RESEARCH ARTICLE

Amelioration of Focal Hand Dystonia via Low-Frequency Repetitive Somatosensory Stimulation

Lorenzo Rocchi, MD, PhD,[1,2*] Anna Latorre, MD, PhD,[2] Elisa Menozzi, MD, PhD,[2] Vittorio Rispoli, MD, PhD,[3] John C. Rothwell, PhD,[2] Alfredo Berardelli, MD,[4,5] and Kailash P. Bhatia, FRCP, MD[2]

[1]*Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy*
[2]*Department of Clinical and Movement Neurosciences, UCL Queen Square Institute of Neurology, University College London, London, United Kingdom*
[3]*Neuroscience, Head and Neck Department, Ospedale Civile di Baggiovara, Azienda Ospedaliero-Universitaria di Modena, Modena, Italy*
[4]*Department of Human Neurosciences, University of Rome "Sapienza", Rome, Italy*
[5]*IRCCS Neuromed, Pozzilli, Italy*

ABSTRACT: Background: Dystonia presents a growing concern based on evolving prevalence insights. Previous research found that, in cervical dystonia, high-frequency repetitive somatosensory stimulation (RSS; HF-RSS) applied on digital nerves paradoxically diminishes sensorimotor inhibitory mechanisms, whereas low-frequency RSS (LF-RSS) increases them. However, direct testing on affected body parts was not conducted.

Objective: This study aims to investigate whether RSS applied directly to forearm muscles involved in focal hand dystonia can modulate cortical inhibitory mechanisms and clinical symptoms.

Methods: We applied HF-RSS and LF-RSS, the latter either synchronously or asynchronously, on forearm muscles involved in dystonia. Outcome measures included paired-pulse somatosensory evoked potentials, spatial lateral inhibition measured by double-pulse somatosensory evoked potentials, short intracortical inhibition tested with transcranial magnetic stimulation, electromyographic activity from dystonic muscles, and behavioral measures of hand function.

Results: Both synchronous and asynchronous low-frequency somatosensory stimulation improved cortical inhibitory interactions, indicated by increased short intracortical inhibition and lateral spatial inhibition, as well as decreased amplitude of paired-pulse somatosensory evoked potentials. Opposite effects were observed with high-frequency stimulation. Changes in electrophysiological markers were paralleled by behavioral outcomes: although low-frequency stimulations improved hand function tests and reduced activation of dystonic muscles, high-frequency stimulation operated in an opposite direction.

Conclusions: Our findings confirm the presence of abnormal homeostatic plasticity in response to RSS in the sensorimotor system of patients with dystonia, specifically in inhibitory circuits. Importantly, this aberrant response can be harnessed for therapeutic purposes through the application of low-frequency electrical stimulation directly over dystonic muscles. © 2024 The Author (s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: dystonia; transcranial magnetic stimulation; evoked potentials; cortical inhibition; hand function; electrical stimulation

*__Correspondence to:__ Prof. Lorenzo Rocchi, Department of Medical Sciences and Public Health, University of Cagliari, 09124 Cagliari, Italy; E-mail: l.rocchi@ucl.ac.uk

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Dystonia, a movement disorder characterized by sustained or intermittent muscle contractions leading to abnormal, often repetitive movements or postures[1,2] poses an escalating concern in light of evolving prevalence insights.[3] Available treatments for dystonia include oral medications, intramuscular injections of botulinum toxin (BoNT), and deep brain stimulation. These are either limited in efficacy, purely symptomatic, or invasive[4-6]; this underscores an unmet need for therapeutic interventions targeting the neural mechanisms underlying dystonia.

Previous research proposed that, in patients with isolated cervical dystonia, high-frequency repetitive somatosensory stimulation (HF-RSS)[7] applied on digital nerves diminishes the effectiveness of sensorimotor inhibitory mechanisms tested with short intracortical inhibition (SICI) and somatosensory evoked potentials (SEPs),[8] whereas low-frequency repetitive somatosensory stimulation (LF-RSS) increases it.[9] These effects, which are opposite to those observed in healthy subjects, have been interpreted as deranged homeostatic inhibitory plasticity[8,9]; if this abnormality were linked to dystonic symptoms, it would be possible to hypothesize a beneficial clinical effect of LF-RSS in dystonia. This was not directly tested in previous studies, because RSS was applied in a body part not affected by dystonia.[8,9] On this basis, we tested whether RSS applied directly on forearm muscles involved in dystonia was able to modulate inhibitory mechanisms and clinical symptoms in patients with focal hand dystonia (FHD). Patients were divided into three groups, according to the stimulation pattern received. In the first two groups, we used either HF-RSS or LF-RSS, based on the results of our previous investigations.[8,9] Previous work has shown that the physiological overlap of cortical representations of upper limb muscles[10] is altered in dystonia.[11] Based on findings from our previous work,[8,9] we predicted that HF-RSS delivered synchronously (ie, at the same time) over dystonic muscles would decrease the effectiveness of inhibition at the boundary of their representation in the somatosensory and motor areas, increasing their simultaneous contraction and thus worsening dystonia. By contrast, we expected LF-RSS to cause an opposite response, ie, a refinement in inhibition and a consequent improvement of dystonia. One caveat is that possible effects of LF-RSS might be confounded by spike timing–dependent plasticity (STDP); in fact, even if muscles were stimulated synchronously, different conduction delays may result in a possible coincidence of ascending volley at the cortical level in a time window compatible with induction of STDP.[12] To exclude this possibility, in a third group of patients, we delivered LF-RSS asynchronously (LFas-RSS) over affected muscles, with an interval (500 ms) sufficient to exclude STDP.[13]

Subjects and Methods

A brief description of methods is provided here; a full account can be found in Supporting Information Data S1.

Patients and Clinical Evaluation

Forty-five patients with a diagnosis of FHD were enrolled in the study (Table S1) and divided in three groups of 15 each, according to the type of stimulation received (HF-RSS, LF-RSS, LFas-RSS). Patients were assessed at least 3 months after their last BoNT injection, and they were not treated with other drugs for their dystonia. Handedness was assessed by the Edinburgh Handedness Inventory,[14] and dystonia was clinically assessed by means of the Unified Dystonia Rating Scale (UDRS) and Arm Dystonia Impairment Scale (ADDS). An informed consent was signed by all participants before the experimental procedures, which were approved by the local institutional review board and conducted in agreement with the Declaration of Helsinki according to international safety guidelines.

Behavioral and Electrophysiological Outcome Measures

Several baseline (T0) measurements were performed, including (1) hand function tests (box and block test [BBT][15] and nine-hole peg test [NHPT][16]); (2) electromyography (EMG) measurement in two blocks of postural activity eliciting dystonia (arms outstretched and holding a pen, 1 minute each); (3) transcranial magnetic stimulation (TMS) (including SICI); and (4) SEPs (single pulse, paired pulse, double pulse). These measures were randomized and repeated after RSS (T1).

EMG activity was recorded using Ag/AgCl electrodes placed over the two muscles (M1 and M2) most affected by dystonia, which were chosen based on BoNT treatment (Table S1) or on clinical observation in case patients received BoNT injection in only one or no muscles. During TMS, we first found the motor hot spot.

During TMS, the motor hot spot and resting motor threshold (RMT) related to the less excitable of the two forearm muscles were found according to standard protocols.[17] Then we found the stimulation intensity to elicit motor evoked potentials (MEPs) of at least 0.5 mV (0.5 mV-int) in both muscles.[18] SICI was obtained through paired-pulse TMS, with an interstimulus interval (ISI) of 3 ms between the conditioning stimulus (CS) and the test stimulus (TS). The TS was set at 0.5 mV-int, whereas the CS was set at 50%, 60% 70%, 80%, 90%, and 100% RMT, to obtain a recruitment curve.[19,20]

The N20/P25 and P14 components of SEPs were recorded using a standard montage, with the recording electrode placed at CP3 or CP4 (contralateral to the dystonic arm).[21,22] Stimulation was performed via

the same electrodes used to record EMG signals from the forearm sites of interest. For paired-pulse SEP (PP-SEP), three blocks of 500 trials were recorded by stimulation at each forearm site separately, one with single-pulse stimulation and the other two with paired-pulse stimulation with ISIs of 5 and 30 ms. Recovery cycles were calculated for both ISIs (R5 and R30, respectively) by subtracting the single-pulse SEP waveform from the PP-SEP one[21] and defined as the ratio between paired and single pulses.[9,23] A further block of 500 stimuli delivered at the two forearm sites at the same time was used for double-pulse SEP, as in previous studies.[8,24] The spatial inhibition ratios of N20/P25 (Q20) and P14 (Q14) were calculated as $M1M2/(M1 + M2) \times 100$, where M1M2 is the SEP amplitude obtained by simultaneous stimulation of the two forearm sites and $M1 + M2$ is the arithmetic sum of the SEP obtained by the individual stimulation at the two sites.[9,23]

Repetitive Somatosensory Stimulation

RSS was delivered at the same sites on the forearm used for SEP and EMG recording and was applied at the maximum tolerable, nonpainful intensity. Each of the three groups of patients underwent a different RSS protocol, but the duration of each was the same (45 minutes). HF-RSS consisted of 20-Hz trains of stimuli (0.2-ms square-wave electrical pulses) of 1-second duration, with 5-second intertrain interval, applied to both forearm sites at the same time. LF-RSS was delivered in a similar way; an exception was made for the different pattern of stimulation, which consisted of one pulse applied every second synchronously over the two muscles. Lastly, in its asynchronous variant (LFas-RSS), electric pulses were applied intermittently on each muscle site, with a 0.5-s interval.

Statistical Analysis

Age, disease duration, scores of UDRS and ADDS, thresholds, and stimulation intensities for RSS were compared by means of one-way between-group analyses of variance (ANOVAs). Several two-way mixed ANOVAs with "group" (HF-RSS, LF-RSS, LFas-RSS) and "time" (T0, T1) as factors of analysis were performed on thresholds and stimulation values, including RMT, 0.5 mV-int, somatosensory threshold, and stimulation intensities for SEP; this was done to compare baseline values in the three groups and to assess the effect of RSS on these variables. ANOVAs with the same factors were performed to investigate the effect of RSS on the following variables: (1) the number of blocks moved in 60 seconds in the BBT, (2) the time to complete the NHPT, and (3) the root-mean-square (RMS) of the EMG activity recorded at sites M1 and M2 during postural activity (arms outstretched, holding a pen).

