BDNF Val66Met polymorphism alters spinal DC stimulation-induced plasticity in humans

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Running head: Altered spinal plasticity in BDNFVal66Met

Key words: H reflex, spinal DC, humans, BDNF Val66Met

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ABSTRACT

The brain-derived neurotrophic factor gene (BDNF) is one of many genes thought to influence neuronal survival, synaptic plasticity and neurogenesis. A common single nucleotide polymorphism (SNP) of the BDNF gene due to valine-to-methionine substitution at codon 66 (BDNF Val66Met) in the normal population has been associated with complex neuronal phenotype including differences in brain morphology, episodic memory or cortical plasticity following brain stimulation and is believed to influence synaptic changes following motor learning task. However, the effect of this polymorphism on spinal plasticity remains largely unknown. Here, we used anodal transcutaneous spinal direct current stimulation (tsDCS), a novel non-invasive technique that induces plasticity of spinal neuronal circuits in healthy subjects. To investigate whether the susceptibility of tsDCS probes of spinal plasticity is significantly influenced by BDNF polymorphism, we collected stimulus-response curves of the soleus (Sol) H reflex before, during, at current offset and 15 minutes after anodal tsDCS delivered at Th11 (2.5mA, 15 min, 0.071mA/cm², 64mC/cm²) in 17 healthy, Met allele carriers and 17 Val homozygotes who were matched for age and sex. Anodal tsDCS induced a progressive leftward shift of recruitment curve of the H reflex during the stimulation which persisted for at least 15min after current offset in Val/Val individuals. In contrast, this shift was not observed in Met allele carriers. Our findings demonstrate for the first time that BDNF Val66Met genotype impacts spinal plasticity in humans, as assessed by tsDCS, and may be one factor influencing the natural response of the spinal cord to injury or disease.
INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is an abundant neurotrophin expressed throughout the central nervous system that has been identified as one of the key neural signals orchestrating neuronal survival, neurogenesis and synaptic plasticity (Lu et al., 2003). A common single-nucleotide missense polymorphism (SNP) at nucleotide 196 (G/A) of the human BDNF gene is associated with decreased activity-dependent BDNF secretion, due to a valine-to-methionine substitution at codon 66 (BDNF Val66Met) (Egan et al., 2003). Lower BDNF levels in Met allele carriers with respect to Val homozygotes have been associated in healthy human subjects to altered cortical morphology (Pezawas et al., 2004; Bueller et al., 2006), memory impairments (Hariri et al., 2003; Egan et al., 2003) or reduced medial temporal lobe activity (Hariri et al., 2003). In addition, several studies, using functional MRI, non-invasive brain stimulation protocols or behavioral endpoints, have explored the impact of BDNF Val66Met on human brain short-term plasticity. Thus, BDNF Val66Met has been associated with deficient activity-dependent cortical plasticity (Kleim et al., 2006) or cortical mapping (McHughen et al., 2010) and with reduced aftereffects of several repetitive transcranial magnetic stimulation (TMS) protocols aimed at inducing LTP/LTD-like plasticity within cortical synaptic connections (Cheeran et al., 2008) suggesting that Val66Met polymorphism would be associated with altered motor cortex plasticity (see Cheeran et al., 2009).

Within the spinal cord, BDNF has been shown to promote spinal plasticity (Baker-Herman et al., 2004). Indeed, BDNF can foster plasticity in cultured spinal neurons and both the stimulation of BDNF release and the application of exogenous BDNF can lead to improved locomotor functions following spinal cord injury (SCI) (Ye and Houle, 1997; McTigue et al., 1998; Sharma et al., 2007). Similarly, exercise-induced upregulation of BDNF mRNA is
associated with functional recovery after spinal cord contusion injury (Gómez-Pinilla et al., 2007) and in vivo model of rats with SCI showed that training increases spinal BDNF expression and engages a BDNF-dependent process that promotes adaptative plasticity (Huie et al., 2012). To what extent Val66Met polymorphism is associated with altered spinal plasticity in humans is unknown and is of great interest given the key role of spinal BDNF in synaptic plasticity in recovery after spinal damage.

