High frequency somatosensory stimulation increases sensori-motor inhibition and leads to perceptual improvement in healthy subjects

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Highlights

1. **Repetitive tactile stimulation improves somatosensory spatial and temporal perception**
2. **The same intervention also modifies cortical sensorimotor interactions**
3. **These changes are likely due to an increased effectiveness of cortical inhibition**

Abstract

Objective: high frequency repetitive somatosensory stimulation (HF-RSS), which is a patterned electric stimulation applied to the skin through surface electrodes, improves two-point discrimination, somatosensory temporal discrimination threshold (STDT) and motor performance in humans. However, the mechanisms which underlie this changes are still unknown. In particular, we hypothesize that refinement of inhibition might be responsible for the improvement in spatial and temporal perception. **Methods**: fifteen healthy subjects underwent 45 minutes of HF-RSS. Before and after the intervention several measures of inhibition in the primary somatosensory area (S1), such as paired-pulse somatosensory evoked potentials (pp-SEP), high-frequency oscillations (HFO), and STDT were tested, as well as tactile spatial acuity and short intracortical inhibition (SICI). **Results**: HF-RSS increased inhibition in S1 tested by pp-SEP and HFO; these changes were correlated with improvement in STDT. HF-RSS also enhanced bumps detection, while there was no change in grating orientation test. Finally there was an increase in SICI, suggesting widespread changes in cortical sensorimotor interactions. **Conclusions**: these findings suggest that HF-RSS can improve spatial and temporal tactile abilities by increasing the effectiveness of inhibitory interactions in the somatosensory system. Moreover, HF-RSS induces changes in cortical sensorimotor interaction.

Significance: HF-RSS is a repetitive electric stimulation technique able to modify the effectiveness of inhibitory circuitry in the somatosensory system and primary motor cortex.

1 Introduction

Godde and coworkers (Godde et al. , 2000) were the first to demonstrate in healthy volunteers that high frequency repetitive stimulation of cutaneous receptors (HF-RSS) improves two point discrimination in the stimulated area. Since previous animal experiments (Godde et al. , 1996) had shown that HF-RSS enlarges cutaneous receptive fields in rat somatosensory cortex, it might have been expected that HF-RSS in humans would reduce spatial discrimination. However, discrimination between the location of two points does not necessarily relate to the receptive field size of individual neurons, but instead reflects the sum total of information present in the discharge of many neurons (Godde et al. , 1996, Godde et al. , 2000, Dinse et al. , 2006). More neurons responsive to inputs from an area of skin with overlapping and slightly different receptive fields code spatial representation with greater precision than any single neuron alone.

HF-RSS also improves somatosensory temporal discrimination threshold (STDT), which is defined as the shortest time interval between two tactile stimuli for them to be perceived as separate (Erro et al. , 2016). However, the reason for this effect is unclear since it is difficult to explain how larger spatial receptive fields can influence temporal discrimination between stimuli. In a previous work using transcranial magnetic stimulation we argued that temporal threshold depends on the effectiveness of short duration inhibition in the somatosensory system, which is used to sharpen temporal processing following the arrival of the initial sensory input (Rocchi et al. , 2016). The aim of the present experiments was to test whether HF-RSS might improve STDT by enhancing this inhibitory effect.[1] If so, it would imply that HF-RSS has two consequences, both of which are spatially

[1] **List of Abbreviations**: ADM: abductor digiti minimi; APB: abductor pollicis brevis; FDI: first dorsal interosseous; HFO: high-frequency oscillations; HF-RSS: high-frequency tactile stimulation; ICF: intracortical facilitation; MEP: motor evoked potential; SEP: somatosensory evoked potentials; SICI: short intracortical inhibition; STDT: somatosensory temporal discrimination threshold; TSD: tactile spatial discrimination: TT: tactile threshold

limited to the area of stimulation: increased size of spatial receptive fields and increased effectiveness of somatosensory inhibition. In fact it could be that both effects are complementary. Thus, increased spatial discrimination between stimuli would benefit both from larger receptive fields as well as increased effectiveness of inhibitory connections between adjacent fields. Similarly, increased temporal discrimination might benefit from engagement of larger numbers of neurons in temporal processing, along with an augmented efficacy of inhibitory connections between them. We therefore correlated changes produced by HF-RSS on spatial and temporal discrimination with our measures of somatosensory inhibition (recovery of P14 and N20-P25 waves with paired-pulse somatosensory evoked potentials and area of high frequency SEP oscillations) to test its relative contribution to temporal and spatial discrimination. We used STDT as our measure of temporal discrimination. For spatial discrimination we employed two different tests: the "bumps" test, which is a simple measure of tactile threshold, and the JVP test, which is a more complex measure of spatial discrimination that assesses the ability to detect the orientation of a tactile grating. Lastly, since HF-RSS has been further shown to improve motor performance (Kalisch et al. , 2008, Smith et al. , 2009, Kalisch et al. , 2010), we also explored possible effects of HF-RSS on processing in the primary motor area (M1), using measures of short intracortical inhibition (SICI; a GABAa-ergic inhibition) and intracortical facilitation (ICF; a glutamatergic excitation).

