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RESEARCH ARTICLE

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# *BDNF* polymorphism and interhemispheric balance of motor cortex excitability: a preliminary study

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## Abstract

Preclinical studies have demonstrated that brain-derived neurotrophic factor (BDNF) plays a crucial role in the homeostatic regulation of cortical excitability and excitation/inhibition balance. Using transcranial magnetic stimulation techniques, we investigated whether BDNF polymorphism could influence cortical excitability of the left and right primary motor cortex in healthy humans. Twenty-nine participants were recruited and genotyped for the presence of the BDNF Val66Met polymorphism, namely homozygous for the valine allele (Val/Val), heterozygotes (Val/Met), and homozygous for the methionine allele (Met/Met). Blinded to the latter, we evaluated inhibitory and facilitatory circuits of the left (LH) and right motor cortex (RH) by measuring resting (RMT) and active motor threshold (AMT), short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF). For each neurophysiological metric, we also considered the interhemispheric balance expressed by the laterality index (LI). Val/Val participants ($n$ = 21) exhibited an overall higher excitability of the LH compared with the RH, as probed by lower motor thresholds, lower SICI, and higher ICF. Val/Val participants displayed positive LI, especially for AMT and ICF (all $P < 0.05$), indicating higher LH excitability and more pronounced interhemispheric excitability imbalance as compared with Met carriers. Our preliminary results suggest that BDNF Val66Met polymorphism might influence interhemispheric balance of motor cortex excitability.

**NEW & NOTEWORTHY** BDNF Val66Met polymorphism might influence interhemispheric balance of motor cortex excitability. Specifically, Val/Val carriers display higher excitability of the left compared with the right primary motor cortex, whereas Met carriers do not show any significant corticomotor excitability imbalance. These preliminary results are relevant to understanding aberrant interhemispheric excitability and excitation/inhibition balance in neurological disorders.

*excitation; GABA; glutamate; inhibition; TMS*

## INTRODUCTION

Brain-derived neurotrophic factor (BDNF), secreted in response to neuronal activity, exercise, and motor learning (1, 2), plays an important role in synaptic plasticity (3). In at least a third of Caucasians, activity-dependent secretion of BDNF is reduced by a valine (Val) to methionine (Met) substitution in the precursor of the BDNF protein producing a common haplotype [Val66Met] (rs6265) (4, 5). Besides Val/Met genotype, homozygous for the Met allele (Met/Met), which is less frequent in Caucasians (6), may also have a negative influence on BDNF activity (4, 7, 8).

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Interestingly, in a functional magnetic resonance imaging study (9), subjects harboring Val66Met polymorphism exhibited a smaller motor area activation than Val/Val genotype at baseline and after motor training. Likewise, transcranial magnetic stimulation (TMS) studies showed that individuals carrying Val66Met polymorphism did not show the expected increase of motor cortex excitability after a single session of motor practice (10) or the plastic changes induced by protocols of repetitive noninvasive brain stimulation (11).

Also, preclinical studies strongly suggest that BDNF has a crucial role in the homeostatic regulation of cortical excitability, by selectively modifying excitation and inhibition balance within cortical networks (12–14). The change in the balance between excitation and inhibition occurs through modifications in many properties of the network. These include changes in the amount of inhibitory current received by pyramidal neurons (13) or, alternatively, promoting synaptic strength adjustments by enhancing excitation onto pyramidal neurons when BDNF release is reduced or by enhancing excitation onto interneurons, which in turn recruit more inhibition onto pyramidal neurons, when BDNF release is increased (14, 15).

TMS is a powerful tool that can probe in vivo multiple aspects of excitatory and inhibitory activity within the human motor cortex (16). Indeed, TMS activates human motor cortex transcranially; specifically, according to the microcircuit model (17), it induces strong depolarization of layer II/III pyramidal and inhibitory cells that in turns lead to highly synchronized recruitment of clusters of excitatory neurons, including pyramidal neurons of layer V, that represent the major output of M1. Single-pulse TMS protocols provide information on the excitability of axons in the primary motor cortex that are directly excited by TMS at around threshold intensity. Motor thresholds are supposed to be dependent on glutamatergic receptors (ionotropic and metabotropic) as well as voltage-gated sodium channels (18). Likewise, motor-evoked potential (MEP) amplitude represents the spatial and temporal summation of different descending pathways activating the α-motoneurons, thus reflecting the excitation state of the corticospinal volley, the motor nerve at periphery, and the muscle (19). On the other hand, protocols of paired-pulse TMS, such as short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF), may provide insights into the function of cortical inhibitory and excitatory motor networks depending on the interval between the conditioning and test stimuli (20). Pharmacological challenges in healthy subjects indicate that SICI might be mediated by GABA(A)-ergic intracortical circuits, whereas ICF might be linked to *N*-methyl-D-aspartate (NMDA)-glutamatergic intracortical circuits, possibly alongside a reduction in GABAergic inhibition (18).