Here, we used transcutaneous spinal direct current stimulation (tsDCS), a novel non-invasive technique that influences spinal functions in humans (see Cogiamanian et al., 2012). We have previously reported in a double-blind crossover randomized study that anodal thoracic tsDCS, but not cathodal or sham conditions, is capable of inducing enduring changes of spinal excitability, as evidenced by a progressive leftward shift of the stimulus-response curve of the soleus (Sol) H reflex during the stimulation, which outlasts the duration of the stimulation by at least 15 minutes (Lamy et al., 2012). Thus, to test the impact of BDNF Val66Met polymorphism on spinal neuronal plasticity, we examined the effects of a single session of anodal tsDCS delivered over the thoracic spine on the stimulus-response recruitment curve of the Sol H reflex in 17 healthy, Met allele carriers and 17 Val homozygotes who were matched for age and sex.

METHODS

Subjects
Seventeen BDNF Met allele carriers (12 Val/Met, 5 Met/Met, 10 women, mean age ± standard error of the mean (SEM): 33.5 ± 2.9) were identified and pairwise matched for age and sex with 17 homozygous BDNF Val/Val carriers (mean age 29.4 ± 1.4). All participants were naïve to DC stimulation protocols. The study was approved by our local ethical committees (University of Louisville and Robley Rex Veterans Affairs Research and
Volunteers provided written informed consent according to the ethical codes of the World Medical Association (Declaration of Helsinki). For each experiment, the subject was comfortably seated in a reclining chair. The examined leg was fixed, with the hip semi flexed (~ 110°), the knee slightly flexed (~ 150°), and the ankle at ~ 100° plantar flexion.

EMG recording and Sol H reflex procedure

Surface electromyography (EMG) was recorded from the belly of the right soleus muscle using pairs of surface Ag-AgCl disposable electrodes. EMG signals were amplified (× 1000), filtered with a band-pass of 100 Hz to 1 kHz (Grass technologies, West Warwick, RI, USA), digitalized at 2 kHz using a Power 1401 data acquisition interface (Cambridge Electronics Design, Cambridge, UK) and stored on a personal computer for offline analysis (Signal software, Cambridge Electronics Design). EMG activity was continuously monitored during the experiment with a visual feedback to ensure complete relaxation of the examined muscle.

Soleus H reflex was elicited by stimulating the posterior tibial nerve with homemade electrodes using constant current square-wave pulses of 1ms duration (DS7A, Digitimer, UK) at a stimulation frequency of 0.33Hz. The active cathode (self-adhesive electrode of 3.68cm²) was positioned in the popliteal fossa, and the anode (self-adhesive electrode of 50cm²) on the anterior aspect of the knee. H reflex and M wave thresholds were determined at the beginning of each experiment and were defined as the minimum stimulus intensity needed to elicit peak-to-peak EMG responses of 100µV in the EMG. To plot the stimulus-response curve of the Sol H reflex, five responses were averaged at each stimulus intensity, which was progressively increased in steps of 0.05-0.1×MT (threshold intensity to evoke an M wave) from below H-reflex threshold and up to the maximum H reflex (H_MAX). M waves
were simultaneously recorded. At the end of the experiment, the maximal compound motor response ($M_{\text{MAX}}$) was also recorded.

**Spinal DC stimulation procedure**

Anodal tsDCS was delivered by a battery-driven constant current stimulator (Eldith DC-Stimulator, Germany) connected to a pair of saline-soaked sponge electrodes ($35\text{cm}^2$) covered with electrolyte gel. The anode was centered over the spinous process of the eleventh thoracic vertebra and also covered adjacent vertebrae due to the large size of the stimulating electrode; it allowed for overlaying the lumbar enlargement which extends from approximately the ninth to the twelfth thoracic vertebrae (Lin et al., 2010). The cathode (reference) was placed over the posterior of the right shoulder (Cogiamanian et al., 2008; 2011; Truini et al., 2011; Lamy et al., 2012). The stimulus intensity was set to 2.5mA and was applied over a 15 minutes period (Cogiamanian et al., 2008; Winkler et al., 2010; Lamy et al., 2012) resulting in a current density of 0.071mA/cm$^2$ and a delivered total charge of 64mC/cm$^2$. The current was ramped up to 2.5mA over a 10s period and similarly ramped down at the end of the stimulation. For each experiment, stimulus-response curves of the Sol H reflex and M wave were collected at five time points: before applying tsDCS (T0), during tsDCS delivery starting 2min after current onset (Per1), during tsDCS delivery starting 9 min after current onset (Per2), immediately after current offset (T1) and 15min after current offset (T2). The investigator (JCL) was blinded to the BDNF genotype.