2 Methods

2.1 Subjects

Fifteen right handed (Oldfield, 1971) subjects (11 male, 4 female, age 54.5) participated in the study. They had no history of any diseases related to the central or peripheral nervous system; they did not have metal or electronic implants and were not on medications on the nervous system. Subjects signed a written informed consent before the experimental session and all experimental

procedures were approved by the local institutional review board and conducted in accordance with the Declaration of Helsinki and according to international safety guidelines.

2.2 Somatosensory temporal discrimination threshold

STDT was tested administering paired electrical stimuli, starting at an interstimulus interval (ISI) of 0 ms (simultaneous pair) and progressively increasing the ISI in steps of 10 ms (Conte et al. , 2012, Rocchi et al. , 2013, Conte et al. , 2014). This ascending method has been reported to yield results similar to common psychophysical assessment (Rocchi et al. , 2016).

Stimulation was delivered to the third phalanx of the right and left thumb and index finger using surface electrodes separated by 0.5 cm (anode placed distally than the cathode). Current was applied by means of a constant current stimulator (Digitimer DS7A) in the form of square-wave pulses. The intensity for STDT testing was the lowest at which each subject could perceive a tactile stimulus in 10 out of 10 consecutive trials (Conte et al. , 2012, Conte et al. , 2014). This was obtained by stimulation of the left index finger starting from 2 mA and increasing the current in steps of 0.5 mA; on the other fingers, the current intensity was adjusted to match the perceived intensity on the left index finger. Before the actual testing subjects had to familiarize with the task, achieving a stable performance. During the procedure, they had to verbally report whether they perceived a single stimulus or two temporally separate stimuli. The first of three consecutive ISI at which participants reported two stimuli was considered the STDT. Each session comprised four separate blocks; the STDT was defined as the average of four STDT values (i.e. one for each block) and was entered in the data analysis. To keep subjects' attention level constant during the test some "catch" trials, consisting of a single stimulus, were introduced randomly during the procedure.

2.3 Tactile tasks

Tactile spatial discrimination (TSD) was measured using a set of JVP domes (Van Boven et al. , 1994). Each dome consists of a circular (20 mm diameter), convex grating surface, mounted on top of a cylindrical handle (30 mm long). The set comprises eight grating domes with equidistant groove and bar widths ranging from 3.0 to 0.35 mm. Testing was performed according to previous recommendations. Subjects were required to judge the perceived orientation of the grating (i.e., either along or across the fingertip) according to a two-alternative forced choice paradigm. The finest grating whose orientations were reported reliably (75% correct) provided a threshold estimate of the limit of spatial resolution in the tested area, as previously suggested (Van Boven et al. , 1994). We avoided using two-point discrimination as a measure of TSD because its threshold often falls under the receptor spacing (Johansson et al. , 1979, 1980, Johnson et al. , 1981). Thus, several investigators have questioned the validity of two-point discrimination as a measure of spatial acuity (Johnson et al. , 1981, Stevens et al. , 1995, Lundborg et al. , 2004, Tong et al. , 2013), while grating orientation can be considered a more rigorous alternative (Craig and Johnson, 2000). Tactile threshold (TT) was tested using the Bumps device (Kennedy et al. , 2011). It is a checkerboard-like smooth surface divided into 12 squares. Each square contains 5 colored circles, of which one contains a coin-shaped bump. All bumps are 550 μm in diameter, but of different heights. The device consists of two such plates (plates A and B), which are identical but for bumps heights: the latter are 2.5 to 8 μm and 8.5 to 14 μm, on plate A and B, respectively (e.g., bump heights on each plate increases in 0.5- μm increments). Participants were asked to locate the bump in each square (testing order: plate B always first). Two trials were performed for each plate and TT was defined as the lowest bump such that it and the next 2 higher bumps were successfully detected in either trial, as previously described (Kennedy et al. , 2011). Both tests were done on the right and the left index finger.

2.4 Somatosensory evoked potentials recording and analysis

To record the N20-P25 component of SEP the active electrode was placed at CP3 and the reference electrode at Fz, while P14 was recorded with the active electrode at Fz and the reference on the

