RESEARCH ARTICLE

Re-emergent Tremor in Parkinson's Disease: The Role of the Motor Cortex

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ABSTRACT: Background: Parkinson's disease patients may show a tremor that appears after a variable delay while the arms are kept outstretched (re-emergent tremor). The objectives of this study were to investigate re-emergent tremor pathophysiology by studying the role of the primary motor cortex in this tremor and making a comparison with rest tremor.

Methods: We enrolled 10 Parkinson's disease patients with both re-emergent and rest tremor. Tremor was assessed by spectral analysis, corticomuscular coherence and tremor-resetting produced by transcranial magnetic stimulation over the primary motor cortex. We also recorded transcranial magnetic stimulation-evoked potentials generated by motor cortex stimulation during rest tremor, tremor suppression during wrist extension, and re-emergent tremor. Spectral analysis, corticomuscular coherence, and tremor resetting were compared between re-emergent tremor and rest tremor.

Results: Re-emergent tremor showed significant corticomuscular coherence, causal relation between motor cortex activity and tremor muscle and tremor resetting. The P60 component of transcranial magnetic stimulation-evoked potentials reduced in amplitude during tremor suppression, recovered before re-emergent tremor, was facilitated at re-emergent tremor onset, and returned to values similar to those of rest tremor during re-emergent tremor. Compared with rest tremor, re-emergent tremor showed similar corticomuscular coherence and tremor resetting, but slightly higher frequency.

Conclusions: Re-emergent tremor is causally related with the activity of the primary motor cortex, which is likely a convergence node in the network that generates re-emergent tremor. Re-emergent tremor and rest tremor share common pathophysiological mechanisms in which the motor cortex plays a crucial role. © 2020 International Parkinson and Movement Disorder Society

Key Words: clinical neurophysiology; motor cortex; Parkinson's disease; TMS-EEG; tremor

Parkinson's disease (PD) patients with rest tremor may present a tremor that appears after a delay while the arms are maintained in an outstretched position, the so-called re-emergent tremor (RET).[1-3] Clinical

observations suggest that RET may have mechanisms similar to rest tremor.[2,4-7]

Only 1 study investigated RET pathophysiology and found that a network converging on the sensorimotor cortex could participate in RET.[8] A better understanding of RET pathophysiology may open up therapeutic strategies for a type of tremor that is often more disabling than rest tremor.[2]

In this article, we investigated the pathophysiological role of the primary motor cortex (M1) in RET using neurophysiological techniques. We first investigated possible correlations between M1 and muscle activity during RET using corticomuscular coherence (CMC) and sought to identify its cortical generator through

current source density (CSD) analysis.[9] We then studied the directionality of any significant correlation between M1 and muscle activity by computing Granger causality (G-causality) between these signals.[10,11] To further address the possible causal relation between M1 activity and RET, we measured the ability of transcranial magnetic stimulation (TMS) to reset RET.[12-14] To investigate a link between M1 excitability and RET, we also combined TMS and electroencephalography (EEG) to study possible changes in the TMS-evoked potentials (TEPs) generated by M1 stimulation across different tremor conditions.[15] Finally, to test whether M1 is similarly involved in generating RET and rest tremor, we compared CMC, G-causality, and tremor resetting between the two tremors in the same patients.

Methods

Participants

Ten PD patients (6 men; mean age ± SD, 67 ± 11 years) were enrolled from the Movement Disorders Outpatient Unit at the Department of Human Neuroscience, Sapienza University of Rome (Table 1). Inclusion criteria were diagnosis of PD confirmed by a movement disorder expert neurologist based on international criteria,[1,16] the presence of RET with an amplitude score ≥ 2 (UPDRS item 3.15), the presence of rest tremor with a summed score for amplitude and constancy ≥ 4 (UPDRS items 3.17 and 3.18), and tremor limited to 1 side. RET was defined as a postural tremor that appeared after at least 3 seconds while maintaining the arm outstretched. Exclusion criteria were: history of other neurological or psychiatric conditions; advanced PD, defined as stage IV or V on the Hoehn and Yahr scale because of possible difficulties in carrying out the experimental procedures; and the presence of head

tremor. Treatment for PD was discontinued at least 12 hours prior to the experiment. Participants provided written informed consent prior to participating in the study. All study procedures were approved by the institutional review board and were in accordance with the Declaration of Helsinki.