With the use of TMS techniques, several studies have also reported lateralization of the excitability in the motor system. Specifically, in right handers the excitability of the left motor cortex is higher respect to the right motor cortex and the magnitude of this imbalance is directly correlated to hand use (21–24). Importantly, BDNF is involved in mediating transient use-dependent plasticity of human motor cortex, and the presence of BDNF Val66Met polymorphism impacts negatively on this phenomenon (10).

In the present study, we aim to investigate whether BDNF Val66Met polymorphism can influence the interhemispheric balance of motor cortex excitability by means of TMS protocols assessing excitatory and inhibitory circuits. Our main hypothesis is that Val/Val participants would exhibit higher excitability of the primary motor cortex contralateral to their dominant hand. On the contrary, Met carriers would exhibit no difference in the excitability of two hemispheres.

To this end, in this exploratory study we compared the excitability of left and right motor cortex to single and paired-pulse TMS in Val/Val participants respect to Met carriers.

## MATERIALS AND METHODS

### Participants

We analyzed the data of 29 healthy volunteers [mean age: 33.17 ± 10.76 (SD) yr, 14 females] who were included in a previous published study and who were genetically assessed for BDNF polymorphism (25). All participants gave their written informed consent. The study was performed according to the Declaration of Helsinki and was approved by the local ethics committee (the Medical Faculty of the Catholic University of Rome). Handedness was determined using the Edinburgh Handedness Inventory (EHI) (26), in which 10 items were administered to assess hand preference and to ensure there were no left-handed participants in the study. We included in the study only individuals with no occupations or habits that require frequent daily use of their hands, such as formal music training or excessive writing behavior. In addition, no participant had contraindications to TMS (27), had previous history of neurological or psychiatric disorders, or was taking drugs acting on the central nervous system at the time of the study. At the time of examination, both participants and examiner were blinded to the genotype.

### BDNF Genotyping

Participants blood samples were genotyped for the BDNF Val66Met polymorphism (rs6265, G > A) as previously reported (25). Briefly, by using standard DNA extraction procedure, we obtained genomic DNA from leukocytes. Polymerase chain reaction (PCR) was performed in 20-μL vol with 50 ng of genomic DNA. GeneAmp PCRsystem 9600 (Perkin-Elmer, Foster City, CA) was used to amplify DNA samples. We then electrophoresed PCR products in 1.5% agarose gels containing 0.5 mg/mL ethidium bromide and visualized with ultraviolet illumination and captured on Polaroid film. Gel electrophoresis analysis of the sequencing products was carried-out in a 3100 Genetic Analyzer (Applied Biosystems, Waltham, MA). The sequences obtained by these procedures were analyzed by the Assign SBT Version 3.2.4 software (Conexio Genomics, Applecross, Western Australia) that detects the heterozygous positions within each electropherogram and assesses the typing based on an alignment of the processed sequence with the sequences of the human BDNF genes retrieved from GenBank.

Lastly, participants were categorized in two groups: homozygous for the Val allele (Val/Val) and Met carriers, namely heterozygotes (Val/Met) and homozygous for the Met allele

(Met/Met). We choose to consider two distinct groups because, according to previous neurophysiological studies, Val/Val participants exhibited different excitability and plasticity profiles compared with Met carriers (Val/Met and Met/Met) (10, 11, 25, 28, 29).