contralateral mastoid according to the international 10-20 EEG system (Klem et al. , 1999, Cruccu et al. , 2008). Digital nerves of the right index finger were stimulated with a constant current stimulator (Digitimer DS7A) through ring electrodes, with the cathode placed at the base of the first phalanx and the anode placed 2 cm distally (Tinazzi et al. , 2000, Kwast-Rabben et al. , 2002). Stimulation was delivered at 250% of the somatosensory threshold and consisted of square wave pulses given every 0.2 s (5 Hz). Signal was recorded from -20 to 100 ms with regard the pulse, digitized with a 5 KHz sampling frequency and band-pass filtered from 3 to 2 KHz (Cruccu et al. , 2008). In a first block 1000 sweeps were averaged and N20 peak latency, N20-P25 peak-to-peak amplitude and P14 baseline-to-peak amplitude were measured. Signal from this block was also used to extract and measure high frequency oscillations (HFO) (see below). Three more recording blocks were performed to measure N20-P25 and P14 recovery cycle. In each block, paired pulses at ISI of 5, 20 and 40 ms were delivered in three separate sequences (Meyer-Hardting et al. , 1983, Valeriani et al. , 2005, Vollono et al. , 2010). Each sequence was made of 750 trials, and the sequences were randomized. In the frames obtained using paired stimuli, the responses following the second stimulus were obtained by subtracting the SEP waveform obtained by the first stimulus from the waveform following each double stimulus (Meyer-Hardting et al. , 1983, Valeriani et al. , 2005, Vollono et al. , 2010). R5, R20 and R40 were defined as the ratio between the second and the first response. The position of the electrodes was kept constant throughout the whole experiment and care was taken to always keep impedance below 5 KΩ. In a further experimental session we recorded SEP by stimulation of digital nerves of the right thumb before and after HF-RSS applied on the right index finger. Recording and stimulation parameters were similar to those used for SEP from the right index finger; 750 trials were recorded.

2.5 High frequency oscillations analysis

To measure HFO from N20-P25 SEP component the pulse artefact was removed from -10 to +5 ms to avoid ringing due to filtering (Katayama et al. , 2010). The SEP original signal was band-

pass filtered (400-800 Hz) and averaged. HFO waveform was divided in two components, early (e-HFO) and late (l-HFO), separated by N20 peak. Onset of e-HFO and offset of l-HFO were defined as their amplitudes exceeding the averaged background noise level by three standard deviations (Murakami et al., 2008). e-HFO and l-HFO area was measured and analysed.

2.6 Transcranial magnetic stimulation and electromyographic recording

EMG activity was recorded through a pair of Ag/AgCl electrodes placed over the right first dorsal interosseous (FDI), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles in a belly-tendon fashion. Raw signal, sampled at 5 kHz with a CED 1401 A/D laboratory interface (Cambridge Electronic Design, Cambridge, UK), was amplified and filtered (bandwidth 20 Hz–2 kHz) with a Digitimer D 360 (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). Data were stored on a laboratory computer for on-line visual display and further off-line analysis (Signal software, Cambridge Electronic Design, Cambridge, UK). To ensure complete target muscle relaxation throughout the experimental sessions we continuously monitored the EMG activity with audio and high-gain visual feedback. Transcranial magnetic stimulation (TMS) was carried out using a Magstim 200 stimulator with a 70mm figure-of-eight coil (Magstim Company Limited, Whitland, UK) which produces monophasic waveform stimuli with pulse width ∿0.1 ms. First, the motor hotspot was found, defined as the site within the primary motor area (M1) in which TMS evoked the largest MEP in the APB muscle. Then, we found the resting motor threshold (RMT), active motor threshold (AMT), and the intensity able to elicit motor evoked potentials of approximately 1 mV amplitude from APB muscle (1mV-int), which was later used for test pulses. RMT was defined as the lowest intensity able to evoke a MEP of at least 50 μV in five out ten consecutive trials during rest (Rossini et al. , 1994), while AMT was defined as the lowest intensity able to evoke a motor evoked potential (MEP) of at least 200 μV in five out ten consecutive trials during a 10-15% voluntary contraction of the target muscle (Huang et al. , 2005). SICI was obtained

through a paired-pulse TMS, with an ISI of 3 ms between the first, conditioning stimulus and the second test stimulus. The test stimulus was set at 1mV-int, while the conditioning stimulus was set at 70%, 80% and 90% AMT, as to obtain a recruitment curve (Kujirai et al. , 1993). Twenty paired stimuli for each different intensity of the conditioning stimuli and twenty single stimuli were delivered in a randomized order. SICI was obtained dividing the amplitude of conditioned MEP by the amplitude of the unconditioned MEP. ICF was obtained in a similar fashion, except that the ISI used was 10 ms and the intensity of the conditioning stimulus was 80% AMT (Kujirai et al. , 1993). Twenty paired stimuli were given during the same recording block used for SICI. ICF was obtained dividing the amplitude of conditioned MEP by the amplitude of the unconditioned MEP.

2.7 High frequency repetitive somatosensory stimulation

HF-RSS consisted of 20 Hz trains of square wave electrical pulses of 200 μs duration delivered for 1s, with 5 s intertrain intervals, for 45 min. Stimuli were delivered with a constant current stimulator (Digitimer DS7A) through surface adhesive electrodes of approximately 1 cm^2 area, with the anode located on the distal phalanx of the right index finger and the cathode located on the proximal phalanx of the same finger. The intensity of the stimulation was set individually at the highest threshold that subjects could tolerate for the whole period of stimulation (Kattenstroth et al. , 2012).

