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Cortical hyperexcitability in patients with *C9orf72* mutations: relationship to phenotype

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Abstract

Introduction—Patients with mutations in *C9orf72* can have amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), or ALS-FTD. The goals were to establish whether cortical hyperexcitability occurs in *C9orf72* patients with different clinical presentations.

Methods—Cortical thresholds and silent periods were measured in thenar muscles in 19 participants with *C9orf72* expansions and 21 healthy controls using transcranial magnetic stimulation (TMS). El Escorial and Rascovsky criteria were used to diagnose ALS and FTD. Fourteen participants with *C9orf72* expansions were re-tested 6 months later. Correlations with finger-tapping speed, timed peg test, the ALS functional rating scale, and Dementia Rating Scale were examined.

Results—Most participants with *C9orf72* expansions had normal or low cortical thresholds. Among them, ALS patients had the lowest thresholds and significantly shorter silent periods. Thresholds correlated with timed peg-test scores. TMS did not correlate with the Dementia Rating Scale.

Conclusion—TMS measures of cortical excitability may serve as non-invasive biomarkers of ALS disease activity.

Keywords

C9orf72; Amyotrophic lateral sclerosis; ALS; Frontotemporal Dementia; Transcranial Magnetic Stimulation; Cortical hyperexcitability; cortical silent period

INTRODUCTION

Cortical hyperexcitability has been measured in sporadic amyotrophic lateral sclerosis (ALS) using transcranial magnetic stimulation (TMS). TMS studies have shown reduced

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thresholds for eliciting motor evoked potential (MEPs) at rest, shortened cortical silent periods, and reduction of intracortical inhibition mediated by short and long intracortical circuits.[1–5] The reductions in intracortical inhibition and the cortical silent period were most apparent in patients with shorter disease durations in cross-sectional[6–9] and longitudinal studies.[10–12] Later, as disease progresses, excitability declines, leading to lengthening of the silent period.[13] These findings have been interpreted as showing impairment of intracortical inhibitory interneurons early in the ALS disease process. In contrast, cortical thresholds remained relatively stable over time[6,7,12] possibly reflecting more prolonged integrity of the corticospinal-motor neuron connection. Cortical hyperexcitability is less certain in TMS studies of patients with sporadic frontotemporal dementia (FTD): cortical thresholds have been reported to be normal[14,15] or increased.[16] Short intracortical inhibition was reported to be normal in FTD patients[14] or slightly reduced, primarily in those with the progressive aphasia variant, with a trend toward shortened cortical silent periods.[15]

Cortical hyperexcitability has been proposed as a therapeutic target in ALS, using TMS to identify the time window for treatment with drugs to reduce hyperexcitability.[17] However, the transition from normal to hyperexcitability may happen over a short period of time. For example, asymptomatic carriers of mutations in the *SOD1* gene for familial ALS had normal measures of cortical excitability, but patients with ALS had increased cortical excitability, as did 3 carriers who developed ALS symptoms shortly after the study.[18,19] Expansion mutations in the gene *C9orf72* are another cause of familial ALS. The same mutation in *C9orf72* causes familial frontotemporal dementia (FTD).[20,21] Patients with the *C9orf72* mutation exhibit a range of phenotypes, from classical ALS to the classical behavioral variant of FTD (bvFTD), to intermediate phenotypes with a variable degree of features of both disorders, even within the same pedigree.[22,23] At autopsy, brains of patients with *C9orf72* expansions have widespread neuronal inclusions containing TDP-43, regardless of whether the clinical phenotype is FTD or ALS.[24,25] A recent threshold tracking study found evidence for reduced short intracortical inhibition in ALS patients with *C9orf72* expansion mutations compared to asymptomatic carriers[26], but it did not assess whether cortical hyperexcitability is associated with both ALS and FTD phenotypes with *C9orf72* expansion mutations. Previous studies of cortical excitability in FTD variants, which mostly predated identification of the *C9orf72* gene mutation, have varied findings.[14,15,27] To examine the relationship between cortical excitability and clinical phenotype, we carried out TMS studies on patients with ALS, bvFTD, and ALS-FTD and on asymptomatic carriers with *C9orf72* expansion mutations.

