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Original Article

Altered Cortical Excitability and Inhibition in Patients with Primary Dystonia: A Transcranial Magnetic Stimulation Study

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ABSTRACT: ***Background:*** The literature on cortical excitability, inhibitory and facilitatory properties of the brain in patients with primary dystonia is not well elucidated. We aimed to study the changes in these neurophysiological parameters in patients with dystonia using transcranial magnetic stimulation (TMS). ***Methods:*** Patients with primary dystonia of presumed genetic etiology ($n = 36$) and an equal number of healthy controls (HC) ($n = 36$) were recruited from May 2021 to September 2022. TMS was done using single and paired pulse paradigms. The left motor cortex was stimulated, and responses were recorded from the contralateral first dorsal interosseus muscle. Resting motor threshold (RMT), central motor conduction time, contralateral silent period (cSP), ipsilateral silent period (iSP), short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF) were recorded. All patients underwent whole exome sequencing. ***Results:*** The mean age of patients was 36.6 ± 13.5 years. There was a significant reduction of cSP (79.5 ± 33.8 vs 97.5 ± 25.4, $p = 0.02$) and iSP (42.3 ± 13.5 vs 53.8 ± 20.8, $p = 0.003$) in patients compared to HC. SICI was significantly enhanced in patients (0.38 ± 0.23) compared to HC (0.51 ± 0.24, $p = 0.006$). RMT was higher (42.1 ± 7.9 vs 37.1 ± 6.4%, $p = 0.032$) with enhanced SICI (0.36 ± 0.21 vs 0.56 ± 0.25, $p = 0.004$) in patients with generalized dystonia ($n = 20$) compared to HC. The genetically determined subgroup ($n = 13$) had significantly enhanced SICI compared to HC (0.23 ± 0.15 vs 0.51 ± 0.23, $p = 0.001$). ***Conclusions:*** Patients with primary dystonia have altered cortical excitability and inhibition with significantly reduced silent period and enhanced intracortical inhibition suggestive of impaired GABAergic neurotransmission.

RÉSUMÉ : Altération de l'excitabilité et de l'inhibition corticales chez les patients atteints de dystonie primaire : une étude par stimulation magnétique transcrânienne. ***Contexte :*** La littérature portant sur l'excitabilité corticale, ainsi que sur les propriétés inhibitrices et facilitatrices du cerveau chez les patients atteints de dystonie primaire, n'est pas encore bien comprise. Nous avons ainsi cherché à étudier les changements de ces paramètres neurophysiologiques chez les patients atteints de dystonie en utilisant la stimulation magnétique transcrânienne (SMT). ***Méthodes :*** Des patients atteints de dystonie primaire chez lesquels on a présumé une étiologie génétique ($n = 36$), de même qu'un nombre égal de témoins en bonne santé (TBS) ($n = 36$), ont été recrutés de mai 2021 à septembre 2022. La SMT a été effectuée en utilisant des paradigmes impulsionnels uniques et appariés. À cet égard, le cortex moteur gauche a été stimulé et les réponses ont été enregistrées à partir du premier muscle interosseux dorsal controlatéral. Le seuil moteur au repos (*resting motor threshold*)), le temps de conduction dans le système nerveux central (*central motor conduction*), la période silencieuse controlatérale (PSC), la période silencieuse ipsilatérale (PSI), l'inhibition intra-corticale à intervalle court (IICIC) et la facilitation intra-corticale (FIC) ont été enregistrés. Notons enfin que tous les patients ont subi un séquençage de l'exome entier (SEE). ***Résultats :*** L'âge moyen des patients était de 36,6 ± 13,5 ans. Une réduction significative de la PSC (79,5 ± 33,8 contre 97,5 ± 25,4 ; $p = 0,02$) et de la PSI (42,3 ± 13,5 contre 53,8 ± 20,8 ; $p = 0,003$) a été observée chez les patients comparés ensuite aux TBS. L'IICIC s'est révélée significativement plus élevée chez les patients (0,38 ± 0,23) par rapport aux TBS (0,51 ± 0,24 ; $p = 0,006$). Le seuil moteur au repos était plus élevé (42,1 ± 7,9 contre 37,1 ± 6,4 % ; $p = 0,032$) avec une IICIC améliorée (0,36 ± 0,21 contre 0,56 ± 0,25 ; $p = 0,004$) chez les patients atteints de dystonie généralisée ($n = 20$) par rapport aux TBS. Finalement, le sous-groupe génétiquement déterminé ($n = 13$) a donné à voir une IICIC notablement plus élevée que celle des TBS (0,23 ± 0,15 contre 0,51 ± 0,23 ; $p = 0,001$). ***Conclusions :*** Les patients atteints de dystonie primaire ont présenté une altération de l'excitabilité et de l'inhibition corticales avec une période silencieuse significativement réduite et une inhibition intra-corticale accrue, ce qui suggère une neurotransmission gabaergique altérée.

