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ORIGINAL ARTICLE

TMS excitability study in essential tremor: Absence of gabaergic changes assessed by silent period recordings

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Summary
Background. — Essential tremor (ET) is thought to emerge from activity in a distributed cerebello-thalamo-cortical network. It has been proposed that the network goes into oscillation because of abnormal GABAergic inhibitory transmission.
Objective. — To test this idea by investigating GABAergic circuitry in motor cortex using transcranial magnetic stimulation (TMS).
Methods. — Motor cortex excitability was examined using TMS in 21 patients with essential tremor and in 20 control subjects. Resting and active motor threshold (RMT, AMT) and input—output curves examined corticospinal excitability. Contralateral silent period (cSP) at a different range of stimulation intensities, and the ipsilateral silent period (iSP) using a stimulus intensity of 150% RMT were used as measures of GABAergic function.
Results. — RMT and AMT were significantly lower in patients than controls and patients had a steeper I/O curve. However, there were no significant differences in either cSP at different intensities or in iSP.
Conclusion. — We found no evidence in favour of the GABA hypothesis in ET.

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Introduction

Essential tremor (ET) is one of the most common neurological disorders and the commonest type of tremor [20]. The 2018 Movement Disorders (MDS) consensus criteria define classic ET as an isolated tremor syndrome of bilateral upper limb action tremor, with a duration > 3 years, with or without tremor in other locations (e.g. head, voice, lower limbs), and without other neurological signs (e.g. dystonia, ataxia or parkinsonism) [1]. Moderate and advanced stages of ET can be physically and socially disabling [18]. The few medications that have been used to treat ET have demonstrated only modest efficacy [6].

Etiology and pathophysiology of ET are not yet well understood. A recent review [12] postulates that ET is a disorder of a cerebello-thalamo-cortical circuit, but what makes this circuit oscillate is still unclear. There are two main theories: the GABA hypothesis and the cerebellar degeneration hypothesis [11]. The former is supported by nuclear imaging showing abnormal 11C-flumazenil binding to GABA-A receptors in ventrolateral thalamus, cerebellar dentate nucleus, and premotor cortex [27]. Furthermore, a postmortem study found reduced levels of GABA-A (35% reduction) and GABA-B (22—31% reduction) receptors in the dentate nucleus of patients with ET [23]. Markers of GABA-ergic dysfunction have also been reported in the locus coeruleus and pons, but these findings are less well established [25]. Mally et al. [22] also found reduced levels of GABA in cerebrospinal fluid analysis of ET patients in one of the first early studies. It has been suggested that reduced GABAergic inhibition of dentate nucleus from Purkinje cells of the cerebellum might destabilize the cerebello-thalamo-cortical network and result in oscillatory activity and tremor [23].

Evidence of cerebellar degeneration has been described by Louis et al., who observed structural changes in Purkinje cells and neighboring neurons, reduced Purkinje cell linear density with ''empty baskets'', and Purkinje cell heterotopias [19]. There were also changes in the distribution of climbing fiber-Purkinje cell synapses [17]. However, not all findings have been replicated by other groups, possibly because of differences in sampling protocols, staining and assessment methods, and subject/control definitions. As with the GABA hypothesis, cerebellar Purkinje cell dysfunction may lead to cerebello-thalamo-cortical network dysfunction.

The purpose of the present study was to look for evidence of abnormal GABA function using TMS methods. Previous studies have failed to show any changes in short-interval intracortical inhibition (SICI), which is thought to test GABAa interaction in motor cortex [24]. However, three studies showed non-significant increases in the duration of the contralateral silent period, which is thought to depend on GABAb pathways [2,24,26]. The silent period duration depends on stimulus intensity, but in the previous studies only a single intensity was used. In addition, the intensity is usually expressed relative to threshold, which assumes that the input/output (I/O) curve is the same in ET as in normal subjects. Here we investigated the I/O curve and the recruitment of the silent period in 21 patients with essential tremor compared with age- and sex-matched healthy volunteers. We also examined the ipsilateral silent period, which is a transcallosally-mediated inhibition of the non-stimulated cortex, also thought to involve a GABAergic pathway.

