Late cortical plasticity in motor and auditory cortex: role of met-allele in BDNF Val66Met polymorphism

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Abstract
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Keywords
bdnf, late, cortical, plasticity, val66met, motor, polymorphism, auditory, cortex, role, met, allele

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Abstract

The brain-derived neurotropic factor (BDNF) Val66Met polymorphism has been associated with abnormalities of synaptic plasticity in animal models, and abnormalities in motor cortical plasticity have also been described in humans using transcranial direct current stimulation. No study has yet been done on plasticity in non-motor regions, and the effect of two Met alleles (i.e. ‘Met dose’) is not well understood. We studied the effect of the BDNF Val66Met polymorphism on the after-effects of transcranial direct current stimulation and tetanic auditory stimulation in 65 subjects (23; Val66Val, 22; Val66Met and 20; Met66Met genotypes). In the first session, motor evoked potentials (MEP) were recorded under stereotaxic guidance for 90 min after 9 min of anodal transcranial direct current stimulation (TDCS). In the second session, auditory-evoked potentials (AEP) were recorded before and after 2 min of auditory 13 Hz tetanic stimulation. There was a difference in MEP facilitation post-TDCS comparing Met carriers with non-Met carriers, with Met carriers having a modest late facilitation at 30–90 min. There was no difference in responses between Val66Met genotype and Met66Met genotype subjects. Tetanic auditory stimulation also produced late facilitation of N1-P2 AEP at 25 min, but there was no apparent effect of genetic status. This study indicates that Met66Met carriers behave like Val66Met carriers for TDCS-induced plasticity, and produce a late facilitation of MEPs. Auditory cortical plasticity was not affected by the BDNF Val66Met polymorphism. This study sheds light on the differences between auditory and motor cortical plasticity and the role of the BDNF Val66Met polymorphism.

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Key words: BDNF, Event related potential, plasticity, transcranial magnetic stimulation, transcranial direct current stimulation.

Introduction

The study of human cortical plasticity has accelerated in the last decade with multiple non-invasive neurophysiological techniques developed to measure plasticity in the motor cortex (Nitsche and Paulus, 2000; Stefan et al., 2002; Huang et al., 2005). These approaches now provide a real opportunity to test synaptic plasticity as a treatment paradigm for a wide variety of central nervous system (CNS) disorders as well as a platform to develop and test new synaptogenic treatments (Nathan et al., 2011; Lu et al., 2013).

The brain-derived neurotropic factor (BDNF) is a modulator of synaptic plasticity in the form of long-term potentiation (LTP) and long-term depression (LTD) in animal studies (Figurov et al., 1996; Woo et al., 2005). BDNF is secreted as a pro-BDNF precursor molecule and the rate of release is activity-dependent (Lu, 2003; Lu et al., 2008). This means that increased pre-synaptic activity increases pro-BDNF release, which in turn allows BDNF to act on post-synaptic TrkB receptors to modulate synaptic efficacy (i.e. plasticity) in an activity-dependent fashion. A common human single nucleotide polymorphism (SNP) – the BDNF Val66Met (rs6265) – lies on the pro-region of BDNF and reduces activity-dependent secretion of the pro-BDNF precursor...
molecule making (−18% for Val66Met and −30% for Met66Met) (Egan et al., 2003; Chen et al., 2006). While the functional consequences in healthy human subjects that have been described include reduced hippocampal volume (Pezawas et al., 2004) and episodic memory (Egan et al., 2003; Pezawas et al., 2004), it is incompletely understood how the expected neurophysiological effects translate to functional changes.

Several studies have shown an effect of the BDNF Val66Met polymorphism on the after-effects of theta-burst transcranial magnetic stimulation (TMS), paired-associative stimulation and use-dependent plasticity (Kleim et al., 2006; Cheeran et al., 2008) indicating a role in human motor cortical synaptic plasticity. However, independent groups have not been able to reliably reproduce the effect of the polymorphism alone with use-dependent plasticity (McHughen and Cramer, 2013), theta-burst stimulation (Li Voti et al., 2011) and to a degree, paired-associative stimulation (Witte et al., 2012). For transcranial direct current stimulation (TDCS), there has only been one retrospective study in humans suggesting an effect of the polymorphism (TDCS), there has only been one retrospective study in humans suggesting an effect of the polymorphism (Antal et al., 2010), but this has not yet been independently verified. The only other relevant study is a study of TDCS in a mouse model (Fritsch et al., 2010).