Experimental Settings

Patients were seated on a chair designed for TMS (EMS, Italy), with their forearms resting on armrests. In all experiments, we assessed rest tremor by asking the patients to leave their affected hand hanging from the edge of the armrest and recording only when the tremor had been present for at least 10 seconds. RET was subsequently assessed by asking the patient to extend the wrist while maintaining the forearm on the armrest to avoid fatigue.[17]

Corticomuscular Coherence and Granger Causality

EEG was recorded from 32 TMS-compatible electrodes mounted on a cap (EASYCAP, Herrsching, Germany) according to the standard 10–20 layout: Fp1-Fp2-AFz-F7-F3-Fz-F4-F8-FC5-FC1-FCz-FC2-FC6-T7-C3-Cz-C4-T8-TP9-CP5-CP1-CP2-CP6-TP10-P7-P3-Pz-P4-P8-O1-O2-Iz. Electrodes were POz-referenced and FPz-grounded. Channel impedance was kept below 5 kΩ. An electromyogram (EMG) was recorded from the extensor carpi radialis muscle (ECR) using a pair of Ag/AgCl surface electrodes connected to a bipolar channel. EEG and EMG were filtered (DC-3.5 kHz) and sampled at 10 kHz using a TMS-compatible system (NeurOne, Bittium, Finland). EMG and EEG were recorded during three 1-minute blocks of RET and rest tremor.

TABLE 1. Patients demographic and clinical characteristics

Pz	Sex	Age (Years)	Duration Disease (Years)	H&Y	Tremor Side	Rest Tremor Severity[a]	RET Severity[b]
1	M	73	2	2	Right	6	3
2	F	80	8	2	Right	4	2
3	F	42	1	1.5	Left	5	3
4	F	79	9	2	Left	4	2
5	M	66	10	2	Right	6	3
6	M	68	8	2	Right	6	3
7	M	62	1	1.5	Right	4	2
8	M	70	2	2	Right	6	3
9	F	61	3	2	Right	5	3
10	M	65	1	1	Right	5	2
Average ± SD		67 ± 11	5 ± 4	1.8 ± 0.4		5 ± 1	3 ± 1

F, female; M, male; H&Y, Hoehn & Yahr.
[a]Sum of tremor scores in UPDRS part III subitems for amplitude and constancy of rest tremor in the more tremulous upper limb.
[b]Tremor score in UPDRS part III subitem for postural tremor amplitude in the more tremulous upper limb.

Tremor Resetting

EMG was recorded as in experiment 1 but using a D360 (Digitimer, Herfordshire, UK) and sampled at 5 kHz with a CED 1401 A/D interface (CED, Cambridge, UK). We used a Magstim 200² stimulator connected to a figure-of-eight coil (Magstim, Whitland, UK) to deliver monophasic single-pulse TMS (spTMS) over the M1 contralateral to the tremor side on the hot spot evoking the largest motor-evoked potential (MEP) in the ECR. We held the coil tangential to the scalp to induce a posteroanteriorly directed current perpendicular to the central sulcus. Neuronavigation (Softaxic, EMS, Italy) was used to monitor coil positioning. We used the intensity that evoked a MEP of 1-mV amplitude at rest. We assessed resetting separately in RET and rest tremor through 5 blocks of spTMS, each consisting of 8 trials with randomly varying intertrial intervals between 7.5 and 12.5 seconds. Blocks were separated by 30 seconds of rest.

TMS-Evoked Potentials

An EEG was recorded as in experiment 1, online filtered (DC-3.5 kHz) but sampled at 80 kHz from FC5-FC1-C3-Cz-CP5 and CP1 for right tremor and from FC2-FC6-C4-Cz-CP2 and CP6 for left tremor. Electrodes were referenced to POz and grounded to FPz. We limited TEP recording to 6 channels over the sensorimotor cortex according to the aim of this study. We used a biphasic stimulator (Magstim SuperRapid²) to be able to deliver TMS pulses with a short interstimulus interval (see below). The stimulator was connected to a figure-of-eight coil to deliver biphasic spTMS over the M1 hot spot with an intensity equal to 80% of the active motor threshold, defined as the lowest stimulus intensity that elicited a MEP ≥ 200 µV in 5 of 10 trials during sustained voluntary wrist extension (0° angle between hand and forearm). We used subthreshold intensity to minimize possible tremor resetting. TMS was delivered every 500 milliseconds ±15% for 60 blocks consisting of rest tremor, wrist extension, and RET. We used this short interstimulus interval to obtain high temporal resolution in probing the cortical dynamics associated with a phenomenon (RET onset) of unpredictable latency. We used noise masking and placed a thin layer of foam between the coil and scalp to avoid TEP contamination by auditory and hypothetical somatosensory responses evoked by TMS.[18]

Analysis
Corticomuscular Coherence and Granger Causality

EEG and EMG recordings were analyzed in Matlab (R2017b, The Mathworks, Natick, MA) using Fieldtrip.[19] A detailed description of CMC and G-causality analyses is provided in the supplemental information section.

CMC was considered significant when its value exceeded the upper 95% confidence limit based on the assumption of independence.[20] To assess the directionality of the interaction between M1 and EMG activity, we computed the nonparametric G-causality between the 2 signals in both M1-to-EMG and EMG-to-M1 directions.[21] We considered G-causality significant when values exceeded a threshold determined by a trial-based random permutation approach.[11] We ran a 2-way repeated-measures analysi of variance (rmANOVA) on CMC peak scores with tremor (RET, rest tremor) and frequency (tremor frequency, double tremor frequency) as main factors and a 3-way rmANOVA on G-causality peak scores with tremor, frequency and direction (M1-to-muscle, muscle-to-M1) as main factors.