## Magnetic Stimulation

### *Motor cortex excitability to single-pulse TMS.*

Single-pulse TMS was applied over the right and left motor cortex by using a figure-of-eight coil (external loop diameters of 9 cm) connected to a high-power Magstim 200 monophasic stimulator (Magstim Co., Whitland, Dyfed). The coil was held tangentially to the scalp, with the handle pointing backward and laterally at 45° from the mid-sagittal line to elicit MEPs in the contralateral first dorsal interosseous muscle (FDI). The induced current flowed in a postero-anterior direction. We evaluated motor thresholds and amplitude of MEPs to single-pulse TMS. Motor thresholds were determined according to published guidelines (19, 30). Briefly, resting motor threshold (RMT) was defined as the minimum stimulus intensity that produced a MEP of ~ 50 μV in at least 5 of 10 trials in the relaxed FDI muscle. Active motor threshold (AMT) was measured as the lowest intensity evoking 5 MEPs of at least 200 μV in 10 consecutive trials during a mild tonic contraction (~ 20% of maximal contraction) of the FDI muscle. A constant level of voluntary contraction was maintained with reference to an oscilloscope display of the EMG signal in front of the subject.

MEP amplitude was assessed in the relaxed FDI muscle by using a stimulus intensity of 120% RMT. Ten data sweeps were collected, and mean peak-to-peak amplitude of the MEPs was calculated. MEPs with background EMG activity were discarded online based on visual inspection, however the amount of rejected MEPs was negligible. Audiovisual feedback, which consisted of subjects' looking at the EMG-oscilloscope screen and listening to the sound produced from spontaneous or evoked muscle activity, was provided during motor thresholds and MEP amplitude assessment. The EMG of resting muscle was tested for spontaneous activity with a gain of 50 μV/division (div) at a sweep speed of 10 ms/div to detect any slightest muscle contraction.

We evaluated RMT, AMT, and MEP amplitude obtained after the stimulation of the right (RH) and left hemisphere (LH).

### *Motor cortex excitability to paired-pulse TMS.*

Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were concurrently evaluated in a single session by applying two magnetic stimuli given through the same coil over the motor cortex and investigating the effect of the first conditioning stimulus on the second test stimulus (31). The intensity of conditioning stimulus was set 95% AMT whereas the test stimulus was adjusted to evoke an MEP of ~1 mV in peak-to peak amplitude by considering the average amplitude of 5 consecutive trials. The interstimulus intervals (ISIs) were 2 and 3 ms for SICI and 15 ms for ICF. Regarding SICI, we considered for the analysis the average of two ISIs (32, 33).

For SICI-ICF paradigm, 10 single-pulse and 10 paired stimuli were delivered at each ISI in a randomized order and constrained to have the same number of pulses at each ISI and the number of ISIs. In addition, the amplitude of the conditioned MEPs was expressed as percentage of the amplitude of the test MEPs. To assist in maintaining complete relaxation, since even slight contraction of the target muscle can affect especially SICI results (34), each subject was provided with audio-visual feedback of the EMG with a gain of 50 μV/div at a sweep speed of 10 ms/div.

Experimental sessions testing the right and left hemisphere excitability were randomized across subjects.

## Statistical Analysis

Data were analyzed using IBM SPSS Statistics v.22.0 for Windows (IBM, Armonk, NY). Normal distribution was verified by means of Kolmogorov and Smirnov test. Since our cohort included only two Met/Met participants, we identified two groups: a Val/Val group and Met carrier group (Val/Met or Met/Met) (25, 28, 35).

Group difference regarding age and gender was tested by means of independent sample $t$ test and $\chi^2$.

BDNF genotype effects on cortical excitability was tested considering the following measures: RMT, AMT, MEP amplitude, SICI, and ICF for both hemispheres. Specifically, we ran analysis of covariance (ANCOVA) for each TMS parameter: RMT, AMT, MEP amplitude, SICI, and ICF with hemisphere (left and right) as within-subjects factor and genotype (Val/Val and Met carriers) as between-subjects factor. Age, gender, and the EHI score were used as covariates to minimize possible effects on excitability measures. Importantly, motor thresholds (RMT and AMT) and paired-pulse paradigms (SICI and ICF) are linked to corticospinal excitability with an opposite behavior: for motor thresholds, the lower the value, the higher is the excitability, on the contrary for SICI and ICF, the higher the value, the higher is the excitability.