Keywords: cortical excitability; dystonia; genetics; intracortical facilitation; short-interval intracortical inhibition; transcranial magnetic stimulation

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Highlights

- Transcranial magnetic stimulation was used to study cortical excitability changes in patients with primary dystonia.
- There is a significant reduction of silent period, increase in resting motor threshold and enhancement of short-interval intracortical inhibition.
- There is impaired GABAergic neurotransmission with differential involvement of $GABA_A$ and $GABA_B$ pathways.

Introduction

Dystonia is one of the common presentations in a movement disorder clinic. Under the aegis of the Movement Disorders Society in 2013, dystonia is defined as a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions resulting in abnormal, often repetitive movements, postures or both that can be patterned, twisting or tremulous.[1] The current classification system has subdivided dystonia into two axes: Axis I classifies it according to age at onset, body distribution, temporal pattern and associated features. Axis II provides etiological classification such as inherited, acquired or idiopathic.[1]

Transcranial magnetic stimulation (TMS) is a non-invasive tool for assessing cortical excitability, facilitatory, inhibitory properties of the brain and neural plasticity.[2] Even though there are no disease-specific signatures of TMS parameters as of now, it serves as a useful ancillary tool in studying the pathophysiology of various neurologic disorders ranging from neurodegenerative to inflammatory etiology.[3] It has found its applications in Parkinson's disease, Huntington's disease, ataxia, dystonia and Tourette syndrome.[3–7] TMS provides critical insights into the integrity of intracortical neuronal structures, as well as conduction along the callosal, corticospinal and corticonuclear fibers, including the peripheral motor pathways.[8] Prior TMS studies have elucidated "loss of inhibition" as the predominant pathophysiological basis of dystonia. Key parameters illustrating this concept include short-interval intracortical inhibition (SICI), long-interval intracortical inhibition (LICI), silent period (SP) and intracortical facilitation (ICF). ICF is due to NMDA receptor-mediated glutamatergic neurotransmission, whereas SP, SICI and LICI involve GABAergic neurotransmission.[7,9] The landscape of primary dystonia has widened enormously over the past few decades with the greater availability of next-generation sequencing techniques. Several pathomechanisms have emerged in each of these genetic subtypes. TMS can reveal further insights into these specific forms of monogenic dystonia with regard to cortical excitability and plasticity.

There is a paucity of literature on changes in various TMS parameters in patients with dystonia. The sample sizes in these studies have been small and are largely restricted to the subtype of focal dystonia.[4,10–16] Moreover, while the majority of the literature on genetically determined dystonia is restricted to *DYT1* and dystonia-myoclonus syndromes,[17–20] most of them have shown inconsistencies in their methodologies, protocols followed and outcome parameters. The number of tested subjects has been far too few to conclude on specific findings in these specific genetically determined dystonia subtypes. We designed a prospective study aimed to study the various neurophysiological parameters in patients with primary dystonia of presumed genetic etiology using TMS and compare them with healthy age and gender-matched subjects.

Methods

This prospective, cross-sectional observational study was conducted at the Department of Neurology, National Institute of Mental Health and Neuro Sciences (NIMHANS), Bengaluru, Karnataka, India. Patients with idiopathic dystonia of presumed genetic etiology with age 12 years or more were included in the study ($N = 36$). Patients with secondary and acquired causes of dystonia and those having epilepsy, metallic implants, pregnancy and organ failure were excluded. A thorough evaluation to exclude the secondary and acquired etiologies of dystonia consisted of brain MRI, copper studies, metabolic screening, tandem mass spectroscopy for inborn errors of metabolism, urine for abnormal metabolites and organic acids, ophthalmological evaluation with slit lamp examination and fundoscopy and other relevant ancillary tests. All patients were classified into axis I and axis II as per the latest consensus.[1] Detailed demographic data were collected, and motor severity was assessed using the Burke–Fahn–Marsden Dystonia Rating Scale and the Unified Dystonia Rating Scale. A well-informed written consent was obtained from all the participants. An equal number of age and gender-matched healthy controls ($N = 36$) were recruited in the study after informed consent.