Tremor Resetting

Tremor resetting was assessed as shown in previous studies by calculating the average resetting index (RI) of the 5 tremor bursts following TMS (Fig. 1; see supplemental information).[12-14] Tremor resetting was considered significant when RI values were statistically different from a hypothesized mean of zero as tested by a 1-sample t test.[14] We measured the stability of tremor resetting by measuring the ratio of the RI between the first and fifth tremor bursts after TMS. Tremor resetting was considered stable when the RI1/RI5 ratio was not statistically different from a hypothesized mean of 1. We used a paired t test to compare the RI and RI1/RI5 ratio between RET and rest tremor.

TMS-Evoked Potentials

TEPs analysis was performed with Matlab using Fieldtrip,[19] EEGLAB,[22] and TESA.[23] A detailed description of TEPs analysis is provided in the supplemental information section. Based on visual inspection of EMG, EEG trials were classified as the following conditions: (1) rest tremor, (2) posture onset, (3) posture holding, (4) RET-onset minus 2, (5) RET-onset minus 1, (6) RET onset, and (7) RET (Fig. 2). TEPs were obtained by averaging across EEG trials for each condition. TEPs at C3/C4 channels were then transformed to the orthogonal source derivation according to the Hjorth referencing technique to reduce the contamination from sources outside M1[24] and were rectified. Analysis was limited to C3/C4 channels and early TEPs as recent TMS-EEG studies showed they are within the region and time of interest respectively, that best reflects changes in M1 local excitability.[25,26] The average RET latency duration measured from posture onset to RET onset was 5.44 ± 0.76 seconds (range, 3.2–9.4 seconds).

Data normality was tested by the Shapiro-Wilk test. A 2-way rmANOVA was run for TEPs amplitude with tremor condition (7 levels; see above) and TEPs component (P30, N45, and P60) as main factors. A
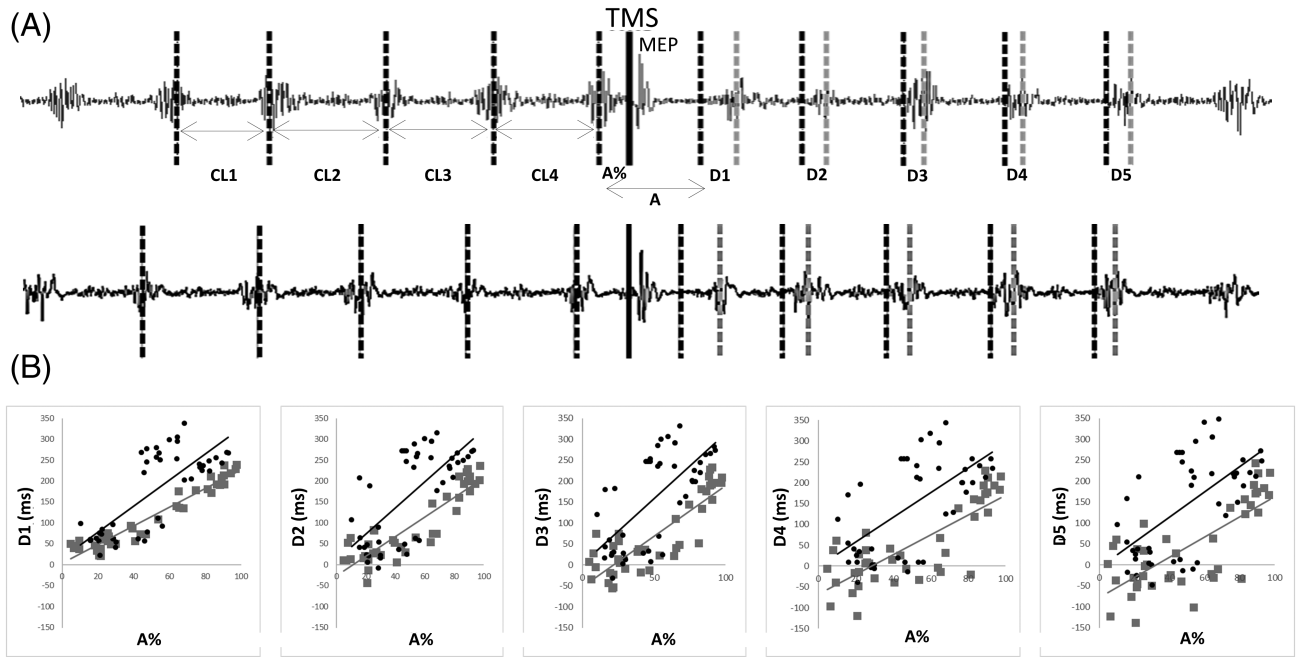
FIG. 1. Tremor resetting analysis. (**A**) Example of tremor-resetting measurement in a single EMG trial from RET (upper trace) and rest tremor (lower trace) in the same patient. CL1–CL4, cycle lengths; A, average tremor cycle length; A%, time from the fifth tremor burst to TMS as a percentage of A; D1–D5, difference between predicted and actual peak times following TMS. (**B**) Example of tremor-resetting index (RI) in 1 patient. Data points represents 1 trial during RET (black circles) and rest tremor (gray squares). The RI of each of the 5 bursts following TMS was calculated as the slope of the regression lines for RET (black line) and rest tremor (gray line).[10-12]
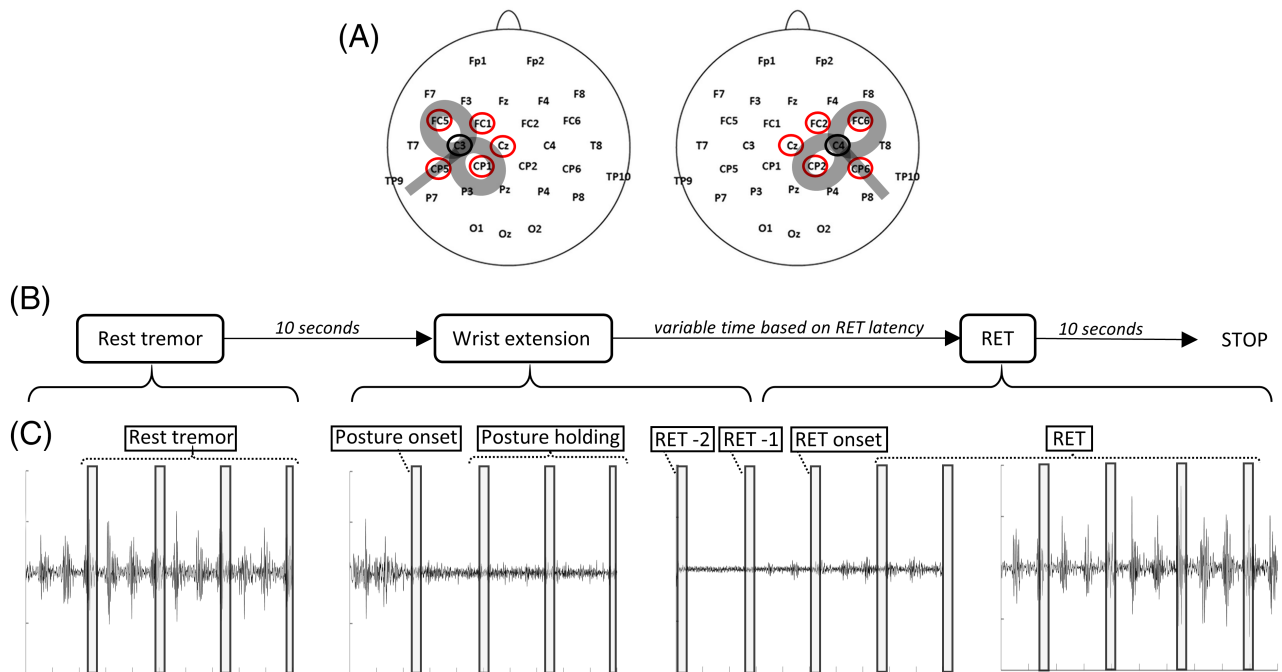