**BDNF genotyping technique**

For DNA extraction, an aliquot from each saliva sample was added to DNAzol Direct (Molecular Research Company) and DNA was extracted according to manufacturer’s instructions. Following DNA extraction, the quality and purity of each sample was examined using the Nano Drop spectrophotometer. Sample DNA extractions were used in subsequent
PCR to amplify the target 274 bp BDNF fragment. PCR was carried out according to the procedure described in Cheeran et al., 2008. Following the PCR procedure, the PCR products were run on a 1.5% agarose gel alongside a DNA size ladder at 90V for one hour. The agarose gel containing the PCR products was stained with ethidium bromide, visualized under UV light and examined for PCR amplification products that represent the amplification of the target 274 bp BDNF fragment. PCR products were used in a restriction enzyme digest for the determination of sample genotype. The restriction enzyme digest using restriction enzyme Hsp92II (Promega) was carried out according to the procedure described in Cheeran et al. 2008. Following restriction digest, the products were run on 2% agarose gels at 90V for 90 minutes. The agarose gels containing the restriction enzyme products were stained with ethidium bromide, placed under UV light and photographed to capture images of the restriction digests. Restriction digest patterns were observed and subsequent genotypes determined for each sample.

Data analysis

Measurements were made on the peak-to-peak amplitudes of the non-rectified EMG responses (±SEM) and mean amplitude was calculated for the responses obtained at each stimulus intensity. All response amplitudes were normalized to $M_{MAX}$, and all stimulation intensities were normalized to the first M wave threshold (MT) prerecorded at T0. Response magnitude was plotted against stimulus intensity and data of each individual curve were fitted with the following three-parameter sigmoid function (Klimstra and Zehr, 2008):

$$H_{reflex}(S) = \frac{H_{MAX}}{1+e^{m(S_{SO}-S)}}$$

Where $S$ is the stimulus intensity, $H_{MAX}$ the maximum H reflex, $m$ the slope parameter of the function and $S_{SO}$ the stimulus needed to obtain 50% of $H_{MAX}$. Because the stimulus intensity
necessary to elicit H reflex threshold ($H_{TH}$) is not an implicit parameter of the function, it was
quantified as the x-intercept of the tangent of the function at $S_{50}$ (Lundbye-Jensen and
Nielsen; 2008).

Given that $m$ represents the slope of the entire function, we instead determined $H_{SLP}$, which
was defined as the slope of the ascending limb of the recruitment curve at $S_{50}$ (see Klimstra
and Zehr, 2008), using the following equation:

$$H_{SLP} = \frac{m(H_{MAX})}{4}$$

Finally, the area under the curve (AUC) of the M wave was calculated using the trapezoid
rule (Lamy et al., 2012).

**Statistical analysis**

Parametric analyses were employed since all data sets successfully passed Kolmogorov-
Smirnov and equality of variance tests. Each H-reflex parameter ($H_{MAX}$, $H_{SLP}$, $S_{50}$ and $H_{TH}$) and
AUC of the M wave were used for statistical analysis. To test for baseline differences
between Val/Val and Met carriers, unpaired t-tests were conducted on raw data. All
measurements were then normalized with respect to baseline levels recorded before the
intervention (T0); therefore, values are interpreted as percentage from baseline. Two-way
analysis of variance (ANOVA) with GENOTYPE as between-subjects factor (2 levels: Val/Val vs
Met) and TIME as within-subject factor (5 levels: T0, Per1, Per2, T1, T2) were first computed
to identify differences between Val/Val and Met groups at different time points. Conditional
on a significant F-value, subsequent one-way repeated-measures ANOVAs were employed to
examine within each condition for significant changes in the time course, using Dunnett’s
multiple comparison tests as post-hocs. In all tests, statistical significance was assumed if $P \leq
0.05$. 
RESULTS

H reflex stimulus-response curves were well fit to the three parameter sigmoid model as evidenced by coefficients of determination $R^2$ exceeding 0.90 (mean 0.99 ± 0.02) for all 170 curves collected (i.e., 34 participants, 5 time points).

To test for baseline bias between Val/Val and Met carriers, unpaired t-tests were conducted on raw data for each H-reflex parameter ($H_{MAX}$, $H_{SLP}$, $S_{50}$ and $H_{TH}$) and for the AUC of the M wave. Here, no a priori differences between baselines could be detected (Table 1).

