Συσχετισμένοι νευρικοί ρυθμοί ως βάση παραγωγής τρόμων σε υγεία και κινητικές διαταραχές. Ο ρόλος τους στο ρυθμικό έλεγχο εκούσιων μυικών συστολών

Διδακτορική Διατριβή

Ορσαλία Μ. Αγαπάκη

Επιβλέπων Καθηγητής Αντώνης Μοσχοβάκης

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Synchronized neural rhythms as a basis of tremor production in health and movement disorders. Their role in rhythmical control of voluntarily produced muscle contraction

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Orsalia M. Agapaki

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To C.N. Christakos

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ΠΕΡΙΛΗΨΗ

Ο τρόμος ηρεμίας καθώς και ο τρόμος θέσης, συνιστούν κοινά χαρακτηριστικά της νόσου του Πάρκινσον. Αποτελούν ακούσιες ρυθμικές ταλαντώσεις ενός τμήματος του σώματος και η συχνότητά τους κυμαίνεται μεταξύ 4 και 8 Hz. Οι νευρικοί μηχανισμοί παραγωγής τους έχουν ευρέως μελετηθεί. Η μεγάλη πλειοψηφία των ερευνητών έχουν εστιάσει την προσοχή τους στις αντίστοιχες ταλαντώσεις υπερνωτιαίων περιοχών και κυρίως στα βασικά γάγγλια, τον κινητικό φλοιό και τον θάλαμο. Παρ' όλα αυτά η κατανόηση αυτών των μηχανισμών παραμένει περιορισμένη.

Στη παρούσα μελέτη, υιοθετώντας διαφορετική προσέγγιση, εξετάσαμε τα πρότυπα πυροδότησης και συγχρονισμού των κινητικών μονάδων σε τρόμους άκρων. Αυτές οι συμπεριφορές καθορίζουν τα γνωρίσματα του μυικού τρόμου δύναμης καθώς και των συσχετιζόμενων νευρικών συνιστωσών των τρόμων άκρων. Επιπροσθέτως, μεταφέρουν πληροφορίες οι οποίες σχετίζονται με τον τρόμο και αφορούν στη συναπτική είσοδο των κινητικών νευρώνων. Για το λόγο αυτό, καταγράψαμε τη στιγμιαία επιτάχυνση άνω άκρων, στην ηρεμία και κατά τη διατήρηση θέσης, ασθενών με τη νόσο του Πάρκινσον και υγιών εθελοντών. Ταυτόχρονα, καταγράψαμε επιφανειακό ΗΜΓ και εκφορτίσεις μεμονομένων κινητικών μονάδων από μύες των άνω άκρων. Τα πρότυπα πυροδότησης μελετήθηκαν με φασματικές αναλύσεις και αναλύσεις των μεσοδιαστημάτων ώσεων. Ο συγχρονισμός μετρήθηκε με τη χρήση υπολογισμών συνάφειας και ετεροσυσχέτισης μεταξύ εκφορτίσεων κινητικών μονάδων και επιφανειακού ΗΜΓ.

Τα αποτελέσματα ανέδειξαν δύο συνιστώσες στα ιδιοφάσματα τρόμου, με απόσταση μεταξύ τους περίπου 1.5 Hz, τόσο στην ηρεμία όσο και στην προσπάθεια διατήρησης θέσης. Αυτές οι συνιστώσες αντανακλούν διαφορετικά διαστήματα περιόδων δραστηριότητας που εναλλάσσονται τυχαία και ονομάζονται περίοδος-I και -II. Η περίοδος-I ομοιάζει φυσιολογικό τρόμο με την έννοια ότι χαρακτηρίζεται από μη ορατό τρόμο συχνότητας 6-10 Hz, συγχρονισμό κινητικών μονάδων με φυσιολογική ισχύ και ρυθμική πυροδότηση κινητικών μονάδων στην ιδιοσυχνότητα της κάθε μονάδας. Από την άλλη, η περίοδος-II χαρακτηρίζεται από ορατό τρόμο συχνότητας 4-8 Hz, ισχυρό συγχρονισμό κινητικών μονάδων και παρουσία δυάδων ή τριάδων ώσεων σε ένα προς ένα σχέση με κάθε κύκλο τρόμου. Η συχνότητα του μη ορατού και ορατού τρόμου, καθώς και των υπόλοιπων χαρακτηριστικών των περιόδων I και II, παρέμειναν αμετάβλητα τόσο κατά την ηρεμία όσο και κατά την προσπάθεια διατήρησης θέσης για κάθε ασθενή. Η επίπτωση των δυάδων και τριάδων ώσεων εμφάνιζε αυστηρή κανονικότητα, ενώ τα μεσοδιαστήματά τους που βρίσκονταν στο εύρος 30-50 ms (βήτα ρυθμός), παρουσίαζαν μέσες τιμές οι οποίες ήταν σταθερές για κάθε ασθενή, χωρίς να σχετίζονται με τη συχνότητα πυροδότησης της κινητικής μονάδας ή τον τύπο του τρόμου κατά τον οποίο είχε καταγραφεί η δραστηριότητα της κινητικής μονάδας. Αντίθετα, οι μέσες αυτές τιμές των μεσοδιαστημάτων των ώσεων σχετίζονταν μόνο με τη διαφορά μεταξύ των περιόδων του ορατού και του μη ορατού τρόμου του κάθε ασθενούς.

Συνοψίζοντας, τα ευρήματά μας αναδεικνύουν την παρουσία κοινών χαρακτηριστικών της πυροδότησης των κινητικών μονάδων για κάθε μία από τις δύο περιόδους, τόσο στην ηρεμία όσο και στην προσπάθεια διατήρηση θέσης, προτείνοντας έτσι ένα μοντέλο που περικλείει δύο εναλλασσόμενες γεννήτριες τρόμου των οποίων οι συχνότητες συνδέονται. Η ασυνήθης πυροδότηση των κινητικών μονάδων στην περίοδο-II αποτελεί τη βάση της ιδιαίτερα αυξημένης

ιδιο-φασματικής συνιστώσας η οποία αντιστοιχεί στον ορατό τρόμο. Κατα συνέπεια, αντανακλά ιδιαίτερα γνωρίσματα της συναπτικής εισόδου στους κινητικούς νευρώνες τα οποία σχετίζονται με τον τρόμο και άρα αποτελεί κατάλληλο πεδίο μελέτης της γεννήτριας του τρόμου. Βάσει των ανωτέρω, προτείνουμε ότι ο βρόχος του νωτιαίου μυοτατικού αντανακλαστικού, ο οποίος στη νόσο του Πάρκινσον παραμένει ανέπαφος, λειτουργεί ως ένας ταλαντωτής δύο φάσεων. Αυτή η θεώρηση θα μπορούσε να επιτευχθεί μέσω διαλείποντως κατερχόμενων, υπερνωτιαία παραγόμενων, βήτα ταλαντώσεων που αλληλεπιδρούν με το βρόχο του νωτιαίου μυοτατικού αντανακλαστικού προκαλώντας την παραγωγή του ορατού τρόμου. Επιπροσθέτως, θα πρέπει να τονιστεί ότι η ανιούσα προσέγγιση που ακολουθήθηκε σε αυτή τη μελέτη, θα μπορούσε να διευκολύνει τη διερεύνηση του τρόμου και σε άλλες κινητικές διαταραχές.

SUMMARY

The emergence of tremor at rest or while a stable posture is maintained are both common signs of Parkinson's disease. These involuntary rhythmical oscillations of a body segment are exhibited in a frequency range between 4 to 8 Hz. The neural mechanisms underlying their generation have been studied extensively. The great majority of the researchers have focused their attention on corresponding oscillations at supraspinal sites, namely basal ganglia, motor cortex and thalamus. However, our understanding of these mechanisms remains limited.

In this study, we alternatively examined the firing patterns and synchrony of motor units during limb tremors. These behaviors determine the features of muscle force tremors and associated neurogenic components of limb tremors. They additionally carry information on the tremor-related synaptic input to the motoneurons. We recorded the instantaneous acceleration of the upper limb of Parkinson's disease patients and control subjects at rest and while they tried to hold a stable posture. Simultaneously, we recorded surface EMG and single motor unit discharges of upper-limb muscles. The firing patterns were studied via spectral and interspike interval analyses. The synchrony was measured using coherence and cross-correlation computations between motor unit discharges and surface EMG.

Our results demonstrated two auto-spectral components, ca. 1.5-Hz apart, for the patients' rest and postural tremor. These components represented different intervals of randomly interchanged activity epochs, termed I and II. Epoch-I resembled physiological tremor in that it was characterized by 6-10 Hz non-overt tremor, motor unit synchrony with normal strength, and rhythmical motor unit firing at the intrinsic rate of the unit. In contrast, epoch-II intervals were

characterized by the presence of 4-8 Hz overt tremor, enhanced motor unit synchrony, and spike-doublets or triplets bearing a one-to-one relation to each tremor cycle. The frequency of non-overt and overt tremor, along with the remaining characteristics of epoch-I and epoch-II intervals, did not differ whether a patient was at rest or held a stable posture. Spike-doublets and triplets had incidences obeying a strict rule, and exhibited interspike intervals in the 30-50-ms (beta) range with mean values that remained roughly constant for any given patient, irrespective of motor unit rate and tremor type. On the contrary, these mean values of the interspike intervals were correlated only with the difference between the periods of overt and non-overt tremor.

Overall, our findings reveal shared features of motor unit firing for either of the two epochs whether a patient was at rest or held a stable posture, thus suggesting an encompassing model of alternating, frequency-linked neural generators of tremor. The abnormal motor unit firing in epoch-II intervals underlies the greatly enhanced auto-spectral component which corresponds to overt tremor. It also likely reflects distinct features of the tremor-related synaptic input to the motoneurons, thus providing a useful basis for studying the generator of such tremor. On this basis, we propose that the spinal stretch reflex loop, which in Parkinson's disease remains intact, acts as a two-state oscillator. This conception could be succeeded through intermittently descending, supraspinally produced, beta oscillations that interact with the spinal stretch reflex loop, inducing a transition to overt tremor. Other than that, our bottom-up approach could in any case facilitate tremor studies in other movement disorders.

INTRODUCTION

Tremor is defined as a rhythmical, involuntary oscillatory movement of a body part (Deuschl et al., 1998). The evaluation of tremor includes several phenomenological components of this movement, such as the condition under which the tremor is exhibited (i.e. at rest or in action), the tremor topography and its frequency. It constitutes the most common motor phenomenon found both as a pathological symptom (i.e. Parkinson's disease, essential tremor, cerebellar tremor, e.t.c.) and in normal individuals. Thus, it has been the subject of numerous neurophysiological and clinical studies. However, the neural basis of tremor constitutes an open question for decades (McAuley & Marsden, 2000).

There exist hypotheses supporting the notion of central generation of physiological tremor. Elble and Randall (1976) were the first to show coherent motor unit (MU) tremor rhythms, implicating the Renshaw cell system in the tremor production. Supraspinal sources include the inferior olive (Llinas and Yarom, 1986), the cortex and subcortical areas where neural rhythmical activities coherent with the tremor have been observed (McAuley and Marsden, 2000; Raethjen et al., 2002). However, any of these generators, is expected to interact with the spinal stretch reflex loop regarding the tremor synchrony and its features. Strong evidence has been presented in support of either of the two major hypotheses on the neural mechanism of the 6-10-Hz physiological tremor generated at the spinal cord level. The first one is concluded in the rhythmical discharges of the last recruited MUs that fire uncorrelated within a narrow frequency band or with weak correlations (Allum et al., 1978; Christakos, 1982b). The second hypothesis implicates action in the monosynaptic stretch reflex loop, which induces

synchronized (in-phase) MU firing (Christakos et al., 2006) at a frequency corresponding to the delays of signal transmission around the loop (Lippold, 1970; Hagbarth and Young, 1979).

Parkinson's disease (PD) is one of the most common neurological disorders, affecting approximately 1% of individuals older than 60 years. The two major pathophysiological findings are the loss of pigmented dopaminergic neurons of the substantia nigra pars compacta and the presence of Lewy bodies. The common motor signs of PD are tremor, bradykinesia, rigidity and dystonia. Resting tremor is one of the cardinal signs of PD while postural tremor is often present as well, both occurring in the 4-8-Hz range (Bain, 2002). Resting tremor is evaluated while patients are asked to relax, being completely supported against gravity. This type of tremor has been studied for the most part separately from postural tremor. The latter coexists with muscle activity opposing gravity maintaining a steady position of a body segment. Several investigators have tried to identify the neural mechanisms underlying various forms of limb tremors in PD (Louis et al., 2001; Brown and Williams, 2005; Helmich et al., 2012).