Methods

Patients

Twenty-one patients were recruited from the outpatient clinic of Aswan University Hospital (14 males and 7 females, mean age 28.6 ± 10.4 years; range, 15—56 years) during the period from Jan 2016-Jan 2017. The consensus statement on the classification of tremors from the Task Force on Tremor of the International Parkinson and Movement Disorder Society was used as the basis for inclusion criteria [1]. According to this consensus statement, tremor is defined along two main axes: clinical features (Axis 1) and etiology (Axis 2).

Table 1 Demographic and clinical data of studied patients with essential tremor (ET).

	ET patients	Controls	*P* value
Sex ratio male/female	14/7 (66.6/33.3%)	13/8 (61.9/38.1%)	0.45
Age (Mean ± SD) (years)	28.6 ± 10.4	32.3 ± 11.3	0.35
Classification of ET according to Tremor Investigation group (TRIG) classification of essential tremor [5]	Definite ET: 14 (66.6%). Probable ET: 7 (33.3%)		
Duration of ET diagnosis: range (mean ± SD) (years)	3—22 (7.7 ± 5.3)		
ET localization	Hands: 14 (66.6%) Hands and feet: 2 (9.5%) Hands and head: 1 (4.8%) Hands, head and jaw: 2 (9.5%) Hands, jaw and feet: 1 (4.8%) Hands, head, jaw, and feet: 1 (4.8%)		
Treatment by propranolol	11 (52.4%)		
Family history of tremor	9 (47.3%)		

The ET syndrome (on Axis 1) is defined as an isolated tremor syndrome of bilateral upper limb action tremor, with a duration > 3 years, with or without tremor on other locations (e.g. head, voice, lower limbs), and without other neurological signs (e.g. dystonia, ataxia, and parkinsonism). The Task Force also defined an ''ET plus syndrome'', where patients have an ET syndrome with additional ''soft'' neurological and systemic symptoms. Exclusion criteria for both ET syndromes are isolated focal tremors (voice, head), orthostatic tremor with a frequency > 12 Hz, task- and position-specific

Table 2 Cortical excitability parameters of essential tremor patients versus controls.

	Patients ($n = 21$) Mean ± SD	Controls ($n = 21$) Mean ± SD	P value	Time X group
Resting motor threshold (RMT)	36.4 ± 5.8	41.5 ± 6.8	0.016	
Motor active threshold (AMT)	30.1 ± 5.8	33.9 ± 6.9	0.04	
Amplitude of MEP (μV)				F = 3.8, df = 1.4,
110%	190.7 ± 92.5	136.3 ± 91.7	0.069	and $P = 0.042$
120%	435.5 ± 439.2	254.1 ± 191.9	0.104	
130%	700.5 ± 782.7	377.5 ± 321.1	0.1	
140%	1159.2 ± 929.8	565.3 ± 385.4	0.014	
150%	1713.4 ± 1573.3	905.2 ± 597.1	0.039	
Cortical silent period duration (ms)				F = 0.19, df = 2.7,
110%	65.2 ± 30.6	60.8 ± 27.3	0.61	and $P = 0.88$
120%	80.79 ± 30.9	81.8 ± 39.5	0.927	
130%	102.0± 37.8	102.8 ± 46.4	0.955	
140%	121.6 ± 33.8	125.8 ± 48.6	0.725	
150%	140.2 ± 41.3	137.6 ± 55.1	0.87	
Transcallosal inhibition duration (ms)	29.1 ± 9.6	26.25 ± 8.2	0.32	