METHODS

Subjects

Symptomatic and asymptomatic carriers with a repeat expansion in the *C9orf72* gene were recruited nationwide for a natural history study (NCT01925196). All subjects gave written informed consent for the study, which was approved by the NIH Combined Neuroscience Institutional Review Board. Symptomatic patients also appointed a surrogate decision maker. An expansion mutation in *C9orf72* (defined as > 44 repeats) confirmed in a CLIA-certified laboratory, was required for inclusion in the study. Healthy controls gave written

informed consent for a separate study (NCT01517087) approved by the Institutional Review Board for physiological studies and clinical rating scales.

Clinical Evaluation

A neurological examination, needle EMG, and cognitive testing were carried out to diagnose motor and cognitive impairment of participants with *C9orf72* expansion mutations (hereafter referred to as "C9+" participants). C9+ participants were classified as C9+ ALS, C9+ bvFTD, C9+ ALS-FTD, or C9+ asymptomatic. The El Escorial criteria-revised[28] were used for diagnosis of ALS, and the Rascovsky criteria were used for diagnosis of bvFTD.[29] The ALS Functional Rating Scale-Revised (ALSFRS-R),[30] finger tapping speed, and timed completion of the 9-hole peg test (9HPT) were measured to determine if physiological measures correlated with motor function. The Mattis Dementia Rating Scale, which provides a profile of cognition in FTD distinct from Alzheimer disease, was used as a measure of cognitive function for correlational analyses.[31] It consists of multiple tasks to measure attention, initiation-perseveration, construction, conceptualization, and memory; a total score of 10 represents the mean for healthy subjects, adjusted for age and education.[32] All healthy controls had normal neurological examinations and cognitive screening with the Montreal Cognitive Assessment (www.mocatest.org).

Physiology

The motor cortex was stimulated using a Magstim 200 transcranial magnetic stimulator (TMS; Magstim, UK) with a hand-held 90-mm round coil. Surface EMG recordings were made from the abductor pollicis brevis (APB) muscles bilaterally using paired 9-mm surface electrodes. The optimal position for obtaining a motor-evoked potential (MEP) from thenar muscles was determined and marked on the scalp. The cortical threshold for each muscle was defined as the lowest intensity producing a motor evoked potential (MEP) of at least 50 microvolts in 5 of 10 trials at rest. Thresholds are given as the percentage of stimulator output. MEPs were elicited using TMS intensities 130% of threshold during moderate contraction. Two sets of 5 MEPs were rectified and averaged. Cortical silent periods were measured from the stimulus artifact to the return of voluntary contraction in rectified traces. Central motor conduction times (CMCT) were calculated by subtracting the peripheral conduction time, estimated from the minimal F-wave latency,[33] from the MEP latency.

Statistics

The Shapiro-Wilk test was used to assess normality. *t*-tests were used to assess group differences between controls and C9+ participants for normally distributed data. A 1-way ANOVA was used to compare C9+ diagnostic subgroups. The average of the right and left silent periods and right and left cortical threshold were used for analysis of differences between C9+ subgroups, using the Tukey test for multiple comparisons, and for correlating with non-lateralized clinical measures. Paired *t*-tests and the Kruskal-Wallis test were used to compare baseline and follow-up of silent periods and thresholds from the same side. Pearson correlations were used to compare TMS measures from the corresponding hemisphere with lateralized clinical variables, such as finger tapping. A threshold of $P < 0.05$ was used to determine significance, corrected for multiple comparisons.