In addition, to better elucidate the interhemispheric excitability balance we computed the laterality index (LI) for each of the previous neurophysiologic metrics (28). For instance, the LI for motor thresholds was expressed by the following equations:

$$LI = \frac{RMT(RH) - RMT(LH)}{RMT(RH) + RMT(LH)} \quad LI = \frac{AMT(RH) - AMT(LH)}{AMT(RH) + AMT(LH)}$$

To get a positive LI meaning higher LH excitability, the numerator of the formula was changed so that RH values were subtracted from LH values in the case of MEP amplitude, SICI and ICF, as expressed by the following equations:

$$LI = \frac{MEP(LH) - MEP(RH)}{MEP(LH) + MEP(RH)} \quad LI = \frac{SICI(LH) - SICI(RH)}{SICI(LH) + SICI(RH)}$$

$$LI = \frac{ICF(LH) - ICF(RH)}{ICF(LH) + ICF(RH)}$$

LI ranges from −1 to +1 and the farther the value is from 0, the higher is the interhemispheric imbalance. Positive values denote higher excitability of the LH, while negative values indicate a higher excitability of the RH. For each parameter, independent sample $t$ test was used to test LI difference between the two groups. Lastly, to explore the significant imbalance of the cortical excitability between hemisphere within each genotype group, we examined whether LI was

statistically different from 0 in each genotype group by applying paired *t* tests for each TMS measure.

Descriptive statistics are reported as means ± SE. Alpha inflation due to multiple comparisons was controlled according to Bonferroni's approach and nonsphericity was corrected with the Greenhousee Geisser method, whenever appropriate. Effects were considered significant if $P < 0.05$.

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

## RESULTS

### BDNF Haplotype Affects Interhemispheric Motor Cortex Excitability Balance

The two groups did not differ with respect to sex ($\chi^2$: $P = 0.91$) and age (independent sample *t* test: $t = 0.772$, $P = 0.46$). See Table 1 for further details.

The ANCOVA analysis revealed a significant hemisphere by genotype interaction for RMT [$F_{(1,24)} = 4.058$; $P = 0.045$], SICI [$F_{(1,24)} = 7.399$; $P = 0.01$], and ICF [$F_{(1,24)} = 5.860$; $P = 0.023$] and a trend toward significance for AMT [$F_{(1,24)} = 3.534$; $P = 0.07$], suggesting a different interhemispheric cortical excitability balance between the two BDNF groups.

Post hoc analysis revealed that in the Val/Val group LH cortical excitability was higher than RH (RMT: $P = 0.04$; SICI: $P = 0.018$; and ICF: $P = 0.022$; Fig. 1). In addition, Val/Val LH excitability was also slightly higher than Met carrier LH excitability (SICI: $P = 0.048$; Fig. 1*C*). The ANCOVA model analysis did not reveal any other significant factor. RH excitability was not different from LH excitability as probed by RMT, AMT, MEP amplitude, SICI, and ICF (factor hemisphere: $P = 0.82$, $P = 0.89$, $P = 0.22$, $P = 0.92$, and $P = 0.91$, respectively); no significant difference was found for the between factor genotype (RMT: $P = 0.75$; AMT: $P = 0.74$; MEP amplitude: $P = 0.64$; SICI: $P = 0.57$; and ICF: $P = 0.94$) and for the interaction between hemisphere with age, gender, or EHI score (all $P > 0.085$).

LI showed a difference in interhemispheric excitability balance between Val/Val (LH > RH) and Met carriers (RH > LH) groups: AMT ($t = 2.194$, $P = 0.04$) and ICF ($t = 2.572$, $P = 0.02$), suggesting a different interhemispheric excitability balance (Fig. 2). A nonsignificant trend was observed for RMT ($t = 2.049$, $P = 0.06$) and SICI ($t = 2.110$, $P = 0.05$; Fig. 2). As for MEP amplitude, LI was not different between the two groups, $t = 0.479$, $P = 0.64$. These findings were partly confirmed when examining whether LI was statistically different from 0 in each genotype group; indeed, we found that in the Val/Val group, the LI of SICI ($t = -3.513$, $P = 0.002$) and ICF ($t = -2.016$, $P = 0.043$) were statistically different from zero, with a statistical trend for RMT ($t = -1.891$, $P = 0.07$). No significant difference was found for AMT ($t = -1.519$, $P = 0.14$) and MEP amplitude ($t = 0.843$, $P = 0.41$). In the Met carrier group, we did not find any statistical difference (all $P > 0.15$), suggesting that this genotype is not associated to any cortical excitability asymmetry.

