-Val66Met-polymorphism, LTD-like plasticity, and intracortical facilitatory networks in healthy subjects. Regarding the inconsistencies between the findings reported by Di Lazarro et al. and Antal et al., as well as our study, differences between the duration of the applied tDCS paradigms should be taken into account. In experimental setups by both Antal et al. and our group, tDCS was performed for 9 minutes (cathodal) and 13 minutes (anodal), whereas cathodal tDCS was conducted for 20 minutes in Di Lazzaro et al.'s study (2012). Furthermore, one should note that none of the foregoing studies (Cheeran et al., 2008; Antal et al., 2010; Di Lazzaro et al., 2012) reported the allele frequency, meaning that we cannot rule out varying allele frequency in accounting for differences across studies.

In healthy met-allele carriers, the observed higher intracortical facilitation at baseline and the increased intracortical inhibition following cathodal tDCS might be explained by higher baseline glutamatergic activity, resulting in increased efficacy of cathodal tDCS due to a larger range for plasticity modulation. Following the assumption of a potentially larger gain between baseline hyperexcitability and cathodal tDCS, we did not observe the same after-effects in schizophrenia met-allele carriers. The reduced SICI following cathodal tDCS could possibly be interpreted as being due to impaired plasticity regulation as a consequence of disturbed homeostatic regulation. Another possible explanation is that the observed effect in schizophrenia met-allele carriers is related to reduced GABAergic interneuron activity. Previous studies have consistently reported a reduction of SICI in subjects at risk of developing psychosis, in first-episode patients, and in chronically ill patients, and have assumed a relationship with reduced GABAergic interneuron inhibition (Wobrock et al., 2010; Hasan, Wobrock, et al., 2012; Bunse et al., 2014; Rogasch et al., 2014). However, the lack of significant differences in baseline SICI would favor a predominant glutamatergic effect. Remarkably, healthy met-allele carriers also displayed ICF baseline alterations without impairments in SICI regulation following cathodal tDCS, while neither schizophrenia nor healthy val-homozygotes displayed these deficits. Thus,

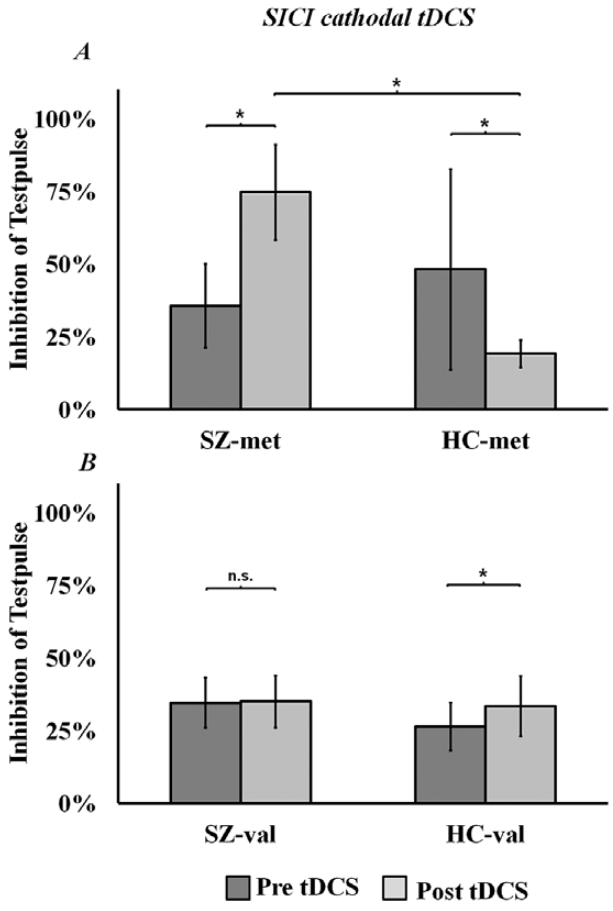

Figure 3. Short-interval intracortical inhibition before and after cathodal tDCS. Panel A shows significant differences between baseline and post tDCS SICI values within the met-SZ and met-HC groups, as well as between met-SZ and met-HC after tDCS. Panel B shows significant differences between baseline and post tDCS SICI values within the val-HC group, but not in the val-SZ group (*$p \leq 0.05$). Data are presented as mean ± standard error of the mean.

one could speculate that the BDNF-met allele in schizophrenia patients reduces the regulation of cortical excitability by means of reduced homeostatic regulation.