RESULTS

Demographics

The demographic and clinical data of all subjects at the baseline visit are shown in Table 1. There was no difference between the mean ages and gender ratios between the group of 19 C9+ participants and 21 healthy controls. At baseline, 13 C9+ participants met criteria for possible, probable, or definite ALS, and 8 C9+ participants met criteria for possible, probable, or definite bvFTD. Of these, 5 C9+ participants met criteria for both ALS and bvFTD, with cognitive symptoms at onset in 3, and motor symptoms at onset in 2. Three C9+ participants were asymptomatic. Ten symptomatic C9+ participants were taking riluzole during the study. Seventeen C9+ participants returned for a follow-up examination 6 months later. Two patients with definite C9+ ALS died before follow-up. One patient with C9+ ALS at the baseline visit met criteria for C9+ ALS-FTD at 6-month follow-up.

Physiology

At baseline, APB MEPs were obtained from hands of all but 1 C9+ participant who had marked atrophy of the left APB muscle. The MEP/CMAP amplitude ratio did not differ between groups. Central motor conduction times were slightly longer in the C9+ group (Table 1). However, the CMCT was within the normal reference range of the laboratory for all but 2 hands. Most C9+ participants had normal or slightly low cortical thresholds, but the group mean did not differ from controls (Figure 1A). Patients with C9+ ALS who did not have bvFTD had the lowest mean thresholds among the *C9+* subgroups, but the difference was not significant (Figure 1A). Cortical silent periods for the C9+ participant group did not differ from controls, but they differed among the C9+ diagnostic subgroups (F=7.279, $P = 0.0007$). The shortest cortical silent periods occurred in the C9+ ALS subgroup (Figure 1B), and were significantly lower than the C9+ asymptomatic subgroup.

Correlation with clinical measures

TMS measures were correlated with the 9HPT time and finger tapping speed in C9+ participants at baseline (Table 2). Lower cortical thresholds were associated with better motor function, with significantly shorter 9HPT times for both hands, and faster finger tapping speed for the left hand. Silent periods, however, were not correlated with these motor measures. Right and left TMS measures were averaged to assess correlations with disease duration, the ALSFRS-R score, and the Dementia Rating Scale score. Silent period durations and cortical thresholds were not correlated with age, disease duration, the ALSFRS-R, or the Dementia Rating Scale.

Follow-up studies

APB MEPs were obtained from the hands of 16 C9+ participants, although 3 APB CMAP amplitudes were less than 1 mV with mildly prolonged distal latencies (4.5–6 ms). There were no significant changes in cortical thresholds (Figure 1C) or silent periods (Figure 1D) of each hand between the baseline and the 6-month follow-up evaluation for the C9+ participants group or in the subgroup with C9+ ALS. Silent periods at 6 months were correlated with silent periods measured at baseline ($r = 0.71$, $P < 0.001$), and thresholds

measured at 6 months were correlated with baseline threshold measurements (r = 0.75, $P < 0.001$). The decline in the ALSFRS-R score was not correlated with the changes in the average of the right and left cortical thresholds or silent periods.

DISCUSSION

In this study, we found evidence for cortical hyperexcitability in participants with *C9orf72* mutations with ALS that was not seen in C9+ participants with only bvFTD or ALS-FTD, or in asymptomatic carriers. Cortical hyperexcitability was evidenced by shortened silent periods following TMS-evoked potentials in hand muscles in patients with ALS, with normal or low cortical thresholds. The finding of a shortened silent period, particularly early in the course of disease, has been noted in some, although not all, previous studies of sporadic ALS patients,[7,13,34] However, our finding that cortical hyperexcitability was associated with the clinical phenotype of ALS, but not with bvFTD or carriers, highlights the specificity of these TMS measures for detecting alterations of inhibition and excitation within the motor cortex. Shortened silent periods were found despite the fact that most of the patients with ALS and ALS-FTD were being treated with riluzole, a drug known to shorten intracortical inhibition in ALS without affecting silent periods.[35] Even though 3 C9+ participants were symptomatic with cognitive impairment, these TMS measures were not different from controls. The lack of changes is notable, since pathological studies have shown widespread degeneration and accumulation of TDP-43 aggregates in projection neurons of layers II-III throughout the cortex and of Betz cells within the motor cortex[36]. Imaging studies also show global brain atrophy in C9+ FTD patients.[37] Asymptomatic C9+ carriers had normal thresholds and silent periods, consistent with studies of asymptomatic carriers with familial ALS.[18,19,26] The findings of cortical hyperexcitability, as detected by single pulse TMS of the motor cortex, coincided with clinical manifestations of upper motor neuron dysfunction.