The effect of RSS on SICI was investigated with two separate three-way mixed ANOVAs with factors "group" (HF-RSS, LF-RSS, LFas-RSS), "time" (T0, T1), and "intensity" (50%, 60%, 70%, 80%, 90%, 100% RMT, indicating the intensity of the conditioning pulse), one for each forearm site (M1, M2). To exclude that possible changes in SICI were biased by changes in test MEP amplitude,[25] we performed two separate two-way mixed ANOVAs with "group" (HF-RSS, LF-RSS, LFas-RSS) and "time" (T0, T1) as factors of analysis on the latter variable, separately for M1 and M2 sites.

The effect of RSS on latencies (P14, N20) and amplitudes (P14, N20/P25) of SEP evoked by single-pulse stimulation at M1 and M2 sites, as well as the effects of RSS on Q14 and Q20, were investigated by several mixed ANOVAs with "group" (HF-RSS, LF-RSS, LFas-RSS) and "time" (T0, T1) as factors of analysis. The recovery cycle of P14 and N20/P25 amplitude was assessed with a similar ANOVA, but with the addition of "ISI" (R5, R30) as a within-subject factor of analysis. Possible correlations between the effects induced by RSS on different variables were investigated with Pearson's correlation coefficient. For this analysis, we considered the maximum SICI obtained at the group level, averaged between M1 and M2. In addition, correlation analyses were run after pooling data from all groups together. Normality of distribution was assessed with the Shapiro-Wilk's test, whereas Greenhouse-Geisser correction was used, if necessary, to correct for nonsphericity (ie, Mauchly's test < 0.05). The P values <0.05 were deemed significant. Homogeneity of variance across groups was tested with Levene's test. Bonferroni's post hoc test was used for post hoc comparisons following ANOVAs and for correlations.

Results

All RSS protocols induced visible twitches in the stimulated muscles. The three groups of patients examined did not significantly differ in terms of age, disease duration, ADDS, UDRS, thresholds, and stimulation values; in addition, the latter two variables were not changed by RSS, as demonstrated by the ANOVAs (the values of the mentioned variables, as well as statistics of the ANOVAs, are summarized in Table S2). Statistics of the other ANOVAs are summarized in Table S3, and a description of the results, including post hoc comparisons, is given in the relevant following subsections.

Hand Motor Function Tests and EMG Activity During Posture

Baseline scores of the BBT and the NHPT were not significantly different across groups. HF-RSS worsened motor performance by decreasing the number of boxes moved in the BBT ($P = 0.016$) and increasing the time

taken by subjects to complete the NHPT ($P = 0.018$). By contrast, after LF-RSS, patients were able to move more boxes in the BBT ($P = 0.021$) and to perform the NHPT in a shorter time ($P = 0.029$). A similar effect was observed after LFas-RSS ($P < 0.01$ for both the BBT and the NHPT) (Fig. 1).

The RMS of the EMG activity, while subjects kept their arms outstretched, increased both in M1 ($P = 0.041$) and M2 ($P = 0.039$) after HF-RSS, whereas it decreased after LF-RSS ($P = 0.02$ for M1, $P = 0.03$ for M2) and after LFas-RSS ($P < 0.01$ for both M1 and M2). Similar changes in EMG RMS occurred when subjects held a pen, with RMS increasing after HF-RSS ($P = 0.025$ for M1, $P = 0.021$ for M2) and decreasing after LF-RSS ($P = 0.01$ for both M1 and M2) and LFas-RSS ($P < 0.01$ for both M1 and M2) (Fig. 1).

Motor Evoked Potentials and Short Intracortical Inhibition

Maximum SICI was found, in all groups and time points, for CS intensity of 80% RMT, consistent with previous reports.[26] There was no effect of RSS on test MEP. HF-RSS decreased SICI at T1 in both M1 and M2; this decrease was statistically significant for 80% ($P = 0.013$) and 90% ($P = 0.018$) CS intensities for M1, and for 60% ($P = 0.022$), 80% ($P = 0.023$), and 90% ($P = 0.025$) CS intensities for M2. LF-RSS induced opposite effects, ie, an overall improvement in SICI. This was statistically significant for 80% ($P = 0.009$), 90% ($P = 0.007$), and 100% ($P = 0.039$) CS intensities for M1, and for 70% ($P = 0.029$), 80% ($P = 0.013$), 90% ($P = 0.015$), and 100% ($P = 0.036$) CS intensities for M2. LFas-RSS caused an improvement in SICI as well, statistically significant, when considering M1, for 60% ($P = 0.026$), 70% ($P = 0.021$), 80% ($P = 0.017$), 90% ($P = 0.012$), and 100% ($P = 0.015$) CS intensities. A similar pattern was observed for M2, where an increase in SICI after LFas-RSS occurred for 60% ($P = 0.024$), 70% ($P = 0.019$), 80% ($P = 0.018$), 90% ($P = 0.016$), and 100% ($P = 0.009$) CS intensities (Fig. 2).

Somatosensory Evoked Potentials

Electrical stimulation of muscles evoked signals that closely resembled SEP obtained by peripheral nerve stimulation, although of smaller amplitude (Fig. S1).

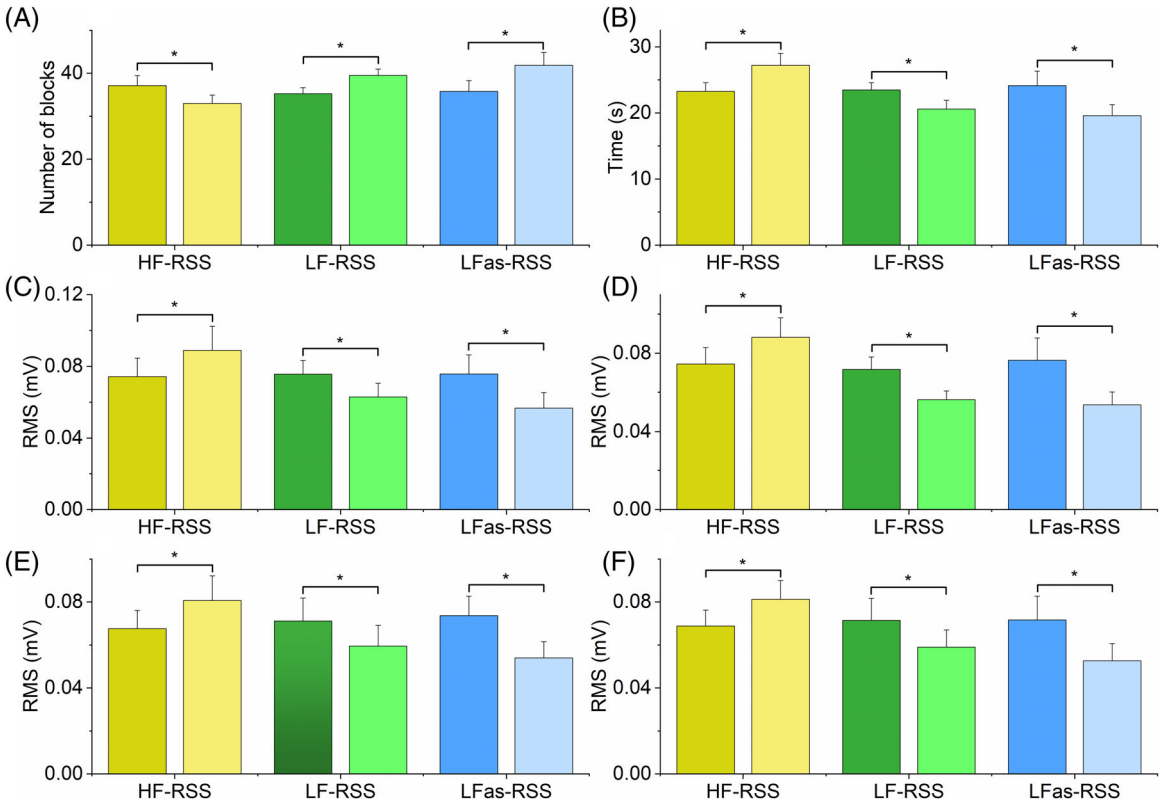

FIG. 1. Effects of repetitive somatosensory stimulation (RSS) on box and block test (BBT), nine-hole peg test (NHPT), and root-mean-square (RMS). Effects of RSS on BBT (**A**), NHPT (**B**), RMS of electromyography (EMG) recorded from M1 (primary motor cortex) when arms were outstretched (**C**), RMS of EMG recorded from M2 when arms were outstretched (**D**), RMS of EMG recorded from M1 while holding a pen (**E**), RMS of EMG recorded from M2 while holding a pen (**F**). Darker bars indicate baseline values; lighter bars refer to poststimulation values. Error bars indicate the standard error of the mean. Brackets with asterisks indicate statistically significant comparisons. LFas, LF-RSS asynchronously. [Color figure can be viewed at wileyonlinelibrary.com]

RSS had no effect on latencies of N20 and P14 SEP components (Fig. S2). HF-RSS increased the amplitude of the P14 (M1: $P = 0.004$; M2: $P = 0.008$) and the N20/P25 complex of SEP (M1: $P = 0.004$; M2: $P = 0.013$), whereas LF-RSS and LFas-RSS had an opposite effect, ie, they decreased the amplitude of P14 (LF-RSS M1: $P = 0.021$; LF-RSS M2: $P = 0.023$; LFas-RSS M1: $P = 0.015$; LFas-RSS M2: $P = 0.008$) and N20/P25 (LF-RSS M1: $P = 0.018$; LF-RSS M2: $P = 0.028$; LFas-RSS M1: $P = 0.004$; LFas-RSS M2: $P = 0.011$) (Fig. 3).

