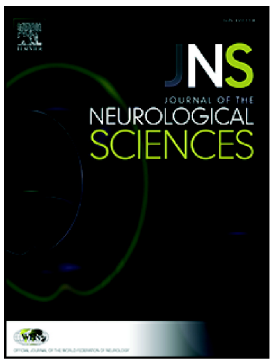

Cortical excitability variability: Insights into biological and behavioral characteristics of healthy individuals

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ABSTRACT

Motor threshold (MT) measured by transcranial magnetic stimulation (TMS) has diagnostic utility in central nervous system disorders. Its diagnostic sensitivity may be enhanced by identification of non-pathological factors which may influence this measure. The aim of this study was to provide a description of MT variability across physiological and non-pathological behaviour characteristics in a large cohort, including hemispheric asymmetries. In a cross-sectional study, age, handedness, physical activity level, body mass index, gender/menstrual cycle phase, glycemic index and degree of stress were collected from 115 healthy participants. The resting MT of the first dorsal interosseous muscle to TMS was recorded in both hemispheres and served as an indicator of the cortical excitability level. Repeated measures ANOVAs revealed higher MT values in the non-dominant hemisphere, elderly people, stressed individuals and women with amenorrhea. Other biological and behavioural individual characteristics did not influence cortical excitability. Although the degree of interhemispheric difference varied (range: 0.2 to 4.3), depending on biological and behavioural characteristics, this variation was not significant ($0.1 \leq p \leq 0.8$). In conclusion, MT varied considerably between subjects. The difference between the hemisphere excitability that was less influenced by external factors, may be an alternative method of TMS measure to identify pathological changes of cortical excitability.

KEYWORDS: Neurophysiology; motor cortex; brain dominance.

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a well-accepted electrophysiological technique which allows a non-invasive evaluation of aspects of cortical excitability in healthy subjects and patients affected by neurological diseases [1]. These aspects provide insight into different neurotransmitter systems, enhance the knowledge of the pathophysiology of neuropsychiatric diseases and may help the development of new therapeutic interventions.

TMS applied at the appropriate stimulus intensity, over the primary motor cortex, induces motor evoked potentials (MEP), which can be recorded as eletromyographic responses in the contralateral extremity muscles [2]. MEP amplitude measurements have been used in research and clinical evaluation of pathological conditions e.g., dystonia [3, 4], Parkinson's disease [5] [6], Huntington's disease [7], and essential tremor [8, 9]. The motor threshold (MT) i.e., the lowest TMS intensity required to evoke MEP in a target muscle in 50% of trials, has been acknowledged as an index of membrane excitability [10, 11]. TMS can used to measure other proprieties of the central nervous system, such as facilitation and intracortical inhibition, recruitment curve and central conduction time. However, these measures are dependent of MT. Recently, studies using MT have provided novel information regarding the pathophysiology of neurological disorders [1, 12], In this way, it is of high relevance to better understand the role of MT, that can be used as a tool in clinical practice, due to its feasibility and applicability.

MEP amplitude and MT can dynamically change over time, e.g., during the sleep-wake cycles [13] or with aging [14]. Moreover, these measures are not static and are relevantly influenced by various factors, such as personality [15], menstrual cycle [16], physical activity [17, 18] that can affect neural function [19, 20]. These factors might increase or decrease the cortical excitability. However, there is no clear consensus regarding the direction in which these changes occur.

The knowledge about which factor influence MT values is crucial for using this parameter in clinical setting as a diagnostic tool. Therefore, the aim of the study was to provide set of data describing intersubject variation in TMS-measured resting MT (rMT) for healthy subjects, and to propose an alternative approach of TMS measurement less variable across subjects for comparison of groups with different biological and behavioral characteristics.

METHODS AND MATERIALS

Subject

One hundred and fifteen healthy subjects participated in this study. Exclusion criteria were pregnancy or history of neurological disease, metallic implants, cardiac pacemakers and seizures. The study was approved by the Research Ethics Committee of the Center for Health Sciences, Universidade Federal de Pernambuco. All subjects gave written informed consent prior to the experiment.

Sample characteristics

Subjects characteristics were collected by means of a self-reported, structured questionnaire, covering gender, age, weight, height, self-related physical activity level (i.e., hours per week) and days from the last menstrual period (LMP). In women, rMTs were measured in different menstrual cycle periods. Subjects were considered sedentary if reported < 3 hours per week of exercise. Body mass index (BMI) was determined for each subject by dividing weight by height squared (kg/m^2).