Neuropathological findings provide evidence for alterations in the GABA synthesis pathway, showing reductions of glutamic acid decarboxylase (GAD67) expression and density of GABAergic interneurons in various cortical areas, including the prefrontal and the motor cortex, of schizophrenia patients (Benes et al., 1991; Lewis et al., 2005; Hashimoto et al., 2008; Benes, 2011). Further neuropathological investigations showed BDNF-related GABAergic deficits in the prefrontal cortices of schizophrenia patients that are regulated by micro-RNA195, indicating deficits in the gene expression of inhibitory interneurons as a result of a complex interplay of coding and noncoding transcripts (Mellios et al., 2009). Beyond that, post-mortem findings displayed reduced BDNF concentrations in cortical and subcortical areas, leading to disturbed neural plasticity mediated by reduced neuronal trophic support (Takahashi et al., 2000; Durany et al., 2001; Weickert et al., 2003). In another study, BDNF expression levels and the mRNA of its receptor tyrosine kinase TrkB were found to be decreased in the prefrontal cortex of two schizophrenia cohorts (Hashimoto et al., 2005). Based on the observation that BDNF-TrkB signaling has an influence on the development of GAD67 and parvalbumin-positive GABAergic neurons (Huang et al., 1999; Cotrufo et al., 2003), the authors hypothesized that the impairments of BDNF-TrkB signaling in schizophrenia might be an upstream event contributing to the altered expression of GABA-related genes in schizophrenia (Takahashi et al., 2000). Another neuropathological investigation failed to reveal the expected difference in GAD67 mRNA expression of schizophrenia patients hetero- or homozygous for the met-allele, but did show the expected reduction when compared to val-homozygotes (Hashimoto and Lewis, 2006). Although the molecular and cellular processes of the observed alterations in ICF at baseline and in SICI following cathodal tDCS in schizophrenia met-allele carriers remain elusive, our results provide the first evidence for BDNF-related alterations with respect to the balance between excitation and inhibition.

Limitations

The major limitation of this study is that subjects were not genotyped before the physiological experiments. Therefore, no matching for number, age, gender, medication, or genotype distribution was conducted. However, no significant group differences were observed with respect to the biometrical data, and chlorpromazine equivalents and the allele frequency met the expectations of the Hardy-Weinberg equilibrium. In general, one should note the impact of any neuroactive medication on tDCS after-effects in patient studies. As a further limitation, the experiments were conducted by two different persons. However, the same stimulation protocol was conducted on all participants using the same stimulator, the same coil types, and the same settings in all experiments. For this reason, all four study groups were considered to be independent, whereas all schizophrenia patients, but only some healthy controls, participated in both experimental sessions. Another limitation is that it is not only BDNF that contributes to the pathophysiology of schizophrenia, but rather an interaction of various genes. Analyses of these gene interactions are beyond the scope and technical possibilities of the experiments presented here. Though being within the sample size of other related physiological studies conducted on healthy subjects (Cheeran et al., 2008; Nitsche et al., 2008; Antal et al., 2010; Jayasekeran et al., 2011; Di Lazzaro et al., 2012; Chaieb et al., 2014), our sample size is rather small, maybe leading only to some numeric differences between groups that did not reach significance. Therefore, our results should be considered as preliminary and need to be replicated in larger samples of schizophrenia patients and healthy controls.

Summary and Outlook

In summary, this proof-of-concept study shows for the first time an impact of BDNF-Val66Met-polymorphism on LTD-like plasticity following cathodal tDCS and on motor-cortical inhibitory and facilitatory networks in healthy controls and schizophrenia patients. Our findings may indicate that, in schizophrenia patients, the met-allele results in impairments of GABAergic and glutamatergic neurotransmission following plasticity induction. These results not only have the potential to provide a new view on the interaction of BDNF and the regulation of the inhibition-facilitation balance in humans, but might have considerable implications for clinical practice. Different techniques of non-invasive brain stimulation, like rTMS or tDCS, have been suggested to offer new possibilities in schizophrenia treatment (Hasan et al., 2013). However, the stimulation after-effects (Ridding and Ziemann, 2010) and the clinical efficacy (Freitas et al., 2009) are subject to large

inter-subject variability. According to the preliminary results of our study, BDNF-Val66Met-polymorphism might contribute to this variability, but future studies with larger sample sizes need to address this hypothesis specifically by linking BDNF genotype to clinical efficacy following non-invasive brain stimulation.

Supplementary Material

For supplementary material accompanying this paper, visit http://www.ijnp.oxfordjournals.org/

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Statement of Interest

The authors did not receive any financial and/or non-financial forms of support for this study and have no conflicts of interest to report related to the content of this report. However, other conflicts of interests are: Dr Wobrock was honorary speaker for Alpine Biomed, AstraZeneca, Bristol Myers Squibb, Eli Lilly, I3G, Janssen Cilag, Novartis, Lundbeck, Sanofi-Aventis, Otsuka, and Pfizer, and has accepted travel or hospitality not related to a speaking engagement from AstraZeneca, Bristol-Myers-Squibb, Eli Lilly, Janssen Cilag, and Sanofi- Synthelabo; he is a member of the advisory board of Janssen-Cilag and has received a research grant from AstraZeneca, I3G, and AOK (a health insurance company). Dr Nitsche is a member of advisory boards of Neuroelectronics, UCB, and Eisai, and was honorary speaker for UCB, Eisai, and Glaxo-SmithKline. Dr Schmitt was honorary speaker for TAD Pharma and Roche and has been member of the Roche advisory board. Dr Falkai was honorary speaker for Janssen-Cilag, Astra-Zeneca, Eli Lilly, Bristol Myers-Squibb, Lundbeck, Pfizer, Bayer Vital, SmithKline Beecham, Wyeth, and Essex. During the last 5 years, but not presently, he was a member of the advisory boards of Janssen-Cilag, AstraZeneca, Eli Lilly, and Lundbeck. Dr Hasan has been invited to scientific meetings by Lundbeck, Janssen-Cilag, and Pfizer, and he received paid speakership by Desitin and BAK. He is member of the Roche Advisory Board. All other authors report no conflicts of interest.

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