The effects of RSS on PP-SEP were variable according to the stimulation pattern used (Fig. 4). HF-RSS increased PP-SEP suppression in both ISIs tested, reflected by a decrease in R5 and R30. The effect was statistically significant for both N20/P25 and P14 obtained by stimulation of M1 and M2 (M1, N20/P25, R5: $P = 0.024$; M1, P14, R5: $P = 0.028$; M1, N20/P25, R30, $P = 0.017$; M1, P14, R30, $P = 0.015$; M2, N20/P25, R5: $P = 0.008$; M2, P14, R5: $P = 0.033$; M2, N20/P25, R30, $P = 0.038$; M2, P14, R30, $P = 0.019$). The effect was opposite (ie, increase in PP-SEP suppression) after LF-RSS (M1, N20/P25, R5: $P = 0.011$; M1, P14, R5: $P = 0.012$; M1, N20/P25, R30, $P = 0.018$; M1, P14, R30, $P = 0.022$; M2, N20/P25, R5: $P = 0.004$; M2, P14, R5: $P = 0.022$; M2, N20/P25, R30, $P = 0.025$; M2, P14, R30, $P = 0.008$) and LFas-RSS (M1, N20/P25, R5: $P = 0.003$; M1, P14, R5: $P = 0.008$; M1, N20/P25, R30, $P = 0.006$; M1, P14, R30, $P = 0.009$; M2, N20/P25, R5: $P = 0.014$; M2, P14, R5: $P = 0.018$; M2, N20/P25, R30, $P = 0.005$; M2, P14, R30, $P = 0.003$).

There were no baseline differences across groups in Q20 and Q14. HF-RSS caused a significant increase in Q20 ($P = 0.002$), whereas it decreased after LF-RSS ($P = 0.005$) and LFas-RSS ($P = 0.003$). The same pattern was observed for Q14 ($P = 0.002$, $P = 0.003$, and $P = 0.005$ for HF-RSS, LF-RSS, and LFas-RSS, respectively) (Fig. S3).

Correlations

RSS induced correlated changes in several variables linked to somatosensory and motor functions (Fig. S4). Specifically, significant correlations were found between

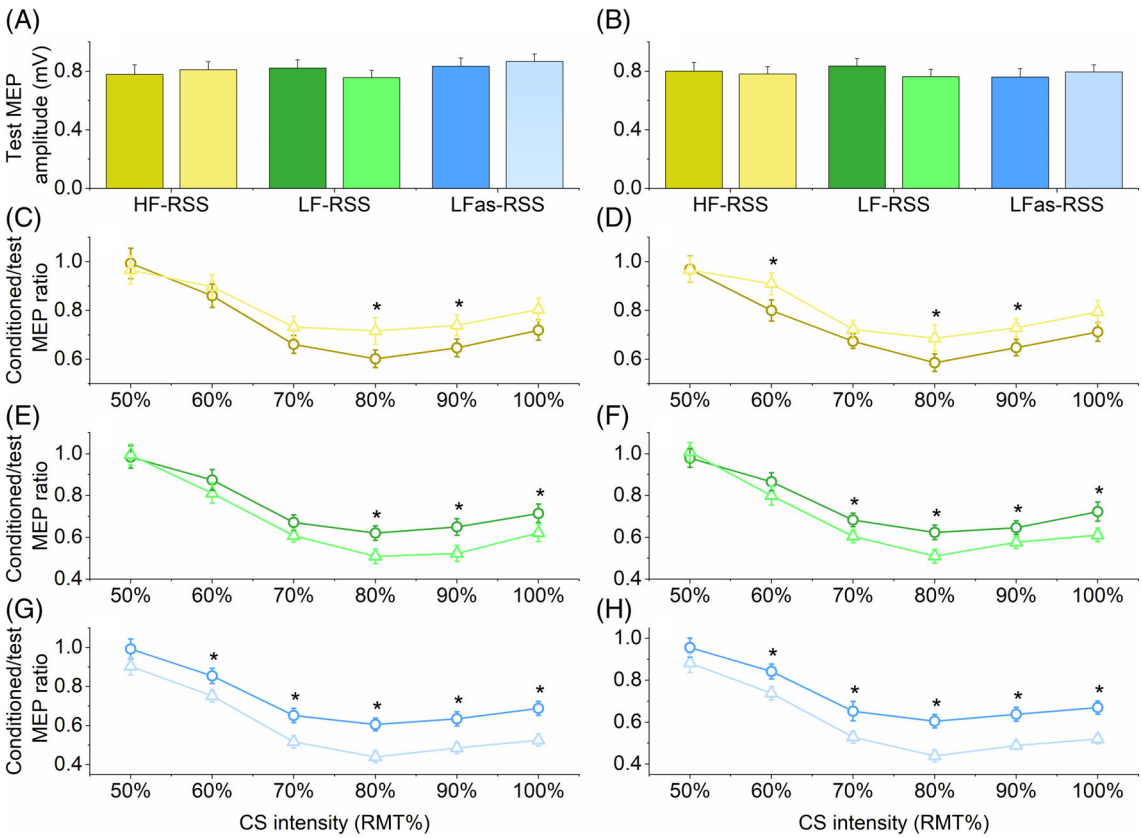

FIG. 2. Effects of repetitive somatosensory stimulation (RSS) on test motor evoked potentials (MEPs). (**A**, **B**) The effects of RSS on test MEP recorded from M1 (primary motor cortex) and M2, respectively. (**C–H**) The effect of RSS on short intracortical inhibition (SICI); in particular, high-frequency RSS (HF-RSS)-M1 (**C**), HF-RSS-M2 (**D**), low-frequency RSS (LF-RSS)-M1 (**E**), LF-RSS-M2 (**F**), LF-RSS asynchronously (LFas)-RSS-M1 (**G**), and LF-RSS-M2 (**H**). Darker bars and darker lines with circles indicate baseline values; lighter bars and lighter lines with triangles refer to poststimulation values. Error bars indicate the standard error of the mean. Asterisks indicate statistically significant comparisons. CS, conditioning stimulus. [Color figure can be viewed at wileyonlinelibrary.com]

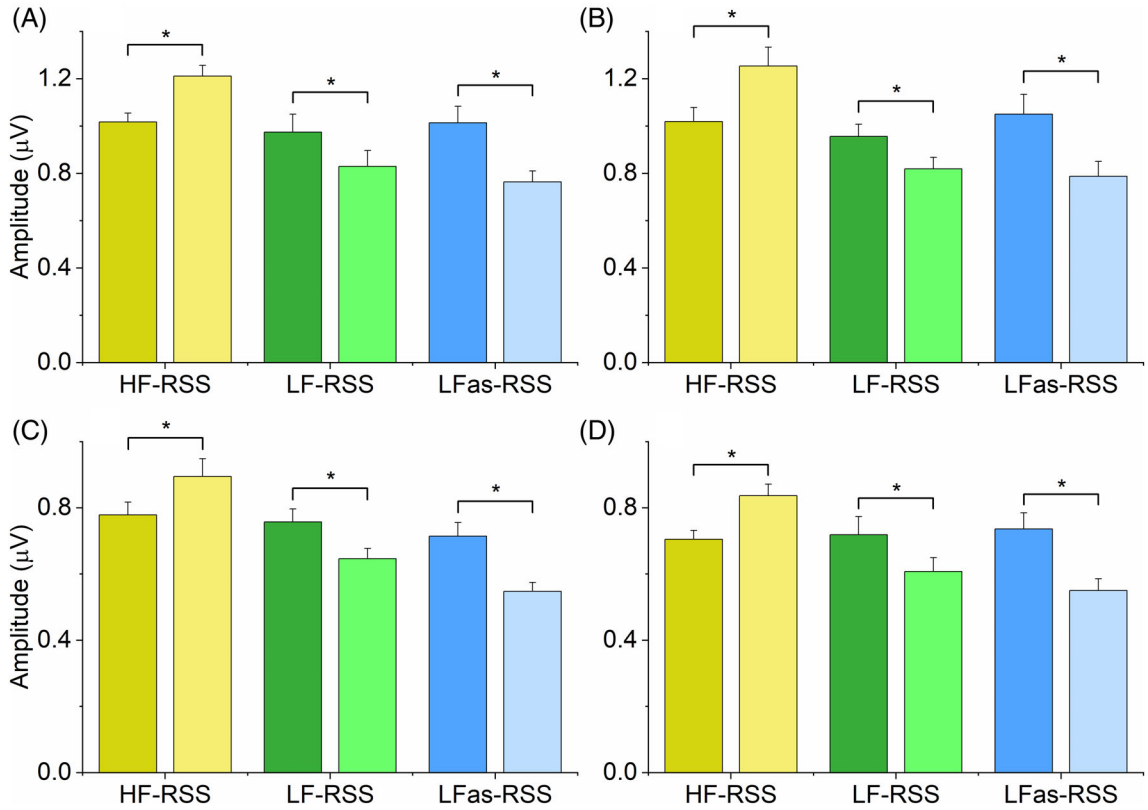

FIG. 3. Effects of repetitive somatosensory stimulation (RSS) on somatosensory evoked potential (SEP) amplitude. (**A**) N20/P25 M1 (primary motor cortex) amplitude. (**B**) N20/P25 M2 amplitude. (**C**) P14 M1 amplitude. (**D**) P14 M2 amplitude. Darker bars indicate baseline values; lighter bars refer to poststimulation values. Error bars indicate the standard error of the mean. Brackets with asterisks indicate statistically significant comparisons. HF-RSS, high-frequency repetitive somatosensory stimulation; LFas, low-frequency repetitive somatosensory stimulation asynchronously; LF-RSS, low-frequency repetitive somatosensory stimulation. [Color figure can be viewed at wileyonlinelibrary.com]

the following variables: SICI and EMG RMS outstretched tested on M1 ($r = 0.734$, $P = 0.018$); SICI and EMG RMS pen tested on M1 ($r = 0.694$, $P = 0.020$); SICI and EMG RMS outstretched tested on M2 ($r = 620$, $P = 0.021$); SICI and EMG RMS pen tested on M2 ($r = 0.571$, $P = 0.027$); Q20 and average EMG RMS outstretched ($r = 0.689$, $P = 0.013$); Q20 and average EMG RMS pen ($r = 0.691$, $P = 0.015$); Q20 and average SICI ($r = 0.634$, $P = 0.028$).