2.8 Procedure

All subjects underwent four measures at baseline (T0), and specifically I – TSD and TT measurement, II – STDT testing, III – SEP recording, IV – TMS. After the baseline evaluation, subjects underwent a single session of HF-RSS, and then repeated the four baseline measurements 5’ after the end of HF-RSS (T1). The sequence of measures I to IV was counterbalanced both at T0 and T1 and across subjects. Notice that at T1 the current intensity used for STDT testing was adjusted to match the

intensity perceived at T0. Before application of HF-RSS it was ensured that subjects practiced the behavioural tests (STDT, TSD, TT) until a stable performance was reached.

2.9 Statistical analysis

A three-way repeated measures ANOVA with "time" (T0, T1), "side" (right, left) and "finger" (thumb, index finger) as factors of analysis was performed to evaluate the effect of HF-RSS on the current intensity used to test STDT. A three-way repeated measures ANOVA with "time" (T0, T1), "side" (right, left) and "finger" (thumb, index finger) as factors of analysis was performed to evaluate the effect of HF-RSS on STDT. Several dependent T-tests were used to evaluate the effect of HF-RSS on the latency and amplitude of N20-P25 and P14 recorded from the right thumb and right index finger. Two two-way repeated-measures ANOVA with "time" (T0, T1) and "ISI" (R5, R20, R40) as factors of analysis were performed to investigate the effect of HF-RSS on N20-P25 and P14 recovery cycle. Two dependent t-tests were also performed to assess the effect of HF-RSS on N20 and P25 separately, while a further independent t-test was performed to compare changes induced by HF-RSS on the two waves (T1/T0 ratios). Two dependent t-tests were used to investigate possible effects of HF-RSS on e-HFO and l-HFO. Two different two-way repeated measures ANOVA with "time" (T0,T1) and "side" (right, left) were used to investigate possible effects of HF-RSS on bumps and domes tests. Pearson's correlation coefficient was used to investigate possible correlations between baseline STDT measured on the right index finger, e-HFO area, l-HFO, SEPs recovery cycle, TSD and TT. The same test was used to investigate whether changes induced by HF-RSS on the same parameters from T0 and T1 were correlated. A three-way repeated measures ANOVA with "time" (T0, T1), "muscle" (FDI, APB, ADM) and "condition" (test pulse, SICI 70%, SICI 80%, SICI 90%, ICF) as factors of analysis was used to disclose possible effects of HF-RSS on SICI and ICF. Normality of distribution was assessed with the Shapiro-Wilks' test. All p values < 0.05 were considered significant. Greenhouse-Geisser correction was used when necessary to correct for non-sphericity (i.e. Mauchly's test < 0.05). Bonferroni post-hoc test was used for post-hoc comparisons.

3 Results

No side effects were recorded during the experimental sessions. The intensity for HF-RSS was 5.13 ± 2.02 mA (average ± standard deviation).

3.1 Somatosensory temporal discrimination threshold (STDT)

There was no significant difference in the threshold for perception of the electrical stimulus in the thumb and index finger in both sides (left and right) and at both time points (T0 and T1). As reported previously (Erro et al. , 2016), HF-RSS improved STDT in a spatially specific manner. This was confirmed by the 3-way ANOVA which revealed a significant "time×side×finger" interaction [$F(1,14) = 8.823$; $p = 0.01$] as well as significant interactions of "time×side" [$F(1,14) = 35.681$; $p < 0.001$] and "time×finger" [$F(1,14) = 8.172$; $p = 0.013$]. There was a significant main effect of "time" [$F(1,14) = 14.624$; $p = 0.002$], but not for "side" [$F(1,14) = 1.104$; $p = 0.311$] or "finger" [$F(1,14) = 2.085$; $p = 0.171$]. Post-hoc analyses showed that STDT significantly decreased in the right index finger from T0 to T1 (87.62±36.01 *vs.* 68.60±37.13; $p < 0.001$), while it remained unchanged in the other fingers (fig. 1).

3.2 N20-P25 and P14 latency and amplitude

HF-RSS had no effect on the latency of these early SEP components recorded by the right index finger (p values of all t-tests > 0.05), but significantly increased their amplitude. Thus, HF-RSS significantly increased the amplitude of N20-P25 [$t(14) = -11.386$; $p < 0.001$] and P14 [$t(14) = -10.862$; $p < 0.001$] obtained by stimulation of the right index finger. This increase in amplitude occurred both in N20 [$t(14) = -6.154$; $p < 0.001$] and P25 [$t(14) = -7.490$; $p < 0.001$], and the changes induced in the two components were not significantly different (1.19±0.14 *vs* 1.22± 0.11 for N20 and P25 respectively), [$t(14) = -0.868$; $p = 0.4$]. No changes were observed in N20-P25 and P14 amplitude

recorded while stimulating the right thumb (all $p > 0.05$) (fig. 2). HF-RSS had no effect on P14 and N20-P25 latency and amplitude recorded by stimulation of the right thumb (all p values > 0.05).

3.3 N20 and P14 recovery cycle

HF-RSS increased the amount of inhibition produced by the first stimulus of the pair on both the N20-P25 and P14 components. Thus, the recovery cycle was suppressed at all three intervals tested (fig. 3).