The study of the uncommon Met66Met genotype in humans is limited due to the relative rarity [frequency of 5% in Caucasians, 20–40% in Han Chinese in Beijing (The International HapMap Consortium, 2007)]. There has only been one study to date of Met66Met humans: a study of seven subjects with the Met66Met genotype who behaved similarly to ten subjects with the Val66Met genotype for paired-associative stimulation and use-dependent plasticity (Cirillo et al., 2012).

The effect of the BDNF polymorphism on cortical activity or plasticity has not been described in other areas of cortex. There have been attempts to develop neurophysiological techniques to probe synaptic excitability and plasticity in other cortical areas: auditory cortex (Clapp et al., 2005) and visual cortex (Cavuş et al., 2012). Abnormalities in auditory cortical plasticity (Mears and Spencer, 2012) and motor cortical plasticity (Hasan et al., 2011) have been detected in schizophrenic patients lending weight to a plasticity-based hypothesis for schizophrenia (Lewis and Gonzalez-Burgos, 2008). However, little is known about what underlies auditory tectal stimulation and whether it truly reflects LTP- or LTD-like processes and whether it operates on similar synaptic processes as measured by TDCS in the sensorimotor cortex.

We proposed to study in greater detail the effect of this polymorphism on motor cortical plasticity and auditory cortical plasticity by recruiting a larger number of BDNF Val66Val, Val66Met and Met66Met genotype individuals to allow a valid comparison and to examine the effect of ‘Met load’ (i.e. the impact of being a Met-allele homozygote). Motor cortical plasticity was assessed using anodal TDCS and auditory cortical plasticity was assessed using tetanic auditory stimulation. We hypothesise that: BDNF polymorphisms would modulate the effect of anodal TDCS; BDNF polymorphisms would modulate the effect of tetanic auditory stimulation; and, there would be a compound effect of the BDNF polymorphism based on animal studies.

Method

Subjects and BDNF genotype grouping

To determine the study population size needed to demonstrate an expected effect, sample size was calculated based on studies of anodal TDCS (Antal et al., 2010). Briefly, Antal et al. (2010) demonstrated an effect of the BDNF genotype with 24 subjects with an effect size of 0.6518. Based on this, a sample size of 65 subjects would be needed to have α of 0.05 and β of 0.10.

Subjects were recruited from databases with information on the BDNF gene polymorphism; NIHR Cambridge BioResource (CBRC), NIHR Oxford BioResource and GlaxoSmithKline Clinical Unit Cambridge Biobank (which, at the time had about 10000, 4000 and 1500 available subjects, respectively). Three groups of subjects were stratified according to the BDNF single nucleotide polymorphism (SNP rs6265) (i.e. Met66Met, Val66Met or Val66Val). Of the 65 subjects recruited with fully evaluable data: 39 were provided by GSK, 25 by CBRC and 1 by Oxford, reflecting the nature of accessibility to subjects and targeting requirements as the study progressed. SNP screening for rs6265 was conducted under ethically approved protocols and results provided to a GSK database manager. Subjects were designated a database code (independent of genetics results) and provided in appropriate proportions to the clinical recruiting team for screening (thus retaining genotype) blind. Subjects who passed screening according to the study protocol were enrolled and randomised to appropriate groups. One person in the team (not involved in recruitment or analysis) was un-blinded such that equal numbers of subjects were included in the groups matched for age and gender.

Subjects were screened for eligibility to participate. Exclusion criteria included (but was not limited to) the following: any current history of Axis I psychiatric disorders as determined by Mini-International Neuropsychiatric Interview (MINI); any previous disease or current medical condition, which, as judged by the investigator, could affect the interpretation of data; personal or family history of epilepsy; positive pre-study HIV, Hepatitis B surface antigen or positive Hepatitis C antibody result within three months of screening; history of alcohol or substance abuse or dependence in the six months prior to screening; regular use of tobacco- or nicotine-containing products within six months prior to screening; use of any centrally acting medication; positive urine
drug test at screening or when tested at any of the study visits; pregnant females as determined by positive urine hCG test at screening and testing days; lactating females; and drug dependence by the DSM-IV criteria within the last six months as assessed by the MINI interview.