Figure 1, obtained in one representative Met allele carrier and in one representative Val/Val subject, displays the effects of anodal tsDCS on the H reflex and M wave recruitment curves. For clarity, only T0 (continuous black line) and T2 (continuous grey line) are illustrated. In the Met allele carrier (Fig 3A), anodal tsDCS failed to modulate the amplitude of the H reflex. In contrast, in the Val/Val subject (Fig 3B), anodal tsDCS induced a leftward shift of the recruitment curve of the H reflex without concomitant changes in the M wave amplitude. These observations were fully confirmed in the whole population of participants enrolled.

Fitted H reflex recruitment curves averaged for 17 Met carriers (right panels) and for 17 age- and sex-matched Val/Val individuals (left panels) for each time point are presented in Figure 2. These curves were obtained by fitting average values of individual $H_{MAX}$, $H_{SLP}$, $S_{50}$ and $H_{TH}$ to a sigmoid function. To test for differences between Val/Val and Met groups at different time points, two-way ANOVAs with factors “GENOTYPE” and “TIME” were computed for each parameter ($H_{MAX}$, $H_{SLP}$, $S_{50}$, $H_{TH}$ and AUC of the M wave) (Table 2) and showed significant “GENOTYPE” × “TIME” interactions for $S_{50}$ ($F_{32,128} = 4.47; P < 0.005$), $H_{TH}$ ($F_{32,128} = 3.27; P < 0.05$) and $H_{MAX}$ ($F_{32,128} = 4.76; P < 0.002$). None of the other parameters tested was significant (Table 2). One-way repeated measures ANOVAs were then computed for $S_{50}$, $H_{TH}$ and $H_{MAX}$ to investigate changes from baseline at each time points in Val/Val and Met
individuals and revealed significant effects for $S_{50}$ ($F_{4,64} = 10.36; P < 0.0001$) and $H_{TH}$ ($F_{4,64} = 7.335; P < 0.0001$) in Val/Val but not in Met individuals and, in contrast, for $H_{MAX}$ ($F_{4,64} = 5.245; P < 0.01$) in Met but not in Val/Val carriers (see Table 3). Dunnett’s multiple comparison post-hoc tests (Figure 3) showed that there was a progressive decrease of both $S_{50}$ and $H_{TH}$ over time in Val/Val (Fig. 3A, B) whereas $H_{MAX}$ progressively reduced over time in Met Carriers (Fig. 3C). On the whole, anodal tsDCS induced a progressive leftward shift of recruitment curve of the H reflex during the stimulation which persisted for at least 15 min after current offset in Val/Val but not in Met individuals. Instead, Met allele carriers exhibited a progressive reduction of $H_{MAX}$.

**DISCUSSION**

In this study, we have shown for the first time that the response of healthy subjects to anodal tsDCS intervention in the spinal cord is influenced by the polymorphism of the BDNF gene that they carry. Indeed, BDNF Val homozygotes exhibited significantly higher tsDCS-induced spinal plasticity than BDNF Met carriers suggesting that genetic variations in healthy subjects can produce significant inter-individual differences in the aftereffects of non-invasive protocols aimed at promoting spinal plasticity. The significance is that if BDNF Val66Met also impacts spinal plasticity physiologically, it may influence activity-dependent plasticity in the spinal cord as well as recovery from spinal cord damages.