The healthy controls were recruited from our outpatient department or hospital staff who were either family friends or relatives of patients with other acquired neurological disorders. These healthy individuals were initially screened for previous head injury, epilepsy, metal implants, etc., and were evaluated with a detailed neurological examination. Healthy controls with a history of head injury, major organ dysfunction or neurodegenerative disorders in the family were excluded. Consecutive healthy controls with age and gender matching were recruited in a one-to-one basis method.

TMS was done for all the participants, and all the patients underwent genetic testing (whole exome sequencing [WES]). The study was approved by the Institute Ethics Committee (No. NIMH/DO/IEC [BS & NS DIV] 2020–21).

Transcranial magnetic stimulation methodology

TMS was performed using a Bistim 200^2 magnetic stimulator connected to a figure-of-eight coil (Magstim 200, UK). Subjects were reassured and comfortably positioned with their arms supported on a chair. Surface electromyographic (EMG) responses were recorded using Ag-AgCl electrodes placed in a belly-tendon configuration. The active electrode was placed on the right first dorsal interossei (FDI) muscle, while the reference electrode was placed on the metacarpophalangeal joint of the right index finger. The left motor cortex (M1) was stimulated using a handheld figure-of-eight coil. The coil handle was positioned at an angle of 45° pointing backward. As a first step, the motor hotspot for right FDI was identified. It was defined as the point on the scalp where a magnetic stimulus would generate the largest amplitude of motor evoked potential (MEP). Subsequently, the spot was marked manually. The stimulus was applied repetitively at the same spot, and the intensity was augmented in 5% increments until a satisfactory graph of MEP was obtained. A total of 10 consecutive trials were recorded. The subjects were repeatedly given audiovisual feedback to ensure adequate relaxation of the FDI muscle. The interval between the consecutive stimuli was more than 3 sec. Both single and paired pulse protocols were performed.

The parameters that were assessed included resting motor threshold (RMT), central motor conduction time (CMCT), contralateral silent period (cSP) and ipsilateral silent period (iSP).[21] The lowest magnetic stimulus intensity required to evoke a MEP of at least 50 μV peak-to-peak amplitude in the relaxed muscle in 50% of the 10 consecutive trials was defined as RMT. CMCT was

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calculated as a difference (L1–L2) in the latency to the onset of MEP obtained by motor cortex stimulation (L1) and lower cervical spinal root stimulation (L2). CMCT was expressed in msec. Silent period (SP), defined as the interval of electromyographic suppression in the ongoing voluntary EMG activity following a single suprathreshold TMS pulse applied over the contralateral motor cortex, was measured. cSP was determined from a partially contracted (30% of maximal voluntary contraction) right FDI by using a stimulus intensity measuring 120% of RMT, and an average response from 10 stimuli was obtained. iSP was determined after applying 100% stimulus intensity (maximal stimulator output) on the ipsilateral side with a fully contracted (100% contraction) muscle.

Paired pulse stimulation studies were also performed to assess intracortical inhibitory and facilitatory changes. The parameters measured included SICI and ICF. In this method, a subthreshold conditioning stimulus (CS) was followed by a suprathreshold test stimulus (TS) at a fixed interval.[7,22] The CS was predefined at 80% of RMT, while the suprathreshold TS was pre-set at 120% of RMT. In our study protocol, SICI was obtained at an interstimulus interval (ISI) of 2 msec, while ICF was obtained at an ISI of 10 msec. Both SICI and ICF were measured as a ratio of the MEP amplitude obtained by paired stimulation (CS + TS amp.) to the MEP amplitude obtained by TS (TS amp.). All the responses were recorded and amplified using the Nihon Kohden, Neuropack 8 device (Nihon Kohden Corp., Osaka, Japan). The data were filtered and band-passed at 10–5000 Hz settings for digitization.

We used these TMS parameters to specifically understand the cortical inhibitory and facilitatory abnormalities in patients with dystonia. These tests were performed in accordance with the guidelines recommended by the International Federation of Clinical Neurophysiology.