Most investigations of Parkinsonian tremor focused on supraspinal sites and argued for their participation as an integral part in an unstable oscillating network that generates tremor. Basal ganglia, owing to the pathophysiological findings that are observed at those nuclei, constitute a place of interest. Studies focusing on subthalamic nucleus and globus pallidus (Bergman and Deuschl, 2002; Liu et al., 2002; Brown, 2003; Moran et al., 2008), observed rhythmical single-unit activity or local field potential oscillations at the tremor 4-8-Hz frequency at these nuclei. Similar findings derived from recordings at thalamus (Lenz et al., 1988). Furthermore, there are studies supporting the participation of cortical areas in the generation of PD tremor. Using a whole-head magnetoencephalography system, they recorded an increase of neural activity in primary motor cortex and other motor cortical areas at the tremor frequency

(Timmermann et al., 2003; Hirschmann et al., 2013). Helmich et al. (2011), using functional magnetic resonance imaging, proposed that transient activity in basal ganglia trigger tremor-related oscillations in cerebello-thalamo-cortical loop which produces resting tremor. In a few cases, the existence of supraspinal activity at the tremor frequency was corroborated by correlation and coherence observations between such rhythms and tremor (Zirh et al., 1998; Hurtado et al., 1999; Timmermann et al., 2003; Amtage et al., 2008; Reck et al., 2010).

Other investigations, however, obtained results strongly suggesting the involvement of proprioceptive input and peripheral loops or spinal motor systems in tremor generation. Rack and Ross (1986) showed that imposed sinusoidal joint-movements entrained resting tremor. Another study demonstrated that an increase in gravitational load did not affect load-independent tremor component while PD patients maintained a posture against gravity (Burne, 1987). Moreover, rhythmical electrical stimulation applied to a peripheral nerve provoked an increase at the frequency of both EMG activity and acceleration signal (Spiegel et al., 2002). Furthermore, the rhythmical electrical stimulation applied to motor cortex and globus pallidus of vervet monkey that underwent 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine treatment, did not induce movement at the corresponding frequency (Rivlin-Etzion et al., 2008). Although hypotheses assuming their supra-spinal origin are far more popular, the neural basis of PD tremor has not been established yet.

Whether PD tremor can be due to spinal or supra-spinal of mechanisms, it and other limb tremors could also be generated, or at least modified, by the inertial and viscoelastic properties of moving body segments. For example, it has been argued that physiological hand and finger tremors are mainly attributable to resonance effects (Lakie et al., 2012; Vernooij et al., 2013) and do not require a synchronous motoneuronal input. Namely, a peak at a certain frequency was

observed at the spectrum of hand or finger tremor at posture condition. The frequency of this peak altered when subjects exhibited slow movements. On the other hand, EMG activity had no distinguishable peak regardless of the tremor frequency. Consistent with the aforementioned, load-dependent frequencies have been reported for the physiological tremor of both the finger and the hand (Stiles and Randall, 1967; Stiles, 1980). In other words, during small displacement oscillations of hand or finger, mass loading decreased physiological tremor frequency. Interestingly, several studies observed twin peaks harmonically unrelated at the power spectrum of PD hand tremor (Stiles and Pozos, 1976; Walsh, 1979; Homberg et al., 1987; Burne et al., 2004) but only one of the two, presumably the mechanical one, is load dependent (Stiles and Randall, 1967; Stiles, 1980). The mechanical/neural interactions in Stiles and Pozos' (1976) parkinsonian "mechanical-reflex oscillator" represent a compromising point of view.

In order to gain further insight into tremor generating mechanisms in PD, we examined the rhythmical firing synchrony and patterns of motor units in upper-limb muscles during force, rest and postural tremors. Two important reasons guided our approach: first, MU firing synchrony and patterns underlie the generation and features of force tremors and hence, the neurogenic components of limb tremors. The study of MU firing may thus enable the identification of neurogenic tremor components and elucidate their formation mechanisms. Second, as the MU firing synchrony and patterns ultimately reflect converging neural influences, they may carry information in respect of the tremor-related synaptic input to motoneurons (MN). This information may be important for the purpose of exploring the PD tremor generator.

Christakos et al. (2009) observed the presence of intermittently overt 4-8 Hz force tremor during quasi-isometric muscle contractions in PD patients. They demonstrated that (i) the tremor-related synchrony of MUs is enhanced and (ii) single motor unit spike-doublets and

triplets with fairly fixed mean interspike interval (ISI), are locked to the tremor. It was argued that these abnormal firing behaviors underlying large-amplitude force tremors may also provide clues for understanding the generation mechanisms of these tremors and hence, of associated neurogenic components of rest and postural tremors.

The present study focuses on the detection and exploration of such characteristics of MU synchrony and discharge patterns during rest and postural tremors in PD. Moreover, it examines whether these two tremor types, which occur under quite different conditions, differ in terms of the enhancement of MU synchrony and the prevalence of spike-doublets and triplets. Further, a preliminary study investigates whether these firing behaviors could be compared with the ones during isometric force tremor.

AIM OF THE STUDY

We here by examine the following major queries: (i) which are the characteristics of the rhythmical MU firing synchrony and patterns during parkinsonian rest and postural tremor? (ii) are there any differences in MU firing between these two tremor types that occur under quite different conditions? (iii) how are these firing behaviors compared with the ones during isometric force tremor? Further, MU firing appeared highly abnormal over distinct epochs of overt 4-8-Hz tremor, for all three tremor types. We, therefore, also considered separately data from overt-tremor epochs, as compared to data from epochs of small and higher-frequency tremor, which were normal-like. We, subsequently, combined our results to draw conclusions on tremor-related synaptic inputs to MNs, and examine possibilities regarding underlying neural generators. Finally, using the coherence of MU activity to the tremor as main criterion for identification of neurogenic components (see Lakie et al., 2012), we examined the relative impact of mechanical effects on parkinsonian tremors.

Preliminary reports have been given in abstracts (Agapaki *et al*., 2011; 2013; 2016) while the main study has been published earlier (Agapaki et al 2018).

METHODS

Patients and control subjects

Forty-eight PD patients examined but only a fraction of them satisfied the requirements or completed the entire proceeding. To be more precise, twenty-three patients (aged between 52 and 82 years, mean 69 years; male:female, 14:9) with idiopathic PD of tremor-dominant type participated in the study (Table 1). They were mild to moderately affected (UPDRS scores in the 'off' state, before measurements 7-48, mean 19; Hoehn and Yahr stages I, II or III). They had been diagnosed between 6 to 96 months (mean 38 months) before testing. Eighteen age-matched (male:female, 12:6), normal volunteers were the control subjects. All participants gave informed consent. The study conformed to the standards of the latest version of the Declaration of Helsinki, and was approved by the Ethical Committee of the University of Athens.

Table 1 Profile of patients with Parkinson's disease

Patient	Disease duration since diagnosis (months)	Most affected upper limb	UPDRS			Hoehn & Yahr score	Anti-parkinsonian medication
			Motor score part III	Rest hand tremor item 20	Postural limb tremor item 21		
1	90	Left	42	3	1	3	Levodopa (100+25)mg, 2 tabs
2	78	Left	32	3	3	2.5	Levodopa (100+25)mg, 2 tabs Rotigotine 4mg, 1 patch
3	84	Right	32	3	2	3	Levodopa (100+25)mg, 2 tabs
4	60	Right	36	2	3	2.5	Levodopa (100+25)mg, 2 tabs Rotigotine 4mg, 1 patch
5	46	Right	21	2	2	1.5	Biperiden 2mg, 2 tabs Levodopa (100+25)mg, 2 tabs
6	6	Left	13	2	1	2.5	Levodopa (100+25)mg, 1 tab Rasagiline 1mg, 1 tab
7	12	Left	9	1	1	1	No
8	12	Right	8	2	1	2	No
9	12	Left	9	1	1	2	No
10	78	Right	48	2	3	1	Levodopa (100+25)mg, 1 tab Carvidopa/Levodopa/Entacapone (100/25/200)mg, 4 tabs
11	24	Left	9	1	1	1	Levodopa (100+25)mg, 0.5 tab Ropinirole 0.25mg, 2 tab
12	12	Right	7	2	1	1	No
13	96	Left	17	1	2	1	Carvidopa/Levodopa/Entacapone (100/25/200)mg, 3 tabs Rasagiline 1mg, 1 tab
14	24	Left	10	2	1	1	Rasagiline 1mg, 1 tab
15	6	Right	7	2	1	2	No
16	90	Left	16	2	2	1	Carvidopa/Levodopa/Entacapone (100/25/200)mg, 3 tabs Rasagiline 1mg, 1 tab
17	24	Right	7	1	1	1	Levodopa (100+25)mg, 1 tab
18	20	Left	21	1	2	1.5	Rasagiline 1mg, 1 tab
19	6	Left	21	2	2	1.5	No
20	18	Right	22	2	2	2.5	No
21	32	Right	25	1	1	1.5	Amantadine 100mg, 2 tabs
22	30	Right	18	1	1	1	Levodopa/Carvidopa (100/250)mg, 2 tabs Pramipexole 700mg, 2 tabs
23	12	Left	10	1	1	1.5	No

Measurements

In all patients, the clinical evaluation and the recordings were performed after overnight withdrawal of anti-parkinsonian medication. The upper limb tremor at rest or while maintaining stable posture was mostly asymmetrical. Recordings were obtained from the most severely affected side.

In the recording sessions, the subjects assumed a comfortable sitting position in front of a rigid table. They were instructed to (i) completely relax their most affected upper limb and have the hand hanging over the edge of the table (limb at rest, 32 trials in patients); or (ii) extend the whole limb, trying to maintain a steady horizontal posture against gravity (limb in posture, 31 trials in patients and 34 trials in control subjects). Recording at rest or in posture started as soon tremulous movement of the limb became obvious. Both tremor types were recorded using an accelerometer (K-Beam 5210B, Kistler Instruments) attached to the dorsum of the hand or the digits, depending on the segment that trembled most. Simultaneous surface EMG (sEMG; using Ag-AgCl disc electrodes, Kendall ARBO) and intramuscular EMG (using disposable concentric needle electrodes, 0.37 mm, 26G, Alpine) were obtained in each patient from an extensor (carpi radialis, digitorum communis) or flexor (carpi radialis, digitorum superficialis) muscle of the wrist or digits. Record duration was generally 2 min. To examine if our findings, obtained from patients, who were resting or maintained a stable posture could also be seen when they exerted isometric force, in an additional preliminary study, we asked four randomly selected PD patients to press their extended fingers against a force transducer and recorded isometric force tremor. A detailed description of the procedures employed to collect this data can be found in Christakos et

al. (2009), while the methods we used to analyze them are described in detail below. The data were digitized with the help of a 16-bit National Instruments DAQ and the program LabView and stored on hard disk for off-line analysis.

Data processing and analyses

Limb position was sampled at 3 kHz and EMG signals at 5 kHz, a sampling rate which is near the upper bound of those used in the field (e.g., 3 kHz: Johnston et al., 2005; 3-5 kHz: McNulty and Cresswell, 2004; 6.4 kHz: Wessberg and Kakuda, 1999). Manual sorting and custom made subroutines in the MATLAB (Math Works, Natick, MA, USA) environment were used to discriminate single-MU spikes from the background activity. Spike trains were subsequently represented as sequences of zeroes and ones. Then, limb position and EMG signals were low-pass filtered at 250 Hz, and resampled at 500 Hz to avoid aliasing. This procedure is an ideal filtering that does not affect spectral analyses and coherence estimates (Christakos et al., 1984). The following analyses were carried out using MATLAB.

Time domain analysis included computations of:

(i) Cross-correlograms of pairs of signals, namely limb acceleration, sEMG and MU spike train.

(ii) Incidence of singlets, doublets and triplets of MU spikes during epoch-II intervals. In cases where the MU exhibited doublets randomly interspersed with single spikes, the proportion of singlets, equaled the ratio of the number of cycles accompanied by single spikes over the total number of cycles. The same applies for the proportion of doublets. In cases where the MU exhibited doublets randomly interspersed with triplets, the incidence of doublets, equaled the

ratio of the number of cycles accompanied by doublets over the total number of cycles. Once more, the proportion of triplets was computed in a similar manner.

In the frequency-domain, spectral analyses (Wang et al., 2004; Christakos et al., 2006), performed via the fast Fourier transform on pairs of simultaneous activities, included:

(i) Segmentation of the time-series in 2-sec segments, combined with mean removal and windowing (Hanning) for each segment;

(ii) Computation of the auto-spectra and the cross-spectrum from each segment (frequency-resolution 0.5 Hz);

(iii) Final estimation of the auto-spectra and the cross-spectra of pairs of signals (limb acceleration, sEMG, MU spike train) by averaging the estimates from the individual segments.

The coherence spectrum was subsequently estimated as the squared modulus of the cross-spectrum divided by the product of the two auto-spectra. This normative (0-1.0) function quantifies the correlation strength between two signals at each frequency. Its significance threshold was set to 99% confidence, determined from the employed number of segments analyzed and the desired smooth data tapering for leakage suppression (Rosenberg et al., 1989). It is represented by a horizontal dotted line in the coherence plots.

The amplitude of the tremor at the frequency of the primary component was estimated as the square root of the total power (integral under the power spectrum curve) within the frequency-band of the corresponding auto-spectral deflection, derived from the entire 2-min record. The amplitude of the tremor at the frequency of the secondary component was estimated in a similar manner. As the deflection corresponding to the secondary component was often

minute relative to the neighboring large, primary component, the central frequencies of the primary and secondary components were confirmed using auto-spectral and coherence estimates separately from epoch-II and epoch-I intervals, respectively. Transition periods were rejected for this analysis.