## DISCUSSION

To the best of our knowledge, this is the first study evaluating the effect of BDNF Val66Met polymorphism on interhemispheric balance of corticospinal excitability in healthy humans. Specifically, we found that in the Val/Val group the left hemisphere displayed a higher cortical excitability compared with the right hemisphere. Indeed, the laterality index, a measure indicating the interhemispheric asymmetry in cortical excitability, was significantly different between the two groups: the Val/Val group showed a significant shift of cortical excitability balance toward the left hemisphere, whereas the Met group did not.

Significant effects were consistently observed for both inhibitory (i.e., SICI) and facilitatory (i.e., ICF) paradigms, thus suggesting that the observed BDNF-related excitability imbalance is likely mediated by GABAergic as well as glutamatergic networks.

Interestingly, preclinical studies have suggested that one of the major functions of BDNF is to regulate the level of excitability of cortical circuits and therefore the inhibitory-excitatory balance within the neocortex (13–15).

A large body of work has independently established BDNF as a positive regulator of neuronal activity involved in the development of GABAergic inhibitory synapse in the cortex (12). For instance, application of recombinant BDNF or BDNF overexpression promotes the development of inhibition (36, 37), whereas the reduction of BDNF-dependent neuronal activity using pharmacological blockers or by sensory deprivation retards the maturation of inhibition (13, 38, 39). Importantly, the reduction of cortical GABA-mediated inhibition onto the pyramidal neurons causes in turn an increase of pyramidal neuron firing rates (13).

Another study also demonstrated the role of BDNF level in the regulation of excitatory glutamate-mediated synapses between pyramidal neurons and inhibitory interneurons in the neocortex (14).

**Table 1.** *Demographics and MEP findings of volunteers included in the study*

| Genotype | Val/Val (*n* = 21) | Met Carrier [Val/Met (*n* = 6); Met/Met (*n* = 2)] |
|---|---|---|
| Mean age, yr | 32.05 ± 9.56 | 36.13 ± 13.72 |
| Sex (F/M) | 11/10 | 4/4 |
| Handedness score (range) | +26 to +100 | +20 to +100 |
| %Right-handed (*n*) (+61 to +100) | 81% (17) | 87.5% (7) |
| %Mix-handed (*n*) (−60 to +60) | 19% (4) | 12.5% (1) |
| Mean MEP amplitude, μV (right hemisphere) | 1,092.6 ± 92.4 | 1,042.8 ± 244.5 |
| Mean MEP amplitude, μV (left hemisphere) | 992.2 ± 78.7 | 947.6 ± 125.5 |
| Mean LI of MEP | −0.04 ± 0.05 | 0.001 ± 0.07 |

Values are ± SD for age and ± SE for MEP amplitude and LI. Handedness score ranging from −100 (full left-handed) to +100 (full right-handed) was assessed by applying the Edinburgh Handedness Inventory. F, female; LI, laterality index; M, male; MEP, motor-evoked potential; met, methionine; val, valine.