Genetic testing

All the recruited patients were subjected to WES. The samples were subjected to genomic DNA extraction using QIAamp DNA Blood Mini Kit (Qiagen Germany, #51104), followed by a quality check. The raw reads were then aligned to the human reference genome (GRCh37) based on the BMA-mem algorithm.[23] The variants were identified using the framework of Genome Analysis Toolkit (Broad Institute, Cambridge, MA, USA).[24] Base quality score recalibration was done for filtration of the variants. The variants were annotated using the ANNOVAR platform (http://www.openbioinformatics.org/annovar/).[25] Common variants and those having a minor allele frequency > 0.01 were not considered. A comparison analysis was performed with the Exome Aggregation Consortium, 1000-genome project and gnomAD database (https://gnomad.broadinstitute.org/). Each individual sequence variant was interpreted using different software including PolyPhen-2, Sorting Intolerant from Tolerant webserver and MutationTaster.[26–28] The mutation effects of the variants on the clinical phenotype were classified following American College of Medical Genetics and Genomics standards and guidelines.[29]

Statistical analysis

The outcome measures of TMS included mean peak-to-peak MEP amplitudes, latencies and duration. Data were represented as mean and standard deviation. Paired *t*-tests were utilized to compare TMS parameters between patients and their age and sex-matched controls, when data were normally distributed. Comparison of non-normally distributed quantitative variables was done using Mann–Whitney *U* test. All statistics were performed using IBM SPSS, version 23. A p-value of < 0.05 was considered statistically significant.

Table 1. Clinico-demographic profile of the study participants

Parameters	Cases	Controls	*p*-value
Number of patients	36	36	
Age at assessment (mean ± SD)	36.6 ± 13.5 years	37.0 ± 13.0 years	0.628
Age at onset (mean ± SD)	29.8 ± 15.7 years	–	–
Duration of illness (mean ± S.D)	6.7 ± 7.3 years	–	–
Gender:			
Males	24 (66.7%)	24 (66.7%)	
Age at onset-wise distribution			
Infantile (up to 2 years)	1 (2.8%)	–	
Childhood (3–12 years)	2 (5.6%)	–	
Adolescent (13–20 years)	11 (30.6%)	–	
Early adulthood (21–40 years)	12 (33.3%)	–	
Late adulthood (>40 years)	10 (27.8%)	–	
Clinical parameters			
Body distribution			
Focal	15 (23.1%)	–	
Segmental	13 (20.0%)	–	
Generalized	36 (55.4%)	–	
Multifocal	1 (1.5%)	–	
Isolated	29 (80.6%)	–	
Combined	7 (19.4%)	–	
Genetics			
Genetically determined cases	13 (36.1%)	–	
Idiopathic cases	23 (63.9%)	–	

Results

Demographic, clinical and genetic data

Of the 36 patients with dystonia who participated in this study, the majority were male ($n = 24$, 66.7%). The mean age at onset was 29.8 ± 15.7 years, and the mean age at presentation was 36.6 ± 13.5 years. Based on axis-I classification for age of onset-wise distribution of dystonia, 2.8% ($n = 1$) was of infantile onset (up to 2 years), 5.6% ($n = 2$) childhood onset (3–12 years), 30.6% ($n = 11$) adolescent onset (13–20 years), 33.3% ($n = 12$) early adulthood (21–40 years) and 27.8% ($n = 10$) late adulthood onset (above 40 years). The mean duration of illness was 6.7 ± 7.3 years, with a range varying from 6 months to 42 years. On the basis of the body distribution of dystonia, the study population was segregated into four groups. The focal, segmental, generalized and multifocal groups comprised 23.1% ($n = 15$), 20.0% ($n = 13$), 55.4% ($n = 36$) and 1.5% ($n = 1$) patients, respectively. Among these, 80.6% ($n = 29$) presented with isolated dystonia, while 19.4% ($n = 7$) of cases had combined dystonia (Table 1). Disease-causing variants in the dystonia-causing genes were identified in 13 cases. These comprised *KMT2B* ($n = 2$) and

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Table 2. Comparison of TMS parameters between patients and healthy controls

TMS Parameter	Cases (n = 36)	Controls (n = 36)	t-score	*p*-value
Single pulse TMS				
RMT (% MSO)	41.1 ± 7.8	38.4 ± 6.4	1.53	0.136
CMCT (msec)	7.9 ± 2.0	7.2 ± 2.2	1.96	0.060
cSP (msec)	79.5 ± 33.8	97.5 ± 25.4	−2.44	**0.020***
iSP (msec)	42.3 ± 13.5	53.8 ± 20.8	−3.16	**0.003****
Paired pulse TMS				
SICI	0.38 ± 0.23	0.51 ± 0.24	−2.91	**0.006***
ICF	1.22 ± 0.18	1.31 ± 0.28	−1.82	0.078

% MSO = percentage of maximal stimulator output. **p*-value < 0.05, ** *p*-value < 0.01. TMS = transcranial magnetic stimulation; RMT = resting motor threshold; CMCT = central motor conduction time; cSP = contralateral silent period; iSP = ipsilateral silent period; SICI = short-interval intracortical inhibition; ICF = intracortical facilitation.