Analysis of rhythmical MU firing synchrony

The method we employed (Christakos, 1994, 1997; Christakos and Giatroudaki, 1998) was previously applied to detailed analyses of synchronous MU rhythms or modulations (e.g., Christakos et al., 2006, 2009; Erimaki and Christakos, 2008). These unit-to-aggregate analyses have been shown to be experimentally and computationally efficient, as they reveal the behavior of a significant fraction of the MU population. They are easy to use in patients with movement disorders as they avoid the discomfort inherent in the simultaneous recording of multiple MUs. Briefly, coherence computations on samples of MU/sEMG (unit/aggregate) pairs were used for:

(i) *Detecting* such synchrony, since the occurrence of a non-zero coherence in a sample indicates the presence of a correlated subset within the population, to which the given MU belongs.

(ii) Estimating the *extent* of the synchrony (proportion of correlated MUs in a muscle), as the fraction of non-zero coherences in the sample.

(iii) Obtaining information on the synchrony *strength*; importantly, for widespread and in-phase synchrony, the MU/MU coherence approaches the squared value of the MU/sEMG coherence (Christakos, 1997) and can thus be easily estimated.

(iv) Further, MU/sEMG cross-correlation computations for the coherent MUs in the sample were used for estimating MU *phases*, in terms of delays relative to the sEMG (common reference signal). Such information on multiple MU phases cannot be obtained from MU/MU analysis which provides information about phase differences between a single MU pair.

The strength of MU to acceleration coherence did not differ significantly from MU to sEMG coherence ($p=0.957$, paired t-test). In relatively few cases the MU/sEMG coherence at the frequency of the secondary component was a little below the significance threshold (99% confidence). These included 14% of the records while patients maintained a stable posture and 20% of those during rest tremor in patients as well as 21% of the records while control subjects maintained a stable posture. Nevertheless, MU/sEMG coherence was significant with 95% confidence in these cases. The sEMG/acceleration coherence provided additional support for the presence of synchrony at the frequency of the secondary component. It is important to note that this aggregate/aggregate coherence analysis, although simple, can be misleading when it comes to estimates of the extent and strength of synchrony. It saturates easily and in cases of widespread and in-phase synchrony it greatly overestimates the true coherence between MUs (Christakos, 1997). Thus is suitable only for synchrony detection.

Statistical analyses

All data were analyzed using the SPSSv20 statistical package. The normality of the distributions of the variables was examined using the one-sample Kolmogorov-Smirnov test. The paired-samples t-test was used to compare the means of two distributions in cases when members of the one could be paired to members of the other (Armitage, 1971). This was done when comparing: (1) MU/sEMG and MU/acceleration coherences estimated for the same MU spike train, (2) the frequencies of the primary and secondary components obtained from the same record, (3) the mean ISI of doublets in different records from the same patient, (4) the mean ISI of doublets from a record and the mean short ISI of all records from the same patient. The independent-samples t-test was used in all other cases; namely: (1) MU/sEMG coherences estimated at the frequency of primary tremor component at rest and while maintaining a stable posture, (2) MU/sEMG coherences estimated at the frequency of secondary tremor component at rest and while maintaining a stable posture, (3) the mean short ISI of MUs characterized by low firing rates and those characterized by high firing rates, (4) the mean short ISI of two MU spike trains which were discriminated from two different simultaneously recorded MUs displaying different firing rates. One-way ANOVA was used to evaluate how the mean short ISI is affected when measured in all relevant records obtained from a single patient and from: (i) doublets, when doublets are interspersed with single spikes, (ii) doublets, when doublets are interspersed with triplets and (iii) triplets, when triplets are interspersed with doublets. Univariate ANOVA was used to determine if the ISI is affected by a number of factors (patient, state of the subject such as resting or holding a stable posture, spike grouping in doublets or triplets, whether doublets are interspersed with triplets or single spikes, etc.). Linear regression was used to examine the

relationship between (i) the proportion of doublets/triplets and the ratio of the mean firing rate of the MU (f_U) to overt tremor frequency, and (ii) the mean short ISI of all records of a patient and the difference between the periods of overt and non-overt tremor. The threshold for rejecting the null hypothesis was set at $p<0.05$ in all statistical tests.

RESULTS

Overview

In agreement with previous observations in PD patients exerting isometric force (Christakos et al., 2009), records obtained from PD patients both at rest and when maintaining a stable posture were characterized by the appearance of overt tremor (epoch-II) randomly interspersed with periods of non-overt tremor (epoch-I). Each epoch could last from around one second to several dozens of seconds. The overt tremor was manifested as a large auto-spectral component, while non-overt tremor, as a higher-frequency and much smaller auto-spectral deflection. It should be stressed out that the frequency of both overt and non-overt tremor, was common in each patient at rest and when maintaining a stable posture. MU firing was normal-like in epoch-I (Figure 1G, 2G), showing a strong, intrinsic rhythm at the f_U of each MU and, additionally, a weaker rhythm exhibiting low coherence to tremor (Figure 1C). There are clear similarities between the characteristics of tremor and the activity of MUs in epoch-I intervals of PD patients (Figure 1F, G) on the one hand, and the characteristics of physiological tremor together with the activity of MUs in control subjects (Figure 3A, B). In contrast, MU firing in epoch-II was highly abnormal, showing spike-doublets and triplets bearing a one to one relationship to each tremor cycle (Figure 1E, 2E). Coherence of MU and sEMG rhythms at the frequency of overt and non-overt tremor was thus always present, being abnormally high at the frequency of the former. It is important to note that f_U remained constant despite epoch transitions (Figure 4) which usually lasted less than half a second. Readers can convince themselves that this is true by counting the number of spikes per second before the transition illustrated in Figure 4 (vertical dashed line),

which is identical to that after the transition. Similar findings were obtained from isometric force tremor recordings in 4 patients.

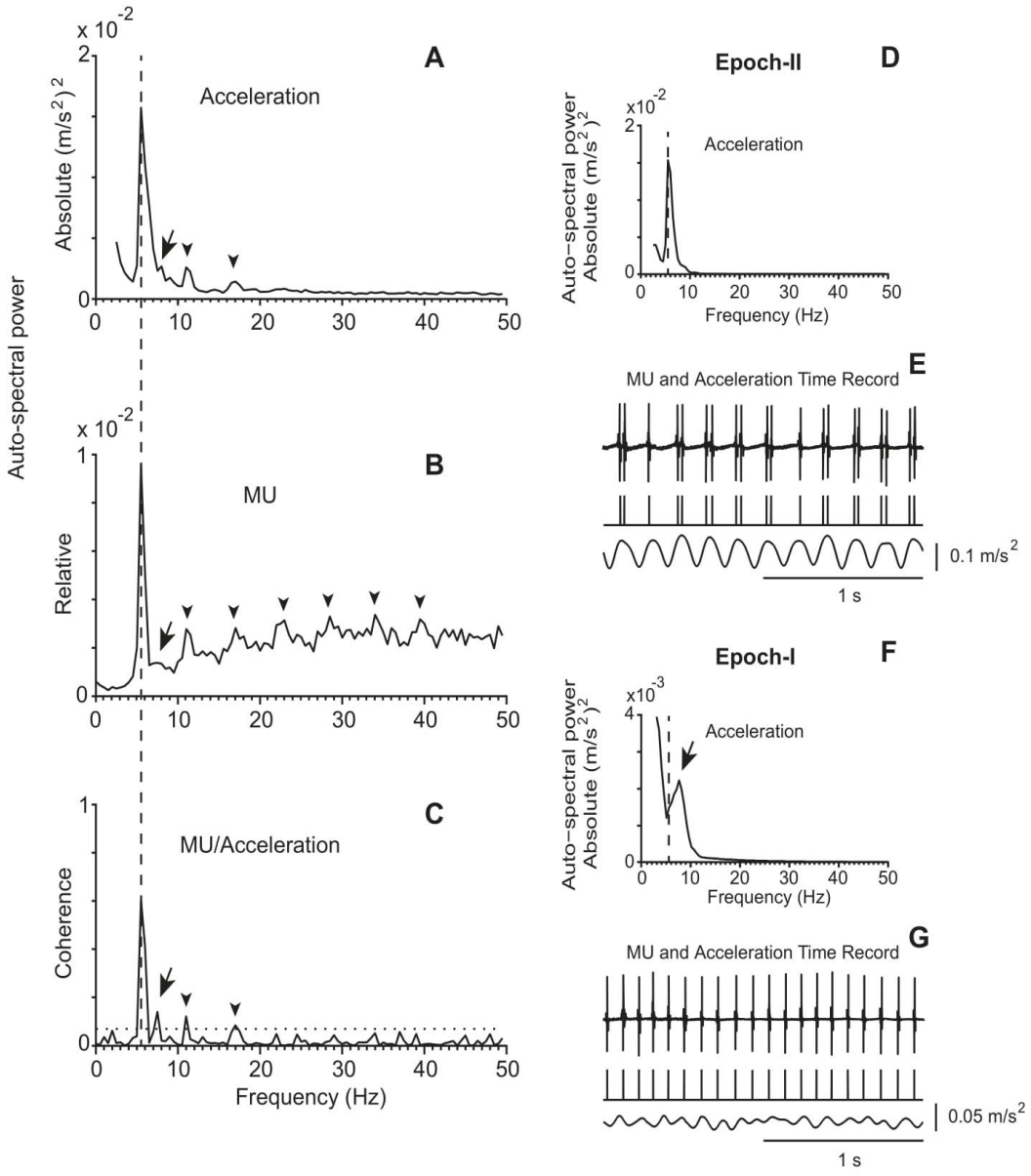

Figure 1 Representative example of the tremor and the firing of a MU observed in a patient (#14) while stable posture was maintained. (**A**) Auto-spectrum of hand acceleration displaying

the primary (5.5 Hz, vertical dashed line) and secondary (7.5 Hz, arrow) components of tremor as well as the first two harmonics of the primary component (arrowhead). (**B**) Auto-spectrum of a MU to display the primary (vertical dashed line) and secondary (arrow) components of tremor as well as several harmonics of the primary component (arrowheads). (**C**) MU/Acceleration coherence displaying the primary (vertical dashed line) and secondary (arrow) components of tremor as well as the first two harmonics of the primary component (arrowheads). The horizontal dashed line represents the significance threshold. (**D**, **F**) Auto-spectra of hand acceleration using only epoch-II (**D**) or epoch-I (**F**) intervals (total duration 67 s and 53 s, respectively). Symbols as in (**A**). Note the absence of the secondary component in (**D**) and of the primary component in (**F**). (**E, G**) Traces corresponding to the accelerometer raw signal (bottom), the MU spike train (middle) and the intramuscular raw EMG signal (top) recorded simultaneously from the patient during epoch-II (**E**) or epoch-I (**G**) intervals. The mean firing rate of the MU was 10.0 Hz in both (**E**) and (**G**).

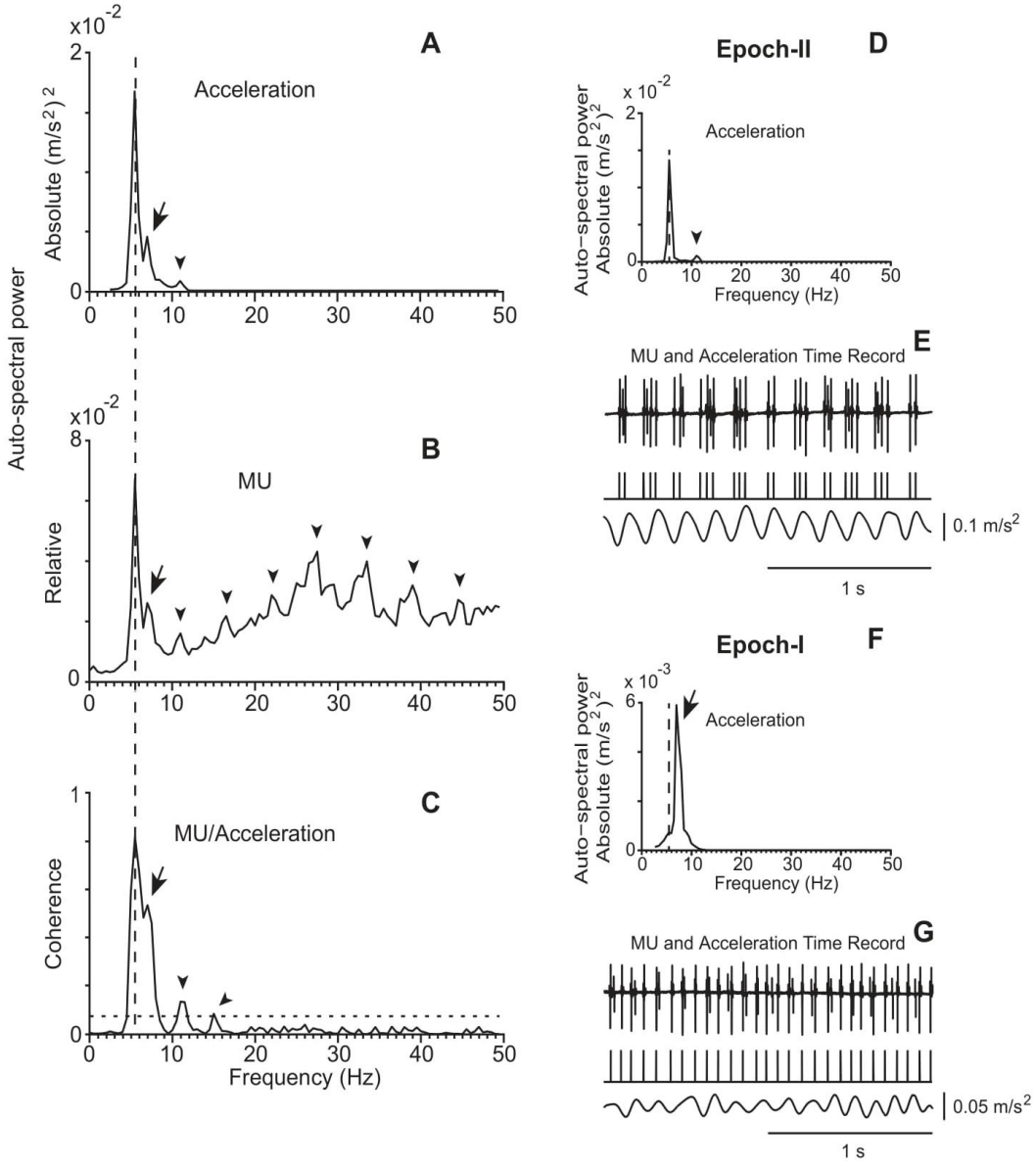

Figure 2 Representative examples of the tremor and the firing of a MU observed in a patient (#21) at rest. (**A**) Auto-spectrum of hand acceleration displaying the primary (5.5 Hz, vertical