In non-genotyped healthy individuals, we previously reported that anodal tsDCS is capable of inducing enduring changes of spinal segmental excitability as evidenced by a progressive leftward shift of the stimulus-response curve of the Sol H reflex that persisted after current offset (Lamy et al., 2012). In the current investigation, we showed that anodal tsDCS-induced spinal plasticity occurred in BDNF Val homozygotes only. To avoid confounding factors such as age and gender that might interact with BDNF (Licinio and Wong, 2010; Nemoto et al., 2011).
2006), subjects were carefully pairwise matched for age and sex. Similarly, our results cannot
be related to either any initial differences in H reflex stimulus-response curve parameters (or
AUC of the M waves) between groups (Table 1) or to an alteration of peripheral
neuromuscular excitability since AUC of the M wave was unaffected by the intervention. This
indicates that BDNF Val/Met heterozygotes individuals are likely less susceptible to the
effects of anodal tsDCS than Val homozygotes. Our findings confirm existing results
demonstrating that the response of healthy subjects to protocols aimed at inducing short-
term plasticity within the primary motor cortex is associated with polymorphisms in the
BDNF genotype and extend these observations to spinal cord plasticity. Enhanced cortical
plasticity-induction in BDNF Val homozygotes with respect to Met allele carriers has been
reported using short-term training learning of a simple motor skill task and cortical map
representations (Kleim et al., 2006; McHughen et al., 2010), theta-burst stimulation
(Cheeran et al., 2008; Antal et al., 2010), ‘homeostatic’ conditioning protocol (Cheeran et al.,
2008) and pharyngeal excitatory stimulation (Jayasekeran et al., 2011) and is usually
interpreted by higher activity-dependent secretion of BDNF in Val homozygotes (Egan et al.,
2003), as BDNF is involved in LTP (Poo, 2001). Others, however, failed to detect significant
differences between BDNF Val66Met genotype groups in measures of cortical plasticity
induced by repetitive TMS, intermittent theta-burst stimulation (Li Voti et al., 2011) and
quadripulse stimulation (Nakamura et al., 2011), whereas only a trend to reduced cortical
plasticity in BDNF Met carriers was reported after paired associative stimulation (Cheeran et
al., 2008; Witte et al., 2012). These divergent results are usually explained in the literature
by methodological differences in stimulation techniques and by the small number of
participants studied.
In contrast with our results, it has been reported that when DC stimulations are applied in a transcranial approach (tDCS), carriers of the BDNF Met allele display enhanced motor cortex plasticity (Antal et al., 2010). However, tDCS and tsDCS techniques differ in important ways. Indeed, DC stimulation-induced aftereffects strongly depend on the distance between electrodes (Moliadze et al., 2010), on the current density reaching the target tissue (Purpura and McMurtry, 1965; Nitsche et al., 2008), on the neural circuits involved (Antal et al., 2010) or on the biophysical properties of neuronal membranes according to the fiber orientation (Creutzfeldt et al., 1962). Because these parameters strongly differ between montages the apparent opposing effects of tDCS and tsDCS in Val66Met individuals are not surprising. Moreover, it is likely that (i) additional genes influence DC stimulation-induced plasticity given that about 100 candidate genes have been shown to influence human brain functions and cognition (de Geus et al., 2008), (ii) BDNF Val66Met interacts with other polymorphisms as recently described (Witte et al., 2012).

The anodal tsDCS produced a leftward shift of the stimulus-response curve of the H reflex, i.e., it reduced $H_{th}$ leading to a concomitant reduction of $S_{50}$ in BDNF Val homozygotes only. These effects are similar to what we previously reported in non-genotyped individuals (Lamy et al., 2012) but effects were much more marked in the present investigation, i.e., when subjects are genotyped. This shift is compatible with observations made in animals showing that anodal tsDCS enhances the resting activity in ventral roots in cats (Fuortes, 1954) or increased both spike frequency and amplitude of spontaneous discharges recorded in the tibial nerve of mice (Ahmed, 2011). Thus, our results are compatible with a direct depolarization of a portion of the motoneuron pool by anodal tsDCS. Other possibilities might include changes in intrinsic motoneuron properties (Carp et al., 2001) or increased
transsynaptic efficacy of the Ia fibre–motoneuron synapse given that post-activation depression is reduced by anodal tsDCS (Winkler et al., 2010).

The fact that anodal tsDCS-related plastic changes in spinal excitability had a gradual development over the course of tsDCS intervention in BDNF Val homozygotes and outlasted the intervention period by at least 15 minutes suggested that complex synaptic changes occurred rather than just a membrane polarizing effect (Stagg and Nitsche, 2011). The effects of DC stimulations have mainly been investigated in transcranial approach (tDCS).