one each case of *AFG3L2, POLG, ATP13A2, CTSA, GNAL, MICU1, MME, PANK2, SGCE, TOR1A and VPS16.*

TMS results

There was no significant difference in RMT and CMCT (41.1 ± 7.8 vs 38.4 ± 6.4, $p = 0.136$ and 7.9 ± 2.0 vs 7.2 ± 2.2 msec, $p = 0.060$, respectively) between the patients and healthy controls. There was a significant reduction of cSP (79.5 ± 33.8 vs 97.5 ± 25.4, msec $p = 0.020$) and iSP (42.3 ± 13.5 vs 53.8 ± 20.8, $p = 0.003$) in patients with dystonia compared to healthy controls. SICI was significantly enhanced in patients compared to healthy controls (0.38 ± 0.23 vs 0.51 ± 0.24, $p = 0.006$), while there was no significant difference in ICF (1.22 ± 0.18 vs 1.31 ± 0.28, $p = 0.078$). However, there was a tendency toward reduced ICF (Table 2).

Subgroup analysis

There was no difference in any of the TMS parameters between isolated and combined dystonia. Based on body distribution, the three subgroups of focal ($n = 10$), segmental ($n = 6$) and generalized dystonia ($n = 20$) had no significant difference in any of the TMS parameters Patients with isolated cervical dystonia ($n = 5$) had significantly reduced iSP (41.5 ± 17.0 vs 59.1 ± 14.9, $p = 0.044$) and cSP (67.3 ± 28.5 vs 107.4 ± 29.9, $p = 0.041$) compared to that of healthy controls. Patients with generalized dystonia ($n = 20$) had higher RMT (42.1 ± 7.9 vs 37.1 ± 6.4%, $p = 0.032$) and prolonged CMCT (8.3 ± 2.3 msec vs 7.4 ± 2.5 msec, $p = 0.044$) compared to healthy controls. In addition, these patients had significantly enhanced SICI (0.36 ± 0.21 vs 0.56 ± 0.25, $p = 0.004$) compared to healthy controls.

The TMS parameters were compared among patients with different ages of onset, but there were no significant changes in any of the TMS parameters between the groups (Table 3).

TMS parameters in genetic dystonia group

There was a significant difference in SICI between the genetically determined dystonia group ($n = 13$) and healthy controls ($n = 13$), with enhanced SICI in patients (0.23 ± 0.15) compared to healthy controls (0.51 ± 0.23, $p = 0.001$). There was no significant change in other TMS parameters. In comparison to healthy controls ($n = 23$), patients with idiopathic dystonia (genetically negative, $n = 23$) showed reduced cSP (75.9 ± 23.0 vs 102.4 ± 27.1 msec, $p = 0.008$), iSP (44.1 ± 14.2 vs 56.5 ± 19.0 msec, $p = 0.015$) and ICF (1.22 ± 0.18 vs 1.38 ± 0.27, $p = 0.017$) (Table 4). There was a significant reduction of cSP in genetically negative patients (75.9 ± 23.0 msec) compared to those with genetically determined dystonia (88.7 ± 44.5 msec, $p = 0.021$). Also, SICI was significantly increased in genetically determined patients (0.23 ± 0.15) compared to genetically negative patients (0.47 ± 0.23, $p = 0.002$). There was no difference in the other TMS parameters between these two groups. The genetically determined classic *DYT* cases included two cases of *KMT2B* and a single case each of *SGCE, TOR1A* and *VPS16*. The other genetically determined cases had disease-causing variants in the genes: *PANK2*., *AFG3L2, POLG, ATP13A2, CTSA, GNAL, MICU1* and *MME* (Table 5).

Table 3. Comparison of TMS parameters between different age at onset

	Adolescent onset (13–20 years) (n = 11)	Early adulthood (21–40 years) (n = 12)	Late adulthood (>40 years) (n = 10)	F-score	p-value
Single pulse TMS					
RMT (% MSO)	42.6 ± 9.1	40.2 ± 6.7	42.2 ± 8.6	0.301	0.742
CMCT (msec)	8.1 ± 1.5	7.7 ± 1.7	7.5 ± 1.9	0.334	0.719
iSP (msec)	41.7 ± 9.8	39.4 ± 15.9	43.7 ± 12.8	0.289	0.751
cSP (msec)	82.3 ± 40.2	69.3 ± 18.9	82.7 ± 33.0	0.663	0.523
Paired pulse TMS					
SICI	0.31 ± 0.17	0.41 ± 0.25	0.49 ± 0.21	1.819	0.180
ICF	1.14 ± 0.13	1.31 ± 0.23	1.23 ± 0.13	2.667	0.086