dashed line) and secondary (7 Hz, arrow) components of tremor as well as the first harmonic of the primary component (arrowhead). (**B**) Auto-spectrum of a MU to display the primary (vertical dashed line) and secondary (arrow) components of tremor as well as several harmonics of the primary component (arrowheads). (**C**) MU/Acceleration coherence displaying the primary (vertical dashed line) and secondary (arrow) components of tremor as well as the first two harmonics of the primary component (arrowheads). (**D**, **F**) Auto-spectra of hand acceleration using only epoch-II (**D**) or epoch-I (**F**) intervals (total duration 66 s and 54 s, respectively). Symbols as in (**A**). Note the absence of the secondary component in (**D**) and of the primary component in (**F**). (**E, G**) Traces corresponding to the accelerometer raw signal (bottom), the MU spike train (middle) and the intramuscular raw EMG signal (top) recorded simultaneously from the patient during epoch-II (**E**) or epoch-I (**G**) intervals. The mean firing rate of the MU was 14.0 Hz in both (**E**) and (**G**).

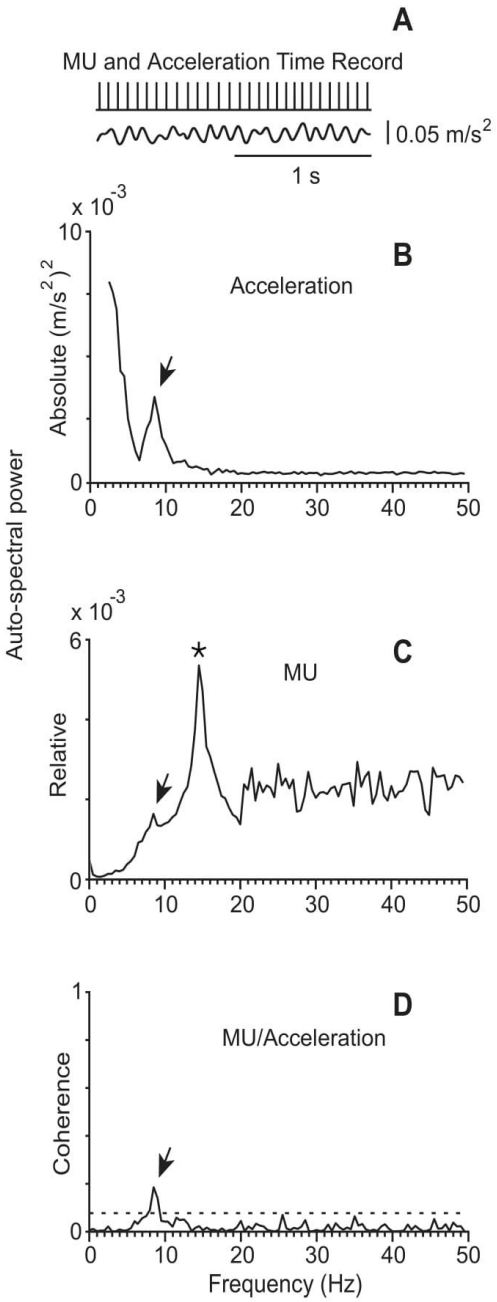

Figure 3 Representative example of physiological tremor and the firing of a MU in a control subject (#5) while maintained stable posture. (**A**) Traces corresponding to the accelerometer raw

signal (bottom), the MU spike train (top) recorded simultaneously from the control subject. The rhythmical firing rate of the MU was at 14.5 Hz (mean firing rate of the MU) and the frequency of the tremor at 8.5 Hz. (**B**) Auto-spectrum of the hand acceleration displaying a single peak at the tremor frequency (arrow). (**C**) Auto-spectrum of the MU displaying a large peak at the frequency of the mean firing rate of the MU (asterisk) and a small deflection at tremor frequency (arrow). (**D**) MU/Acceleration coherence displaying a small but significant peak at tremor frequency (arrow).

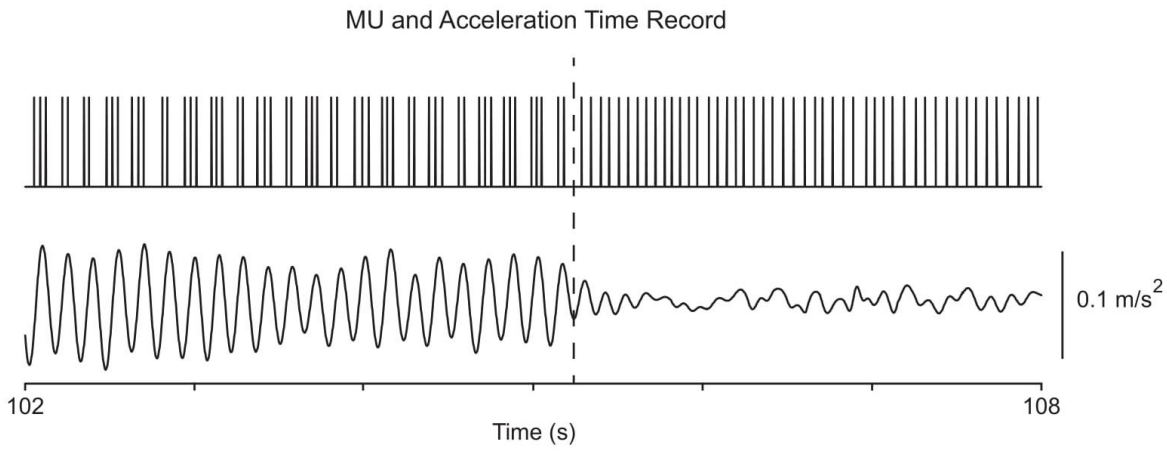

Figure 4 Transition from epoch-II to epoch-I in a patient (#8) at rest. The top trace illustrates the MU spike train and the bottom the acceleration of the hand. Note that the MU firing rate (17 Hz) before the transition (dashes) is identical that after the transition.

Components of tremor

Figure 1A provides an example of the power density of the fast Fourier transform of an acceleration record lasting for 2 minutes. It was obtained from a PD patient while stable posture was maintained and contains both epoch-I and epoch-II intervals. It is characterized by a prominent (primary) component (dashed vertical line; 5.5 Hz) and an additional, harmonically unrelated, smaller peak at a higher frequency (secondary component; Figure 1A, arrow; 7.5 Hz). Although the latter was often (N=56) barely discernible (Figure 1A, arrow), it was always clearly visible in auto-spectra obtained exclusively from intervals characterized by non-overt tremor (epoch-I; Figure 1F, 2F). In contrast, a large solitary component appeared in tremor auto-spectra obtained exclusively from epoch-II intervals at the relatively low frequency typical of the primary component (compare Figure 1D with Figure 1A and Figure 2D with 2A). Similar results were obtained from another 30 trials during postural and 32 trials during rest tremor obtained from 23 patients.

Table 2 Summary of experimental results

	Patients				Control Subjects
f_U (Hz)					
Range	6.0-18.5				7.0-20.0
Mean (SD)	10.9 (2.9)				14.6 (3.4)
Recordings	Rest (*n* = 32)		Postural (*n* = 31)		Postural (*n* = 34)
n of MUs	35		31		35
Tremor Component	Primary	Secondary	Primary	Secondary	Single
f_T (Hz)					
Range	4.5-7.0	6.0-9.0	4.5-7.0	6.0-9.5	6.5-10.0
Mean (SD)	5.9 (0.80)	7.7 (0.91)	6.1 (0.84)	7.9 (0.92)	8.2 (0.93)
A_T (m/s^2)					
Range	0.252-2.309	0.015-0.340	0.376-3.085	0.046-0.344	0.034-0.328
Mean (SD)	0.843(0.056)	0.144(0.112)	0.958(0.076)	0.164(0.084)	0.156(0.072)
MU/EMGCoh					
Range	0.23-0.96	0.05-0.75	0.21-0.97	0.04-0.80	0.04-0.77
Mean (SD)	0.73 (0.18)	0.20 (0.19)	0.76 (0.16)	0.24 (0.19)	0.19 (0.17)

A_T: amplitude tremor, f_T: frequency of tremor, f_U: firing rate motor unit, MU: motor unit, MU/EMGCoh: MU/EMG coherence

For both postural and rest tremor, the frequency of the primary component was in the 4-8 Hz range which is typical of the PD tremor. In contrast, the frequency of the secondary component was in the range of the physiological tremor, i.e. between 6-10 Hz. The difference between these two ranges is illustrated in the cumulative frequency histogram of Figure 5 and is highly significant ($p<0.001$, paired *t*-test). Taking each patient separately, the secondary component of tremor was on the average 1.72 ± 0.42 Hz (mean$\pm$SD) higher than the primary (Figure 6, Table 2). Figure 7A plots the relationship between the frequency of the primary component of tremor at rest against the frequency of the primary component of tremor while maintaining a stable posture for each one of the 23 PD patients we studied. As shown here, most of the data (N=18) lies along the diagonal which implies that the frequency of the primary component at rest is identical to that during stable posture. In the remaining five cases the difference did not exceed the resolution of our technique (0.5 Hz). With the exception of 4 patients, the frequency of the secondary component of tremor at rest was also identical to that during stable posture (Figure 7B).

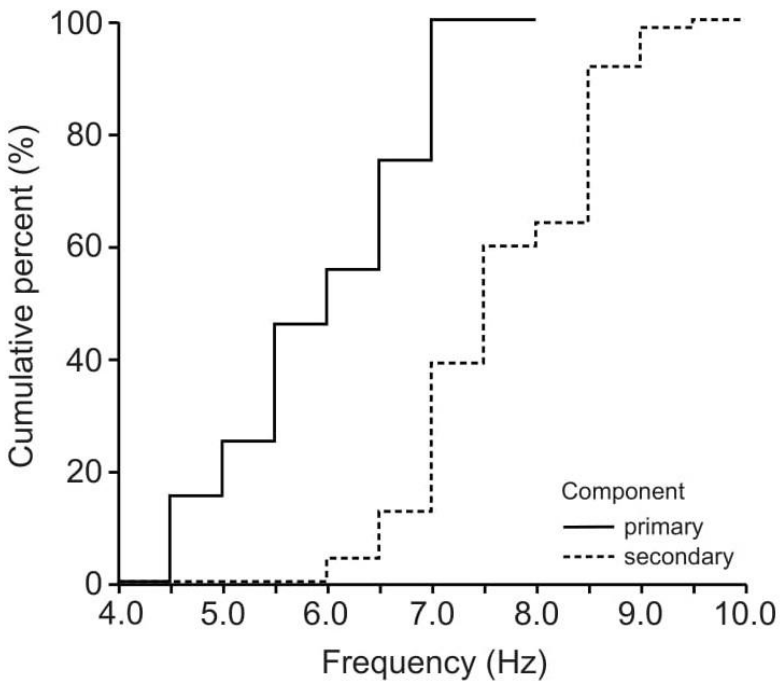

Figure 5 Cumulative curves of the frequencies of the primary (solid) and secondary (dashed) components of tremor in the population of PD patients.