FIG. 2. TEPs methods. (**A**) TMS EEG setup. EEG was recorded during TMS from the circled channels in patients with right-dominant tremor (left) and left-dominant tremor (right). (**B**) Each block consisted of: (1) 10 seconds of rest tremor, followed by (2) voluntary wrist extension (variable duration), and finally (3) re-emergent tremor (RET), 10 seconds. (**C**) TEPs conditions. Vertical bars represent time range of TMS delivered during 1 exemplificative block; labels show how TEPs conditions were defined based on the EMG from the ECR muscle. [Color figure can be viewed at wileyonlinelibrary.com]

$P < 0.05$ was considered significant. Multiple paired t tests were carried out as post hoc analysis with a false discovery rate correction threshold of 0.1. Spearman's rank order was run to assess the correlation between TEP changes during tremor suppression (calculated as (1 − [posture holding/rest tremor]) and RET latency duration.

Results

Corticomuscular Coherence and Granger Causality

Data were expressed as mean ± standard error. RET showed a main EMG peak at 4.74 ± 0.13 Hz (tremor frequency) and a second peak at 9.40 ± 0.22 Hz (double tremor frequency). The EEG over C3/C4 contralateral to the RET side showed a peak corresponding to RET frequency (4.60 ± 0.12 Hz) and a second peak at around double tremor frequency (9.40 ± 0.22 Hz). All patients showed significant CMC at tremor frequency and double tremor frequency during RET.