All subjects were Caucasian except one individual who was of mixed ethnicity in the Val66Met group. The genotypes in our sample were not in Hardy-Weinberg Equilibrium. This was intentional, as we wanted to recruit an equal number of subjects from each of the genotype groups to look at gene-dose effects in a balanced design. The mean age of the subjects was 40 yr (range 19–55 yr), but subjects were not age-matched or gender-matched due to the difficulties in identifying suitable numbers of Met66Met individuals.

Seven subjects did not complete experiment one, while one subject did not complete experiment two. Non-completion was due to one failed drug screen, two were unable to enter into MRI due to claustrophobia (and therefore withdrawn from study as they were unable to complete all tasks), two withdrew due to illness and three withdrew for personal reasons that they did not provide.

All participants gave written informed consent for participation in the study, which was approved by the National Research Ethics Service (NRES) Committee – East of England, UK. This study comprised part of larger study of various behavioural, neuro-imaging, neuropsychometric and neurophysiological assessments of the BDNF polymorphism in human behaviour.

**Genotyping**

Genotyping was conducted by LGC Genomics (http://lgcgenomics.com). SNPs were genotyped using the KASPar SNP genotyping system. KASPar is a competitive allele-specific PCR incorporating a FRET quencher casette (see http://www.lgcgenomics.com/kaspar-genotyping-reagents) and genotyped for BDNF Val66Met SNP via TaqMan 50exonuclease assay (Applied Biosystems, USA). See Supplemental Methods for more detail.

**Experiment one**

**Transcranial magnetic stimulation**

Nineteen subjects with Val66Val, nineteen subjects with Val66Met, and twenty subjects with Met66Met genotypes completed experiment one. Surface electromyography (EMG) was recorded from the right first dorsal interosseus muscle (FDI), with Ag/AgCl electrodes using a tendon-belly montage. EMG signals were amplified with Digitimer D360 amplifiers (Digitimer, UK) with 1000× gain, band-pass filtered (30–1000 Hz) and sampled at 5000 Hz using a CED1401 laboratory interface and Signal software (Cambridge Electronic Design, UK).

Magnetic stimuli were delivered with a Magstim-200 magnetic stimulator (The Magstim Co., UK). A figure-of-eight coil (diameter 80 mm) was adjusted over the optimal scalp position (‘hotspot’) to induce a motor-evoked potential (MEP) in the right FDI with the coil handle pointed postero-laterally at a 45° angle to the sagittal plane. The position of the hotspot was registered using a stereotaxic camera system (Brainsight, Rogue Resolutions, UK), and a pen-mark was also marked on the scalp.

The resting motor threshold (RMT) was defined as the lowest intensity capable of inducing at least five out of ten MEPs of >50 μV peak-to-peak amplitude.

The motor cortical excitability was measured at rest at 120% RMT over ten trials by a coil-operating technician who monitored coil position and ensured the distance from the hot-spot was always <5 mm and <5°. A second technician monitored subject relaxation of surface EMG for online rejection of trials with EMG activation.

**Transcranial direct current stimulation**

The experimental protocol was identical to previously described studies in the literature (Nitsche et al., 2003). Subjects were sat in semi-reclined chair. Subjects had MEP recorded in two baseline blocks of twelve trials. Anodal TDCS was then delivered at 1.0 mA intensity for 9 min using a battery-driven electrical stimulator (DC-Stimulator-Plus, NeuroConn GmbH, Ilmenau, Germany) using conductive-rubber electrodes placed in 2 saline-soaked sponges (5 cm × 7 cm size; 35 cm² area). The motor cortical anodal electrode was fixed over the representational field of the right FDIas identified by TMS and the cathodal electrode placed contra-laterally above the right orbit. This stimulation protocol was chosen to induce after-effects lasting about 15 min (Nitsche and Paulus, 2000, 2001). After TDCS, MEP recordings were then obtained at 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75 and 90 min.

**Experiment two**

**Recording session of tetanic auditory stimulation**

Twenty-three subjects with BDNF Val66Val polymorphism, twenty-two subjects with BDNF Val66Met polymorphism and nineteen subjects with BDNF Met66Met polymorphism completed experiment two.