This was confirmed in the two-way ANOVA on N20-P25 amplitude which showed a significant main effect of "time" [$F(1,14) = 70.02$; $p < 0.001$] and "ISI" [$F(1.479,17.234) = 38.816$; $p < 0.001$] and a significant interaction of "time×ISI" [$F(1.949,27.282) = 4.014$; $p = 0.031$]. Post-hoc comparisons showed that inhibition increased from T0 and T1, and this was true for R5 (0.53 ± 0.19 *vs.* 0.37 ± 0.16; $p < 0.001$), R20 (0.72 ± 0.11 *vs.* 0.52 ± 0.12; $p < 0.001$) and R40 (0.92 ± 0.06 *vs.* 0.67 ± 0.14; $p < 0.001$) (fig. 4). Similarly, the two-way ANOVA on P14 amplitude showed a significant main effect of "time" [$F(1,14) = 59.48$; $p < 0.001$] and "ISI" [$F(1.540,21.561) = 136.85$; $p < 0.001$] and a significant interaction of "time×ISI" [$F(1.618,22.649) = 5.883$; $p = 0.012$]. Again, post-hoc comparisons showed an increase in inhibition from T0 and T1 for R5 (0.56 ± 0.15 *vs.* 0.40 ± 0.09; $p < 0.001$), R20 (0.78 ± 0.10 *vs.* 0.55 ± 0.08; $p < 0.001$) and R40 (0.92 ± 0.04 *vs.* 0.80 ± 0.06; $p < 0.001$) (fig 3).

3.4 Early and late high-frequency oscillations

The paired t-tests showed a significant increase of e-HFO [$t(15) = -5.860$; $p < 0.001$] and l-HFO [$t(15) = -5.279$; $p < 0.001$] after HSS (fig. 4).

3.5 Tactile tasks

The two-way ANOVA on the bumps test showed a significant main effect of "time" [$F(1,14) = 16.227$; $p = 0.001$], a non-significant effect of "side" [$F(1,14) = 1.720$; $p = 0.211$] and a significant interaction of "time×side" [$F(1,14) = 18.026$; $p = 0.001$]. Post hoc analyses showed that HF-RSS significantly reduced tactile threshold (TT) from T0 to T1 in the right hand (6.43 ± 0.59 *vs.* 4.80 ± 0.56; $p = 0.001$), while there was no effect on the left hand (6.35 ± 0.58 *vs.* 6.43 ± 0.60; $p > 0.05$). By contrast, performance on the JVP domes (tactile spatial discrimination, TSD) did not change after HF-RSS (all p values > 0.05) (fig. 5).

3.6 Correlation between the effect of HF-RSS HFS on STDT and neurophysiological - behavioural measures

There was a strong correlation between changes induced by HF-RSS in physiological measures of inhibition of the N20-P25 and l-HFO and in STDT (fig. 6). The correlations were marginal for TT, and not significant for TSD.

At baseline (i.e. T0) there were significant correlations between STDT and R5 of the N20 ($r = 0.830$; $p < 0.001$); STDT and l-HFO area ($r = -0.887$; $p < 0.001$); and R5 (N20) and l-HFO area ($r = -0.690$; $p = 0.004$). In addition, the changes induced by HF-RSS in STDT were significantly correlated with the changes induced by HF-RSS on R5(N20) ($r = 0.795$; $p < 0.001$) and on l-HFO area ($r = 0.746$; $p = 0.001$) (fig. 8). There was also a significant correlation between changes induced in R5 and in l-HFO ($r = 0.765$; $p = 0.001$). No correlations were found between STDT and SEP recovery at ISIs other than 5 ms, and no correlation was found between STDT and e-HFO. Notably, the changes induced by HF-RSS on R5 of the N20-P25 and P14 were not correlated. Neither was there any correlation between STDT and P14 recovery at any of the ISI explored.

STDT was not correlated with TSD assessed with the JVP domes test at any time point (all p values > 0.05). Although not significant, there was a trend towards correlation between the STDT and TT at

T0 (r = 0.466, p = 0.08) and T1 (r = 0.424, p = 0.074), and also the changes induced on the two variables by HF-RSS showed the same tendency (r = 0.466, p = 0.08).

3.7 Effect of HF-RSS on inhibitory circuitry of the primary motor area

HF-RSS produced a focal increase of SICI in APB, but had no effect on other muscles nor on ICF (fig. 7).