Given that long-lasting effects after brain polarization involve (i) modulation of both glutamatergic and GABAergic synapses and, (ii) changes in N-methyl-D-aspartate (NMDA) receptor efficacy (Liebetanz et al., 2002; Nitsche et al., 2003) resulting in increased intracellular Ca$^{2+}$ in the postsynaptic neuron, it is believed that DC stimulation-induced aftereffects arise through synaptic changes via LTP/LTD-like processes as well as through non-synaptic mechanism of action based upon changes in neural membrane function (Ardolino et al., 2005). In contrast, the physiological basis of tsDCS has been less investigated. However, a recent study in mice reported that tsDCS aftereffects on spinal neuronal circuits also involve changes in glutamatergic neurotransmission (Ahmed and Wieraszko, 2012) leading to the assumption that tsDCS aftereffects are likely to occur via activation of neuronal processes terminating in the spinal cord, which are responsible for expression of LTP in postsynaptic spinal cord motoneurons (Arvanov et al., 2000; Ahmed and Wieraszko, 2012).

In our study, anodal tsDCS-induced failed to modulate the stimulus-response curve of the Sol H reflex in BDNF Met allele carriers. Interestingly, recent evidence suggests that anodal tDCS applied to the primary motor cortex enhances BDNF secretion and TrkB activation (Fritsch et al., 2010). In addition, BDNF Val66Met polymorphism likely exerts its effects by impacting
intracellular trafficking and activity-dependent secretion of BDNF, given that lower depolarization-induced BDNF secretion in Met-BDNF transfected neurons has been reported (Egan et al., 2003). Together, as a tentative view, it might be suggested that if similar processes occur at the spinal cord level, this will result in different responses to anodal tsDCS protocol depending on the polymorphism of the BDNF that individuals carry as observed in this study. In addition, BDNF Met allele carriers exhibited a progressive reduction of $H_{\text{MAX}}$. Such reduction of $H_{\text{MAX}}$ has also been reported after spinal associative stimulation (Cortes et al., 2011) but has not been clearly interpreted. Obviously, from our results, it is not possible to conclude about the lack of tsDCS-induced plasticity and reduced $H_{\text{MAX}}$ in BDNF Met allele carriers. Further studies using genotyped animal models may clarify these issues.

In animal models of SCI, up-regulating the H reflex, as observed in the present study, is important for functional recovery after SCI as it encourages recovery of EMG activity (Chen et al., 2010) and changes locomotor kinematics (Chen et al., 2011). After SCI, neurotrophic factors, such as BDNF, are highly regulated (Ikeda et al., 2001) and can rescue neurons (Novikova et al., 2002), promote regeneration (Ye and Houlé, 1997) and facilitate functional recovery including locomotor functions (McTigue et al., 1998; Sharma et al., 2007). Thus, the fact that BDNF Val66Met impacts anodal tsDCS-induced spinal plasticity in humans suggests that this polymorphism could influence the natural response of the spinal cord to injury and/or functional recovery. For example, this polymorphism has been associated with poor outcome of recovery at 3 months in patients suffering from aneurysmal subarachnoid hemorrhage (Siironen et al., 2007). Given that tsDCS has recently been shown to also powerfully modulate the responsiveness of spinal cord neurons in spinal cord injured animals (Ahmed and Wieraszko, 2012), it remains to determine whether anodal tsDCS can
improve motor functions in humans with SCI. This should be done by taking into account BDNF Val66Met polymorphism of participants.

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**LEGENDS TO THE FIGURES**

**Table 1:** Mean values (±SEM) of baseline for each H reflex parameter ($H_{MAX}$, $H_{SLP}$, $S_{50}$ and $x$-intercept) and for AUC of the M wave in Val/Val and Met individuals and results of unpaired t-tests (with $P$ values). MT: Motor Threshold. AUCs of the M wave are expressed in arbitrary units because they were computed from normalized curves (i.e., ordinate was the amplitude of the M wave expressed in percentage of $M_{MAX}$ and abscissa was the stimulus intensity expressed in $\times MT$).

**Table 2:** Results of two-way repeated measures ANOVAs (with $F$ and $P$ values) with factors GENOTYPE (2 levels: Val/Val vs Met) and TIME (5 levels: T0, Per1, Per2, T1, T2) for each H reflex parameter ($H_{MAX}$, $H_{SLP}$, $S_{50}$ and $H_{TH}$) and for AUC of the M wave. MT: Motor Threshold. AUCs of the M wave were expressed in arbitrary units (see legend to table 1). Values in bold indicate significant statistical differences ($P \leq 0.05$).