Discussion

In this study, we reaffirm that HF-RSS prompts a paradoxical response in sensorimotor inhibition in dystonic patients, whereas LF-RSS has opposite effects.[8,9] Because LF-RSS and LFas-RSS yielded similar results, it is likely that the effects are attributable to the stimulation frequency itself and not to STDP. This modulation occurs when stimulation is directed toward muscles affected by dystonia, moving beyond the confines of digital nerve application.[8,9,23] Importantly, the effects of RSS on somatosensory and motor intracortical circuitry are associated with changes in EMG activation and hand function tests. Key findings and their proposed physiological interpretation are summarized in Figure 5. Overall, this work supports the use of low-frequency RSS as a means for ameliorating motor symptoms in dystonia.

Effects of RSS on Somatosensory Evoked Potentials

Similar to digital nerve stimulation,[8,9] RSS in this study led to a global enhancement in the excitability of S1, as manifested by increased single-pulse SEP. In contrast, LF-RSS and LFas-RSS induced the opposite effect. These results are likely due to changes in the excitability of postsynaptic neurons responsible for generation of SEPs.[22]

Notably, RSS exerted distinct effects on somatosensory inhibition assessed through PP-SEP. After HF-RSS, PP-SEP suppression decreased, whereas it increased after LF-RSS and LFas-RSS. These effects were widespread across the somatosensory system, evidenced by changes in suppression at both short (5 ms) and long (30 ms) ISIs; the former is indicative of local inhibitory mechanisms, whereas the latter suggests more intricate inhibitory loops involving distant structures.[27-31] This conclusion is also supported by the observation that

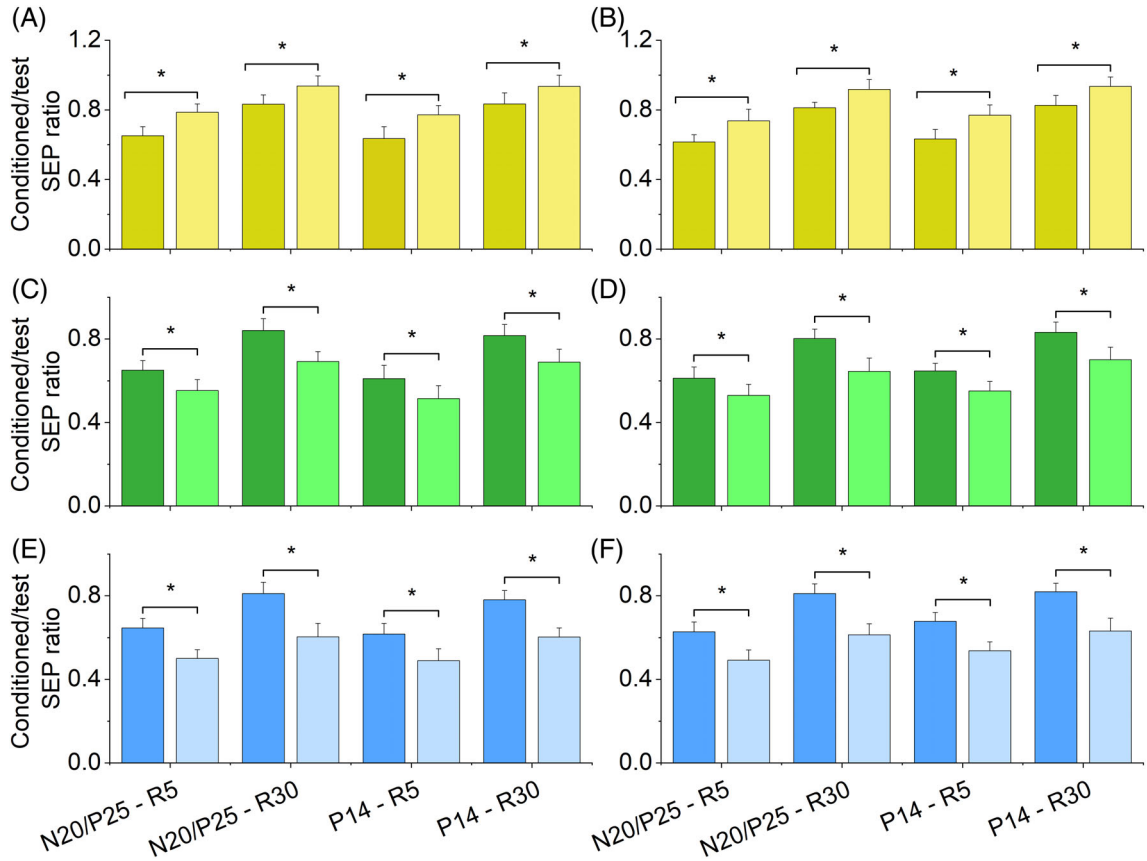

FIG. 4. Effects of repetitive somatosensory stimulation (RSS) on PP-SEP suppression. (**A**) High-frequency RSS (HF-RSS) on N20/P25 and P14, M1 (primary motor cortex). (**B**) HF-RSS on N20/P25 and P14, M2. (**C**) Low-frequency RSS (LF-RSS) on N20/P25 and P14, M1. (**D**) LF-RSS on N20/P25 and P14, M2. (**E**) LF-RSS asynchronously (LFas)-RSS on N20/P25 and P14, M1. (**F**) LFas-RSS on N20/P25 and P14, M2. Darker bars indicate baseline values; lighter bars refer to poststimulation values. Error bars indicate the standard error of the mean. Brackets with asterisks indicate statistically significant comparisons. SEP, somatosensory evoked potentials. [Color figure can be viewed at wileyonlinelibrary.com]

SEP changes occurred both for N20/P25 and P14 components, whose generators are thought to reside in S1 and in the nucleus cuneatus/medial lemniscus, respectively.[22] Drawing on insights from previous work,[8,21,24,32,33] it is plausible that the effects of RSS on PP-SEP suppression, particularly at 5-ms ISI, signify changes in the efficacy of feedforward inhibition mediated by fast-spiking inhibitory interneurons. However, this might not be the only mechanism through which RSS exerts its effects. Our investigation showed alterations in somatosensory lateral inhibition after RSS, as reflected in the degree of suppression of double-pulse SEP, obtained by simultaneous stimulation of dystonic muscles. Specifically, this inhibition decreased after HF-RSS and increased after LF-RSS and LFas-RSS. It is known that some interneurons have axons that extend beyond the local area where their soma is located, terminating in different cortical columns,[34] and that they can provide feedback inhibition to neighboring populations of principal cells located at a certain distance that may not have provided excitation to that particular interneuron population. We speculate that this phenomenon, better known as surround inhibition, might explain the observed changes in Q20 and Q14. In the present setting, principal cells receiving afferent from one stimulated muscle would activate interneurons responsible for the suppression of activity generated within the representation of the other muscle in S1 and in the cuneate nucleus.

Effects of RSS on Motor Evoked Potentials

Similar to prior investigations,[8,9,23,32] our findings demonstrated that the impact of RSS extends beyond the somatosensory system to the motor cortex. Although there was no discernible effect on MEPs evoked by single TMS pulses, SICI decreased after HF-RSS, whereas it increased after LF-RSS and LFas-RSS. We posit that the processes underlying the relay of RSS effects to the GABAergic interneurons mediating SICI[35] are similar to those hypothesized in previous work for the stimulation of digital nerves,[8,9,23] but this time involving proprioceptive information. In brief, it is likely that changes in excitability are transmitted by somatotopic connections between S1 and motor cortex, either directly targeting layer V pyramidal tract neurons[36] or terminating in cortical layers II/III,[37] in a way

	Significance/proposed physiological basis	Effects of HF-RSS in dystonia	Effects of LF-RSS in dystonia
Box and block test, nine-hole peg test	Manual dexterity	Decreased performance	Improved performance
EMG activity from dystonic muscles	Activation of dystonic muscles	Increased activity	Decreased activity
Amplitude of MEPs obtained with single-pulse TMS	Net excitability of M1	No changes	No changes
Short intracortical inhibition	Measure of M1 intracortical inhibition	Decreased inhibition	Increased inhibition
Amplitude of P14 and N20/P25 SEP components obtained with single pulses	Excitability of post-synaptic cells in the nucleus cuneatus (P14) and S1 (N20/P25)	Increased amplitude	Decreased amplitude
Recovery of P14 and N20/P25 SEP components obtained with paired-pulse stimulation	Feedforward inhibition within S1, possibly mediated by fast-spiking inhibitory interneurons (ISI 5 ms)/ S1 inhibition involving longer loops (ISI 30 ms)	Decreased inhibition	Increased inhibition
Spatial inhibition ratio obtained with double-pulse SEP	Surround inhibition within S1, possibly mediated by feedback mechanisms	Decreased inhibition	Increased inhibition

FIG. 5. Summary of key findings and their physiological interpretation. EMG, electromyography; ISI, interstimulus interval; M1, primary motor cortex; MEP, motor evoked potential; SEP, somatosensory evoked potential. [Color figure can be viewed at wileyonlinelibrary.com]

similar to the induction of long-term potentiation in layers II/III of the primary motor area after tetanic stimulation of S1.[38,39] Considering that SICI was modulated by RSS, but MEPs induced by TS alone were not, it is likely that inhibitory interneurons in the motor cortex are more susceptible to modulation than the principal cells and connected excitatory interneurons responsible for MEP generation. Prior literature has proposed that, in comparison with excitatory interneurons, inhibitory ones possess faster time constants and stronger excitatory inputs, facilitating more significant and rapid membrane potential changes; consequently, they can reach threshold more frequently, exhibiting heightened spiking activity for nonpreferred stimuli and broader tuning.[40] Excitatory inputs to inhibitory interneurons also tend to be stronger[41,42] and have more convergence than those to pyramidal cells.[43,44] It is plausible that one or more of the mentioned factors contribute to the differential effects of RSS on single-pulse MEPs and SICI.