The three-way ANOVA on SICI and ICF showed a non-significant main effect of "time" [$F(1,14) = 3.028$; $p = 0.104$], significant main effects of "muscle" [$F(1.907,26.702) = 33.952$; $p < 0.001$] and "condition" [$F(1.828,25.589) = 344.620$; $p < 0.001$] and significant interactions of "time×muscle" [$F(1.761,24.658) = 3.771$; $p = 0.042$], "time×condition" [$F(1.925,26.945) = 7.781$; $p = 0.002$], "muscle×condition" [$F(2.938,41.135) = 136.131$; $p < 0.001$] and "time×muscle×condition" [$F(2.885,40.391) = 5.816$; $p = 0.002$]. Post hoc analyses showed that HF-RSS had no effect on unconditioned MEP, SICI and ICF recorded in FDI and ADM (all $p > 0.05$). On APB, by contrast, while HF-RSS had no effect on test MEP and ICF (all $p > 0.05$), the amount of SICI increased from T0 to T1 (i.e. there was a decrease in the amplitude of the conditioned MEP), and this was true with a conditioning pulse set respectively at 70% (0.76 ± 0.10 mV *vs.* 0.63 ± 0.06 mV; $p < 0.001$), 80% (0.53 ± 0.10 mV *vs.* 0.43 ± 0.09 mV; $p < 0.001$), and 90% (0.38 ± 0.07 *vs.* 0.29 ± 0.09; $p < 0.001$) of AMT.

4 Discussion

The present data show that improvements in STDT produced by HF-RSS are associated with correlated increases in two measures of somatosensory inhibition (N20-P25 recovery curve and l-HFO area). HF-RSS also improved tactile threshold (TT), as measured by the bumps test. Notably, the changes in STDT and TT were confined to the stimulated finger, which rules out any non-specific changes due to retest effect, impaired levels of alertness or attention. The fact that the improvement

in TT was weakly correlated with changes in the somatosensory recovery curves may indicate that it partially depends on inhibitory interactions. However, there was no effect on tactile spatial discrimination (TSD) as assessed by the JVP domes, suggesting that HF-RSS may have less influence on more complex types of spatial processing. Surprisingly, HF-RSS also increased SICI in the APB but not other muscles, pointing to a focal transmission of HF-RSS effects to the motor system. Overall, we conclude that HF-RSS focally increased the excitability of inhibitory circuitry in the somatosensory system and that this sharpens temporal and some types of spatial processing. These effects are transmitted to M1.

4.1 Electrophysiological results

Somatosensory recovery curves reflect an inhibitory influence of the first stimulus on the response to the second stimulus. The present results suggest that HF-RSS increases the effectiveness of these interactions at both cortical and subcortical levels of the somatosensory afferent pathway. The N20 and P25 components of the SEP are generated in the posterior bank of the central sulcus and in the anterior crown of the postcentral gyrus respectively (Allison et al. , 1989, Allison et al. , 1991, McCarthy et al. , 1991). N20 suppression at ISI 5ms is generally thought to be due to inhibitory interactions in sensory cortex (Schwartz et al. , 1964, Emori et al. , 1991, Ugawa et al. , 1996, Frasson et al. , 2001, Mochizuki et al. , 2001, Valeriani et al. , 2005). Thus, increased R5 may indicate increased effectiveness of S1 inhibition. At longer ISIs inhibition of N20 may involve subcortical structures within the somatosensory pathway, such as dorsal column nuclei or thalamus (Luders et al. , 1984, Hoffken et al. , 2010). Thus, increased inhibition at these intervals suggests that HF-RSS may also increase inhibitory interactions at subcortical levels. In the same way, P14 is a subcortical component of the SEP that is recorded as a far-field potential from the scalp, and is probably generated at or near the first synaptic relay of the dorsal column-medial lemniscus system (Cruccu et al. , 2008). Increased suppression of P14, which was independent from suppression of N20, is also consistent with an increase in inhibition at subcortical stages of the somatosensory system. Our findings are

apparently in contrast with those reported by Hoffken and co-workers (Hoffken et al. , 2007), who found decreased suppression of PP-SEP after repetitive tactile stimulation. However, it is worth noting that in their study tactile stimuli were given at a random ISI based on a Poisson distribution (average frequency of 1 Hz) and the total stimulation duration was 3 hours. This is very different from our HF-RSS (see methods section).

HFOs are small wavelets with a frequency around 600 Hz superimposed on the N20 component of SEP. l-HFO are thought to depend on the activity of S1 inhibitory interneurons (Hashimoto et al. , 1996, Curio, 2000, Jones et al. , 2000, Klostermann et al. , 2001, Ozaki et al. , 2001, Ikeda et al. , 2002, Gobbele et al. , 2004, Ozaki et al. , 2011) possibly producing feedforward inhibition of cortical pyramidal neurons (Ozaki et al. , 2011). Since HF-RSS produced an increase in l-HFO, it is likely that excitability of S1 inhibitory interneurons was increased. Alternatively, it has also been proposed that S1 pyramidal chattering cells participate in the generation of l-HFO (Restuccia et al. , 2003), so an increase in excitability of these neural elements should also be considered. In contrast with l-HFO, e-HFO are supposed to originate from activity of thalamocortical fibers directed to areas 3b and 1 within S1 (Ozaki et al. , 2011). As such, the increase in e-HFO area produced by HF-RSS might be interpreted as an increase in excitability of thalamo-cortical relay cells.