Rest tremor showed a main EMG peak at 4.27 ± 0.14 Hz and a second peak at 8.73 ± 0.27 Hz. The EEG over C3/C4 contralateral to rest tremor side showed a peak corresponding to rest tremor frequency (4.30 ± 0.15 Hz) and a second peak at around double tremor frequency (8.50 ± 0.31 Hz). All patients showed significant CMC at tremor frequency and double tremor frequency during rest tremor. RET had a significantly higher EMG frequency compared with rest
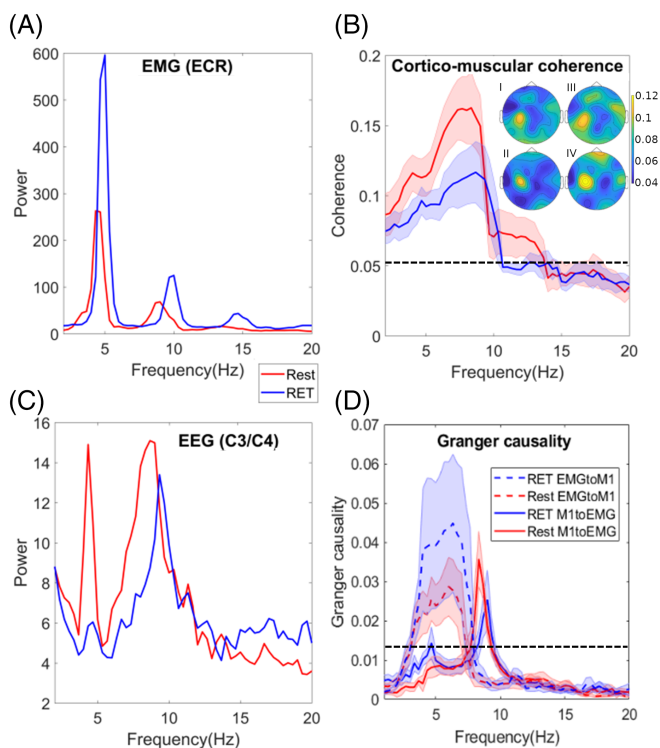
tremor both at tremor frequency ($t = 4.11$, $P = 0.003$) and double tremor frequency ($t = 3.38$, $P = 0.008$); see Figure 3A. Also, RET showed a significantly higher EEG frequency peak compared with rest tremor at double tremor frequency ($t = 3.02$, $P = 0.015$), but only a trend toward a higher frequency at tremor frequency ($t = 2.21$, $P = 0.055$); see Figure 3B. ANOVA for CMC showed a nonsignificant tremor × frequency interaction ($F_{1,9} = 2.05$, $P = 0.186$). There was a significant frequency main effect ($F_{1,9} = 7.36$, $P = 0.024$) explained by significantly higher CMC at double tremor frequency compared with tremor frequency (0.146 ± 0.02 vs 0.106 ± 0.01, $P = 0.024$). There was a nonsignificant trend for lower CMC values during RET than rest tremor both at tremor frequency (0.09 ± 0.01 vs 0.12 ± 0.12) and double tremor frequency (0.12 ± 0.02 vs 017 ± 0.02, $F_{1,9} = 4.46$, $P = 0.064$). Topographical plots of both RET and rest tremor showed that maximal CMC values were over the centrolateral cortex contralateral to the tremor side. Compared with rest tremor, RET showed a slightly wider distribution of CMC values, with a more posterior extension toward the parietal cortex (Fig. 3C). G-causality in the M1-to-EMG direction was significant at tremor frequency in 6 patients during RET and in 4 patients during rest tremor, whereas at double tremor frequency it was significant in all but 1 patient in both tremors. G-causality in the EMG-to-M1 direction was significant at tremor frequency in 8 patients during RET and in all 10 patients during rest tremor, whereas at double tremor frequency it was significant only in 1 patient in both tremors. ANOVA for G-causality showed a significant direction × frequency interaction ($F_{1,9} = 10.70$, $P = 0.010$), but no significant main effect of tremor ($F_{1,9} = 1.25$, $P = 0.293$), frequency ($F_{1,9} = 2.43$, $P = 0.154$), or direction ($F_{1,9} = 0.05$, $P = 0.822$), and no significant direction × tremor ($F_{1,9} = 3.10$, $P = 0.112$), frequency × tremor ($F_{1,9} = 3.89$, $P = 0.080$), or direction × tremor × frequency ($F_{1,9} = 0.41$, $P = 0.541$) interactions. Post hoc analysis showed that G-causality at tremor frequency was significantly lower in the M1-to-ECR direction than in the ECR-to-M1 direction (0.014 ± 0.003 vs 0.040 ± 0.007, $F_{1,9} = 5.32$, $P = 0.047$), whereas at double tremor frequency G-causality was higher in the M1-to-EMG direction (0.033 ± 0.007 vs 0.006 ± 0.002, $F_{1,9} = 21.45$, $P = 0.001$); see Figure 3D.