Effects of RSS on Electromyographic Activity and Hand Function Tests

In line with our hypothesis, our observations indicated that RSS effectively modulated muscle activity during two postural tasks eliciting dystonia. EMG activity from dystonic muscles increased after HF-RSS and decreased after LF-RSS and LFas-RSS. Although the exact mechanisms driving this modulation remain speculative, we can formulate hypotheses based on the observed associations between changes in EMG activity and electrophysiological indices of S1 and motor cortex inhibition. Specifically, we identified correlations between RSS-induced modulation in EMG from dystonic muscles, engaged in simple postures, and parameters such as the Q20 and SICI. Notably, the effects of RSS on Q20, but not on PP-SEP suppression, demonstrated a correlation with changes in RMS. In this regard, it is possible that our protocol tested two different interneuronal circuits in S1, each having distinct relationships with the clinical manifestation of dystonia. The first subset potentially acts on principal cells through a feedforward mechanism, assessable through PP-SEP suppression and late high-frequency oscillations (l-HFO). This ensemble is likely associated with somatosensory deficits, such as the reported higher somatosensory temporal discrimination threshold, but does not appear to contribute directly to the manifestation of dystonia.[45,46] In contrast, a different subset of inhibitory interneurons tested with double-pulse SEP and responsible for surround inhibition in S1, possibly through a feedback mechanism,[47] may play a more prominent role in the expression of motor symptoms in dystonia.

Changes in RMS were also correlated with those observed in SICI, which is believed to hinge on the activity of gamma-aminobutyric acid GABAergic interneurons within the primary motor area.[35,48] Although the precise connection between SICI and motor function remains incompletely understood, it is established that GABAergic interneurons contribute to shape the

activity of the motor cortex by determining selective output from pyramidal cells during voluntary movement,[49] a mechanism partially compromised in dystonia.[50] It is plausible that the same class of interneurons targeted by SICI is involved in this phenomenon. Because we postulated that the effects of RSS in S1 are transmitted to the motor cortex via somatotopic connections,[36,37] it is conceivable that changes in excitability in the latter area predominantly occur within circuits responsible for lateral inhibition in the same muscles. This notion is reinforced by the correlation between RSS-induced changes in Q20 and SICI, potentially resulting in correlated modifications in the simultaneous activation of the two muscles in dystonia. The impact of RSS on muscle activity proved to be behaviorally significant, extending beyond simple postures to influence performance in hand function tests. HF-RSS resulted in a decline in performance in the BBT and NHPT, whereas LF-RSS and LFas-RSS improved scores in these tasks. It is plausible that the heightened involuntary muscle activity induced by HF-RSS hindered the execution of motor programs required for the BBT and NHPT, whereas the opposite was observed with low-frequency stimulation.

In conclusion, our findings suggest that the abnormal homeostatic plasticity in response to RSS in the sensorimotor system of patients with dystonia[8,9] can be harnessed for therapeutic purposes through the application of low-frequency electrical stimulation directly over dystonic muscles. It is important to note that, by applying electrical stimulation over muscles, cutaneous nerve fibers are inevitably stimulated. Consequently, it is challenging to ascertain whether the observed effects stem from the stimulation of muscle afferents alone or from a mixed population of muscle and skin afferents. Also, the application of RSS by means of surface electrodes means that nondystonic muscles surrounding the target ones may have occurred to a degree; therefore, we cannot rule out that the observed effects are at least partly due to nonspecific afferent stimulation. In this regard, we acknowledge that our set of experiments was not exhaustive due to time limitations, and that more thorough control conditions involving stimulation of nondystonic muscles and low-intensity RSS, to selectively stimulate skin afferents, may have helped to clarify the mechanisms underlying our protocol.

Another limitation of the study is the limited exploration of the time course of effects; therefore, more data are needed to ascertain the feasibility of RSS as a therapeutic intervention for dystonia. Regardless, low-frequency stimulation demonstrated the ability to enhance hand function in patients with FHD, presenting itself as a promising option for therapeutic intervention in FHD. Further endeavors are warranted to fine-tune stimulation parameters (eg, duration and intensity) and explore responses on more extensive patient cohorts. ■

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Data Availability Statement

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

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

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Author Roles

Design: L.R., A.L., J.C.R., A.B., K.P.B.
Execution: L.R., A.L., E.M., V.R.
Analysis: L.R., A.L., E.M., V.R.
Writing: L.R., A.L., E.M., V.R.
Editing of final version of the manuscript: L.R., A.L., E.M., V.R., J.C.R., A.B., K.P.B.

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Supplementary methods

Patients and clinical evaluation

Forty-five patients with a diagnosis of FHD (23 female, mean age 55.13 ± 13.04) were enrolled in the study (their characteristics are summarized in supplementary table 1). They were divided in three groups of 15 each, according to the type of stimulation received (HF-RSS, LF-RSS, LFas-RSS). The appropriateness of our sample size was established by a power calculation based on the effect size of previous studies using similar patterns of RSS in idiopathic cervical dystonia [1, 2]. The power analysis was performed with G*Power software, which indicated that 15 participants would be required to detect an effect with a power of 0.85 and an alpha level of 0.05. Patients were recruited from the outpatient botulinum toxin clinic of the Clinical Neurophysiology Department of the Institute of Neurology, University College London, and from the Movement Disorders outpatient clinic of Sapienza University of Rome. Patients were assessed at least 3 months after their last BoNT injection, and they were not treated with other drugs for their dystonia. Handedness was assessed by the Edinburgh handedness Inventory [3] and dystonia was clinically assessed by means of the Unified Dystonia Rating Scale (UDRS) [4] and Arm Dystonia Impairment Scale (ADDS) [5]. An informed consent was signed by all participants before the experimental procedures, which were approved by the local institutional review board and conducted in agreement with the Declaration of Helsinki according to international safety guidelines.

Hand motor function tests

Hand dexterity was assessed with the box and block test (BBT) and the nine-hole peg test (NHPT). In the BBT, patients had to move, one by one, the maximum number of blocks from one compartment of a box to another of equal size, within sixty seconds [6]. The number of blocks moved was used as a variable for following analyses. In the NHPT, subjects were asked to put, as fast as possible, nine wooden pegs from a container into holes in a board, and then back [7]. The behavioural variable of interest, which was measured and used for further analyses, was the time in seconds to complete the task.

Electromyographic recording and transcranial magnetic stimulation

EMG activity was recorded using Ag/AgCl electrodes placed over the two muscles (M1 and M2) most affected by dystonia and were primarily chosen based on BoNT treatment (supplementary table 1). In case patients received injection in only one, or no muscles, recording sites where chosen based on clinical observation of dystonia (i.e., muscles where involuntary contraction was more clearly seen during clinical examination). No patients had BoNT injections in more than two forearm sites. Electromyographic (EMG) signals were digitized at 5 kHz with a CED 1401 A/D laboratory interface (Cambridge Electronic Design Ltd, Cambridge, UK) and bandpass filtered (5 Hz - 2 kHz) with a Digitimer D360 (Digitimer Ltd., Welwyn Garden City, 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). EMG activity was monitored throughout the experiment.

TMS was performed using a Magstim 200 monophasic stimulator with a 70 mm figure-of-eight coil (Magstim Company Ltd, Whitland, UK). First, the motor hotspot was found, defined as the location in the primary motor area where the largest MEP in the forearm muscles from which the EMG signals were recorded were found. Then, we measured the resting motor threshold (RMT) related to the less excitable of the two forearm muscles, and we found the intensity able to elicit motor evoked potentials (MEPs) of at least 0.5 mV (0.5 mV-int) in both of them. 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 [8]. SICI was obtained through paired-pulse TMS, with an interstimulus interval (ISI) of 3 ms between the conditioning stimulus (CS) and the test stimulus (TS). The TS was set at 0.5 mV-int, while the CS was set at 50%, 60% 70%, 80%, 90% and 100% RMT, to obtain a recruitment curve [9-11]. Fifteen paired stimuli for each different intensity of the CS and fifteen single pulses were delivered in a randomized order. SICI was calculated dividing the amplitude of conditioned/unconditioned MEP.

Somatosensory evoked potentials recording and analysis

To record the N20/P25 component of SEP, the active electrode was placed at CP3 or CP4 (contralateral to the dystonic arm) and the reference electrode at Fz, while the P14 was recorded with the active electrode at Fz and the reference electrode on the contralateral mastoid [12, 13]. Stimulation was performed via the same electrodes used to record EMG signals from the forearm sites of interest, connected to two constant current stimulators (Digitimer DS7A,

Digitimer Ltd, Welwyn Garden City, UK). Monophasic square wave pulses of 200 μs duration were delivered at a frequency of 3 Hz. Since the stimulation was not applied on a nerve trunk, as for standard SEP, the brain potential recorded would be due to afferent activity due to muscle contraction, with some contribution from stimulation of cutaneous nerve endings. This would result in a smaller signal; to try to overcome this limitation, the stimulation intensity was the highest that subject could tolerate without generating pain. The somatosensory threshold was also recorded at each site and was defined as the minimum current intensity able to elicit a consistent percept. Signals were recorded from -20 to +100 ms around to the pulse, digitized at a 5 KHz sampling frequency and band-pass filtered (3 Hz - 2 KHz) [13, 14]. Three blocks of 500 trials each were recorded by stimulation at each forearm site separately, one with single pulse stimulation, and the other two with paired-pulse stimulation with ISIs of 5 and 30 ms. In the frames where two stimuli were delivered, responses following the second stimulus were obtained by subtracting the single-pulse SEP waveform from the PP-SEP one [15, 16]. R5 and R30 were calculated as the ratio between the second and the first response for ISIs of 5 and 30 ms, respectively. In a further block of 500 trials stimulation was given at the two forearm sites at the same time. As in previous work [2, 17], the spatial inhibition ratios (SIR) of N20/P25 (Q20) and P14 (Q14) were calculated as M1M2/(M1+M2) x 100, where M1M2 is the SEP amplitude obtained by simultaneous stimulation of the two forearm sites and M1+M2 is the arithmetic sum of the SEP obtained by the individual stimulation at the two sites [1, 18, 19]. All blocks were recorded in a randomized order. The position of the electrodes was kept constant throughout the whole experiment and care was taken to always keep impedances below 5 KΩ.