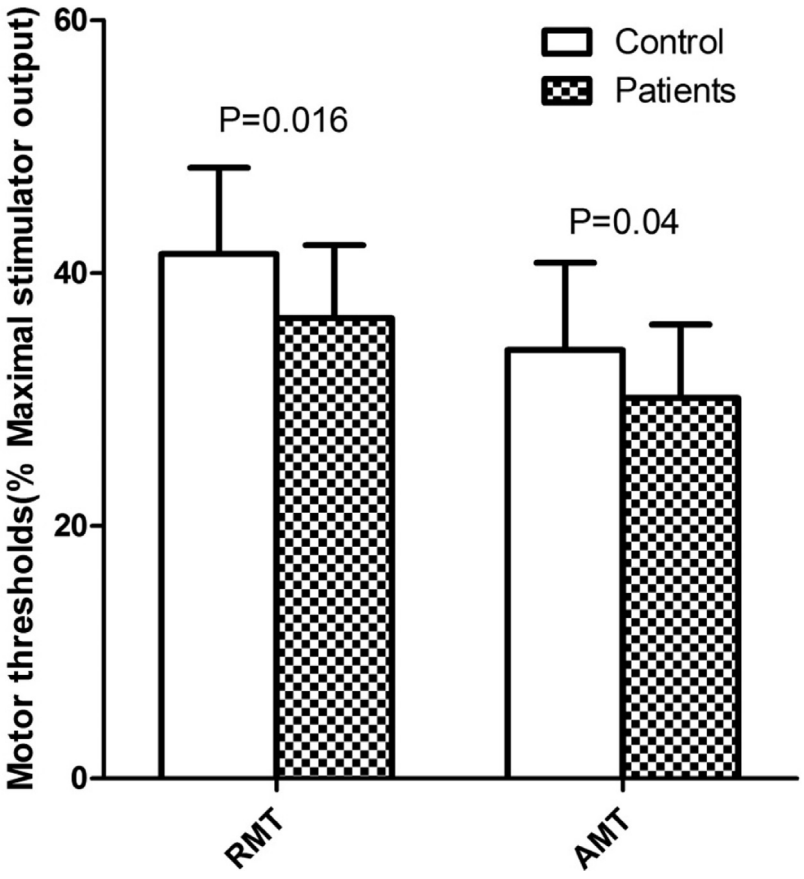

Figure 1 Resting and active motor threshold (rMT and aMT) among studied groups. There were significant lower both rMT and aMT in patients group compared with control group ($P = 0.016$, and 0.04 respectively).

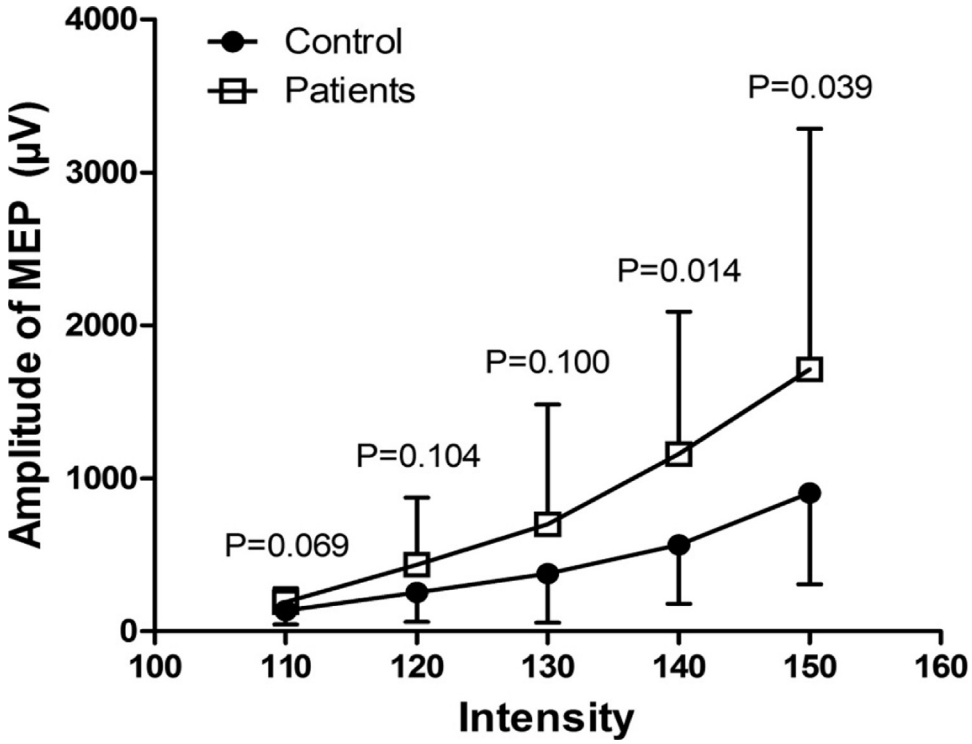

Figure 2 Input-output curve (I/O) among studied groups. There was a significant TMS intensity (at 110, 120, 130, 140, and 150% of resting motor threshold) X group (patients versus control group) interaction ($P = 0.042$) that was caused by a steeper I/O relationship in patients compared with controls.

tremors, and sudden onset and stepwise deterioration [5]. Subjects with recent exposure to tremorgenic drugs, signs of drug withdrawal state or historical or clinical evidence of psychogenic tremor were also excluded.