Note: There was a single case with infantile onset and two cases with childhood onset. Due to low sample size in these two groups, comparative analysis was not performed. % MSO = percentage of maximal stimulator output; CMCT = central motor conduction time; cSP = contralateral silent period; ICF = intracortical facilitation; iSP = ipsilateral silent period; RMT = resting motor threshold; SICI = short-interval intracortical inhibition; TMS = transcranial magnetic stimulation.

Discussion

Electrophysiological evaluation of patients using TMS was performed in 36 cases of primary dystonia and an equal number of controls, which represents one of the largest TMS studies in patients with primary dystonia. Compared to age and gender-matched healthy controls, iSP and cSP were found to be significantly reduced with an enhancement of SICI. The results of our study are concordant with the previously published literature.[7,9,14,30] The subgroup of patients with isolated cervical dystonia showed significantly greater reduction of cSP and iSP. SP represents two of the important physiologies. While at the cortical level, it depicts the $GABA_B$ receptor-mediated inhibition of cortical pathways, at the spinal level, it represents the inhibitory reflex pathway of Renshaw inhibition.[13] The reduced SP duration, both ipsilateral and contralateral, suggests greater influence of inhibitory pathways at play in patients with cervical dystonia. This is concordant with previous observations on focal dystonia.[31] Unlike previous studies, where the authors have demonstrated reduced

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Table 4. Comparison of TMS parameters between patients of genetic positive and negative dystonia with healthy controls

	Genetically determined (n = 13)	Healthy controls (n = 13)	t-score	*p*-value	Genetic negative (n = 23)	Healthy controls (n = 23)	t-score	*p*-value
Single pulse TMS								
RMT (%)	41.8 ± 8.5	37.0 ± 5.5	2.035	0.063	41.2 ± 7.8	38.8 ± 7.0	0.903	0.378
CMCT (msec)	8.6 ± 2.5	7.5 ± 3.2	1.738	0.106	7.4 ± 1.7	6.9 ± 1.5	1.012	0.325
iSP (msec)	38.0 ± 13.0	49.0 ± 23.3	−1.581	0.138	44.1 ± 14.2	56.5 ± 19.0	−2.698	**0.015**
cSP (msec)	88.7 ± 44.5	90.3 ± 22.1	−0.129	0.899	75.9 ± 23.0	102.4 ± 27.1	−2.989	**0.008**
Paired pulse TMS								
SICI	0.23 ± 0.15	0.51 ± 0.23	−4.358	**0.001**	0.47 ± 0.23	0.49 ± 0.26	−0.225	0.825
ICF	1.24 ± 0.19	1.24 ± 0.29	0.023	0.982	1.22 ± 0.18	1.38 ± 0.27	−2.639	**0.017**

Note: Genetic data of three patients were not available for three patients. TMS = transcranial magnetic stimulation; RMT = resting motor threshold; CMCT = central motor conduction time; cSP = contralateral silent period; iSP = ipsilateral silent period; SICI = short-interval intracortical inhibition; ICF: intracortical facilitation.

Table 5. TMS parameters in specific genetic cases (n = 13)

Gene	RMT (%)	CMCT (msec)	iSP (msec)	cSP (msec)	SICI	ICF
AFG3L2	48.0	6.1	22.3	71.3	0.330	1.67
POLG	54.0	10.7	24.5	30.4	0.500	1.50
ATP13A2	52.0	10.4	38.3	119.2	0.450	1.01
CTSA	42.0	6.1	46.5	125.7	0.070	1.33
GNAL	40.0	9.2	25.5	45.5	0.310	1.19
KMT2B	36.0	15.1	72.2	155.2	0.27	1.28
KMT2B	36.0	5.8	32.6	34.0	0.120	1.06
MICU1	36.0	7.7	38.3	85.5	0.180	1.23
MME	25.0	9.7	41.5	132.2	0.090	1.09
PANK2	53.0	10.1	44.2	100.4	0.200	1.40
SGCE	34.0	5.7	49.8	107.7	0.030	1.06
TOR1A	40.5	6.8	30.7	95.0	0.190	1.15
VPS16	48.0	8.3	35.5	44.2	0.290	1.30

TMS = transcranial magnetic stimulation; RMT = resting motor threshold; CMCT = central motor conduction time; cSP = contralateral silent period; iSP = ipsilateral silent period; SICI = short-interval intracortical inhibition; ICF = intracortical facilitation.