Cortical hyperexcitability in ALS is hypothesized to involve a loss of input from cortical inhibitory interneurons onto corticospinal neurons, with subsequent alterations in the complement of post-synaptic receptors and channels on corticospinal neurons.[2,3,5,38] This sequence – loss of synaptic input and changes in expression of ion channels – has been recapitulated *in vitro* with iPS-derived motor neurons from C9+ ALS patients.[39,40] Loss of inhibitory synaptic inputs and postsynaptic receptors is followed by death of the iPS-derived motor neurons. Reduced expression of inhibitory receptors and ion channels may be directly related to impairment of RNA processing of transcripts caused by the expanded repeat.[41,42] The relative integrity of the corticomotoneuronal connection during the period of hyperexcitability has been postulated to permit anterograde spread of degeneration to homotopic lower motor neurons.[17,43] In our data, the preservation of MEP/CMAP amplitudes and CMCT in the face of cortical hyperexcitability would be compatible with a relatively intact corticomotoneuronal connection that would be necessary for this proposed mechanism of the spread of degeneration.

The single pulse TMS techniques for measuring cortical thresholds, CMCTs, and silent periods used in this study are basic and were easily carried out in a clinical EMG laboratory with experience in this technique. Paired pulse TMS and threshold tracking techniques[3] are

alternative methods for probing cortical excitability. They have the advantage of being independent of a patient's ability to make a voluntary contraction but require additional equipment and software. We recognize that the relatively small sample size, particularly upon dividing the cohort into diagnostic subgroups, is a limitation of this study. These data emerged from an ongoing longitudinal study and will need to be confirmed as more subjects and longer time points are accrued. An important question to be answered is whether silent periods remain stable beyond 6 months or lengthen as disease progresses.[13] However, given the rapid pace of therapeutic development for disease caused by *C9orf72* expansion mutations,[44,45] there is an urgent need for biomarkers of disease activity to serve as surrogate measures in clinical trials.[46] We suggest that TMS measures of cortical excitability are candidates for a role as non-invasive biomarkers of disease activity in the motor cortex. Other measures, such as neuroimaging,[37] may be better positioned to be biomarkers of disease activity that has not spread beyond non-motor areas, such as the frontal and temporal cortex.

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Disclosure: The NIH and Dr. Traynor have applied for a patent for the diagnostic and therapeutic uses of the C9ORF72 hexanucleotide repeat expansion used in this research.

ABBREVIATIONS

9HPT	9-hole peg test
ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic lateral sclerosis functional rating scale-revised
ANOVA	Analysis of Variance
APB	Abductor pollicis brevis
bvFTD	behavioral variant Frontotemporal dementia
C9+	person with expansion mutation in *C9orf72*
CLIA	Clinical laboratory improvement amendment
CMAP	Compound muscle action potential
CMCT	Central motor conduction time
EMG	Electromyography
FTD	Frontotemporal dementia
MEP	Motor evoked potential
SICI	Short-interval intracortical inhibition