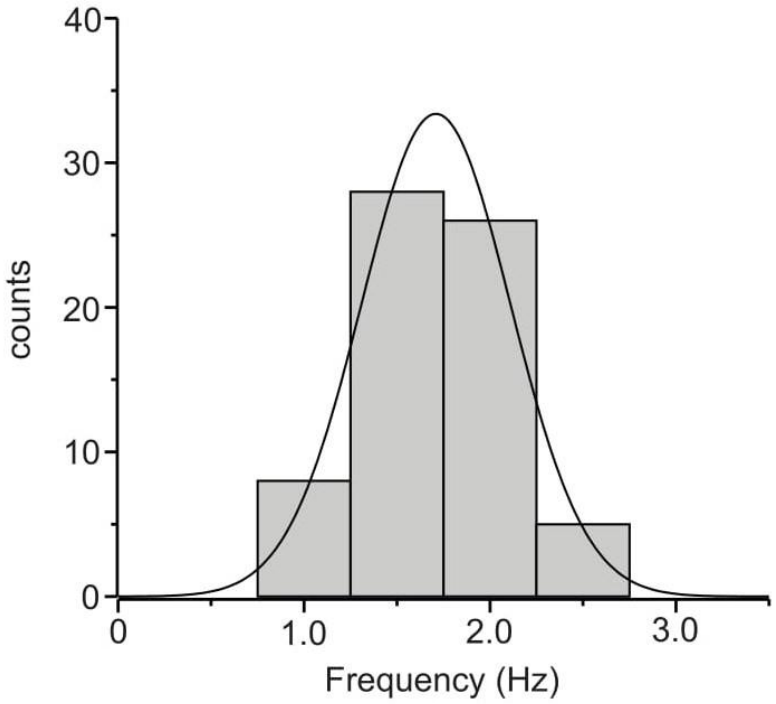

Figure 6 Frequency histogram of the difference between the primary and the secondary component of tremor in all of the records we collected (N=63).

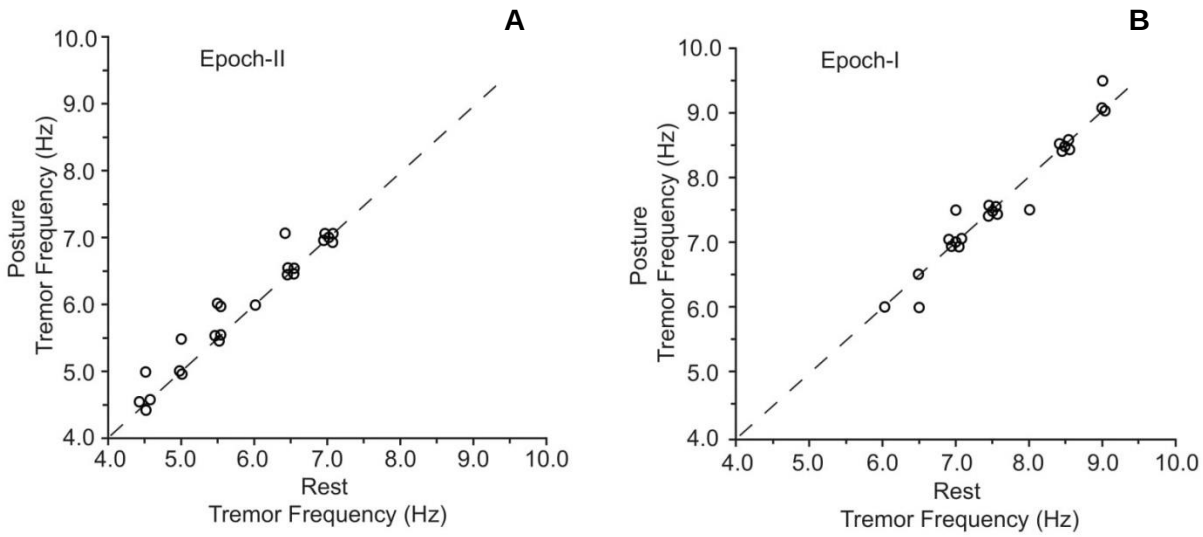

Figure 7 Scatterplot of tremor frequency at rest (abscissa) versus tremor frequency while holding a stable posture (ordinate) measured from epoch-II (**A**) and epoch-I (**B**) intervals. Each data point is from a different patient.

The large difference between the primary and secondary components of tremor in terms of power density (Figure 1A, 2A) reflect the large differences between epochs I and II in terms of tremor amplitude. During epoch-I (dominated by the secondary component) the amplitude of the postural (0.164 ± 0.084 m/s^2, mean$\pm$SD) and rest (0.144 ± 0.112 m/s^2) tremor in patients was small and comparable to that of the postural tremor in control subjects (0.156 ± 0.072 m/s^2). In contrast, during epoch-II, dominated by the much larger primary component, the amplitude of the postural and rest tremor of patients was abnormally large (0.958 ± 0.076 and 0.843 ± 0.056 m/s^2 respectively), exceeding thus the physiological tremor amplitude by a factor of around 6.

Characteristics of the motor unit firing synchrony

The strength of the tremor-related MU synchrony, as judged from the coherence of MUs to acceleration and sEMG (see Methods), varied considerably within the same patient and between patients (Table 2). In general, it obtained large values when evaluated at the frequency of the primary component and smaller at the frequency of the secondary. In Figure 1C, it equals 0.62 when MU to acceleration coherence was evaluated at 5.5 Hz (primary component) and only 0.13 at 7.5 Hz (secondary component). Similarly, in Figure 2C, the MU to acceleration coherence at rest equaled 0.82 when it was evaluated at 5.5 Hz (primary component) and much lower at 7.0 Hz (0.54; arrow; secondary component). Patients displaying increased MU synchrony (i.e., high coherence values at the primary component frequency) during rest also displayed increased MU synchrony while maintaining stable posture. Figures 8A and 8C provide an illustrative example. At the frequency of the primary component (6.5 Hz), the strength of MU to sEMG coherence was 0.70 at rest and 0.81 while maintaining stable posture. In PD patients, the strength of the MU to the sEMG coherence was high at the frequency of the primary component, both at rest and while maintaining stable posture; the former obtained values (0.73 ± 0.18) that did not differ significantly ($p=0.543$, independent t-test) from those of the latter (0.76 ± 0.16). The much smaller values of the MU to sEMG coherence at the frequency of secondary components were also similar ($p=0.360$, independent t-test) at rest (0.20 ± 0.19, Table 2) and while maintaining stable posture (0.24 ± 0.19). More importantly, they were similar to those obtained from control subjects maintaining stable posture (0.19 ± 0.17).

The cross-correlation between the spikes of individual MUs and sEMG records indicates that MU discharges were, on average (-2±12 ms, N=66), in phase with the sEMG and thus with one another (Methods). Figures 8B and 8D provide typical examples of two different motor units, recorded from the same patient at rest (Figure 8B) and while maintaining stable posture (Figure 8D), respectively. An estimate of the MU to MU coherence can be obtained by squaring the MU to sEMG coherence (see Methods). When measured at the frequency of the secondary component, MU to MU coherence equaled 0.10±0.14 in PD patients maintaining a stable posture and 0.08±0.16 in the same patients at rest. Similar values were seen in control subjects 0.08±0.12 maintaining a stable posture. On the contrary, MU synchrony in PD patients was much stronger at the frequency of the primary component as surmised from the MU to MU coherence estimated at rest (0.56±0.23) or while they maintained a stable posture (0.59±0.21).

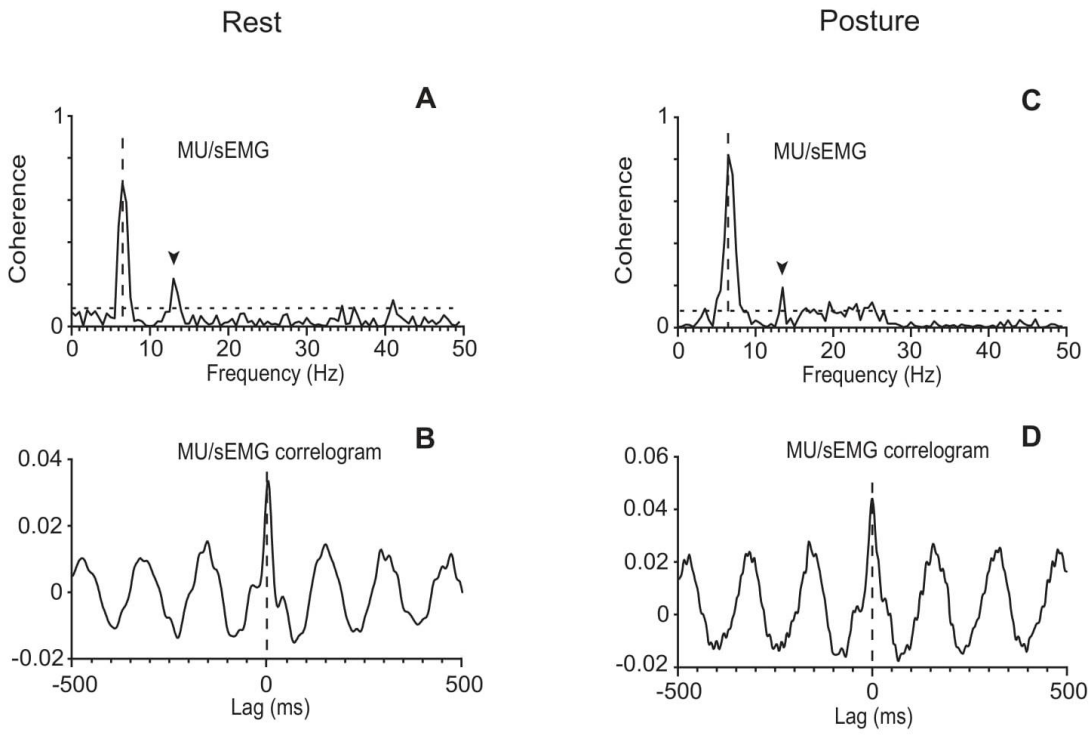

Figure 8 Analysis of MU synchrony in a patient (#12) at rest (A, B) or while he maintained a stable posture (C, D). (**A, C**) The very high MU/sEMG coherence at 6.5 Hz. (**B, D**) The zero-lag central peak (in-phase synchrony) in the oscillatory MU/sEMG cross-correlogram at 6.5 Hz.

Firing patterns of motor units

MU firing patterns

The mean firing rates of the MUs we studied in PD patients at rest or maintained a stable posture ranged between 6.0-18.5 Hz (f_U; Table 2). It ranged between 7.0-20.0 Hz in control subjects (Figure 3A). During epoch-I intervals, each MU displayed rhythmic activity at its intrinsic f_U (e.g., Figure 1G, 2G). This rhythm appears as an easily discernible peak in the auto-spectrum of the MU illustrated in Figure 3C (asterisk). Additionally, the auto-spectrum of the MU displayed a second component (e.g., Figure 1B, 2B; arrow). On the frequency axis it occupies a position identical to that of the secondary component of the MU to acceleration coherence plot (Figure 1C, 2C; arrow). It also corresponded to the dominant component of the physiological tremor of control subjects maintaining a stable posture (Figure 3D).

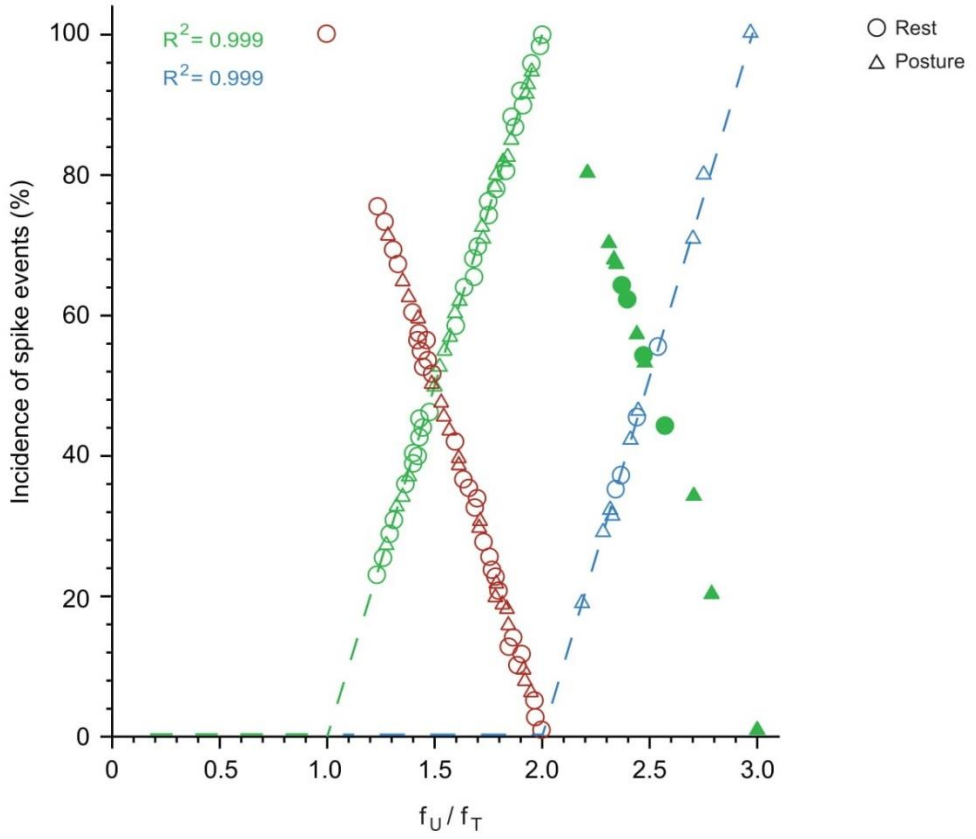

Figure 9 The proportion of single-spikes, spike doublets and triplets, depends on the firing rate of the MU (f_U) and the frequency of the tremor (f_T) during epoch-II intervals. Note the linear relationship between the incidence of single-spikes (red; N=49) and the ratio f_U/f_T. Also note the linear increase of the incidence of doublets (open green; N=48) up to $f_U/f_T=2$ and its decrease above this value (solid green; N=13). Finally note the linear relationship of the incidence of triplets (blue; N=13) to the ratio f_U/f_T.