Standard electrolyte panel, thyroid function tests, blood urea nitrogen and creatinine levels, liver function tests were obtained for each patient to exclude other causes of tremors. Cerebral tomography (CT) and or magnetic resonance imaging (MRI) was performed if needed to exclude lesions.

All cases were diagnosed by neurologists. The duration of ET ranged from 3 to 22 years. The location of tremor is reported in Table 1. Eleven patients were on medication (all on propranolol) with no satisfactory results and the other 10 were taking no medication. Nine patients had a positive family history of ET. One patient had benign prostatic hyperplasia.

Twenty-one age-matched healthy volunteers (13 males and 8 females; mean age, 32.3 ± 11.3 years; range, 17—55 years) represented the control population for assessment of cortical excitability. Patients and controls were asked not to take drugs that affect motor cortex excitability (dopaminergic drugs, tranquillizers or antiepileptic drugs, selective serotonin reuptake inhibitor antidepressant drugs) for at least one week before the study.

The study was approved by the Institutional Ethical Committee of Aswan University Hospital, and subjects gave their informed consent according to the declaration of Helsinki.

Experimental setup and design

Subjects sat in a comfortable chair. Electromyographic (EMG) recordings (Nihon Kohden 9400, Japan) from the abductor digiti minimi muscle of the right hand was acquired with silver—silver chloride surface electrodes, using a muscle belly—tendon set-up, with a 3-cm-diameter ground electrode placed on the wrist. The EMG parameters included a band pass of 20 to 1000 Hz and a recording time window of 200 ms. The TMS was performed with a 90-mm figure-of eight coil connected to a Magstim (UK) super rapid magnetic stimulator. Motor thresholds (MT) were determined after localization of the motor ''hot spot'' for the abductor digiti minimi muscle in left hemisphere as described in previous reports [7,13]. EMG signals were monitored and recorded for 20 ms before stimulation. Both resting motor threshold (RMT) and active motor threshold (AMT) were expressed as a percentage of maximum stimulator output (equal to 100%).

Input—output curve was evaluated at rest by increasing the intensity of stimulation in steps of 10% from 110% to 150% of RMT. Any trials in which there was detectable prestimulus EMG activity were discarded from the analysis on the basis that this indicated that the participants were not completely relaxed [14,15].

The contralateral cortical silent period (cSP) of left hemisphere was evoked with stimuli of different intensities (110, 120, 130, 140, and 150% of RMT) during isometric 50% maximum voluntary contraction of the contralateral abductor