The experimental protocol was similar to previous description in the literature (Clapp et al., 2005; Zaehle et al., 2007). For auditory evoked potentials, 121 pure auditory tones (50 ms duration at 1000 Hz) presented binaurally to subjects at pseudo-random inter-stimulus intervals 1800–2600 ms (auditory recording block). Subjects were requested to focus on a cross presented on a screen and listen to the selection of tones. Two baseline recordings were performed to confirm consistency of response and confirm no effect of auditory recording block on subsequent response. Tetanic auditory stimulation was then delivered using the same stimuli (50 ms duration 1000 Hz auditory tones) but presented at 13 Hz for 2 min.
Subjects were then given a break of 1 min before another auditory recording block (similar to baseline). Subjects then performed a non-auditory task, which lasted approximately 23 min and then another auditory recording block.

Auditory evoked potential components were identified in the averaged data for each subject. N1 peak was determined as the most negative voltage reversal occurring at Cz during the interval 75–150 ms after stimulus onset. The P2 peak was determined as the most positive voltage reversal at Cz between 100–250 ms after stimulus onset.

Data analysis

Experimenters (GB, PL and FS) performed the collection and processing of individual subject data. All were blinded to the genotype of the subjects during the analysis.

For experiment one, peak-to-peak amplitude of MEP was used as the primary measure of motor cortical excitability. For auditory evoked potentials, 110 traces were averaged and the N1-P2 peak-to-peak amplitude was used as the primary measure of auditory cortical excitability.

Statistical analysis was performed by separate investigators (JT, SM and DW). Subjects were initially divided by ‘genotype’ (Val66Val, Val66Met, Met66Met) and repeated-measures analysis of variance (ANOVA) was performed using ‘genotype’ (Val66Val, Val66Met and Met66Met; between-subject factor) and ‘time’ (Baseline, 1, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90 min; within-subject factor) to examine time effects (specifically change from baseline to later time-points) and genetic effects at each individual time-point, in particular focusing on the pairwise difference Val66Val vs. Met66Met (as this was expected to yield the largest signal). An additional repeated-measures ANOVA was performed for experiment one with ‘genotype’ as the factor of interest, having averaged the post-baseline MEP into early and late ‘intervals’ (Baseline, 1–25 and 30–90 min).

Three further ANOVAs were conducted to determine which of three candidate genetic models (Met-dominant, co-dominant and Val-dominant) fitted the data best by assessing the magnitude of the dominance term in an additive-dominant model. The approach was analogous to that described by Lettre et al., as a ‘co-dominant model with two degrees of freedom’ (Lettre et al., 2007), with the difference that we decided the ‘best fit’ based on the magnitude of the estimated dominance term relative to the additive, rather than relying on the p-value (i.e. if the dominance term divided by the additive term was approximately 1 this corresponded to Met-dominance, -1 corresponded to Val-dominance, and 0 to additive/linear).

All ANOVA models also included covariates to adjust for gender and age. Statistical analysis was performed using SAS 9.2 (SAS Inc). The threshold for statistical significance (α) was set at p<0.05, two-sided.

Results

Baseline characteristics of subjects

Baseline characteristics of subjects in experiment one and experiment two are described in Table 1 and Table 2, respectively. There were no significant differences between the genetic groups.

Effect of BDNF genotype on transcranial direct current stimulation

A repeated-measures ANOVA using the between-subject factor ‘genotype’ and within-subject factor ‘time’ showed no effect of ‘genotype’ (p=0.699), ‘time’ (p=0.555) or

Table 1. Baseline characteristics of subjects by genotype for experiment one with anodal transcranial direct current stimulation (anodal TDCS). Figures provided represent mean±S.E.M. Numbers in parenthesis represents ranges. MSO represents mean stimulator output