During epoch-II intervals, and while subjects displayed overt tremor, MUs exhibited abnormal discharge patterns. Twenty of the MUs that we recorded from PD patients while they maintained a stable posture exhibited spike-doublets randomly interspersed with single spikes (e.g. Figure 1E). As shown here, either single or double spikes occurred in a one-to-one relation to the tremor cycle. As described before (Christakos et al., 2009), the sum of one short and one long ISI corresponds to the tremor period. In these MUs, f_U was maintained at a frequency intermediate between that of the tremor and twice its value. Similar data were obtained in 28 MUs recorded while PD patients were at rest and in 3 MUs recorded while they produced isometric force. The firing pattern of another 4 (9) MUs recorded from PD patients at rest (while they maintained a stable posture) was a series of spike-triplets randomly interspersed with doublets as shown for example in Figure 2E. Once again, either doublets or triplets occurred in a one-to-one relation to tremor cycles. In this case, it is the sum of two short and one long ISI that corresponds to the tremor period (Christakos et al., 2009). The f_U obtained in these MUs between twice and three times the value of the tremor frequency. Similar findings were obtained from 2 MUs recorded when patients produced isometric force. In two patients, 4 MUs exhibited spike-quadruplets interchanged randomly with triplets at rest (2 units) or while they maintained a stable posture (the remaining two units). Here, it is the sum of three short and one longer ISI that corresponds to the tremor period. The f_U of these MUs was higher than three times the value of the tremor frequency. The firing pattern of only one MU recorded at rest exhibited single-spikes in a one-to-one relation to the tremor. Evidently, in this case f_U equaled the tremor frequency. To summarize, MUs discharged at a rate equal to or higher than that of epoch-II tremor and therefore their recruitment rate is equal to the frequency of overt tremor.

Spikes are grouped into doublets, triplets, etc. and the proportion of these depends on the firing rate of the unit in relation to the frequency of the tremor. This is shown in Figure 9, which plots the proportion of doublets (open green) as a function of the ratio f_U/f_T (where f_T is the frequency of the overt tremor). As shown here, the proportion of tremor cycles accompanied by doublets increased linearly together with f_U/f_T in all 48 of the relevant MUs and equaled 100% (doublets present in all tremor cycles) when $f_U = 2 \times f_T$. The excellent relationship between the proportion of doublets and the ratio f_U/f_T is indicated by the fact that it accounts for 99.9% of the variance of the dependent variable. From the regression equation, the proportion of tremor cycles with doublets is $(f_U-f_T)/f_T$. Accordingly, *all* spikes in excess of f_T can be thought of as combining with other spikes to form doublets that are randomly interspersed with the remaining single spikes. As expected, the proportion of tremor cycles accompanied by single-spikes decreased linearly with f_U/f_T (Figure 9, red). Similarly, the proportion of tremor cycles accompanied by triplets (Figure 9, blue) increased linearly together with f_U/f_T in all 13 relevant MUs and again equalled 100% (triplets in all tremor cycles) for $f_U = 3 \times f_T$. Here again the relationship between the two variables was perfect as it accounted for 99.9% of the variance of the dependent one and once again *all* spikes in excess of $2 \times f_T$ can be thought of as combining with doublets to form triplets randomly interspersed with the remaining doublets. Here again, the proportion of tremor cycles accompanied by doublets decreased linearly with f_U/f_T (Figure 9, solid green).

Interspike intervals within doublets/triplets

The short ISIs within doublets (Figure 10A; top) and triplets (Figure 10A; bottom) were concentrated around mean values in the 30-50 ms range. The mean ISI of doublets in any 2 min record did not differ significantly from that of the triplets ($p>0.10$, one-way ANOVA) found in the same record (compare the two plots of Figure 10A). Nor did it differ from that of the doublets found in any other 2 min record from the same patient as shown by the fact that the difference between the two (Figure 11; left) was not significantly bigger than zero ($p=0.944$, paired *t*-test) when we considered all possible pairs of records obtained from all patients. Finally, it did not differ significantly from that of all short ISIs in all records of the same patient again shown by the fact that the difference between the two (Figure 11; right) was not significantly bigger than zero ($p=0.453$, paired *t*-test). Thus, the mean short ISI in any 2-min record was practically invariant across different records obtained from a patient and thus represented a robust personal characteristic or 'idiosyncratic' feature of his tremor-related MU firing. Univariate ANOVA showed statistically significant effect only for the factor 'patient' ($F=656.064$, $P<0.0001$) and not for other factors such as state of the patient (resting or holding a stable posture), spike grouping (doublets or triplets) and firing pattern (single spikes interspersed with doublets or doublets interspersed with triplets).

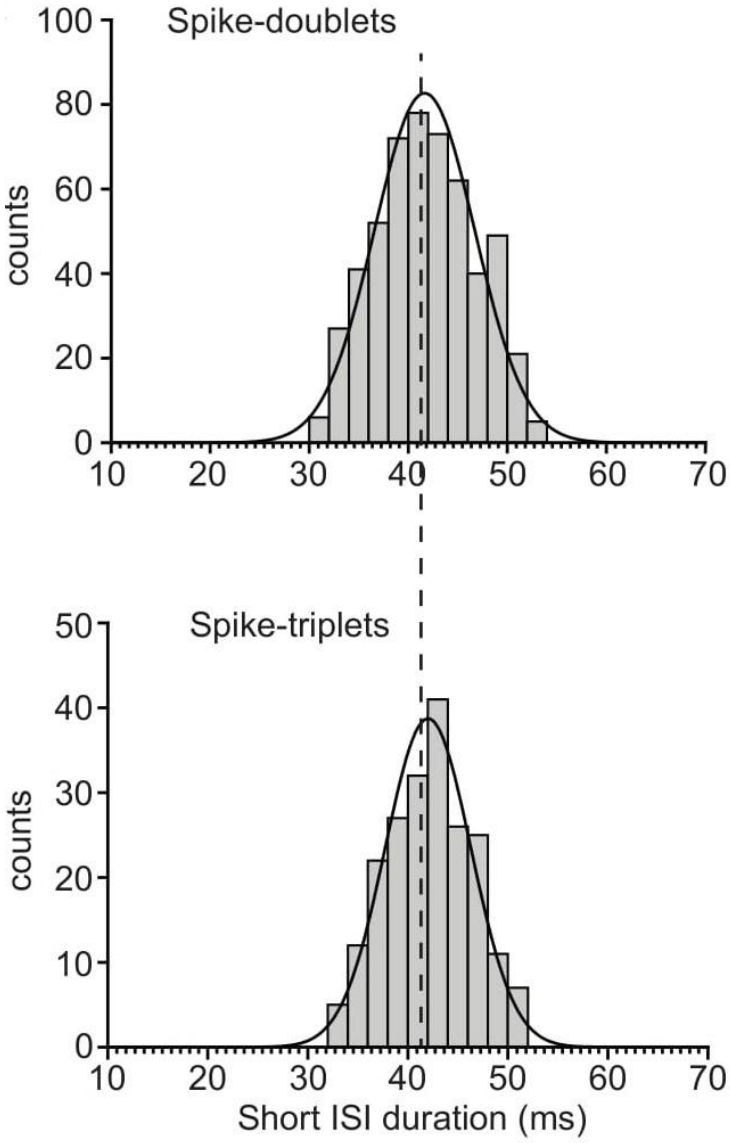

Figure 10 Frequency histogram of the duration of the short ISIs measured from the doublets (top; N=526) or the triplets (bottom; N=208) found in the same MU spike train. Note the statistically indistinguishable means (vertical dashed line) of the two distributions (41.6±5.1 ms and 42.0±4.3 ms, respectively).

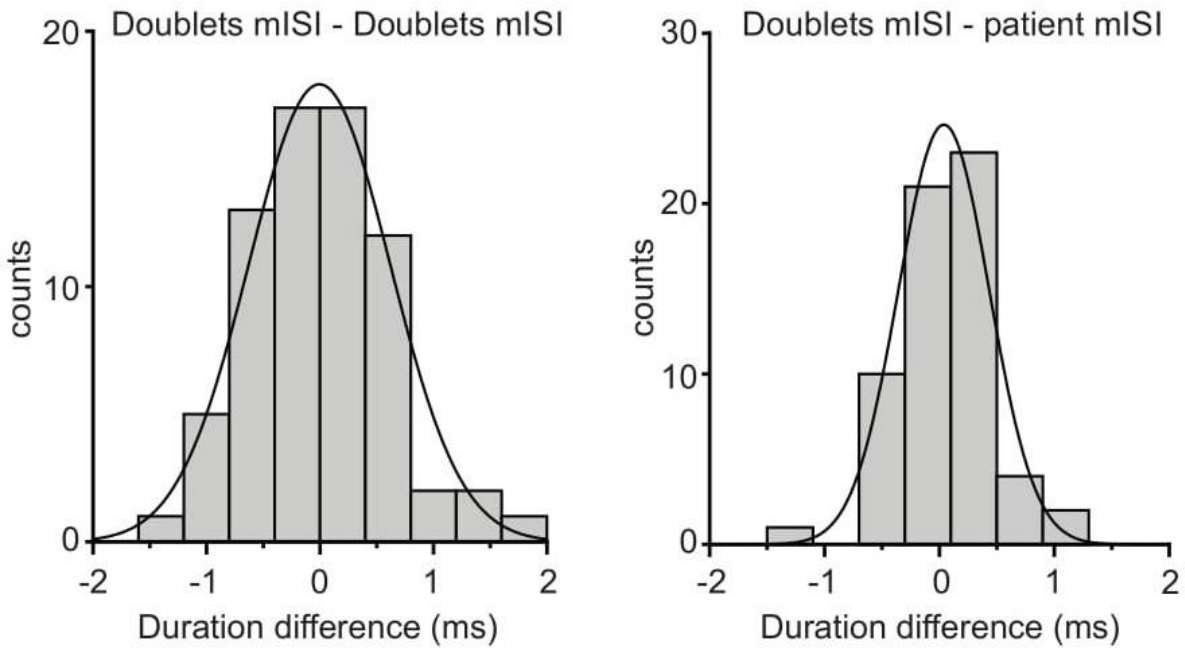

Figure 11 Left: Frequency histogram of the difference between the mean ISI (mISI) of doublets found in any 2 min record and that of doublets from any other 2 min record from the same patient. Note that the average difference (-0.005±0.62 ms, N=70) was statistically indistinguishable (p=0.944) from zero. Right: Frequency histogram of the difference between the mISI of doublets from any 2 min record and that of all short ISIs in all records of the same patient. Note that the average difference (-0.038±0.39 ms, N=61) was statistically indistinguishable (p=0.453) from zero.

One might argue that the mean short ISI depends on the excitation level of the unit expressed in its f_U. To examine if this is the case we separated the MUs we encountered into two groups, those with relatively high mean rates (mean±SD: 13.5±2.3 Hz; range: 11-18.5 Hz, N=28) and those with relatively low mean rates (mean±SD: 9±1.2 Hz; range: 7-10.5 Hz, N=38) and examined if the mean short ISI of the former is smaller than that of the latter. The average short ISI of MUs characterized by high firing rates was equal to 37.2±5.6 ms while the mean short ISI of MUs characterized by low firing rates was equal to 36.5±4.4 ms. The small difference between the two, albeit in the wrong direction, was insignificant (p=0.58, independent t-test). The fact that the mean short ISI does not depend on MU firing rate was also demonstrated in records obtained from 3 pairs of MUs that were simultaneously recorded in three different patients and which exhibited the same mean short ISI despite large differences in the rates with which they fired at rest. For example, the upper plot of Figure 12 illustrates the distribution of the short ISIs seen in a MU that was firing at a rate of 7 Hz while the lower plot, illustrates those seen in a second MU that was firing at a rate of 12 Hz and was recorded at the same time. The mean short ISI of the former (mean±SD: 32.53±4.0 ms) did not differ significantly (p=0.868, independent t-test) from that of the latter (mean±SD: 32.47±4.2 ms).