SICI, our patients had preserved or enhanced SICI.[7,9] While certain parameters, like SP and SICI, were consistently found to be shortened in dystonia patients in previous studies, the results have varied with regard to other measures like ICF.[30]

Both SP and SICI are measures of cortical inhibition. SICI represents a complex phenomenon occurring at the level of the motor cortex, mediated by $GABA_A$ receptors, while SP is mediated by $GABA_B$ receptors. The studies that have demonstrated reduced SICI have primarily included patients with focal dystonia, task-specific dystonia and cervical dystonia. In addition, some of these studies on SICI in dystonia have been confounded by the inclusion of data collected across a wide range of ISIs (1–6 milliseconds). There are other mechanisms such as abnormal plasticity and sensorimotor integration that are responsible for causing dystonia. Similar to our findings, some studies have shown normal or preserved SICI in patients with dystonia.[31,32,33] It has also been postulated that different types of motor cortical inhibition are produced by different inhibitory circuits. Hence, in our patients with generalized dystonia, there may be differential involvement of the inhibitory circuits that may explain the discrepancy observed between reduced SP and enhanced SICI.

This discrepancy could also be due to TMS methodological differences, patient selection and variability in sample characteristics. Our study differed from previous research in several key aspects. First, our patient cohort had a presumed genetically determined etiology, a factor that has been underrepresented in prior studies. Second, although all patients underwent TMS after a 24-hour drug washout, the potential influence of anti-dystonic medications – known to enhance SICI – could not be entirely ruled out due to the high burden of disability and the absence of drug level testing.[32] Third, voluntary muscle contraction, which is known to enhance SICI as a reflection of surround inhibition in motor pathways, may have played a role. This effect was particularly relevant since some patients had suboptimal intelligence, making it difficult for them to strictly follow test instructions.

Previous studies have largely demonstrated a normal RMT in dystonia patients, which is similar to our findings.[30]

In a study on six subjects with myoclonus dystonia, there was no difference in the TMS parameters (SICI, ICF and LICI) compared to healthy controls.[20] Similar findings have been replicated in the subsequent two studies on *DYT11* cases.[34,35] Studies on *TOR1A*-related dystonia have shown decreased SICI in patients as well as asymptomatic carriers.[17] The role of cerebellar pathways in genetic dystonia was revealed in a TMS study on a single *THAP1*-related dystonia, which detected absent cerebellar inhibition.[36] The genetically determined subgroup in our study was heterogeneous. The combined analysis of the TMS parameters in genetically determined dystonia subgroups was at par with the TMS results of previous studies, which were largely confined to *DYT1* and *DYT11* (Table 6).

While the anatomical basis of dystonia is still a matter of a lot of controversy, the current understanding of its pathophysiology revolves around the concept of a network model involving the basal ganglia-cerebello-thalamocortical circuit.[37] Recent theories suggest that the pattern of spatial and temporal activity within globus pallidus interna and substantia niagra pars reticulata modulates the excitation and inhibition within these nuclei, leading to normal movements. Impairment in the excitability of these inhibitory pathways at this network level contributes to the development of dystonia.[37–39] The balance between the inhibitory and excitatory pathways within this network is altered. Loss of inhibition and increased facilitation within this network are the basis for dystonia. Reduced SP suggests loss of inhibition.

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Table 6. Summary of the TMS findings in studies involving genetically determined dystonia patients

Authors	Study participants	Methods	Results	Conclusions
Edwards et al. 2003[17]	10 manifesting DYT1 gene carriers, 7 non-manifesting DYT1 gene carriers, 13 HC	ICI, ICF, SP, RI	MDYT1: Decreased ICI, SP, presynaptic spinal RI. NMDYT1: Decreased ICI, SP, normal spinal RI	Reduced cortical inhibition
Li et al. 2008[20]	6 cases of myoclonus dystonia	SICI, ICF and LICI	No significant difference of all the parameters compared to HC	Preserved functioning of GABAergic pathways
Meunier et al. 2008[34]	5 DYT11 and 10 HC	aMT, SICI, SAI	Higher aMT. SICI and SAI normal.	Impairment of cortical membrane excitability
Salm et al. 2009[35]	15 DYT11	MEP, SP, SICI, ICF and SICF at different ISIs varying from 1.2 to 3.2 msec	Normal SP, SICI and ICF. Polyphasic MEPs in SICF	Abnormality in cortical membrane excitability
Nikolov et al. 2019[36]	1 case of DYT-THAP1-related dystonia	Cerebellar inhibition	Absent cerebellar inhibition	Alteration in cerebellar pathways

