Neuroscience Letters 509 (2012) 39–43

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Effects of low and high frequency repetitive transcranial magnetic stimulation of the primary motor cortex on contingent negative variations in normal subjects

Marina de Tommaso*, Claudia Serpino, Katia Ricci, Giovanni Franco, Vito Devitofrancesco, Paolo Livrea

Neuroscience and Sensory System Department, Aldo Moro Bari University, Neurological Building, Policlinico General Hospital, 70124 Bari, Italy

ARTICLE INFO

Article history:
Received 27 August 2011
Received in revised form
21 November 2011
Accepted 22 December 2011

Keywords:
Contingent negative variation
Low frequency TMS
Motor cortex inhibition

ABSTRACT

The state of primary motor cortex (M1) excitability is crucial for the processing of voluntary movement. We aimed to test the modulation induced by 1 Hz and 5 Hz repetitive transcranial magnetic stimulation (rTMS) of M1 on both early and late components of the contingent negative variation (CNV) and on the motor reaction in normal subjects.

The CNV was evaluated in basal, and after 15 min of real or sham 1 Hz and 5 Hz stimulation of the left motor cortex in 7 right handed volunteers. Inhibition of motor cortex, due to rTMS stimulation, resulted in an amplitude increase of early and late components of CNV, and a slight reducing effect on motor reaction times, while 5 Hz stimulation did not change CNV amplitude. In normal subjects transient inhibition of motor cortex causes an increase of cortical events preceding external-cued voluntary movements, as a probable compensatory phenomena able to maintain an efficient motor performance.

1. Introduction

The state of primary motor cortex (M1) excitability is crucial for motor control and change in the process of generation of a voluntary movement [1]. In healthy humans, preparatory cortical activity can be assessed non-invasively by electroencephalography (EEG). The contingent negative variation (CNV) reflects neuronal activity during preparation of externally cued movements. It is a slow negative cortical potential that occurs between two successive contingent stimuli, a warning stimulus followed by a response stimulus [14]. The CNV is generally divided into an early component (eCNV), which largely reflects sensory information of the warning stimulus, and a late component (lCNV), which mainly represents motor readiness and preparatory activity of the forthcoming motor response [5,12]. Animal experiments using depth recordings demonstrated the origin of potentials, corresponding to the human CNV, between the primary (S1) and secondary (S2) sensory cortex, in the prefrontal and supplementary Motor Areas (SMA), and in the primary motor cortex contralateral to the limb involved in the motor task [4]. In particular, a peculiar origin of CNV from the dorsal prefrontal cortex was supposed in respect to the specific involvement of the supplementary motor area in the preparation of spontaneous voluntary movement [5]. The effects of M1 excitability changes on the preparation of externally cued movements has been poorly examined, despite in the condition of chronic M1 dysfunction, changes in primary motor cortex excitability may subtend an enhancement of secondary motor areas activation as a possible phenomenon contributing to the recovery process [7].

Repetitive transcranial magnetic stimulation (rTMS), induces cortical modulation that lasts beyond the time of stimulation [10,11,16], with an effect depending on the frequency of the stimulation used: increased and decreased excitability result from low-frequency (LF) and high-frequency (HF) TMS, respectively. Real but not sham 5 Hz rTMS to left M1 induced a site-specific increase in amplitude of the late component of the CNV at the electrode C3 overlaying the site of stimulation, suggesting a facilitation of premovement activity under primary motor cortex activation [6]. Only one study is available on the effect of primary cortex 1 HZ rTMS on CNV, showing no effect of low intensity 1 Hz rTMS of M1 on CNV [9]. However, in the same study, the inhibition of the dorsal premotor cortex resulted in an amplitude increase of lCNV, suggesting a compensatory activity in other parts of the network to maintain a constant motor performance, or increased functional or effective connectivity within the neuronal network involved in externally cued movement preparation [9]. In this sense, the lack of effects of M1 inhibition on CNV amplitude seems not in line with the supposed compensatory resources of the cortical networks devoted to motor preparation, partly expressed by early and late CNV [13]. Also the site-specific increasing effect observed on the late CNV after M1 high frequency stimulation, needs to be clarified in order to establish the behavior of other cortical areas involved in motor preparation. These were the reasons why we aimed to test the effects of M1 modulation via 1 Hz and 5 Hz rTMS on CNV in healthy subjects, employing high stimulation intensity and an automatic

* Corresponding author. Tel.: +39 080 5478565; fax: +39 080 54784532.
E-mail address: m.detommaso@neurol.uniba.it (M. de Tommaso).

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doi:10.1016/j.neulet.2011.12.043

waves detection software, in order to improve the analysis of CNV amplitude changes across basal, sham and rTMS conditions.

2. Materials and methods

2.1. Subjects

Seven healthy volunteers were selected. They were 4 females and 3 males, aged 27.8 ± 4.34. Exclusion criteria were CNS acting drugs taking in the last three months, general medical or psychiatric and neurological diseases, including epilepsy and migraine. The study was approved by the Ethic Committee of the Bari Policlinico General Hospital, and the subjects gave their informed consent before selection.