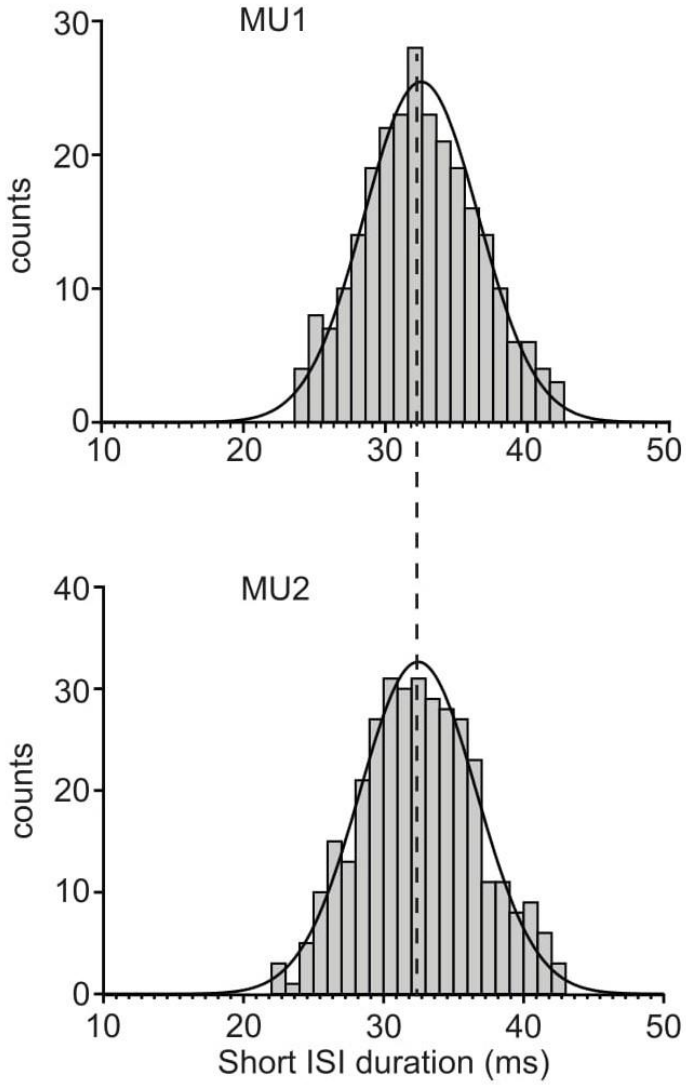

Figure 12 Frequency histogram of the short ISIs in doublets (top; N=257) and in doublets/triplets (bottom; N=442) observed in two different MUs that were recorded simultaneously and discharged at a rate of 7.0 Hz (top) and 12.0 Hz (bottom), respectively. Note the statistically indistinguishable means (vertical dashed line) of the two distributions (32.53±4.0 ms and 32.47±4.2 ms, respectively) despite the very different firing rates of the two MUs.

Interestingly, the ‘idiosyncratic’ mean short ISIs of our 23 patients were nevertheless correlated (R^2: 0.43, p=0.0003) to the difference between the periods that correspond to the primary and secondary components (Figure 13). The implications of this finding are considered in the Discussion together with the possible role of the stretch reflex loop and descending beta-range oscillatory signals in the generation of PD tremor.

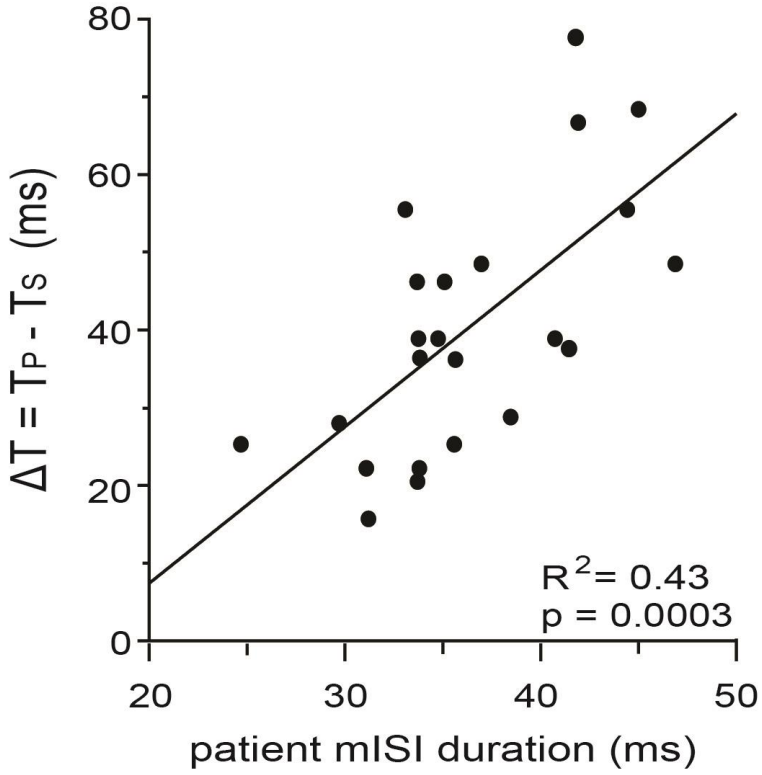

Figure 13 The difference (ΔT, ordinate) of the period of non-overt tremor (T_S) from the period of overt tremor (T_P) depends on the mean short ISI (abscissa) of the patient. Each data point is from a different patient and the line through them is the linear regression line.

DISCUSSION

Our results provide a broad and detailed view of tremor-related MU firing synchrony and patterns in Parkinson's disease. Apart from disclosing two common motoneuronal bases of formation of tremor components at rest, when stable posture is maintained but also when force is produced, these results also represent vital data for considering possible pairs of underlying neural generators. Specifically, the present findings demonstrate epoch-II intervals randomly interspersed with epoch-I intervals. Epoch-II intervals are characterized by enhanced MU synchrony at the frequency of the primary component of tremor and spike doublets/triplets exhibiting fixed, beta-range, patient specific, mean short ISIs bearing a one to one relationship to each tremor cycle. On the other hand, epoch-I intervals are reminiscent of the physiological tremor of normal subjects and are characterized by rhythmical MU firing and weak MU synchrony at the frequency of the secondary component of tremor. Finally, the frequency of the primary (4-8 Hz) and secondary (6-10 Hz) components, ca. 1.5 Hz apart, did not change appreciably among the different tremor types in each patient.

Characteristics of PD tremor and associated MU firing

Components of PD tremor

A 6-10 Hz rhythm (defined as the secondary component) appeared only during epoch-I intervals randomly interspersed with epoch-II intervals characterized by the presence of 4-8-Hz overt tremor (defined as the primary component). The secondary component of non-overt tremor was not a harmonic of the primary PD tremor component. Both at rest and while maintaining a stable posture, its auto-spectral peak was usually small and often obscured by the peak of the primary component. The small size of the secondary component at the auto-spectra was due to the small amplitude of the tremor itself rather than the small duration of epoch-I since it was roughly the same as that of epoch-II. Moreover, the frequencies of the two components did not differ by more than 1.0-2.5 Hz. This and the small duration of records obtained from patients could shift estimates of the actual frequency of parkinsonian tremor from values in the neighborhood of the primary component to values closer to the secondary. As an example, in "classic parkinsonian tremors" (Consensus Statement, Deuschl *et al*., 1998), the frequencies of rest and postural tremors per patient are considered "similar" or "same" for differences ≤1.5 Hz; but, this is the typical frequency difference between primary and secondary components. As another example, in the original study by Findley *et al*., (1981), there was difficulty in interpreting twin tremor components presumably representing different epoch categories.

Predominance of neurogenic tremor components over mechanical ones

Given the reported load-dependent frequency for one of twin tremor components in Parkinson's disease (Stiles and Pozos, 1976; Burne *et al*., 2004), one of the here observed paired components, which nevertheless showed coherence to MU activities, could reflect mechanical/neural interactions. Such interactions would preserve tremor coherence to MU activity, which cannot be due to mechanical action alone (see Lakie *et al*., 2012). More generally, interactions of neural tremor generating mechanisms with oscillations due to mechanical resonance seem unlikely because: (i) the frequency of the primary component at rest did not change when patients were asked to maintain a stable limb posture against gravity despite the large differences of the two situations in terms of limb "masses" and the same is true of the secondary component; (ii) the transition from epoch-I to epoch-II intervals and back could not be due to mechanical factors, in particular during isometric force tremor, and therefore is clearly neurogenic.

Finally, the reported load-dependent f_T appears to contrast the constant f_T observed with added mass (Homberg *et al*., 1987) or externally applied rhythmical forces (Walsh, 1979). In view of our results, such apparent discrepancies may simply reflect a variable and restricted impact of interacting mechanical components. Thus, the present identification of (even partially) neurogenic limb tremor components, using the coherence and doublets/triplets criteria, contributes towards the clarification of seemingly conflicting observations.

MU firing synchrony

In agreement with previous observations in PD patients exerting isometric force (Christakos et al., 2009), the strength of the widespread, in-phase MU synchrony at the frequency of the primary component of the tremor was higher at rest as well as while patients maintained a stable posture. In contrast, PD patients did not differ from control subjects in terms of MU synchrony at the frequency of the secondary component. Notably, given the often minimal synchrony in category-I epochs, such small tremors primarily reflect MN firing rhythmicity (see Christakos, 1986), in analogy to the small physiological force tremor resulting from uncorrelated contributions of last-recruited MUs, or, from synchronized MU action potentials due to stretch reflex loop activity (Taylor, 1962; Allum *et al*., 1978; Christakos, 1982b,c; Christakos et al., 2006). The above mentioned findings, combined with the unaltered frequency of each tremor component per patient, argue for a common neural generator, irrespective of the different conditions under which the tremor appears. The appropriateness of the employed method, compared to short-term cross-correlation analyses suggesting normal strength (Baker et al., 1992), is noteworthy.

MU firing patterns

The spike-doublets/triplets, phase-locked to primary tremor component, constitute an unusual feature, as the one that was previously described for isometric force tremor in PD patients (Christakos et al., 2009). For all tremor types, their incidences are determined by a simple and strict rule. Specifically, *all* spikes per sec in excess of f_T combine with other spikes to form doublets that are randomly interchanged with the remaining singlets; there is thus one doublet per tremor cycle when $f_U = 2 \times f_T$. Further, for spike-rates $> 2 \times f_T$, spikes in excess combine with tremor-related doublets to form triplets; consequently, there is one triplet per tremor cycle when $f_U = 3 \times f_T$. An extension to this rule is our observation of triplets/quadruplets for spike-rates $> 3 \times f_T$.

For all recorded tremors, the observed minimal spike-rates in category-II epochs were equal to f_T (see also Christakos *et al*., 2006, 2009; Erimaki and Christakos, 2008). Given the rules of MU activation, the MU recruitment rate therefore coincided with f_T. This finding follows the rationale of the common recruitment rate for the MUs of a muscle (e.g., Milner–Brown *et al*., 1973). A preliminary study of our laboratory (Erimaki et al., 2011) suggested that tremor-related neural rhythms seem to provide a timing mechanism (clock) for rhythmical control of MU activation, initiation of muscle contractions and co-ordination of muscles acting around a joint and sharing a common tremor frequency. Further, according to the size principle governing MU recruitment and rate coding (Henneman 1979; Glendinning and Enoka, 1994), MUs exhibiting doublets/triplets are small and medium-sized ones, thus representing the great majority of active MUs.

Significance of ISI features

Our data demonstrate that the average duration of the short ISIs (i.e., the interval between spikes that belong to a doublet or triplet) is nearly fixed for all tremor types, is idiosyncratic and patient specific, is in the beta range (30-50 ms) and does not depend on the mean firing rate of the unit. It has been argued that such spike-events may represent normal MN firing in response to increased net excitation (Dietz et al., 1974), as for example when strong synaptic input leads to fast contractions (see Discussion in Dengler et al., 1986). On the other hand, doublets / triplets / quadruplets, as in our records, could simply represent frequency-modulated MN discharges in response to the rhythmical input from the tremor generator. Such discharges would be analogous to the ones during voluntary force variations (De Luca and Erim, 1994; Erimaki and Christakos, 2008), including voluntary tremor (Logigian *et al*., 1988; Elek *et al*., 1991 – for differences between parkinsonian and voluntary tremor, see Morrison *et al*., 2008, 2013). If this were the case, the spikes of doublets emitted by smaller MUs, characterized by higher f_U, would be separated by shorter ISIs than those of bigger MUs in response to the same synaptic drive (Granit et al., 1966; Matthews, 1996). As a consequence, the average duration of the short ISIs would drop for MUs with high f_U, and this we did not observe. It would also be shorter for the triplets relative to the doublets emitted by a certain unit, the quadruplets relative to the triplets, etc. Our findings demonstrate that these expectations do not reflect reality either.

Alternatively, the spike-doublets of PD tremor could be due to "successive facilitatory stimuli" as suggested by Das Gupta (1963) for the generation of force tremor in PD. If this was the case, the herein observed doublets/triplets would reflect rhythmical, beta range, MN inputs from a tremor generator in the brain. Consistent with this, the average short ISI is in the middle of the values expected of the period of beta range oscillations. This is not the first time that beta-

rhythms have been implicated in the generation of tremor (e.g., Levy et al., 2002; Brown, 2003; Weinberger *et al*., 2009). By analogy to the high frequency (60-100 Hz) oscillations of phrenic MNs in response to medullary descending inputs (Christakos et al., 1988; Huang *et al*., 1996) and in line with a previous hypothesis from this laboratory (Christakos et al., 2009), the intermittent beta-range input would ride on top of a slower rhythm such as that underlying physiological tremor. The combination of the two input rhythms would push the envelope of MN membrane potential modulation towards smaller frequencies (4-8 Hz; see next subsection, below) and give rise to rhythmical local peaks, ca. 40 ms apart, in each cycle. The membrane potential of the MN would thus repeatedly surpass (2-4 times/cycle) the threshold for initiating action potentials.