Stress level and handedness assessment

The subjective stress level of subjects was measured by the Perceived Stress Scale (PSS)-10 item [21]. PPS is a self-report questionnaire with 10 items used to measure the degree of common situations experienced by the subject as stressful in the last 30 days. The recorded score was compared with a normative table for the Brazilian population [21]. The total score was divided into three aged group, and subjects were considered subjective to stress state if

the score was greater than 21 for subjects aged 18 to 29 years, greater than 18 for adults aged 30 to 44 and greater than 17 for individual between 45 and 54 years old.

Handedness was assessed using the 10-item version of the Edinburgh Inventory [22]. Subjects were considered left-handed if their score was ≤ -70, or right-handed if the score was ≥ 70.

Blood glucose Level

Blood glucose levels were measured by means of a glucometer (G-TECH FREE 1 manufactured by Biosensor - South Korea) immediately prior to TMS stimulation. A small drop of blood, obtained by pricking the skin with a lancet, was placed on a disposable test strip, and was used to calculate the blood glucose level. The glucometer then displayed the blood glucose levels measured in mg/dl. Each strip was used once and then discarded.

Transcranial magnetic stimulation

Subjects were seated in a comfortable chair with head and arm rests. Single-pulse TMS was applied with a magnetic stimulator (Neurosoft Ltd., Russia; peak magnetic field=2.2 tesla) using a figure-of-eight magnetic coil (diameter=70mm). The coil was held tangentially to the skull, with the handle pointing backwards and laterally at an angle of 45° from midline. Centred over the primary motor cortex, the coil position was determined at the site which the TMS stimulation consistently evoked the largest MEP amplitudes in the relaxed first dorsal interosseous (FDI) muscle [23]. Raw signals were amplified, filtered over a time constant of 80 ms and a low-pass filter of 5.0 Hz, then digitized at an analogue-to-digital rate of 20 kHz, and further relayed into a computer for off-line analysis using Neuro-MEP-Micro software (Neurosoft Company, Russia).

RMT was defined as the minimum single pulse TMS intensity needed to produce a MEP peak-to-peak amplitude larger than 50 µV in at least half of 10 consecutive trials in the relaxed FDI. RMTs were determined in both hemispheres and expressed as a percentage of the maximal stimulator output [11].

Data processing and analysis

rMT was recorded for each hemisphere of each participant. Data were averaged across subjects for each group, as outlined below.

To investigate age-related rMT changes, subjects were grouped into three groups according their age: (i) < 25 years, (ii) between 25 and 50 and (iii) > 50 years. The rTM changes related to the gender/stages of the menstrual cycle were analyzed for male, women with amenorrhea (associated with menopause or induced by medication); women in reproductive age were divided into four menstrual cycle phases: early (0-7days from LMP) and late (8-14 days from LMP) follicular phases, and early (15-21 days LMP period) and late (>21 days from LMP) luteal phases. To identify blood glucose level- related rMT differences, subjects were grouped into two groups: above and below 99 mm/dl. For BMI, subjects were grouped into two groups: $\geq$ 25 kg/m^2 (i.e., overweight and obesity) and < 25 kg/m^2 (i.e., underweight and normal weight). To investigate the effect of physical activity, stress and handedness on RMT, the subjects were divided into sedentary / non-sedentary, with / without stress, and right- / left-handers, respectively.

Dara were tested for normally distribution (Kolmogorov- Smirnov test, $p > 0.05$) before performing parametric statistics. To evaluate significant differences of rMT between groups with different biological and behavioral individual characteristics, a multifactorial repeated-measures analysis of variance (ANOVA) was performed with *hemisphere*"(dominant and non-dominat) as a within-subject factor and "*subject characteristic"* as a between-subject factor. ANOVA was used for each group comparison: age (ANOVA 2x3), gender/stages of the menstrual cycle (ANOVA 2x6), blood glucose Level (ANOVA 2x2), body mass index (ANOVA 2x2), physical activity level (ANOVA 2x2), handedness (ANOVA 2x2) and stress level (ANOVA 2x2). ANOVAs were followed by Bonferroni-corrected post hoc tests, if appropriate. In all the repeated measures ANOVA, the Greenhouse-Geisser correction was applied if the data did not satisfied the Mauchly's test of sphericity.

The degree of interhemispheric asymmetry (non-domintant hemisphere - dominant hemisphere) was determined for each group of subject characteristics and compared using t-test (2 groups-comparisons) or one-way ANOVA (more than 2 groups-comparisons). For all statistical tests, the significance level was set at $p < 0.05$.

RESULTS

No participants reported adverse effects during or after TMS. Table 1 shows the characteristics of the sample. Table 2 shows all values of rMT for each hemisphere according to the analyzed variable. Hemisphere asymmetry scores are also presented in Table 2.