<table>
<thead>
<tr>
<th>BDNF genotype</th>
<th>Val66Val</th>
<th>Val66Met</th>
<th>Met66Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>19</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.2±2.6 (19–54)</td>
<td>40.0±2.3 (24–54)</td>
<td>41.8±2.2 (20–55)</td>
</tr>
<tr>
<td>Gender (number of females)</td>
<td>11 females</td>
<td>12 females</td>
<td>13 females</td>
</tr>
<tr>
<td>RMT (%MSO)</td>
<td>51±2.7 (33–67%)</td>
<td>50±2.4 (35–70%)</td>
<td>56±3.5 (38–86%)</td>
</tr>
<tr>
<td>Baseline MEP amplitude (mV)</td>
<td>1.03±0.120</td>
<td>0.92±0.133</td>
<td>0.88±0.095</td>
</tr>
</tbody>
</table>
genotype × time interaction (\(p = 0.543\)). There were no significant changes from baseline (all \(p>0.06\)). The Val66Val vs. Met66Met comparison did not reach statistical significance at any time-point (all \(p>0.054\)) (See Supplementary Figure 1). These results were supported by the additional ANOVAs having averaged the post-baseline MEP into two intervals, with no significant changes from baseline (all \(p>0.10\), or between the Val66Val and Met66Met groups (all \(p>0.20\)).

The ANOVA fitting an additive-dominant model indicated that Met-dominance was the best fitting genetic model (see Methods). Fitting a repeated-measures ANOVA using the between-subject factor ‘carrier’ (Met carriers, Val66Val) and within-subject factor ‘interval’ showed a significantly increased cortical excitability from baseline for Met carriers in the 30–90 min interval after anodal TDCS (\(p=0.038\)) (see Fig. 1), although there was no significant difference between Met carriers and Val66Val subjects (\(p>0.21\)).

**Effect of BDNF genotype on tetanic auditory stimulation**

A repeated-measures ANOVA using the between-subject factor ‘genotype’ and within-subject factor ‘time’ showed no effect of ‘genotype’ (\(p=0.994\)) or ‘genotype’×‘time’ interaction (\(p=0.282\). There was an effect of ‘time’ (\(p=0.009\)) with no significant changes from baseline at 1 min (\(p=0.159\), but significant changes from baseline at 25 min (\(p<0.0001\). The Val66Val vs. Met66Met

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**Table 2.** Baseline characteristics of subjects by genotype for experiment two with auditory tetanic stimulation. Figures provided represent mean±S.E.M. Numbers in parenthesis represents ranges. MSO represents mean stimulator output.

<table>
<thead>
<tr>
<th>BDNF genotype</th>
<th>Val66Val</th>
<th>Val66Met</th>
<th>Met66Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>23</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>39.1±2.2 (19–54)</td>
<td>41.0±2.1 (24–54)</td>
<td>41.8±2.3 (20–55)</td>
</tr>
<tr>
<td>Gender (number of females)</td>
<td>14 females</td>
<td>16 females</td>
<td>16 females</td>
</tr>
<tr>
<td>Baseline N1-P2 amplitude (μV)</td>
<td>6.82±0.48</td>
<td>6.77±0.54</td>
<td>7.01±0.66</td>
</tr>
</tbody>
</table>

**Educational attainments**

- No qualifications: 1, 2, 1
- Secondary school (CSE/ GCE/ GCSE): 4, 5, 5
- High school (A’Levels): 5, 3, 2
- Vocational qualifications: 6, 4, 4
- Tertiary degree: 7, 8, 7

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**Fig. 1.** (a) Motor-evoked potentials after 9 min of anodal TDCS across each time-point for subjects grouped according to Met-carrier status (solid line and squares represent Met-carriers, dashed lines and triangles represent non-Met-carriers). Black-filled squares represent time-points \(p<0.05\), while grey-filled squares represent time-points \(p<0.10\) (uncorrected student’s t-test, compared with baseline). (b) Motor-evoked potentials after 9 min of anodal TDCS with time points grouped into early (<30 min) and late (≥30 min). *represents \(p<0.05\) with an uncorrected student’s t-test compared with baseline.
subject factor using the between-subject factor with the TCDS analysis. A repeated-measures ANOVA dominance was therefore investigated for consistency time-point (both p comparison did not reach statistical significance at either time-point (both p > 0.72) (See Supplementary Figure 2).

The ANOVA fitting an additive-dominant model resulted in no clear well-fitting genetic model. Met-dominance was therefore investigated for consistency with the TCDS analysis. A repeated-measures ANOVA using the between-subject factor ‘carrier’ and within-subject factor ‘time’ showed no effect of ‘carrier’ (p = 0.835) or ‘carrier’ × ‘time’ interaction (p = 0.547). There was an effect of ‘time’ (p = 0.011). There was significant change from baseline in N1-P2 amplitude at 25 min after tetanic auditory stimulation (p < 0.0001) (see Fig. 2). There was no evidence of a difference between the Val66Val and Met66Met groups at either time-point (both p > 0.67).