We have previously suggested that both short latency paired pulse interactions at R5 and l-HFOs reflect activity in GABAa-ergic neurones that are known to produce feedforward inhibition, at least at cortical level, of excitatory somatosensory inputs (Rocchi et al. , 2016). These neurones sharpen the temporal profile of the incoming input by preventing overlap with later-arriving dispersed inputs in the same pathway. We speculate that repetitive activation of these neurons during HF-RSS increased the effectiveness of this feedforward inhibition, thus increasing the suppression of N20 and P14 components of the SEP produced by the second stimulus of a pair. HF-RSS may also increase the excitability of post-synaptic neurons responsible for N20 and P14 generation, consistent with the observed increase in amplitude of the cortical N20 (Hashimoto et al. , 1996) and in the P14 from the nucleus cuneatus (Cruccu et al. , 2008). However, increased amplitude of the SEP did not correlate

with changes in STDT suggesting that the change in temporal inhibition was the main factor influencing temporal discrimination. Interestingly, both behavioral and electrophysiological changes were spatially specific to input from the stimulated finger, with no effect on the SEP recorded by stimulation from the thumb, as previously found (Pleger et al. , 2001).

4.2 Relation between temporal and spatial perception and electrophysiological inhibition

STDT showed the same spatial specificity as the N20 and P14. It improved (i.e. decreased) only on the stimulated finger (i.e. the right index finger), while it showed no changes when tested on the right thumb or on the contralateral hand. As proposed by Rocchi and coworkers (Rocchi et al. , 2016) we suggest that increased excitability of feedforward somatosensory inhibition sharpens the temporal profile of afferent somatosensory stimuli; this, in turn, can contribute to the observed decrease in STDT (Conte et al. , 2012, Rocchi et al. , 2016). The correlation between the changes produced by HF-RSS in STDT and electrophysiological inhibition further supports this explanation.

We examined two tests of spatial perception: the bumps test, which is a threshold detection task, and the JVP domes, which is an edge detection task. Given the relatively advanced age and wide distribution of our participants' sample, TSD and TT values are higher than reported in younger subjects (Van Boven et al. , 1994, Kennedy et al. , 2011). However, in line with our results, it has been demonstrated that somatosensory perceptual abilities can also be modulated by repetitive tactile stimulation in subjects of advancing age (Dinse et al. , 2006, Erro et al. , 2016). The JVP domes test involves activity in slowly-adapting (SA) cutaneous afferents from Merkel cells, which have a high sensitivity to edge detection (Johnson et al. , 1981, Phillips et al. , 1981). In contrast, the bumps test, which entails the detection of small raised dots on a flat surface, is thought to depend on the activity of rapidly adapting (RA) skin receptors, represented by Meissner corpuscles (Johansson et al. , 1983, LaMotte et al. , 1986). Although RA receptors have a lower spatial acuity than the SA system, they

are optimal for the detection of very small surface variations, such as small dots embedded in a flat surface (Johnson et al. , 1992).

Why did HF-RSS modulate bumps perception but did not influence grating discrimination? It is known that cutaneous afferents project mainly to area 3b within S1. All neurons within each column in S1 respond to the same class of receptors, i.e. columns that receive afferents from cutaneous SA and RA receptors are distinct (Sur et al. , 1981, 1984). Accordingly, they are called, respectively, SA and RA cortical neurons (Sur et al. , 1981). A possible explanation is that HF-RSS preferentially modulated the excitability of RA neurons in S1. This might be due to peripheral factors: while the receptive fields of RA and SA are more or less the same (11-12 sq mm) (Johansson et al. , 1980), the density of RA is considerably higher than SA on the volar surface of the fingers (Johansson et al. , 1979, Johnson et al. , 1992). According to this view, a greater number of RA skin receptors might have been stimulated by HF-RSS. This could have two possible consequences. It could increase the excitability of cortical RA cells in S1 which, like skin RA receptors, increase their discharge in correspondence of application and removal of tactile stimuli (Phillips et al. , 1981). This increase in cortical excitability might then contribute to the improved performance in the bumps test. It would also be consistent with the increase in amplitude of SEPs to single stimuli. A second possibility is raised by the finding that there was a weak correlation between the change produced by HF-RSS in the bumps test and electrophysiological inhibition. RA cells involved in the bumps test fire with brief bursts of activity at the beginning and end of the stimulation as the finger pad is swept over the target area. It may be that repeated activation of peripheral RA input during HF-RSS can enhance feedforward inhibition in cortical RA columns. This may then sharpen detection of phasic sensory inputs as the finger pad is swept across the surface and improve perception.

4.3 HF-RSS effects on motor cortex inhibition

Since HF-RSS has been reported to affect motor performance (Kalisch et al. , 2008, Smith et al. , 2009, Kalisch et al. , 2010), we also investigated its effect on M1 excitability and inhibitory circuitry.