Repetitive somatosensory stimulation

RSS was delivered at the same sites on the forearm used for SEP and EMG recording (see above). Each of the three groups of patients underwent a different RSS protocol, but the duration of each was the same (45 minutes). Similar to SEP, the somatosensory threshold was measured for each protocol, and conditioning was applied at the maximum tolerable, non-painful intensity. HF-RSS consisted of 20 Hz trains of stimuli (0.2 ms square wave electrical pulses) of 1 s duration, with 5 s inter-train interval, applied to both forearm sites at the same time. LF-RSS was delivered in a similar way, exception made for the different pattern of stimulation, which consisted of 1 pulse applied every second synchronously over the two muscles. Lastly, in its asynchronous variant (LFas-RSS), electric pulses were applied intermittently on each muscle site, with a 0.5 s interval.

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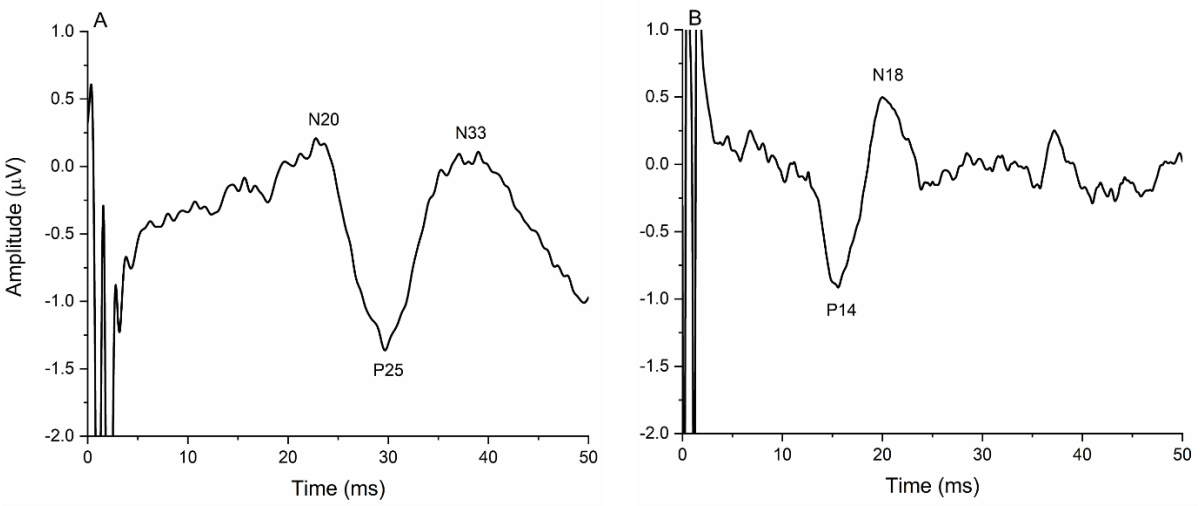
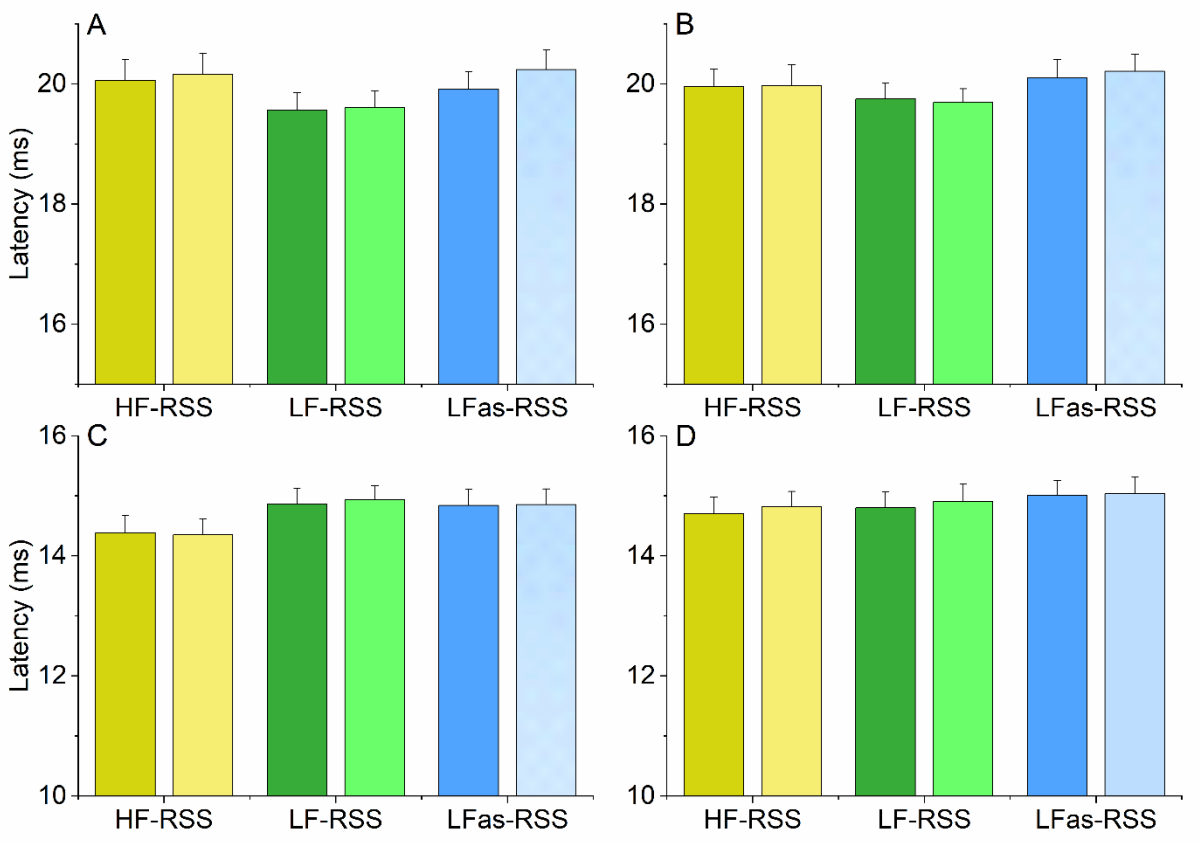
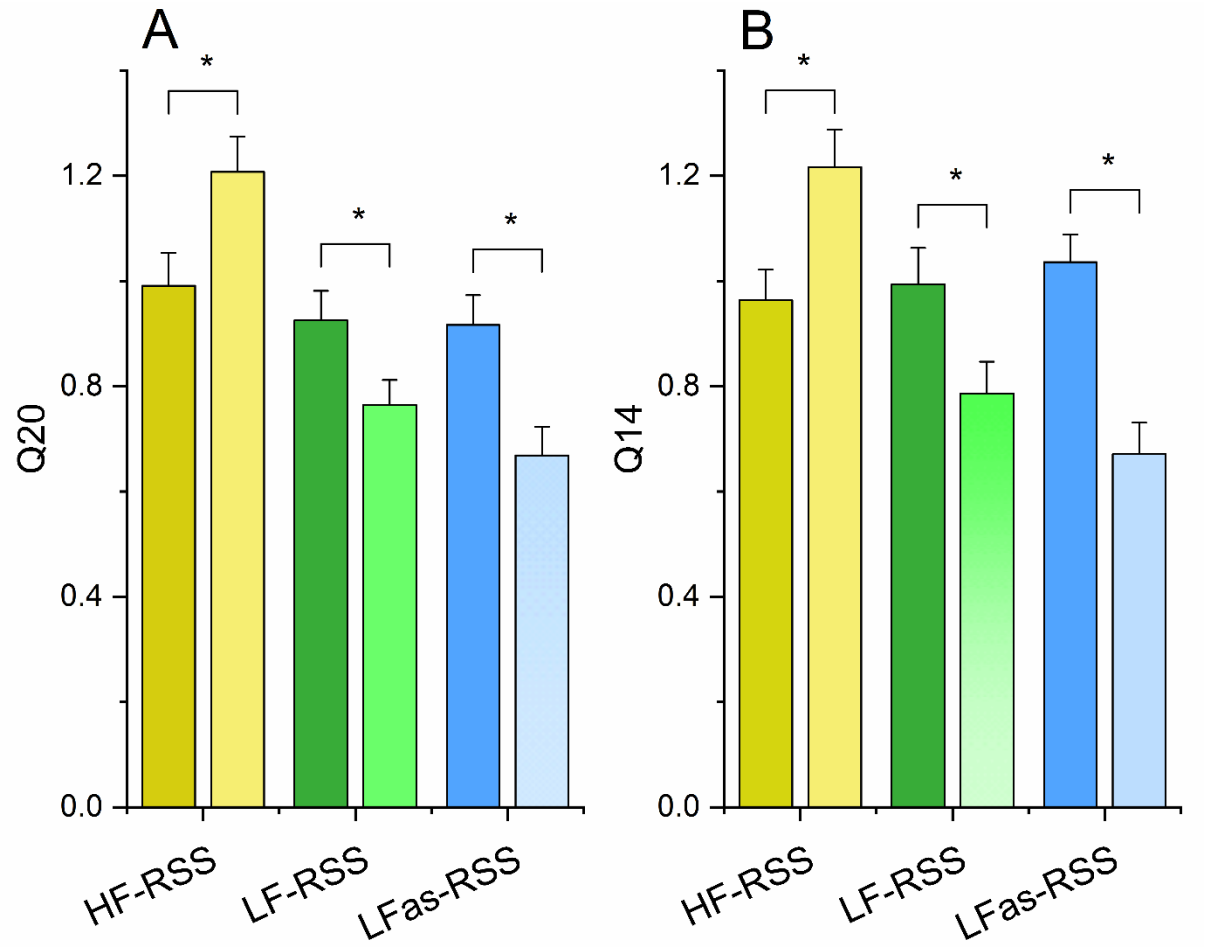
Supplementary figures



Supplementary Figure 1: **SEP obtained by single pulse stimulation over one muscle site in a representative subject.** Signals resemble SEP obtained by stimulation of a peripheral nerve, although they are of smaller amplitude. (**A**): parietal components (N20, P25, N33) measures with CP3/4-FCz montage. (**B**): central components (P14, N18) recorded with Fz-contralateral mastoid montage.