FIG. 3. Corticomuscular coherence and Granger causality results. All figures represent mean values across all patients. Shaded regions, standard error; dashed horizontal lines, threshold for statistical significance. (**A**) EMG power spectrum from the ECR muscle. (**B**) EEG power spectrum from C3/C4 channel. (**C**) corticomuscular coherence spectra and topoplots for values at tremor frequency in RET (I), end rest tremor (II), and at double tremor frequency for RET(III), and rest tremor (IV), in patients with left tremor. EEG channels were reversed with respect to the midline before plotting topoplots). (**D**) Granger causality spectrum. [Color figure can be viewed at wileyonlinelibrary.com]

Tremor Resetting

The average 1-mV MEP intensity was $68\% \pm 8\%$ of the maximum stimulator output. Data from 1 representative subject is shown in Figure 1. The average RI of RET was significantly different from zero (2.07 ± 0.10; $t = 12.352$, $P < 0.001$), whereas the RI1/RI5 ratio proved not to be statistically different from 1. Rest tremor also showed a significant RI (2.16 ± 0.17, $t = 20.387$, $P < 0.001$) and
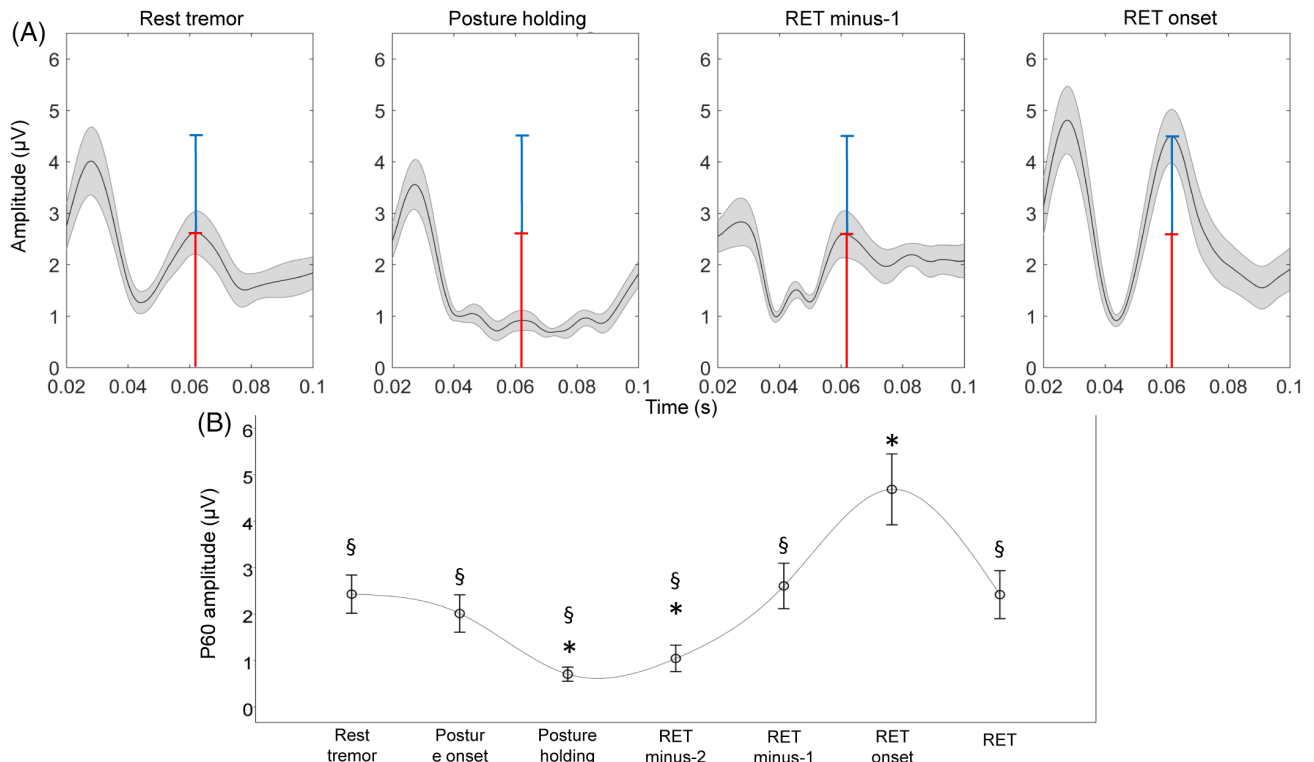
FIG. 4. TMS-evoked potential (TEP) results — Hjorth reference. (**A**) Grand average rectified TEPs at electrode C3/C4 in 4 representative conditions after TMS. Vertical lines indicate P60 amplitude during rest tremor (red) and RET onset (blue). Shaded regions, standard error. (**B**) P60 mean amplitudes across conditions. Error bars, standard error. *Post hoc significant versus rest tremor; §post hoc significant versus RET onset. See Figure 2C for definition of conditions. See Figure S3 for results with POz reference. [Color figure can be viewed at wileyonlinelibrary.com]

a RI1/RI5 ratio not statistically different from 1 (1.00 ± 0.08, $t = 0.01$, $P = 0.992$). Paired t test showed nonsignificant differences between RET and rest tremor in RI ($t = 0.70$, $P = 0.504$) and the RI1/RI5 ratio ($t = 0.58$, $P = 0.574$).