**Table 3:** Results of one-way repeated measures ANOVAs (with $F$ and $P$ values) for $S_{50}$, $H_{TH}$ and $H_{MAX}$ for Val/Val and Met individuals. MT: Motor Threshold. Values in bold indicate significant statistical differences ($P \leq 0.05$).
Figure 1: Stimulus-responses curves and waveforms of Sol H reflex and M wave obtained at T0 (continuous black line) and T2 (continuous grey line) in a representative Met allele carrier (A) and in a representative Val/Val (B) subject. Five H reflexes and M waves were collected at each stimulus intensity using increments of 0.1 × MT. Sigmoid curve-fitting analyses were used to plot H reflex recruitment curves. Values are expressed in percentage of $M_{MAX}$. In the Met allele carrier (A), anodal tsDCS failed to modulate the amplitude of the H reflex (i.e., the amplitudes of waveforms are comparable between T2 and T0). In contrast, in the Val/Val subject (B), anodal tsDCS induced a leftward shift of the stimulus-response curve of the H reflex (i.e., note the increase in the amplitude of the Sol H reflex at T2 when compared to T0 – see arrows). In both individuals, M waves amplitude were unchanged by the intervention.

Figure 2: Modeling of the effects of anodal tsDCS on H reflex stimulus-responses curves collected at T0 (black line) and at each time point (grey line, row1: Per1; row2: Per2; row3: T1; row4: T2) in 17 Met (right column) and 17 age and sex-matched Val/Val (left column) subjects. Hypothetical H reflex recruitment curves were built using averaged values of individual $H_{MAX}$, $H_{SLP}$, $S_{50}$ and $H_{TH}$ values.

Figure 3: Results of Dunett’s multiple comparison post-hoc tests illustrating anodal tsDCS-induced changes in $S_{50}$ (A), $H_{TH}$ (B) and $H_{MAX}$ (C) at each time point (± SEM) in Val/Val (open circles, continuous line) and Met (filled grey circles, dashed line) individuals compared to baseline (T0). Values in bold indicate significant statistical differences (* $P \leq 0.05$; ** $P \leq 0.01$; ***$P \leq 0.001$).

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FIGURE 1

(A) H reflexes and M wave amplitudes (%Mmax) vs. intensity (× MT).

(B) H reflex and M wave amplitudes (%Mmax) vs. intensity (× MT).
FIGURE 2
FIGURE 3
<table>
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<tr>
<th></th>
<th>Val/Val</th>
<th>Met</th>
<th>P value</th>
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<tr>
<td>$H_{\text{MAX}}$ (% of $M_{\text{MAX}}$)</td>
<td>37.78 ± 3.06</td>
<td>35.82 ± 3.62</td>
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<tr>
<td>$S_{50}$ (×MT)</td>
<td>1.043 ± 0.05</td>
<td>1.081 ± 0.07</td>
<td>0.666</td>
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<tr>
<td>x-intercept (×MT)</td>
<td>0.935 ± 0.05</td>
<td>0.932 ± 0.07</td>
<td>0.968</td>
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<tr>
<td>$H_{\text{SLP}}$</td>
<td>178.2 ± 18.4</td>
<td>128.3 ± 17.3</td>
<td>0.067</td>
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<tr>
<td>AUC M wave</td>
<td>3.096 ± 0.38</td>
<td>4.05 ± 0.53</td>
<td>0.151</td>
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**TABLE 1**
<table>
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<th>GENOTYPE</th>
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<tr>
<td></td>
<td>F(_{1,128}) value</td>
<td>P value</td>
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<td>H(<em>{\text{MAX}}) (% of M(</em>{\text{MAX}}))</td>
<td>8.64</td>
<td><strong>0.0291</strong></td>
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<td>S(_{50}) (×MT)</td>
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<td>H(_{\text{TH}}) (×MT)</td>
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<td>AUC M wave</td>
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**TABLE 2**
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F_{4,64} value</td>
<td>P value</td>
<td>F_{4,64} value</td>
<td>P value</td>
</tr>
<tr>
<td>$S_{50} \times MT$</td>
<td>10.36</td>
<td>&lt;0.0001</td>
<td>0.284</td>
<td>0.887</td>
</tr>
<tr>
<td>$H_{TH} \times MT$</td>
<td>7.335</td>
<td>&lt;0.0001</td>
<td>0.158</td>
<td>0.959</td>
</tr>
<tr>
<td>$H_{MAX} \times M_{MAX}$</td>
<td>1.135</td>
<td>0.34</td>
<td>5.245</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**TABLE 3**