Supplementary Figure 2: **effects of RSS on latencies of SEP.** (**A**): N20 M1 latency; (**B**): N20 M2 latency; (**C**): P14 M1 latency; (**D**): P14 M2 latency. Error bars indicate the standard error of the mean.



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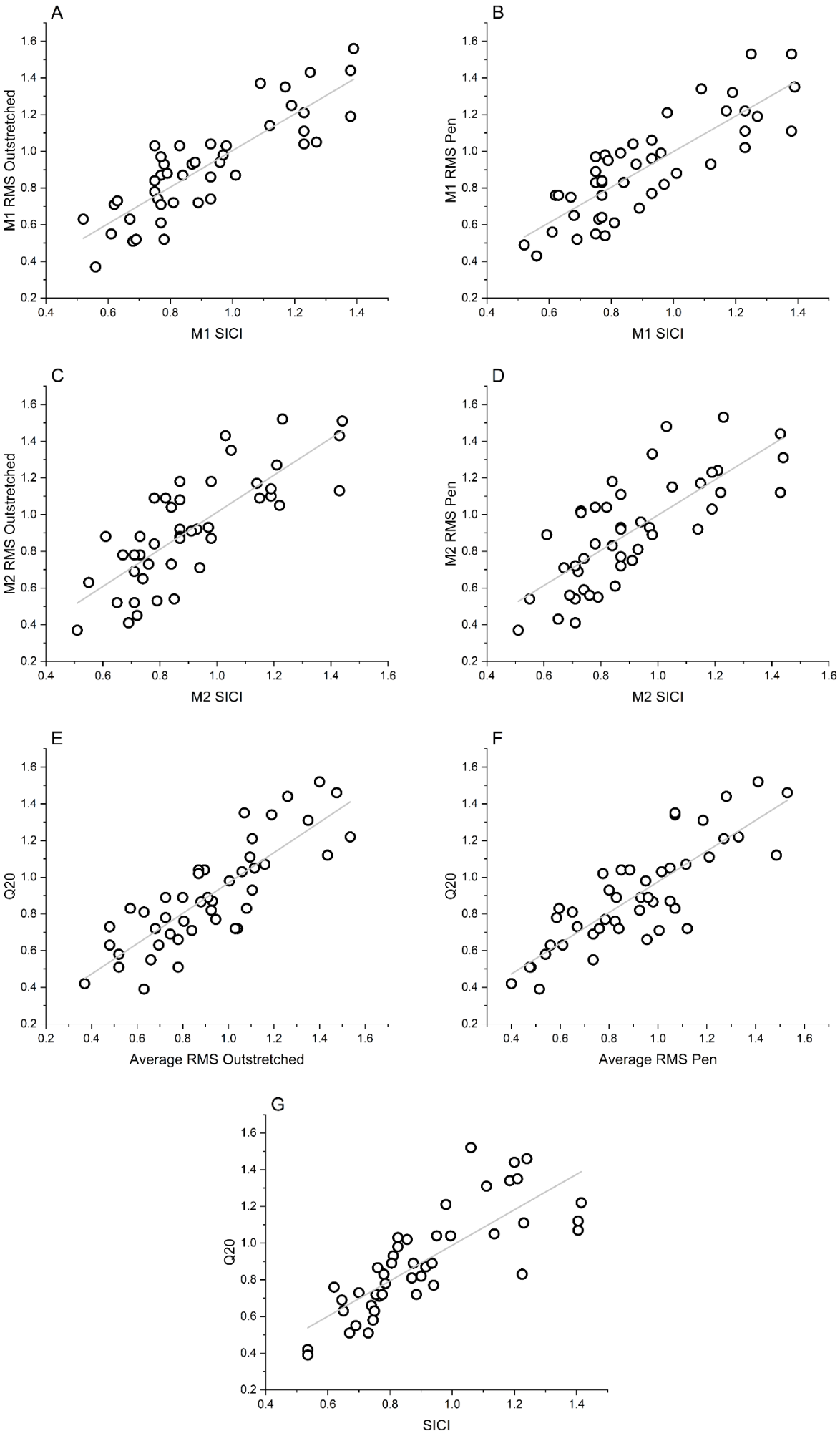
158 **Supplementary Figure 3**: **effects of RSS on Q20 and Q14**. A. Effects of RSS on Q20. B.
159 Effects of RSS on Q20. B. Darker bars indicate baseline values, while lighter ones refer to post-
160 stimulation values. Error bars indicate the standard error of the mean. Brackets with asterisks
161 indicate statistically significant comparisons.

162


A
M1 RMS Outstretched
M1 SICI
B
M1 RMS Pen
M1 SICI
C
M2 RMS Outstretched
M2 SICI
D
M2 RMS Pen
M2 SICI
E
Q20
Average RMS Outstretched
F
Q20
Average RMS Pen
G
Q20
SICI

Supplementary Figure 4: **correlations**. (**A**): M1 SICI, RMS outstretched; (**B**): M1 SICI, RMS pen; (**C**): M2 SICI, RMS outstretched; (**D**): M2 SICI, RMS pen; (**E**): Q20, average RMS outstretched; (**F**): Q20, average RMS pen; (**G**): Q20, average SICI.

Supplementary tables

Group 1 – HF-RSS								
	Age	Gen	Hand	Disease duration	UDRS	ADDS	M1	M2
1	50	F	R	1	2.50	85.95	FCR	FDS
2	74	F	R	18	6.50	72.86	PT	FCU
3	74	M	R	11	14.00	42.86	FCU	BR
4	68	M	R	20	4.50	55.71	FCR	ECR
5	50	F	L	15	3.50	77.14	EDC	FCR
6	63	M	R	7	8.50	68.57	ADM	FCU
7	58	F	L	6	9.00	81.43	FCR	FDI
8	55	F	R	15	6.00	90.48	FCR	ECR
9	58	F	R	12	12.00	47.14	FCR	FPL
10	51	M	R	7	8.00	58.81	PT	FCU
11	42	M	R	8	4.00	85.71	FDS	FPL
12	49	F	L	4	8.50	42.86	FCU	BR
13	55	M	R	5	9.00	47.14	FCR	ECR
14	51	F	L	6	6.00	85.95	ADM	FCU
15	48	M	R	14	14.00	47.14	FDS	FPL
AV	56.4			9.9	7.73	65.98		
SD	9.6			5.5	3.57	17.92		
Group 2 – LF-RSS								
	Age	Gen	Hand	Disease duration	UDRS	ADDS	M1	M2

1	58	F	L	6	8.50	81.43	FCR	FDI
2	52	M	R	10	26.50	68.57	FCU	ECR
3	53	F	R	6	8.00	95.24	FPL	APL
4	41	M	R	15	4.50	72.38	FCR	FPL
5	45	F	R	15	2.50	72.38	FDS	FPL
6	44	F	R	10	4.00	90.48	FDS	FPL
7	66	M	R	20	7.00	38.57	FDS	FCR
8	58	F	R	7	14.00	55.71	FCR	ECR
9	58	M	R	9	9.00	55.71	FCU	ECR
10	53	F	R	10	5.50	38.57	FCR	ECR
11	49	M	R	5	5.00	85.95	PT	FCU
12	67	F	R	7	8.50	47.14	FDS	ECR
13	64	F	R	9	9.50	60.00	ADM	FCU
14	66	M	R	12	11.50	72.38	FCR	PT
15	74	M	L	13	12.00	42.86	FDS	FPL
AV	56.5			10	9.07	65.16		
SD	9.6			4	5.78	18.62		

Group 3 – LFas-RSS

	Age	Gen	Hand	Disease duration	UDRS	ADDS	M1	M2
1	71	M	R	6	5.00	34.29	FCR	FCU
2	45	F	R	10	3.00	55.71	FCR	FDS
3	63	M	R	7	8.50	68.57	ADM	FCU
4	45	M	R	2	7.00	30.00	EPL	EDC
5	66	M	R	20	7.00	38.57	FDS	FCR
6	48	M	L	1	13.00	17.14	FDS	ECR
7	71	F	R	13	2.00	85.71	FCU	PT
8	45	F	R	8	6.00	85.95	FCU	ADM

9	63	M	R	6	14.00	64.29	FCR	ECR
10	45	F	R	9	6.50	90.48	FCR	ECR
11	66	M	R	13	14.50	54.29	FCR	EDC
12	52	F	L	16	2.50	85.71	FDS	ECR
13	76	F	R	11	8.50	76.90	FDS	FPL
14	72	F	R	7	10.00	72.38	PT	FCU
15	65	M	R	11	13.50	68.57	FDS	EDC
AV	59.5			9	8.07	61.9		
SD	11.5			4	4.2	22.96		

Supplementary Table 1: patient variables. Disease duration is expressed in years. Abbreviations for muscles: ADM: abductor digiti minimi; APL: abductor pollicis longus; BR: brachioradialis; ECR: extensor carpi radialis; EDC: extensor digitorum communis; EPL: extensor pollicis longus; FCR: flexor carpi radialis; FCU: flexor carpi ulnaris; FDI: first dorsal interosseous; FDS: flexor digitorum superficialis; FPL: flexor pollicis longus; PT: pronator teres. Other abbreviations: AV: average; Hand: hand affected by dystonia; Gen: gender; AV: average; SD: standard deviation.