TMS-Evoked Potentials

TEPs at C3 showed average peak times across conditions of 28.1 ± 0.2, 45.5 ± 0.4, and 63.0 ± 0.6 milliseconds, consistent with the previously described components P30, N45, and P60, respectively (Fig. S2).[27,28] Peak amplitude was normally distributed in each condition, as assessed by the Shapiro-Wilk test ($P > 0.05$). ANOVA showed a significant main effect of condition ($F_{6,54} = 6.474$, $P = 0.003$), component ($F_{2,12} = 11.311$, $P = 0.001$), and a significant condition × component interaction ($F_{12,108} = 4.426$, $P < 0.001$). Follow-up ANOVA showed a simple main effect of condition for the P60 component ($F_{6,54} = 12.947$, $P < 0.001$), which was explained by a significantly higher P60 amplitude during rest tremor (2.43 ± 0,4) compared with posture holding (0.71 ± 0.15, $P = 0.004$), and RET-onset minus 2 (1.05 ± 0.29, $P = 0.008$), and a significantly higher P60 amplitude at RET onset (4.69 ± 0.76) compared with rest tremor ($P = 0.003$), posture onset (2.01 ± 0.4, $P = 0.004$), and posture holding ($P < 0.001$), RET-onset minus 2 ($P < 0.001$), RET-onset minus 1 (2.61 ± 0.49, $P = 0.014$), and RET (2.42 ± 0.52, $P = 0.004$). No significant effect of condition was found for P30 ($F_{6,54} = 1.289$, $P = 0.278$) and N45 amplitude ($F_{6,54} = 1.684$, $P = 0.143$); see Figure 4 and Figures S3–S4. There was a significant positive correlation between P60 suppression during posture holding and latency duration ($rs_{10} = 0.936$, $P < 0.001$; Fig. S5).

Confidence intervals and effect sizes are provided in the supplemental information section.

Discussion

In the present article, we demonstrated several neurophysiological features of RET. Frequency analysis of RET showed a first EMG peak at the basic tremor frequency and a second peak at about double tremor frequency. Similarly, the EEG over M1 contralateral to the tremor side showed two peaks that corresponded to the frequency of the EMG peaks. A significant CMC was present between EEG and EMG peaks. G-causality at tremor frequency was predominant in the muscle-to-M1 direction, whereas at double tremor frequency it was predominant in the M1-to-muscle direction. TMS over M1 induced a significant resetting of RET. TEP recording over M1 showed that the suppression of rest

tremor during posture holding and RET appearance was associated with significant modulation of the TEP P60. Compared with rest tremor, RET had slightly higher EMG frequency, but the two tremors had similar CMC and G-causality values and showed similar reset after TMS over M1.

We took several precautions to minimize confounding biases. We excluded patients with head tremor to avoid bias in CMC analysis because of mechanical transmission to EEG channels. TMS EEG was performed according to the most recent standards to minimize the possibility of TEP contamination by nontranscranial TMS-associated evoked potentials.[29,30] In addition, because we kept the TMS parameters consistent during the experiment, any possible contamination by sensory potentials would have been stable across tremor conditions and could not explain the modulation of TEPs we observed. TEPs were elicited with TMS below the motor threshold to limit bias because of corticospinal activation such as tremor resetting.

The first result of this study is that during RET, EMG and EEG activity were coherent at tremor-frequency. We localized the source of the oscillatory activity coherent with RET over EEG electrodes corresponding to the contralateral sensorimotor cortex. Tremor analysis also showed a second CMC peak at about double tremor frequency in both RET and rest tremor.[31,32] Previous authors have interpreted this second peak as either a technical artifact[33,34] or a physiological phenomenon.[8,35-37] G-causality showed that at tremor frequency, the information flow was in the muscle-to-M1 direction, whereas at about double tremor frequency, it was predominantly in the M1-to-muscle direction. These results suggest that corticomuscular coupling at tremor frequency mainly reflects proprioceptive input generated by limb movements,[38,39] whereas the coupling at double tremor frequency reflects the M1 output that participates in driving the tremor.[34,40-42]

TMS over M1 produces a tremor resetting that cannot be entirely explained by sensory feedback associated with MEP elicitation,[43,44] but rather by interference with M1 intracortical or basal ganglia activity through cortical-subcortical connections such as the hyperdirect pathway.[12-14,45,46] However, previous evidence has directly linked tremor resetting to M1 intracortical mechanisms, thus limiting the possibility that the reset was obtained solely via the activation of M1-to-basal ganglia connections.[12] We found that the resetting of the first tremor burst after TMS was similar to the resetting of the fifth tremor burst after TMS, indicating a stable rather than a transient resetting. The stable resetting we found suggests that TMS over M1 directly affected the RET-generating network by setting its oscillatory activity to a new fixed point in its cycle, whereas a transient resetting would have indicated that the oscillator itself was not affected. Therefore, stable resetting indicates that M1 is a very critical node in the corticosubcortical network that generates RET and is not just an output. Finally, the results on resetting suggest that RET originates from instability of network activity involving M1 and is not from an independent oscillator.