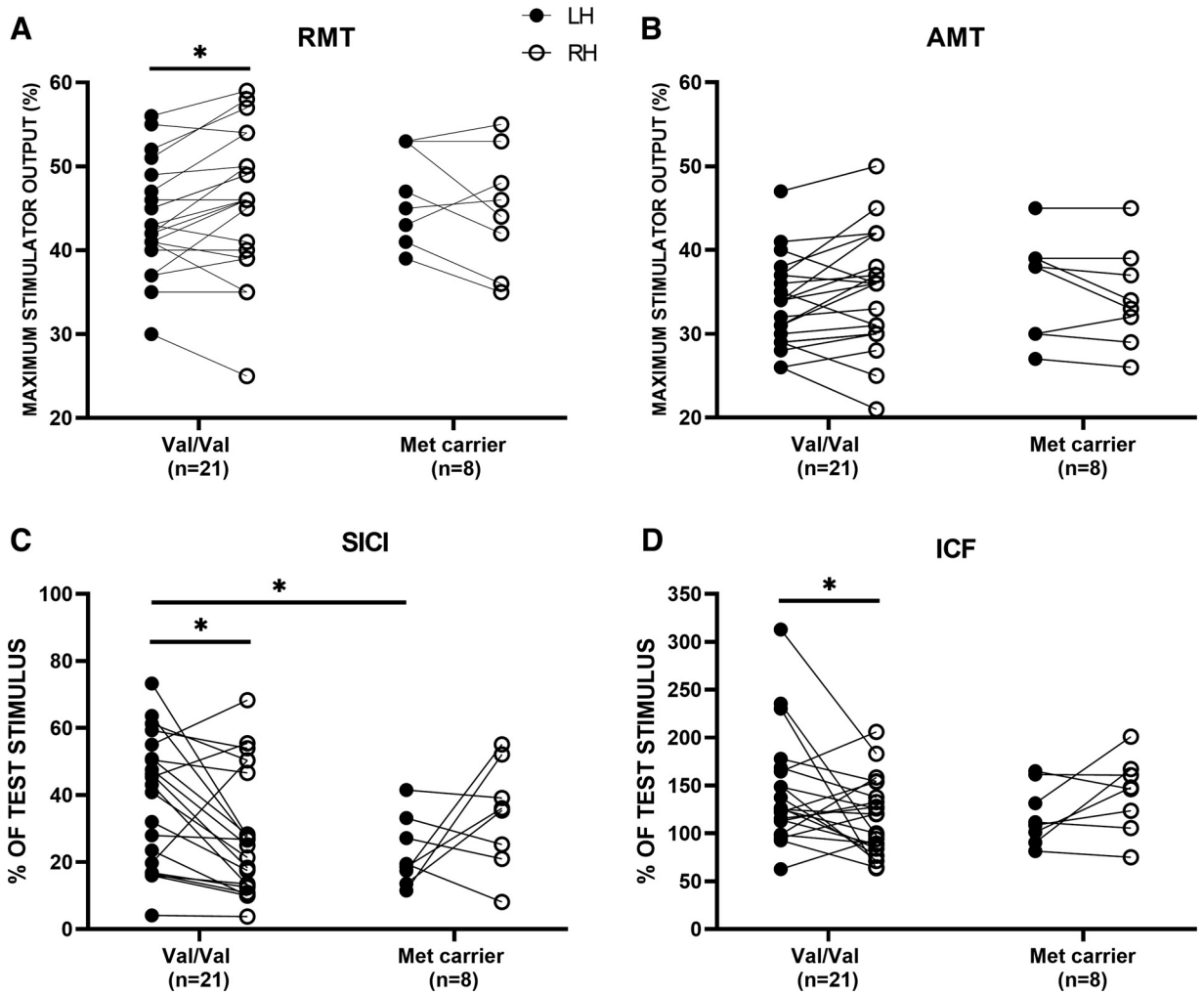


**Figure 1.** Spaghetti plots representing different excitability profiles of the left hemisphere (LH) and right hemisphere (RH) in valine/valine (Val/Val) and methionine (Met) carrier groups probed by resting motor threshold (RMT), active motor threshold (AMT), short interval intracortical inhibition (SICI), and intracortical facilitation (ICF). RMT (*A*) and AMT (*B*) are expressed as the mean percentage of the maximum stimulator output and SICI (*C*) and ICF (*D*) as percentage of test stimulus. *$P < 0.05$, significant difference. Open black circles indicate RH, and close black circles indicate LH.

Overall, these preclinical data suggest that BDNF modulates the relative balance of cortical glutamatergic excitation and GABAergic inhibition by coordinately regulating the strengths of different types of cortical synapses. Such results could be translated in humans as well, since the expression of BDNF messenger RNA (mRNA) can be found in almost every area of the human brain, especially in pyramidal cells of the hippocampus and neocortex (40). In our study, we have systematically evaluated in vivo the effect of BNDF Val66Met polymorphism on the relative balance of both glutamatergic excitation, indexed by motor thresholds and ICF, and GABAergic inhibition by means of SICI. Our results showed that Val/Val genotype is associated to a greater excitability of the dominant hemisphere compared with the contralateral side as the result of an increased facilitation likely mediated by higher glutamatergic and reduced GABAergic activity. In contrast, Met carriers did not exhibit any hemispheric excitability asymmetry.

Importantly, pharmacological manipulation of both NMDA and GABAergic receptors can transiently interferes with the induction of synaptic plasticity in a bidirectional way and therefore influencing the mechanisms operating in use-dependent plasticity in intact human motor cortex (41). This could fit with the concept that Val/Val people, as opposed to Met carriers, may efficiently drive use-dependent plasticity, resulting in higher excitability of the primary motor cortex contralateral to their dominant hand.