	Group 1 (HF-RSS)		**Group 2 (LF-RSS)**		**Group 3 (LFas-RSS)**		**Group**	**Time**	**Group × Time**
	T0	**T1**	**T0**	**T1**	**T0**	**T1**			
Age	56.4 ± 9.6		56.5 ± 9.5		59.5 ± 11.5		F=0.45 p=0.64		
DD	9.9 ± 5.6		10.3 ± 4.1		59.5 ± 4.9		F=0.13 p=0.88		

UDRS	7.7 ± 3.6		9.1 ± 5.8		8.1 ± 4.2		F=0.34 p=0.71		
ADDS	65.9 ± 17.9		65.2 ± 18.6		61.9 ± 22.9		F=0.18 p=0.84		
RMT	51.20 ± 11.82	51.67 ± 11.87	53.0 ± 6.55	53.67 ± 6.44	51.60 ± 10.93	52.27 ± 10.96	F=0.15 p=0.87	F=0.41 p=0.65	F=0.95 p=0.91
0.5 mV-int	64.07 ± 14.39	64.20 ± 13.70	65.60 ± 7.93	64.93 ± 8.06	64.60 ± 13.52	63.80 ± 12.91	F=0.04 p=0.96	F=1.18 p=0.42	F=1.92 p=0.16
SEP ST M1	3.1 ± 0.9	3.2 ± 0.9	2.8 ± 0.8	2.9 ± 0.8	3.2 ± 0.8	3.4 ± 0.7	F=1.28 p=0.29	F=1.10 p=0.48	F=2.18 p=0.09
SEP ST M2	3.1 ± 1.1	3.2 ± 1.1	2.9 ± 1.0	3.0 ± 1.0	3.2 ± 0.8	3.3 ± 0.8	F=0.38 p=0.68	F=1.56 p=0.15	F=1.15 p=0.33
SEP STIM M1	10.1 ± 2.6	10.4 ± 2.5	10.2 ± 5.0	10.5 ± 4.8	11.1 ± 4.6	11.3 ± 4.5	F=0.21 p=0.21	F=1.23 p=0.32	F=0.66 p=0.53
SEP STIM M2	9.2 ± 2.3	9.6 ± 2.4	10.3 ± 4.7	10.8 ± 4.9	10.6 ± 3.5	10.9 ± 3.5	F=0.57 p=0.57	F=1.41 p=0.22	F=0.44 p=0.65
RSS ST M1	2.6 ± 1.3		2.8 ± 0.8		3.2 ± 0.8		F=1.51 p=0.23		

RSS ST M2	2.5 ± 1.3	3.2 ± 1.2	3.3 ± 0.8	F=2.01 p=0.15
RSS STIM M1	8.7 ± 3.0	11.4 ± 5.6	13.1 ± 6.8	F=1.53 p=0.22
RSS STIM M2	8.9 ± 3.1	11.3 ± 5.1	13.6 ± 4.3	F=2.01 p=0.15

Supplementary Table 2: effects of RSS on thresholds and stimulation intensities. DD: disease duration; ST: somatosensory threshold: STIM; stimulation condition (refers to both SEP and RSS). M1 and M2 indicate the two forearm sites where stimulation was delivered (see text for details). Values are expressed as mean ± standard deviation. Note that degrees of freedom and error were 1,42 for main effects of "group" and "time", and 2,42 for "group×time" interaction.

	Main effects and interactions	**F statistics**	**P values**
BBT	Group	$F_{2,42} = 0.803$	$p = 0.455$
	Time	$F_{1,42} = 16.490$	$p < 0.001$
	Group × time	$F_{2,42} = 39.787$	$p < 0.001$
NHPT	Group	$F_{2,42} = 1.442$	$p = 0.248$
	Time	$F_{1,42} = 9.131$	$p = 0.004$
	Group × time	$F_{2,42} = 45.853$	$p < 0.001$
RMS M1 outstretched	Group	$F_{2,42} = 0.700$	$p = 0.502$
	Time	$F_{1,42} = 6.849$	$p = 0.012$

	Group × time	$F_{2,42} = 22.773$	$p < 0.001$
RMS M1 pen	Group	$F_{2,42} = 0.349$	$p = 0.708$
	Time	$F_{1,42} = 9.271$	$p = 0.004$
	Group × time	$F_{2,42} = 24.223$	$p < 0.001$
RMS M2 outstretched	Group	$F_{2,42} = 1.560$	$p = 0.222$
	Time	$F_{1,42} = 8.475$	$p = 0.006$
	Group × time	$F_{2,42} = 15.700$	$p < 0.001$
RMS M2 pen	Group	$F_{2,42} = 0.593$	$p = 0.557$
	Time	$F_{1,42} = 9.218$	$p = 0.004$
	Group × time	$F_{2,42} = 20.541$	$p < 0.001$
Test MEP M1	Group	$F_{2,42} = 0.806$	$p = 0.453$
	Time	$F_{1,42} = 0.612$	$p = 0.439$
	Group × time	$F_{2,42} = 0.392$	$p = 0.678$
Test MEP M2	Group	$F_{2,42} = 0.949$	$p = 0.395$
	Time	$F_{1,42} = 1.645$	$p = 0.217$
	Group × time	$F_{2,42} = 0.094$	$p = 0.910$
SICI M1	Group	$F_{2,42} = 1.850$	$p = 0.170$
	Time	$F_{1,42} = 90.217$	$p < 0.001$
	Intensity	$F_{5,210} = 382.88$	$p < 0.001$
	Group × time	$F_{2,42} = 140.995$	$p < 0.001$
	Group × int	$F_{10,210} = 3.563$	$p < 0.001$
	Time × int	$F_{5,210} = 0.988$	$p = 0.426$
	Group × time × int	$F_{10,210} = 13.225$	$p < 0.001$
SICI M2	Group	$F_{2,42} = 2.257$	$p = 0.117$
	Time	$F_{1,42} = 33.375$	$p < 0.001$
	Intensity	$F_{5,210} = 305.61$	$p < 0.001$
	Group × time	$F_{2,42} = 63.899$	$p < 0.001$

	Group × int	$F_{10,210} = 2.872$	$p < 0.001$
	Time × int	$F_{5,210} = 2.657$	$p = 0.024$
	Group × time × int	$F_{10,210} = 5.168$	$p < 0.001$
SEP N20 latency M1	Group	$F_{2,42} = 0.963$	$p = 0.390$
	Time	$F_{1,42} = 2.912$	$p = 0.425$
	Group × time	$F_{2,42} = 0.872$	$p = 0.425$
SEP N20 latency M2	Group	$F_{2,42} = 0.711$	$p = 0.497$
	Time	$F_{1,42} = 0.031$	$p = 0.861$
	Group × time	$F_{2,42} = 0.187$	$p = 0.830$
SEP P14 latency M1	Group	$F_{2,42} = 1.759$	$p = 0.185$
	Time	$F_{1,42} = 0.106$	$p = 0.746$
	Group × time	$F_{2,42} = 0.265$	$p = 0.768$
SEP P14 latency M2	Group	$F_{2,42} = 0.348$	$p = 0.828$
	Time	$F_{1,42} = 1.517$	$p = 0.225$
	Group × time	$F_{2,42} = 0.190$	$p = 0.828$
SEP N20/P25 amp M1	Group	$F_{2,42} = 1.657$	$p = 0.214$
	Time	$F_{1,42} = 36.647$	$p < 0.001$
	Group × time	$F_{2,42} = 146.281$	$p < 0.001$
SEP N20/P25 amp M2	Group	$F_{2,42} = 2.541$	$p = 0.125$
	Time	$F_{1,42} = 8.201$	$p = 0.007$
	Group × time	$F_{2,42} = 60.386$	$p < 0.001$
SEP P14 amplitude M1	Group	$F_{2,42} = 1.984$	$p = 0.127$
	Time	$F_{1,42} = 25.436$	$p < 0.001$
	Group × time	$F_{2,42} = 65.569$	$p < 0.001$
SEP P14 amplitude M2	Group	$F_{2,42} = 2.781$	$p = 0.103$
	Time	$F_{1,42} = 50.082$	$p < 0.001$
	Group × time	$F_{2,42} = 151.986$	$p < 0.001$

SEP Q20	Group	$F_{2,42} = 1.293$	$p = 0.264$
	Time	$F_{1,42} = 8.709$	$p = 0.005$
	Group × time	$F_{2,42} = 43.183$	$p < 0.001$
SEP Q14	Group	$F_{2,42} = 2.447$	$p = 0.139$
	Time	$F_{1,42} = 7.032$	$p = 0.011$
	Group × time	$F_{2,42} = 21.401$	$p < 0.001$
SEP N20/P25 recovery M1	Group	$F_{2,42} = 17.874$	$p < 0.001$
	Time	$F_{1,42} = 137.991$	$p < 0.001$
	ISI	$F_{1,42} = 196.593$	$p < 0.001$
	Group × time	$F_{2,42} = 322.446$	$p < 0.001$
	Group × ISI	$F_{2,42} = 0.894$	$p = 0.417$
	Time × ISI	$F_{1,42} = 32.061$	$p < 0.001$
	Group × time × ISI	$F_{2,42} = 1.146$	$p = 0.328$
SEP N20/P25 recovery M2	Group	$F_{2,42} = 21.037$	$p < 0.001$
	Time	$F_{1,42} = 256.769$	$p < 0.001$
	ISI	$F_{1,42} = 213.856$	$p < 0.001$
	Group × time	$F_{2,42} = 578.0.7$	$p < 0.001$
	Group × ISI	$F_{2,42} = 1.141$	$p = 0.329$
	Time × ISI	$F_{1,42} = 24.448$	$p < 0.001$
	Group × time × ISI	$F_{2,42} = 3.103$	$p = 0.055$
SEP P14 recovery M1	Group	$F_{2,42} = 33.025$	$p < 0.001$
	Time	$F_{1,42} = 175.700$	$p < 0.001$
	ISI	$F_{1,42} = 239.572$	$p < 0.001$
	Group × time	$F_{2,42} = 512.329$	$p < 0.001$
	Group × ISI	$F_{2,42} = 2.149$	$p < 0.001$
	Time × ISI	$F_{1,42} = 22.409$	$p < 0.001$
	Group × time × ISI	$F_{2,42} = 0.413$	$p = 0.664$

SEP P14 recovery M2	Group	$F_{2,42} = 9.914$	$p < 0.001$
	Time	$F_{1,42} = 167.258$	$p < 0.001$
	ISI	$F_{1,42} = 211.008$	$p < 0.001$
	Group × time	$F_{2,42} = 488.688$	$p < 0.001$
	Group × ISI	$F_{2,42} = 3.065$	$p = 0.057$
	Time × ISI	$F_{1,42} = 22.235$	$p < 0.001$
	Group × time × ISI	$F_{2,42} = 0.499$	$p = 0.611$

Supplementary Table 3: statistics relative to main effects and interactions of the ANOVAs. Values are expressed as mean ± standard deviation. In the statistics for SICI, "int" refers to the intensity of the conditioning stimulus. Bold characters indicate statistically significant p values.