In our study, we observed that the amplitude of TEP P60 reduced during tremor suppression by holding the wrist extended. P60 recovered fully before tremor reappeared and was facilitated at the onset of RET. However, the P60 facilitation observed at RET onset disappeared during the tremor, and P60 amplitude returned to values similar to rest tremor. Some evidence has shown that TEPs generated by M1 TMS are a reproducible index of cortical excitability and connectivity.[15,27,29] In normal subjects P60 is mainly distributed over the precentral gyrus with posterior spread toward the somatosensory cortex,[47] and its amplitude is affected by TMS protocols known to reflect M1 excitability[24,25,48,49] and sensory input.[50,51]

Because we delivered TMS below the threshold for eliciting a MEP, the P60 modulation we observed cannot be explained by sensory feedback because of muscle twitch.[46] The direct correlation we found between RET latency and P60 inhibition during posture holding likely suggests that P60 modulation reflects changes in M1 excitability associated with tremor. Therefore, the possibility that P60 amplitude changes reflect different neurophysiological properties in the different experimental conditions is unlikely. Despite recent evidence of an inverse correlation between RET latency and RET amplitude,[52] we found that P60 had its maximum amplitude at RET onset, when tremor amplitude is minimal. Therefore, we believe that P60 modulation reflects changes in M1 excitability directly related to tremor, independent of its amplitude. In this context, P60 decrement indicates M1 inhibition during tremor suppression; when P60 recovers, tremor returns as RET, indicating a very critical role of M1 excitability in the tremor-generating network. M1 inhibition during RET latency may be because of either increased intracortical inhibition or proprioceptive input elicited by postural change affecting the cerebello-thalamo-cortical network via the spinocerebellar pathway.[53] Similarly, M1 facilitation at RET onset may indicate either an increased intracortical facilitation, or it could first arise in the GPi, the nucleus that has been suggested to "switch on" tremor by activating the cerebello-thalamo-cortical circuit via M1.[54,55]

Finally, novel findings were provided by the comparison between RET and rest tremor in the same patients. We found that RET and rest tremor had similar EMG peaks, EEG peaks, CMC, and G-causality. However, compared with rest tremor, RET showed a slightly

higher EMG frequency of the tremor peaks[4] and a slightly wider CMC distribution extending toward the parietal cortex. The higher frequency of the RET EMG peaks compared with that in rest tremor may be because of the interaction of a central oscillator with different mechanical resonance of the limbs during posture compared with rest. In fact, small differences between RET and rest tremor frequency could be from a different proprioceptive input associated with different mechanical conditions of the limb during posture holding and rest.[56] Concordantly, the different distribution of CMC, which extends toward the parietal cortex in RET, suggests a larger contribution of somatosensory input. Because we found a similar amount and stability of reset for RET and rest tremor, our results suggest that M1 plays a similar role in RET and rest tremor and that resetting was most likely achieved by the perturbation of a common central oscillator.

We acknowledge several limitations. Movements related to limb tremor may be mechanically transferred to the head, thus possibly producing spurious coupling between EMG and EEG signals distributed over the whole scalp. However, the observation that maximal CMC was distributed over the sensorimotor cortex contralateral to the tremor side limits the possibility that CMC may be biased by mechanical transmission of tremor from the arm to the head. Albeit TEPs recorded at the C3/C4 level are thought to be from M1 activity, a contribution from the primary somatosensory cortex because of volume conduction cannot be ruled out. However, because TEPs were evoked using low-intensity anterior-posterior directed, and neuronavigated TMS over a motor hot spot, we believe that our stimulation was highly focal on M1. Moreover, the Hjorth rereferencing technique we used on C3/C4 channels attenuates the contribution of sources in neighboring areas. Therefore, we believe our TEPs primarily reflected M1 activity. Recording from a larger number of channels could have provided additional information on the topographical distribution of TEP changes, and we therefore recognize this as a limitation of our study. Also, because we did not record peripheral channels, residual muscle activity, and eye artifacts could not be removed by independent component analysis. However, the effect of these artifacts on our channels of interest (C3/C4) would have been minimal and conveyed only through volume conduction, a phenomenon that we reduced by Hjorth rereferencing our TEPs. The relatively low number of TEP trials could result in a low signal-to-noise ratio. However, a larger number of blocks would have induced fatigue, and our TEP analysis had enough power to identify significant changes in P60 amplitudes. Although TMS was delivered with a random jitter, possible bias from repetitive TMS cannot be completely ruled out. However, any confounding effect produced by repetitive TMS would have shown a linear trend over time. The nonlinear effect we observed thus likely reflects a specific effect of the conditions investigated.

In conclusion, G-causality on CSD EEG provided evidence that source activity at the M1 level participates in RET.[21] We also found that M1 TMS caused stable RET resetting. Finally, our experiment using TEPs demonstrated M1 excitability changes before and during RET. Overall, our findings support the hypothesis that M1 plays a role in the RET-generating circuit.[54,55,57-60] Knowing the mechanisms underlying tremor-related changes in M1 activity may open new perspectives for possible therapeutic interventions. ■

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