TMS = transcranial magnetic stimulation; RMT = resting motor threshold; CMCT = central motor conduction time; cSP = contralateral silent period; iSP = ipsilateral silent period; SICI = short interval intracortical inhibition; ICF = intracortical facilitation; SAI = short afferent inhibition; HC = healthy control; aMT = activated motor threshold; MDYT1 = manifesting DYT1 carriers; NMDYT = non-manifesting; DYT1 = carriers; MEP = motor evoked potential; RI = reciprocal inhibition.

However, the SICI was enhanced in our study, which could be due to the differential involvement of the GABAergic pathways within the network and/or the nodes of the network. Additional neural circuitry that is also involved includes the pathways of spinal reciprocal inhibition, which particularly explain the genesis of limb dystonia.

Most of the studies have shown a reduction in SP in patients with dystonia, suggesting a reduction in the GABAergic neurotransmission. This shows that there is a loss of cortical inhibition in patients with dystonia. Other electrophysiological tests such as the blink reflex and somatosensory evoked potentials have shown a reduction in the cortical inhibition.[40] In addition, the RMT, which is a measure of neuronal hyperexcitability, is normal. This suggests that dystonia is induced by a loss of inhibition of the motor circuitry rather than a change in the neural membrane excitability.[41] The balance between excitatory and inhibitory circuits is altered in patients with dystonia, with a reduction in inhibitory neurotransmission. The surround inhibition is also lost in these patients.[42] The cerebellum also plays an important role in modulating the cortical excitability and SICI. This cerebellar brain inhibition is lost in patients with dystonia.[32] Hence the reduction in SP seen in our patients suggests a loss of inhibition, leading to dystonia. However, SICI is preserved in our patients differing from previous studies. This could be due to differential involvement of $GABA_A$ and $GABA_B$ motor circuitry in these patients, methodological differences, patient selection and variability in the dystonia.

The difference in study protocols, heterogeneity in experimental approaches, lack of uniform measurement standards and nonavailability of normative data are some of the challenges related to TMS's applicability. In addition, the majority of the studies have been based on focal hand dystonia, task-specific dystonia, blepharospasm, cervical dystonia and psychogenic dystonia, with very little literature available on generalized dystonia. This is probably attributable to the fact that many of the patients with generalized dystonia are significantly disabled, hence not amenable to undergoing TMS. With regard to TMS parameters on individual genetically determined etiologies, further research involving a greater number of subjects in each of the genetic subgroups is required to conclude on any specific pattern of TMS signatures in each of the genetic variants. The findings of our study not only strengthened the previously known attributes but also served to add new findings in generalized dystonia and in a diverse genetically determined group.

We acknowledge several limitations of our study. Our study includes a heterogeneous group of genetically determined dystonia patients, with some genetic subgroups having small sample sizes. This represents an important limitation that reduces the power of the study in drawing conclusions. This warrants further dedicated TMS research on specific genetically determined etiologies to determine the pattern of alterations in cortical excitability and delve further into the pathophysiological mechanisms at play. Given the rarity of these genetically determined dystonias, collaboration with other centers can enhance the sample size of each of these genetic subgroups and provide more robust data. There were considerable dropouts, primarily attributed to the high degree of severity of dystonia and the greater disability burden in these patients. As predicted from our study, genetically determined subgroups were significantly more disabled that interfered with their participation in TMS testing. Hence, the majority of our patients who underwent procedure-based assessments were genetically negative. In the absence of normative data for the Indian population in TMS, we had to rely on findings from healthy controls, which reduces the generalizability of our study results. Furthermore, our study didn't include a paired association protocol that could have added value by enabling assessment of measures of synaptic plasticity, another key element of dystonia pathogenesis.

Conclusions

The electrophysiological correlates showed findings suggestive of altered cortical inhibition and impaired cortical excitability as has been suggested in previous studies. Our findings not only reaffirm established aspects of dystonia but also contribute novel insights, particularly in the context of generalized dystonia. The use of TMS in genetically determined dystonia has shed light on spared GABA-mediated pathways in some forms of the disorder. However, this needs to be confirmed in a larger and more homogeneous genetic dystonia cohort. These results enhance our understanding of the complex neurophysiological basis of dystonia and provide a foundation for further investigations and potential therapeutic strategies targeting inhibitory pathways.

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