The main finding was that HF-RSS increased SICI tested in APB, while leaving SICI in FDI and ADM and the unconditioned MEP unchanged. The lack of change of SICI in ADM is not entirely surprising according to the somatotopic organization of motor cortical input-output relationship described in previous investigations. Several authors have reported that in monkeys M1 receives sensory information from portions of limbs in close relation to the muscle to which it projects (Rosen et al. , 1972, Asanuma, 1981). In humans, MEP amplitude is also modulated by stimulation of cutaneous fields close to the muscle involved (Classen et al. , 2000, Tamburin et al. , 2001). Since HF-RSS was applied on skin closer to APB than ADM, it is plausible that modulation of SICI was clearer in APB. However, this does not explain why SICI in FDI was unaffected. The reason might be that TMS was centered over APB representation in M1; this means that activity in M1 evoked by TMS conditioning pulse was probably less effective in FDI representation and thus the effects of HF-RSS were less clear.

We can only speculate on how HF-RSS effects were transmitted to M1. There are extensive and somatotopic connections between S1 and M1 directly targeting layer V pyramidal tract neurons (Porter, 1996) or relaying in MI cortical layers II/III (Kaneko et al. , 1994). It is also known that tetanic stimulation of S1 produces long-term potentiation in layers II/III of M1 (Sakamoto et al. , 1987, Keller et al. , 1990)(Sakamoto et al., 1987, Keller et al., 1990). This could represent one pathway whereby HF-RSS might somatotopically increase excitability of the M1 GABAergic interneurons involved in SICI (Kujirai et al. , 1993). The lack of changes in MEP and ICF might be interpreted considering the higher sensitivity of SICI to low-intensity repetitive stimulation (McAllister et al. , 2009).

5 Conclusion

In conclusion, we suggest that HF-RSS increases the effectiveness of inhibition at cortical and subcortical nodes of the somatosensory pathway. This leads to improved performance in behavioural tests of temporal discrimination and contributes to improved performance in some tests of spatial detection (i.e. STDT and the bumps test). Surprisingly, HF-RSS also affects short latency GABAa-ergic inhibition in M1. Together these changes in S1 and M1 may underlie reported improvements in manual motor performance such as the pegboard test. HF-RSS might therefore be a suitable therapeutic tool in neurological disorders characterized by a loss of inhibition, such as dystonia (Hallett, 2011).

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Figure legends

***Figure 1*:** STDT values obtained from the thumb (I) and the index finger (II) of both hands before (T0) and immediately after (T1) HF-RSS applied on the right index finger. HF-RSS produced a significant decrease of STDT tested on the right index finger only ($p < 0.001$). Asterisks indicate statistical significance. Error bars indicate standard error.

Figure 2: latency and amplitude of N20-P25 and P14 components of SEP obtained by stimulating the thumb (I) and the index finger (II) of the right hand before (T0) and immediately after (T1) HF-RSS applied on the right index finger. HF-RSS induced an increase in the amplitude of N20-P25 ($p < 0.001$) and P14 ($p < 0.001$) SEP components obtained from stimulation of the index finger but not the thumb. No changes in N20 or P14 latency were observed. Error bars indicate standard error. Asterisks indicate statistical significance. Error bars indicate standard error.

***Figure 3*:** recovery cycle of N20-P25 (panels A, B, C) and P14 (panels D, E, F) components of SEP at ISIs of 5, 20 and 40 ms before (T0) and immediately after (T1) HF-RSS applied on the right index finger. HF-RSS increased the amplitude of unconditioned N20-P25 and P14 whereas it decreased the amplitude of PP-SEP (thus increasing the effectiveness of inhibition). For visualization purposes the raw signal was bandpassed between 20 and 500 Hz. Artefact from electric stimulus (at 0.05 s) was removed. Error bars indicate standard error.

***Figure 4*:** HFO area before (left panel, T0) and immediately after (middle panel, T1) HF-RSS applied on the right index finger. HF-RSS induced a significant increase of both early ($p < 0.001$) and late

HFO ($p < 0.001$). HFO area in the right panel is expressed in $\mu V^2 \times 10^{-4}$. Artefact from electric stimulus (at 0.05 s) was removed. Asterisks indicate statistical significance. Error bars indicate standard error.

Figure 5: tactile spatial acuity assessed with the domes (left panel) and bumps (right panel) test before (T0) and immediately after (T1) HF-RSS applied on the right index finger. HF-RSS induced a significant decrease of threshold evaluated on the right index finger ($p = 0.001$), while no effect was observed on the contralateral index finger. By contrast, tactile threshold assessed by the domes test was not changed by HF-RSS on either side.

Figure 6: correlations between STDT, R5 and l-HFO. The upper panels show a significant correlation between baseline values of STDT and R5 (left) and between baseline values of STDT and l-HFO (right). There was also a significant correlation between the changes induced by HF-RSS on STDT and the changes induced, respectively, on R5 (left) and on l-HFO (right).

Figure 7: effect of HF-RSS on SICI on APB. Raw signal from a single subject before (left panel) and after (middle panel) on APB using different intensities of the conditioning TMS stimulus (CS) (70, 80 and 90% of AMT). HF-RSS induced an increase in SICI irrespective of the strength of the conditioning TMS pulse (all p values < 0.001). Right panel shows SICI averaged among all subjects. Asterisks indicate statistical significance. Error bars indicate standard error.