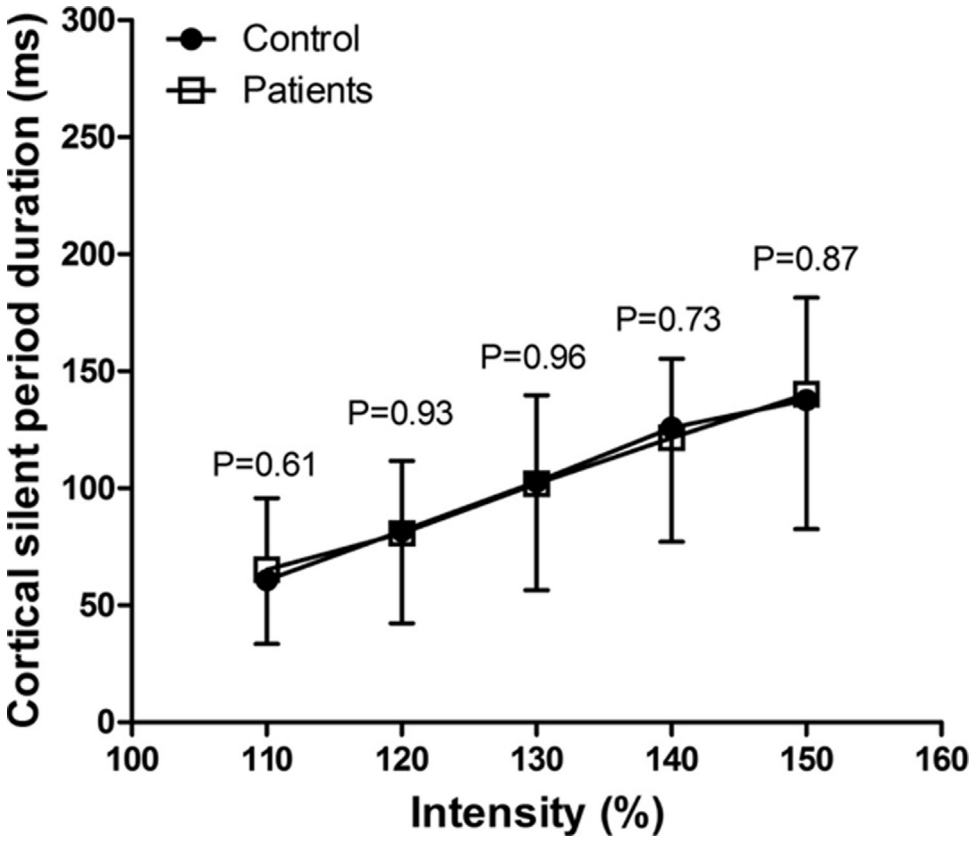

Figure 3 Duration of contralateral silent period (cSP) at different TMS intensities. There was no significant interaction in the duration of cSP at different TMS intensity (110, 120, 130,140, and 150% of RMT) and ''group'' (patients and controls) and no significant differences at any stimulus intensity.

digiti minimi muscle [14,15]. Ipsilateral silent period (iSP) was assessed as previously described [14,15].

Statistical analysis

One- or two-way analysis of variance (SPSS version 16) was used to compare measures between patients and controls. Means ± standard deviation (SD) were used to represent data. The level of significance was set at $P < 0.05$. A two factor repeated measures analysis of variance (ANOVA) with ''groups'' (patients versus control) and ''intensity'' as main factors was conducted for the I/O and cSP curves. When necessary, a Greenhouse—Geisser correction was applied to correct for non-sphericity. Post hoc unpaired t tests were carried out for specific comparisons of data from the two groups.

Results

Motor thresholds

Both RMT (36.4 ± 5.8) and AMT (30.1 ± 5.8) for patients with ET were significantly lower than for controls (41.5 ± 6.8 and 33.9 ± 6.9, respectively; *P* value significance for intergroup comparison: 0.01 and 0.04 for RMT and AMT, respectively) (Table 2, Fig. 1,).

Input—output (I/O) curve

A two-way repeated measures analysis of variance with main factors of ''TMS intensity'' (110, 120, 130,140, and 150% of RMT) and ''group'' (patients and controls) showed a significant intensity X group interaction ($F = 3.8$ and $P = 0.042$) that was caused by a steeper input—output (I/O) relationship in patients compared with controls. This was attributable to significantly higher amplitudes of MEP at 140, and 150% of RMT (unpaired t-test $P = 0.014$ and 0.039 uncorrected values respectively) (Table 2, Fig. 2).

Duration of contralateral silent period (cSP) at different TMS intensities

A two-way repeated measures analysis of variance with main factors of ''TMS intensity'' (110, 120, 130,140, and 150% of RMT) and ''group'' (patients and controls) showed no significant group X intensity interaction ($F = 0.19$ and $P = 0.88$) and no significant differences at any stimulus intensity (Table 2, Fig. 3).

Ipsilateral silent period (iSP)

The iSP was not significantly different between the patient group (29.1 ± 9.6) and controls (26.25 ± 8.2; $P = 0.32$) (Table 2, Fig. 4).