2.2. r-TMS procedure

All subjects were comfortably seated in a chair and instructed to be as relaxed as possible. The rTMS was delivered over the hand motor cortex of the left hemisphere through a water-cooled figure eight coil powered by a MagPro X 100 (MedTronic) magnetic stimulator. The stimulating coil was placed over the site that optimally elicited responses in the contralateral abductor pollicis brevis (ABP) target muscle (termed the APB hotspot). The motor threshold (MT) was measured as the minimum stimulus intensity that elicited a motor evoked potential (MEP) of at least 50% in 5 or more of 10 consecutive stimulations in the right APB hot spot. To establish the motor threshold, electromyography (EMG) signals were recorded from the right APB muscle using 0.9-cm diameter Ag–AgCl surface electrodes placed 3 cm apart over the center and tendon of the muscle. The EMG activity was recorded with a band-pass filter between 10 and 1000 Hz and a display gain ranging from 50 to 200 lV/cm. In the 1 Hz rTMS task, each rTMS session consisted of 900 stimuli at a frequency of 1 Hz, at 90% MT intensity. Sham (control) rTMS was performed at the same stimulation position with the coil tilted approximately 45° over the scalp. In the 5 Hz rTMS task, the motor threshold was evaluated again, and each rTMS session consisted of 1800 stimuli divided in 12 trains at a frequency of 5 Hz and 90% MT intensity, with and 10-s pause between trains. Sham (control) rTMS was performed at the same stimulation position with the coil tilted approximately 45° over the scalp.

2.3. Experimental procedure

The CNV task was first recorded in basal condition (basal), after 1 Hz TMS (rTMS) and sham rTMS sessions (sham). The basal, rTMS and sham tasks were performed in different days in random order (starting at about 11 a.m.). After six months from this procedure, the same subjects were submitted again to CNV in basal condition, after 5 Hz rTMS and sham rTMS, in random order (starting at the same time in the morning).

2.4. CNV recording

All subjects were seated on a comfortable chair inside a sound attenuating and electrically shielded room at about 30 cm from a computer screen, bilaterally armed of aforesaid speakers, holding a small box with a button in their right hand. Two acoustic stimuli were delivered through opposite speakers placed in front of the patient with a fixed intensity of about 70 dB SPL. The first stimulus (warning stimulus, S1) was a tone burst of 1000 Hz. The second stimulus (imperative stimulus, S2) was a tone burst of 2000 Hz. On hearing S2, the subjects pressed the button on the box as fast as possible. The interval between S1 and S2 was fixed at 3 s. Forty-eight trials were done with a randomly varying inter-trial interval from 4 to 6 s, in each of the three experimental conditions.

Three electrodes were positioned, on the Fz, Cz, Pz, derivations, according to the 10–20 international system, with linked mastoids as reference and the ground electrode positioned on the nasion. Impedance was kept at 10 kΩ or less.

The EEG signals were amplified using a Micromed amplifier with a bandpass filter settled at 0.03–35 Hz digitized at a rate of 250 Hz for each channel. Vertical eye movement artefacts were excluded by parallel recording of the electrooculogram (EOG) using electrodes (Ag/AgCl) positioned 1–1.5 cm above and below the right eye. The trial was rejected if EOG deflections greater than 20 mV interfered with 5 s of the EEG recording. A protocol listed the number of rejected trials for each recording. A 200 ms period preceding the S1 served as a baseline for the CNV: trials containing values exceeding 70 μV were automatically excluded from the averaging. The CNV was calculated over a total time of 4 s, including the baseline. An automatic wave scoring program was used, according to ASA software, vers. 4. The program calculated the maximum negativity in the time interval of 550–750 ms for the early CNV, and in the time interval 2700–3000 for the late CNV, after a baseline correction and the subtraction of the pre-stimulus signal.

A grand average of initial and late CNV components was also obtained for the 7 subjects in the three conditions.

The CNV amplitude and reaction times (RT), were evaluated and compared across the basal, sham and rTMS conditions, by the means of Bonferroni test for multiple comparisons, after two-ways ANOVA with condition and channel as factors was run out separately for the 1 Hz and 5 Hz rTMS tasks.

3. Results

The amplitude of the early and late CNV appeared to be significantly higher when the left motor cortex was inhibited by low frequency TMS modulation, compared to the sham and basal conditions (ANOVA with eCNV amplitude as variable and condition as factor: F 5, df 2, p 0.010; channel as factor: F 0.2, df 2, n.s.; channel × condition as factor: F 1.2, df 4, n.s.; ANOVA with lCNV as variable and condition as factor: F 7.85, df 2, p 0.001; channel as factor: F 0.21, df 2, n.s.; channel × condition as factor: F 0.4, df 4, n.s) (Figs. 1a, 1c and 2a). The reaction times (RT) appeared to be only slightly reduced in the condition of inhibition of the motor cortex (ANOVA with RT as variable and condition as factor: F 2.75, df 2, n.s.) (Fig. 3a). The M1 stimulation by 5 Hz rTMS did not change either early or late CNV (Figs. 1b, 1d and 2b) (ANOVA with eCNV amplitude as variable and condition as factor: F 0.47, df 2, n.s.; channel as factor: F 0.12, df 2, n.s.; channel × condition as factor: F 0.009, df 4, n.s.; ANOVA with lCNV as variable and condition as factor: F 2.34, df 2, n.s.; channel as factor: F 1, df 2, n.s.; channel × condition as factor: F 0.084, df 4, n.s). Also in this case, RT were slightly and not significantly reduced (ANOVA with RT as variable and condition as factor: F 1.94, df 2, n.s.).

4. Discussion

Our results showed that low frequency rTMS of left primary motor cortex, produced a clear increase of both early and late CNV in healthy subjects, without affecting, even slightly improving, motor reaction times. Giving that the excitability reducing effect of LF rTMS is widely accepted [2], we can suggest that inhibition of left primary motor cortex resulted in an activation of early and late cortical processes preceding external paced voluntary movement. These results are not in line with the increase of lCNV after excitability increasing 5 Hz rTMS [6], as in those studies the sign of movement related potential change was in the same direction as the sign of excitability change. The late CNV tracts partly origin from the M1 [4], so its amplitude increase may be a direct effect