Indeed, the reduction of activity-dependent BDNF release that has been associated with BDNF Val66Met polymorphism (4) might be associated to the lack of responsiveness of motor cortex excitability following use-dependent plasticity (10, 42) as well as to a decreased susceptibility to induced plasticity by noninvasive brain stimulation (11, 28, 42–45).

Notably, the hemispheric excitability asymmetry between motor cortices seems to be affected by hand use, since higher cortical excitability (46) and larger cortical motor representation (47, 48) have been found in the contralateral hemisphere of the preferred hand.

However, the studies on hemispheric excitability lateralization have produced conflicting results: some authors suggested that the dominant hemisphere exhibits larger cortical representation areas with lower excitability (48, 49), whereas

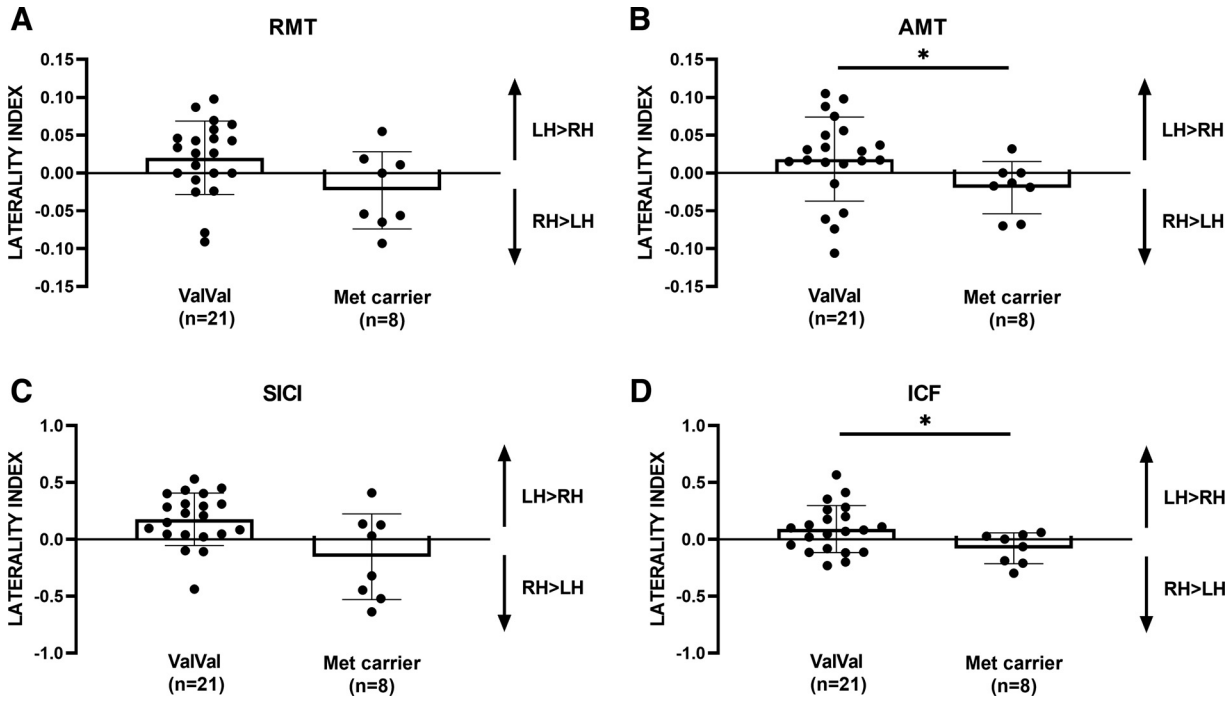


**Figure 2.** Laterality index (LI) of each neurophysiological parameter for valine/valine (Val/Val) and methionine (Met) carrier groups. Positive values of LI indicate higher cortical excitability of the dominant hemisphere [left hemisphere (LH) > right hemisphere (RH)], whereas negative values denote a higher cortical excitability for the nondominant hemisphere (RH > LH). Val/Val and Met carrier groups exhibited a significant different interhemispheric cortical excitability balance for active motor threshold (AMT; *B*) and intracortical facilitation (ICF; *D*) but not for resting motor threshold (RMT; *A*) and short interval intracortical inhibition (SICI; *C*). *$P < 0.05$, significant difference. Error bars indicate SD. Close black circles indicate individual values of LI.

others reported higher cortical excitability in dominant cerebral hemisphere compared with the contralateral side (47, 50) or even could not find any interhemispheric difference (49, 51, 52).