Correlation of TDCS and tetanic auditory stimulation

There was no apparent correlation between the effect at 25 min after anodal TDCS and the effect at 25 min post-tetanic auditory stimulation when performing for all subjects together (r = 0.21, p = 0.11) or just for Met-carriers (r = 0.25, p = 0.12).

Discussion

Effect of anodal TDCS on Met-carriers

This study showed a late facilitation of MEPs after anodal TDCS in Met-carriers similar to a previous retrospective study of Val66Met genotype (Antal et al., 2010). Another recent study of seven subjects with Met66Met genotype used paired associative stimulation (PAS) and showed that the Met66Met genotype behaved similarly to Val66Met genotype for PAS (Cirillo et al., 2012); this is similar to our study, which also shows similar responses after TDCS for both Val66Met and Met66Met subjects.

Our study and the previous study (Antal et al., 2010) demonstrate the opposite direction of effect than would be predicted by animal studies: Fritsch et al. (2010) showed that BDNF gene knockout blocks the after-effects of anodal TDCS on mouse cortical slices so human Met-carriers would be expected to have less MEP facilitation rather than more longer-lasting facilitation (>25 min after stimulation). The most parsimonious explanation for the reversal of direction of effect is that this reflects a secondary phenomenon and is likely to be linked to why the effect is so late after the stimulation.

Late effects of anodal TDCS

NMRA-receptor-dependent long-term-potentiation (LTP) is thought to underlie the effects of artificially induced plasticity such as anodal TDCS (Nitsche et al., 2003) but classical LTP begins immediately after the stimulation ends. Other forms of non-invasive artificially induced plasticity also produce facilitation, which builds up over 10 min (PAS, Stefan et al., 2002) or 20 min (intermittent theta-burst rTMS, Huang et al., 2005) and the reason for this late build-up remains to be elucidated.

The effect of the BDNF polymorphism on late MEP facilitation after anodal TDCS hints at the role of late-phase processes that BDNF modulates, like synaptic scaling (Rutherford et al., 1998; Turrigiano et al., 1998), late-LTP (Kelleher et al., 2004; Bramham and Messaoudi, 2005) and neuronal growth (Anastasia et al., 2013). Late LTP, in particular, provides an attractive explanation as the BDNF-TrKB signalling system is involved in synaptic tagging, a key step in late LTP (Lu et al., 2008, 2011). Speculatively, anodal TDCS would enhance the amount of pro-BDNF release but the pro-BDNF would be then cleaved into BDNF and the pro-domain. The BDNF molecule would activate the TrKB signalling system for synaptic scaling, synaptic tagging and late LTP (greatest extent in Val66Val genotype and least in Met66Met genotype). Simultaneously, the pro-domain induces acute neuronal pruning in Met-carriers but not in non-Met carriers (Anastasia et al., 2013). As our study shows that the BDNF Val66Met polymorphism behaves like a dominant allele rather than an additive allele on anodal TDCS, this favours that neuronal pruning is an important component to the late effect.

The results of this study therefore highlight the complexity of human cortical plasticity measured by non-invasive stimulation with effects operating at different timescales via different molecular mechanisms.

Lack of effect of TDCS in BDNF Val66Val group

This study failed to demonstrate MEP facilitation after anodal TDCS in Val66Val subjects: there is only a hint
of early facilitation in the Val66Val group (Fig. 1), which is not statistically significant. This stands in contrast to a previous study (Antal et al., 2010). This difference could be methodological, but the protocol used is identical with stricter quality safeguards (e.g. TMS delivered under stereotaxic control and all patients having a structural MRI verifying the lack of any underlying structural abnormality in the motor cortex).

The explanation favoured is that the mean age of the subjects in this study (age range 19–55 years) was substantially older compared with the previous retrospective TDCS study of BDNF (age 20–31 years, Antal et al., 2010) and with usual TMS populations (20–35 years reflecting the post-graduate and post-doctoral population). Age has been shown to play a role in paired-associative-stimulation cortical plasticity (Müller-Dahlhaus et al., 2008). Although age was included as a covariate in our models and did not show any clear evidence for age-dependency ($p=0.44$), age-related cortical atrophy would be expected to increase the distance from the scalp TDCS electrode to the cortex thereby reducing the potency of any stimulation.