There was large variability in rMT measured by TMS between subjects and between hemispheres. Values ranged from 32% (minimum) to 87% (maximum), i.e., a variability of 55% of the maximal stimulator output.

Repeated measures ANOVA revealed significant main effects of hemisphere (within subject factor) in all biological and behavioural variables analysed ($8.29 \leq F \leq 13.56$; $0.0001 \leq p \leq 0.005$), except for gender/stages of the menstrual cycle ($F=8.02$, $p=0.05$) and handedness ($F=1.30$, $p=0.25$), with higher rMT in the non-dominant than dominant hemisphere. No significant interactions between within- and between-subject factors were found for any of biological and behavioural variables studied ($0.2 \leq F \leq 2.09$, $0.13 \leq p \leq 0.79$). Significant main effects of subject characteristic group (between subject factor) were found only for age ($F=13.80$, $p=0.000$), gender/stages of the menstrual cycle ($F=5,73$, $p=0.00$) and stress level ($F=9.35$, $p=0.003$). Post hoc tests showed significantly higher rMT in elderly subjects, in women with amenorrhea compared to women in the early follicular phase and in individuals with stress (self-perception of stress).

Although the degree of difference between hemisphere (hemisphere asymmetry) largely varies among subject characteristic groups (mean range: 0.6 to 4.3), these rMT fluctuations were not significant ($0.1 \leq p \leq 0.8$) for all the variables analyzed (Table 2).

DISCUSSION

We have explored the influence of inter-individual variability on cortical excitability of dominant and non-dominant hemispheres measured by TMS-measured rMT in healthy subjects. Results revealed significant influence of some subject characteristics on rMT of hemispheres. In addition, an inter-hemispheric asymmetry was found in all groups of the subject characteristic analyzed. Despite, this asymmetry varies depending on the biological and behavioural characteristics of individual examined (e.g., greater asymmetry in the elderly people and smaller asymmetry in the younger subjects), this variation was not significant, suggesting that interhemispheric asymmetry can be used to reduce between-subject variability over rMT.

Age

We found that aging is associated with a decrease of cortical excitability, given that rMTs were higher in older than younger people in both hemispheres, which is in agreement with previous studies [24-26]. In line with aging-related excitability reduction, MEP amplitude decrease has been demonstrated [27-29] and higher stimulus intensities seem to be required for reaching the maximal motor output in elderly subjects[14, 29].

The understanding of the mechanisms underlying these differences in cortical excitability between older and younger subjects is unclear. Central and peripheral mechanisms could be taken into account, such as age-related loss of cortical and spinal motor neurons, and decline of the neuromuscular system [14, 30].

Gender and stage of menstrual cycle

According to previous studies, women shown better responses to TMS therapies and higher interhemispheric connectivity than men due to a larger corpus callosum [31, 32]. Our study found no difference on rMTs correlated with sex. To the best of our knowledge, no study has directly investigated comparisons between males and females in each menstrual cycle phase, separately. Due to the lack of studies, discussions about this issue might be speculative. Previous studies demonstrated that the estrogen variations are related with cortical excitability alteration [18, 25] and this hormone is

associated with increase of cortical excitability [26]. Men and women in menopause showed higher values of rMT and, consequently, lower cortical excitability level, so the low level of estrogen found in these subjects could help to understand this pattern of cortical excitability.

Furthermore, when we analyzed the values of the rMT of women in different menstrual phases, no effect was observed. This feature could indicate that cortical excitability in women in the reproductive period is not modulated by changes in ovarian hormones levels during the menstrual cycle. However, the rMT values were slightly higher in the presence of amenorrhea. It is known that during the menstrual cycle, ovarian hormones level varies depending on cycle phase; in the follicular phase, estrogen is elevated and progesterone is reduced, In the luteal phase, both are elevated [33]. Previous studies have shown that cortical excitability in women is modulated by changing ovarian hormones level [16, 34, 35], probably due to modulation of ion channels induced by hormonal fluctuation [36]. While estrogens enhance the cortical excitability, progesterone decreases [34]. However, phases of the menstrual cycle were determined based on self-reported days from the last menstrual period and not measured by hormones blood level concentrations, possibly some recall bias has occurred.

Blood glucose level

Previous studies demonstrated that cortical excitability is influenced by fluctuations in blood glucose levels, even when glucose levels remain within normal ranges [37]. This was not confirmed in our study. In congruence with our findings, Andersen et al [38] show that excitability of the motor cortex in Type 1 diabetic patient are unaffected by short-term moderate hyperglycemia as compared with normoglycemia. This difference could be partly explained by the TMS technique used to measure cortical excitability. Indeed, Badawy et al [12] found cortical excitability changes following fluctuations in blood glucose levels when applied paired-pulse TMS. However, authors found that the blood glucose level did not interfere on rMT values in each hemisphere. Also, discrepancies in results might simply reflect the difference of glucose intake time among studies.