A possible explanation for such controversial results could be in part due to the lack of genotype screening of BDNF in the population recruited in the above studies, and therefore BDNF polymorphism could represent an unmodifiable factor influencing interindividual variability in cortical excitability asymmetry. This explanation is also corroborated by the fact that Met carriers suffer of a reduced enlargement of the muscle map in the motor cortex following a single session of motor training (10) and might fit with our findings, where mostly right-handed Val/Val participants displayed hemispheric excitability asymmetry, being the dominant hemisphere more excitable compared with the contralateral side, whereas Met carriers did not.

Similarly, the effects of BDNF polymorphism on interhemispheric excitability balance could also explain why noninvasive brain stimulation (NIBS) has no plasticity-inducing effects in people with BDNF polymorphism (11, 28, 35, 42–45, 53).

Indeed, the level of motor cortex intracortical excitability has been associated to the effectiveness of plasticity induced by NIBS. For instance, the baseline level of SICI is related to the effectiveness of paired-associative stimulation (54, 55); the baseline level of ICF correlated positively with MEP facilitation following anodal transcranial direct current stimulation (56). However, besides baseline motor cortex excitability, physiological, technical, and statistical factors can influence interindividual variability response to NIBS of the motor cortex (57).

There are some main shortcomings of our research to be considered. First, the overall small sample size as well as the significant imbalance between the two groups (21 Val/Val vs. 8 Met carriers). This aspect could have influenced the analysis of interhemispheric balance of motor cortex excitability, since differences between groups could have been driven by the values of just a few individuals in either group. This limitation reflects the prevalence of BDNF polymorphism in Caucasian population (4–6) even if our sample size is in line with previous published studies (25, 56, 58, 59).

Anyway, future TMS studies should be performed after having determined BDNF haplotype in advance, selecting a matched and balanced group of Val/Val, Val/Met and Met/Met. Despite the small sample size, we observed a rather consistent excitability behavior across several different excitability measures. In addition to providing evidence on the inhibition/excitation mechanisms at play in the motor cortex, such consistency improved our confidence and trust in the results.

Second, we did not consider individual anatomical features such as the scalp to cortex distance (60, 61), which could have driven the difference between the two groups in AMT values and in turn could have also affect both SICI and ICF. In addition, merging the results of SICI at 2 ms and 3 ms is common practice (32, 33, 62) and provides a global estimation of GABA inhibition while reducing variability and increasing statistical power. Nonetheless, it should be acknowledged that SICI at 2 ms and at 3 ms ISI relies on different physiological mechanisms (63).

Third, the number of collected trials for each protocol (i.e., 10) is not sufficient for a reliable readout within or between

sessions, but it may suffice for cluster comparisons or cross-sectional studies. This is likely the case of our study which relies on a paired/within subject design and where multiple and diverse measures of cortical excitability consistently show the same behavior. Nonetheless, recent studies (64, 65) suggest a higher (i.e., 20) number of pulses for better reliability and future studies should comply with such a suggestion.

Lastly, to better elucidate interhemispheric imbalance of cortical excitability in both groups, it would have been interesting considering additional neurophysiological measures such as interhemispheric inhibition (IHI). In addition, the lack of behavioral results should prompt future studies in investigating the relationship between genotype-dependent difference in interhemispheric balance of motor cortex excitability and use-dependent plasticity.

In summary, we demonstrated that BDNF polymorphism could influence and drive interhemispheric differences of motor cortex excitability. In humans, this preliminary study further supports the role of BDNF in the development of cortical inhibition and more generally the notion of BDNF as a genetic signature of excitatory/inhibitory balance in the cortex. Present findings, even if preliminary, could pave the way for our understanding of neurological diseases characterized by abnormal interhemispheric cortical excitability balance such as stroke (28, 66, 67) or by altered excitatory/inhibitory balance such as neurodevelopmental and autism spectrum disorders (68).

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

V.D.L. conceived and designed research; R.D., M.D., and L.R. performed experiments; R.D., G.D.P., and S.T. analyzed data; G.P., F. R., G.D.P., A.U., F.M., and V.D.L. interpreted results of experiments; R.D., G.P., and S.T. prepared figures; R.D. drafted manuscript; F.C., A.U., F.M., and V.D.L. and edited and revised manuscript; R.I., A.U., F.M., and V.D.L. approved final version of manuscript.

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