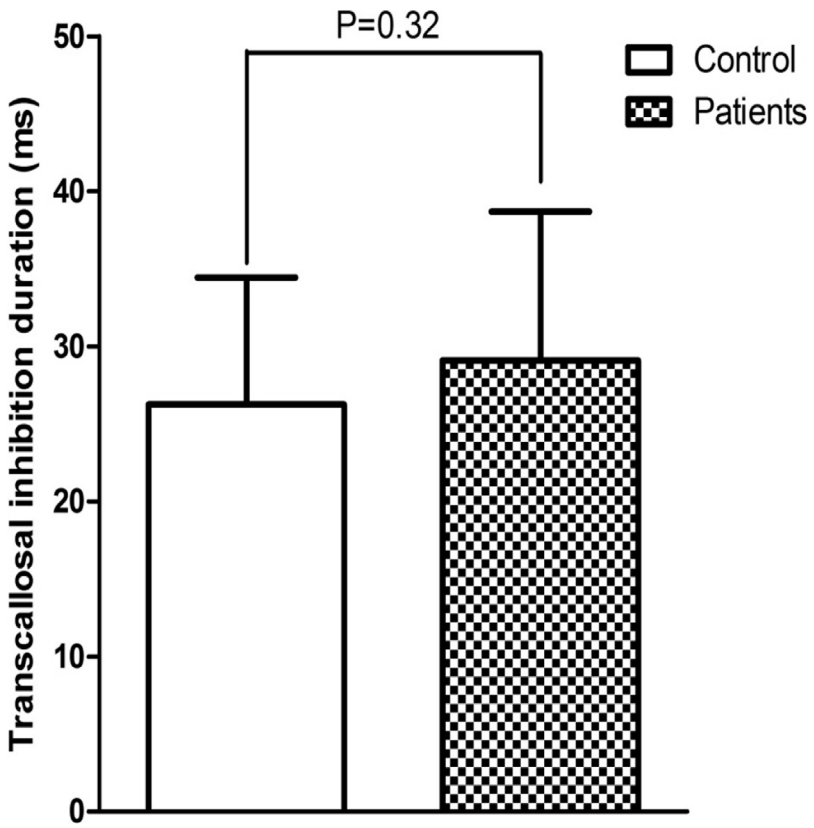

Figure 4 Ipsilateral silent period (iSP). There was no significant difference in the duration of iSP between the patient group and controls ($P = 0.32$).

Discussion

The present experiments were designed to examine in detail whether patients with ET displayed abnormalities in two measures of cortical GABA function: cSP and iSP. Two previous studies of patients with ET [2,26] found non-significant increases in cSP duration, recorded at only one given intensity relative to the patients' RMT. Since the cSP duration increases with stimulus intensity, we explored a larger range of intensities to test if more subtle changes could be observed. However, there was no difference in cSP duration compared to normal subjects at any stimulus intensity. There was also no difference in the duration of the iSP, which has not been previously studied in ET and is thought to assess transcallosal GABAergic connection. The present results therefore fail to support the GABA hypothesis for ET.

Several other arguments have failed to find evidence in favour of the GABA hypothesis in ET. First, one previous study found no significant alteration of SICI in ET patients [24], a paired-pulse TMS parameter thought to reflect GABAa cortical activities. Deng et al. investigated the association between GABAa receptor alpha-1 (GABRA1) gene expression and ET in 76 familial ET patients, which proved to be non-significant. The authors concluded that missense, nonsense, or splice site mutations in the coding regions of the GABRA1 gene were not a major genetic cause of ET [4]. A larger study including 503 ET patients and 818 controls did not find evidence of an association between polymorphisms in 15 GABAa receptor genes and four GABA transporter genes and ET [27]. The inability of several drugs targeting the GABA system to control ET in various clinical trials also tends to suggest that GABA function may not be the primary deficit in ET. Such drugs include progabide [16], gabapentin [9], topiramate [3], tiagabine [10] and pregabalin [8,28].

However, we did note that our patients had lower AMT and RMT compared with the control group as well as an increased I/O slope, which suggests increased corticospinal excitability. No previous studies of ET have reported differences in motor thresholds [21,24,26] and none have examined the I/O characteristics. It is possible that RMT and I/O curves could be influenced by ''sub-threshold'' tremor activity in the sampled muscle, meaning that although at rest, the motoneurons were closer to firing threshold than those of the relaxed control subjects and hence more readily excitable. However, the fact that AMT, which cannot be influenced by ''sub-threshold'' motor activity, also appeared to be slightly reduced in patients suggests that some cortical excitability changes occur in ET that would be useful to explore in future studies.

Disclosure of interest

The authors declare that they have no competing interest.

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