**Effect of BDNF Val66Met genotype on the effect of tetanic auditory stimulation**

Tetanic auditory stimulation generated an enhancement of the cortically generated N1-P2 auditory evoked potential, but there was no modulation of BDNF polymorphism on this enlargement, which is not what we initially hypothesised.

There are several possible explanations: the problem could be methodological. The older age group may be a factor as a recent study showed that there was a lack of effect of the BDNF polymorphism in motor plasticity in older subjects (McHughen & Cramer, 2013).

Another possible explanation is that the nature of the stimulus provided in tetanic auditory stimulation may be inappropriate. Cirillo et al., demonstrated that the BDNF polymorphism did not modulate plasticity when performing a simple motor task, but when subjects had to perform a much more complex motor task, there was clear modulation (Cirillo et al., 2012). Tetanic auditory stimulation with a single featureless tone as used in this study is analogous to ‘use-dependent plasticity in a simple task’, as the stimulation is physiological and naturalistic but also fairly featureless. The role of BDNF may be only evident on more complex auditory cortical activation tasks like acoustic discrimination tasks, which also modulate N1-P2 auditory evoked potentials (Tremblay et al., 2001; Atienza et al., 2002). This is unsurprising since the effect of BDNF polymorphism is not consistent across various motor cortical plasticity measures (rTMS, TDCS, PAS, use-dependent plasticity) with each method providing different patterns of stimulation and different levels of complexity.

Alternatively, it is also reasonable to propose that the processes underlying event-related potential enlargement after tetanic auditory stimulation do not involve the pro-BDNF molecule and that more work will need to be done to understand the mechanisms.

**Limitations**

Studying only the BDNF Val66Met polymorphism is prone to confounds from other polymorphisms that have an effect on synaptic plasticity or interact with the BDNF Val66Met polymorphism [e.g. COMT Val158Met polymorphism as described by Witte et al. (2012) or dopamine neurotransmission genetic variation as described by Pearson-Fuhrhop et al. (2013)], but are non-uniformly distributed in the study population. This is inherent in the study design, and all human neuroplasticity studies to date are limited in this degree and only large-scale genome-wide association studies would be able to overcome this (Fernandez del Olmo et al., 2010).

Our study did not fully match the three groups for gender and age due to rarity of the individual with the necessary genotypes, which could introduce additional variability. We have accommodated for this by introducing these covariates into all statistical models.

**Implications on this study in depression and schizophrenia**

While early studies suggested a role for low BDNF levels in depression (Shimizu et al., 2003; Sen et al., 2008) and TDCS has been shown to be a treatment modality in depression (Brunoni et al., 2013a), it remains unproven if TDCS produces this effect through BDNF. More recent studies did not find a change in serum levels of BDNF after TDCS (Palm et al., 2013) and another genetic study suggests a role for serotonergic mechanisms (Brunoni et al., 2013b). The BDNF phenomenon detected could therefore reflect epiphemonon (Molendijk et al., 2013).

For schizophrenia, a number of recent studies have shown abnormalities of plasticity in the motor cortex (Hasan et al., 2011), visual cortex (Cavuş et al., 2012) and auditory cortex (Mears and Spencer, 2012), lending weight to the hypothesis of schizophrenia being a disorder of plasticity (Haracz, 1985; Lennox et al., 2012; Steiner et al., 2013) and disconnection (Stephan et al., 2006). Our study suggests that the abnormalities in cortical plasticity measured by these measures of cortical plasticity have heterogeneous root causes and abnormalities in one measure of cortical plasticity do not necessarily translate into abnormalities in other measures of cortical plasticity.

**Conclusion**

This study highlights the role of BDNF in TDCS in humans, and provides useful insights into the
mechanisms of late-phase effects. It also contrasts the differences of motor cortical plasticity as induced by TDCS with auditory cortical plasticity induced by tetanic auditory stimulation.

Supplementary material

For supplementary material accompanying this paper, visit http:\/\slash{}/10.1017/S1461145713001636.

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Statement of Interests

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