Andersen et al [38] reported that glucose differences are not able to alter cortical excitability, since occur within 3 hours. In our study, 79.8% of subjects (95 subjects) had less than 3 hours of fasting, which could explain the lack of variation in cortical excitability related to blood glucose level.

Stress level and body mass index

Cortisol levels, main stress hormone in human, could influence cortical excitability [39]. Indeed, a prior study suggested that higher circulating levels of cortisol rapidly increase corticospinal excitability [40]. In disagreement with previous findings, our study found greater rMT values for both hemispheres in stressed individual. The self-reported stress scale employed in our study, might not precisely reflect physiological changes in plasma cortisol level. Therefore, lack of measurement of cortisol level in the present study limits the potential of comparison with other studies.

Our data not showed a significant interaction between BMI and cortical excitability. Due to the lack of studies on this issue, discussions are limited.

Physical activity level

It is known that cortical excitability could be affected by physical activity, depends if the motor training is passive [41] or active [42]. Previous studies have demonstrated this excitability modification is associated with many factors, such as increased cerebral blood flow [43], angiogenesis [44] and increase in neurotrophic factors [45]. In our study, were not found difference between sedentary and non-sedentary subjects. Besides, physical activity level was not evaluated through a validated questionnaire as an International Physical Activity Questionnaires (IPAQ) and this could limit our discussion.

Furthermore, no difference was shown in the comparison between the dominant and non-dominant hemisphere. These finding were not in line with previous studies which observed interhemispheric asymmetry during uni- and bimanual motor training [46, 47].

Inter-hemispheric asymmetry and handedness

We observed a significant inter-hemispheric asymmetry, with higher rMT in non-dominant hemisphere than dominant hemisphere. This result is

consistent with previous findings [48-50], although others found no difference [51].

According to our results, this inter-hemispheric asymmetry appears to be affected by hand preference. Indeed, while right hand preference has no impact on the asymmetry, in left-handers the difference between the hemispheres disappears. Reid and Serrien [52] showed that hemispheric inhibitory connections would be distinct in left- and right-handers. They demonstrated that right-handers have a better dominant motor organization than left handers. Furthermore, structural and functional imaging studies showed that hemispheric organization of left handers is more heterogeneous [53, 54]. The reason for this difference in asymmetry between left- and right-handers might be related to fact that left-handers tend to use their hand non dominant more than right-handers [55].

The most likely reason for this discrepancy of results may simply be explained by no representative number of left-handers' subjects in our study. Our sample size (9 left-handers) might not allow an examination of the effect of handedness on inter-hemispheric asymmetry.

Implications

We demonstrated rMT variability dependent on biological and behavioral individual characteristics. Since the TMS-measured motor threshold has been used to explore the pathophysiology of neurological and psychiatric disorders and help in the clinical diagnostic of these conditions [1], our findings suggest that between subjects variability may be a primary contributor to interpretation error of TMS measure in a clinical setting. Surprisingly, difference of rMT between groups may be normal due solely to regular across-subject variability. We pointed out that the measure obtained from stimulation of the two hemispheres (degree of side-to-side asymmetry) is much less variable across subjects, i.e., minimize the influence of between subject factors that may affect excitability of the brain as a whole, such as age and stress level of subjects. Therefore, it may be an alternative approach and more powerful measure when comparing groups under different conditions or for evaluating longitudinal changes over time within the same group.

The present study has few limitations that should be discussed. First, the skull to cortex distance was not take into account, since this measurement is acquired through Magnetic Resonance scans, that was not performed in our study. However, previous studies [56-59], shown the correlation between the skull to cortex distance and TMS motor threshold. Further, the small number of left-handed participants might limit the probability to find a significant difference between hemispheres. Lastly, analysis focused on the difference of rMT between hemispheres across subject's characteristics rather than model the degree to which factors explain rMT variability.

In conclusion, our results indicate that rMT measured by TMS is influenced by some biological and behavioural characteristics of the individual and therefore it isolated analysis may lead to misinterpretation. The difference between the hemisphere excitability that was less influenced by external factors may be used to identify changes of cortical excitability.