Coherent beta-rhythms have been observed in cerebello-thalamo-cortical loops (Timmermann et al., 2003) showing coherence to sEMG records (Marsden et al., 2000). A recent hypothesis implicates this loop in the genesis of PD tremor (Helmich et al., 2011, 2012; Helmich, 2013; Hallet, 2014) and ascribes to the basal ganglia a role as a parkinsonian tremor "switch", intermittently triggering activity in the cerebello-thalamo-cortical loop which co-fluctuates with the tremor amplitude. This model could reflect the herein observed transition to epoch-II intervals. Beta-range oscillations have been found in low frequency potentials recorded from the subthalamic nucleus, and were patient specific enough to merit the term "signature rhythm" (Bronte-Stewart et al., 2009). Moreover, deep-brain stimulation of the ventral intermediate thalamic nucleus (Fukuda et al., 2004; Swan et al., 2020), leading to reduction of cortical and cerebellar activity, causes drastic decrease in the amplitude of tremor and increases its frequency from abnormal (4-8 Hz) to normal (6-10 Hz) values, i.e., changes similar to those that take place during transitions from epoch-II to epoch-I intervals. Similarly, deep-brain

stimulation of the subthalamic nucleus, globus pallidus or ventral intermediate thalamic nucleus causes tremor attenuation (Bronte-Stewart et al., 2009; Birdno et al., 2012) through cortical-beta suppression (Whitmer et al., 2012; Menon et al., 2013; Wang et al., 2018). Finally, it is observed that the intake of L-dopa medication suppresses beta-band oscillation while improves tremor (Tinkhauser et al., 2017).

General implications for the genesis of parkinsonian tremors

The presence of 4-8-Hz rhythms at basal ganglia and thalamus nuclei, which sometimes exhibited coherence to cortical activities and sEMGs, have led to proposals on parkinsonian tremor genesis that directly involve these affected regions (dopamine depletion). There are thus various attractive hypotheses using supraspinal models of tremor-underlying neural rhythms. The recorded tremor-locked rhythmic bursts from neurons located mainly at posterior ventral lateral nucleus of thalamus supported the model of thalamic pacemaker of tremor in Parkinson's disease (Jahnsen and Llinas, 1984; Magnin et al., 2000; Plaha et al., 2008). Other studies propose the emergence of PD tremor when 12-15-Hz oscillations in the basal ganglia transformed into 4-6-Hz rhythmicity by thalamic anterior ventral lateral neurons (Pare et al., 1990). The tremor pacemaker in this hypothesis is located in basal ganglia while pallido-thalamic interactions determine the frequency band of tremor. Plenz and Kital (1999), based on in vitro data, proposed that the subthalamic nucleus and external globus pallidus constitute the central tremor generator which is modulated by striatal inhibition. Finally, the increased correlations between remotely situated pallidal neurons could result in peripheral tremor. Based on these observations, Bergman et al. (1998) suggested the basal ganglia circuit as the tremor generator.

According to the present observations of common tremor frequencies and shared, abnormal MU firing behaviors in category-II epochs, the neural generator of primary components seems to be the same for all tremor types. Further, the rules determining the incidences of doublets / triplets, and the features of the respective ISIs, may profitably guide its

exploration. The situation is analogous for the secondary components (epochs-I), where normal-like MU behaviours are common among the different tremor types.

The here observed differences between primary and secondary components are compatible with two alternating rhythm generators that operate at frequencies differing by ca. 1.5 Hz, or, otherwise, one generator with two states of operation. The former possibility is not directly supported by existing data on alternating cerebral rhythms 1.5-Hz apart; but it could also represent one cerebral oscillator and another oscillator in spinal-peripheral motor systems, the second oscillator being analogous to those discussed by Burne (1987) and Rivlin-Etzion *et al*. (2008). The alternative possibility of a two-state oscillator seems more plausible if one considers the intermittently modified rhythmical action of the spinal stretch reflex loop through the doublets/triplets.

Possible role of spinal stretch reflex loop oscillations in parkinsonian tremor genesis

Oscillatory action of the spinal stretch reflex loop was implicated in earlier hypotheses on parkinsonian tremor genesis (Rack and Ross, 1986; Burne, 1987; Spiegel, 2002). Such action has long been considered as an inevitable generator of physiological force tremor, depending on the loop's gain and the degree of associated MU synchrony (Lippold et al., 1957; Lippold, 1970; Hagbarth and Young, 1979; Rack and Ross, 1986). More recent studies (Christakos *et al*., 2006; Erimaki and Christakos, 2008) indeed presented strong evidence, through the ischemic Ia afferent block suppressing both 6-10-Hz synchrony and tremor, for a crucial role of spindle feedback in tremor generation. They further described a simple model of spinal stretch reflex loop action, which, appropriately modified, seems also applicable to parkinsonian tremor genesis.

Oscillations due to the operation of the stretch reflex loop occur at a frequency corresponding to the *overall delay* around the loop which normally consists of (i) a roughly 100-ms *muscle contraction delay* from the onset of lumped MU-twitches to the onset of grouped muscle spindle discharges during the decay of the muscle-twitch, i.e. around the time of peak lengthening velocity (Lippold et al., 1957), and (ii) a roughly 30-ms afferent and efferent *conduction delay*. The resulting 130 ms overall delay corresponds to a tremor frequency of 7.7 Hz (Oppenheim et al., 1983), which lies roughly in the middle of the 6-10 Hz range of physiological tremor frequencies (Christakos et al., 2006), as well as of non-overt tremor frequencies during epoch-I intervals in PD patients. Figure 14A illustrates a schematic

representation of simulated rhythmic spikes, force-twitches and force oscillation of a hypothetical MU (Christakos, 1982a) during epoch-I intervals.

During epoch-II intervals, it is MUs with relatively high f_U that are likely to contribute to the tremor. They emit spikes about 40 ms apart which are grouped into doublets and triplets. The summation of their twitches results into 'broadened' and larger force-pulses (Figure 14B). Because of the increase of the contraction delay, the overall delay increases proportionately (Figure 14B). As a consequence, the frequency of the primary component of tremor would drop to the 4-8 Hz range that characterizes Parkinson's disease. This line of argument is supported by the positive correlation between the mean short ISI and the difference T_P-T_S, where T_P is the period of the primary component of the tremor and T_S that of the secondary. Likewise, the presence of broader MU-twitches in leg muscles (Belanger and McComas, 1985) relative to those in arm muscles could be responsible for the lower frequency of parkinsonian leg tremor, compared with that of arm tremor (O'Suilleabhain and Matsumoto, 1998). Moreover, summation of two (doublet) or more (triplet, quadruplet) twitches of the same MU leads to contraction decays with steeper slope (Fig. 9B; Fig. 3 of Elek et al., 1991; Fig. 2 of Thomas et al., 1999). In turn, muscle spindles respond more vigorously, leading to enhanced MU synchrony and additional tremor enhancement. Thus, the occurrence of spike doublets/triplets leads to a reduction of tremor frequency and increase of tremor amplitude.

Taking into account the aforementioned arguments, the coherent 4-8 Hz rhythms found in the brain could instead represent ascending projections of muscle spindles and would thus co-vary with the tremor (Hirschmann et al., 2013). This is consistent with the results of Florin et al. (2012) who used partial directed coherence analysis to conclude that 4-8 Hz rhythms are propagated from the periphery to the center in the tremor-dominant Parkinson's disease. It also

agrees with the results of Cagnan et al., (2014), where stimulation of basal ganglia or thalamic nuclei at or near f_T (e.g., 4.0-Hz vs. 3.8-Hz or 4.6-Hz) left tremor amplitude and f_T unaltered. Cerebral influences may, however, play a complementary role (see "phase-entrainment" in Cagnan et al., 2014, study) or a modulating one (see cortical stimulation results by Brittain et al., 2013).

As discussed above, doublets and triplets likely reflect a beta-rhythm generated in the cerebello-thalamo-cortical loop, and controlled by the basal ganglia "switch" for intermittent cerebello-thalamo-cortical loop outflow. This is consistent with the two epoch categories and the two states of operation of the spinal stretch reflex loop. The present two-state loop model thus fully accounts for all three tremor types and MU behaviours, including the ca. 1.5-Hz frequency difference between primary and secondary components.

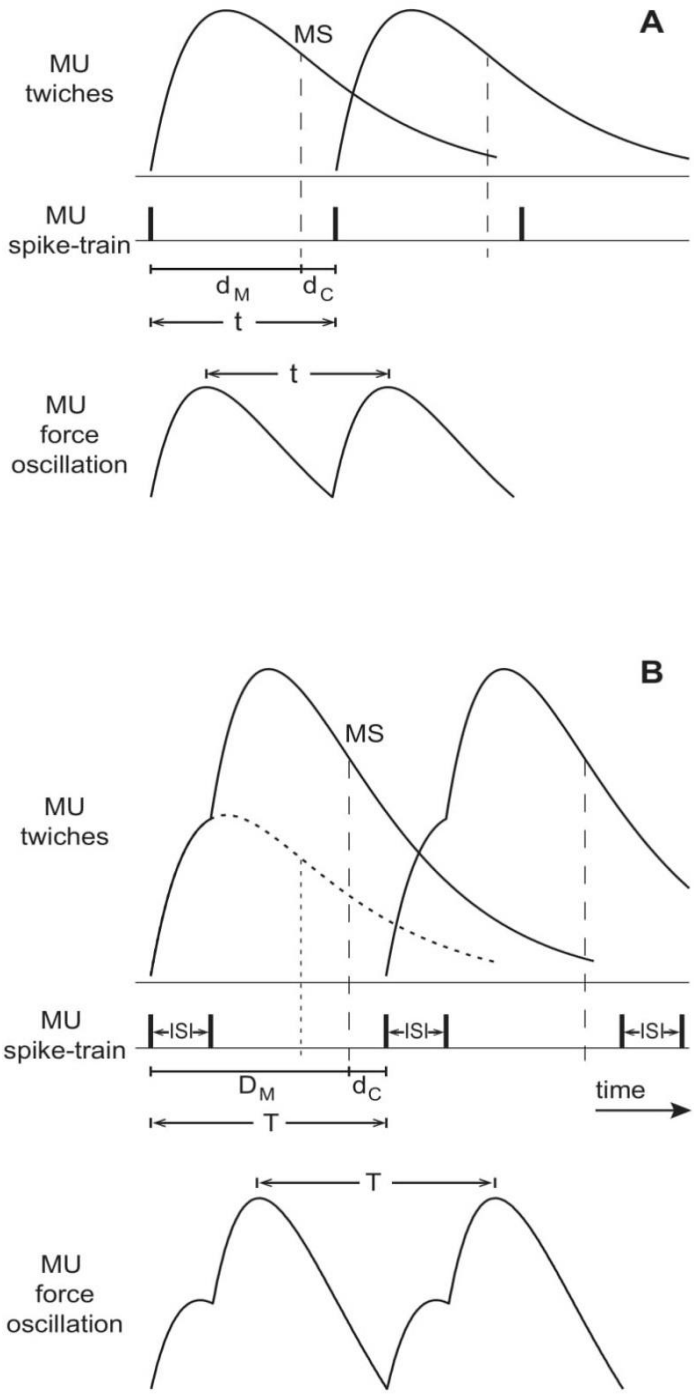

Figure 14 Conceptual model of the interaction between the stretch reflex loop and descending high-frequency oscillations that could account for our observations. (**A**) Top: MU-twitches

resulting from rhythmical discharges and associated muscle spindle discharge during the decaying phase of the MU-twitch (MS, vertical dashed line, i.e. occurring at peak lengthening velocity); contraction delay d_M, from the onset of this MU-twitch to the moment of spindle discharge, and the conduction delay d_C (afferent and efferent) from MS to the onset of the subsequent MU-twitch. Bottom: The period of the resulting MU force oscillation t, is equal to the sum of d_M and d_C. (**B**) Top: Large and broadened force-pulses resulting from the summation of two MU-twitches (solid line), caused by spike-doublets characterized by a 40 ms interspike interval (ISI); for comparison, the profile of a single MU-twitch is depicted as dotted line; the contraction delay D_M is thereby prolonged and its decay phase slope steeper as compared with that of the single twitch. Bottom: The period of the resulting MU force oscillation T, is in this case prolonged and its amplitude increased.

Conclusions

Parkinsonian tremor exhibits an abnormal, low frequency component (4-8 Hz), which is abnormally large due to the presence of enhanced MU synchrony. It also exhibits spike doublets / triplets with beta-range (30-50 ms) mean ISIs. Parkinsonian tremor is randomly interspersed within segments of higher frequency (6-10 Hz) physiological tremor. For each patient, the frequencies of the two components differ by about 1.5 Hz, but also the synchrony enhancement and the mean ISIs, are the same for all tremor types. Moreover, these fixed, patient-specific mean ISIs seem to link the two generators. These data are consistent with a neural origin of parkinsonian tremor and severely constrain models of its generation, whether cerebral or spinal-peripheral. The spinal stretch reflex loop, acting as a two-state oscillator influenced by intermittently descending high-frequency (beta-range) oscillations, provides a model that could explain our data as well as previous observations on tremor-related cerebral rhythms. Finally, our analysis of MU activity links tremor appearance to underlying neural generators and could facilitate tremor studies in other movement disorders.

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