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Table 1. Characteristics of the participants for biological and behavioral variables.

	n (%)	Range	Mean ± SD
Age			
< 25 year	71(63%)	18-24	21.6±1.4
25-50 year	25 (21%)	25-50	31.4±7.1
>50 year	19 (16%)	53-83	66.6±6.8
Gender			
Male	23 (20%)	-	-
Female	92 (80%)	-	-
Stages of the menstrual cycle (days from LMP)			
<7days	23 (25%)	0-7	3.7±2.5
8-14 days	14 (15.2%)	8-14	10.7±2.2
15-21 days	11 (11.9%)	15-21	18.4±2.3
>21 days	14 (15.2%)	22-60	29.7±9.1
Amenorrhea	32 (34.8)	41-87	-
Blood glucose Level *			
<99mg/dl	50 (43.5%)	11-99	89.0±14.5
≥100mg/dl	65 (56.5%)	100-208	118.2±18.2
Body mass index ** (BMI)			
<25	68 (59.1%)	17.1-24.9	21.4±2.0
≥25	47 (40.9%)	25.0-43.3	29.3±4.6
Physical Activity* **			
Sedentary	61 (53%)	-	-
Non sedentary	54 (47%)	-	-
Handedness ** **			
Right-handed	106 (92.2%)	-	-
Left-handed	9 (7.8%)	-	-
Stress Level*** **			
With stress	46 (40%)	11-35	-
Without stress	69 (60%)	3-23	-

*American Diabetes Association (2005)

** *Body mass index* (weight/height^2) in Anjos (1992)

*** Physical activity (hours per week) -WHO

**** Edinburgh Inventory

**** *Perceived Stress Scale* normative data for the Brazilian population (REIS; PETROSKI, 2005)

Table 2: Mean, standard deviation (SD) and range of resting motor threshold (rMT) for biological and behavioral variables in the dominant and non-dominant hemispheres and mean differences between hemispheres.

	Dominant Hemisphere			**Non-dominant Hemisphere**			**Difference between Hemispheres**	
	rTM	**SD**	**Range**	**rTM**	**SD**	**Range**	**mean**	**P-value***
Overall	57.2	10.2	33-87	59.2	10.3	32-88	1.95	
Age								
< 25 year (1)	54.7	8.6	33-75	55.9	9.1	32-88	1.14	0.1
25-50 year (2)	58.3	11.4	39-81	61.1[1]	9.9	43-84	2.8	
>50 year (3)	64.9 [1,2]	11.0	45-87	69.2[1,2]	8.5	47-84	4.3	
Gender/ Stages of the menstrual cycle (days from LMP)								
Male (1)	58.9	9.6	44-81	60.4	8.7	43-84	1.4	0.8
Female, <7 days (2)	52.7	9.0	33-75	54.9	11.6	32-88	2.1	
Female, 8-14days (3)	53.7	8.0	39-71	54.3	8.1	36-67	0.67	
Female,15-21 days (4)	54.6	9.8	38-70	57.9	9.5	42-74	3.3	
Female, >21 days (5)	57.4	10.9	40-74	58.1	7.8	48-75	0.7	
Female, amenorrhea (6)	61.7[2,3]	10.7	41-87	64.5[2]	10.5	46-84	2.9	
Blood glucose Level								
<99mg/dl (1)	58.6	10.0	41-83	60.4	10.2	36-84	1.8	0.6
≥100mg/dl (2)	55.5	10.5	33-87	57.8	11.0	32-88	2.4	
Body Mass Index								
<25 kg/m^2 (1)	55.1	10.5	33-87	56.0	9.9	32-77	0.9	0.04
≥25 kg/m^2 (2)	59.1	9.9	39-83	62.9	10.5	46-88	3.8	
Physical Activity level								
Sedentary (1)	55.7	8.8	38-74	58.1	9.6	36-80	2.3	0.7

Non sedentary (2)	58.3	12.2	33-87	60.1	12.1	32-88	1.8	
Stress level								
With stress (1)	59.9	12.2	38-87	63.0	11.9	42-88	2.9	0.2
Without stress (2)	54.6[1]	8.2	33-72	56.0[1]	8.8	32-74	1.3	
Handedness								
Right (1)	56.3	10.2	33-83	58.7	10.9	32-88	2.3	0.1
Left (2)	63.2	11.7	51-87	61.7	8.1	54-75	1.1	

Superscript numbers indicate significant difference ($p < 0.05$) using repeated measure ANOVA between correspondent groups in left column for each biological and behavioral variable. *P-value of difference of interhemispheric asymmetry among each subgroup of subject.

HIGHLIGHTS

- There was a large variability in cortical excitability between healthy subjects.
- **Resting motor threshold** (rMT) is influenced by some subject characteristics.
- The interhemispheric asymmetry is less influence by subject characteristics.