TMS Transcranial magnetic stimulation

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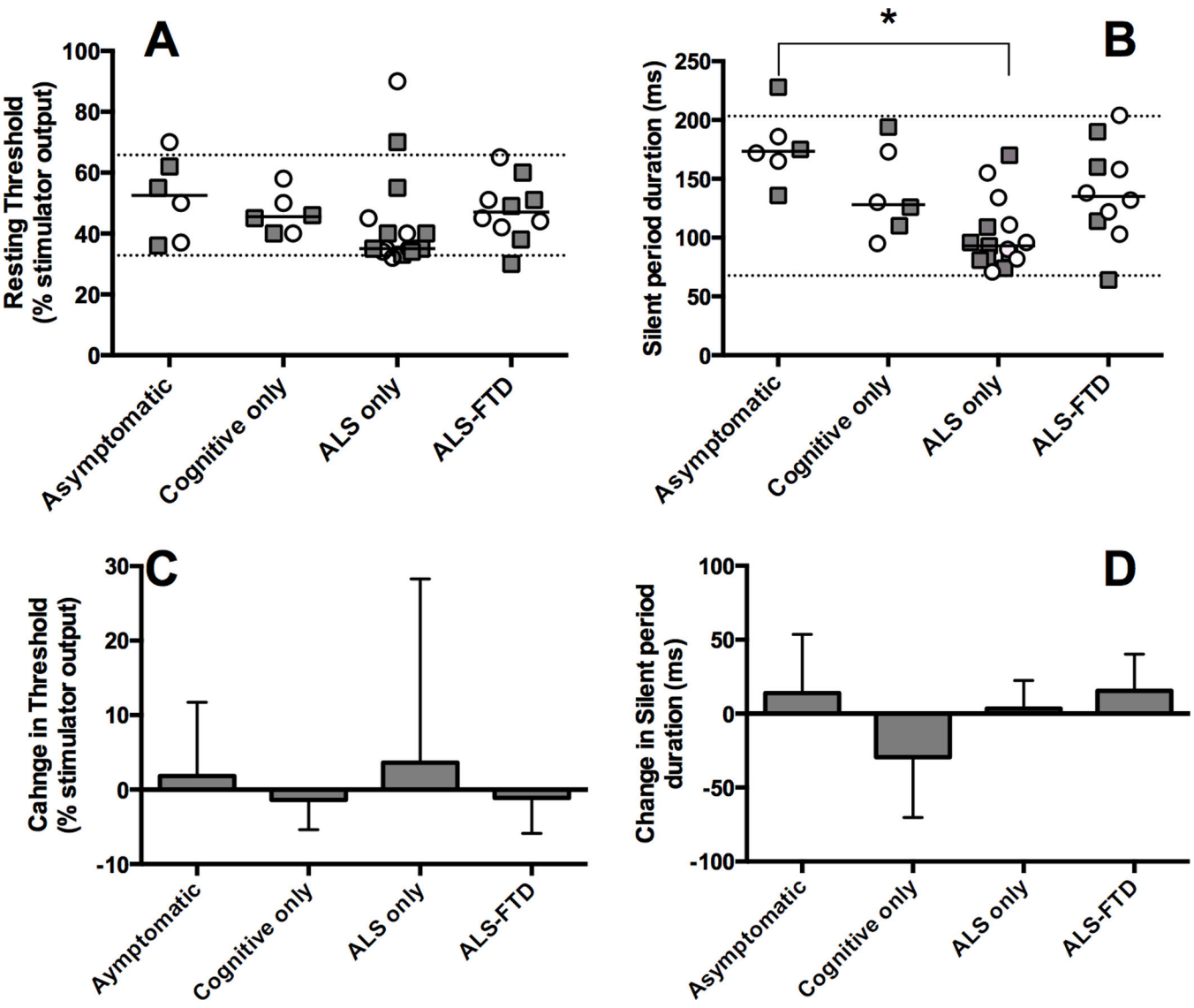

Figure 1.

Transcranial magnetic stimulation measures in C9+ participants. C9+ participants were diagnosed as having ALS or FTD according according to the El Escorial and Rascovsky criteria, with level of certainty as possible or greater. Right (squares) and left (circles) APB measures are plotted for each C9+ participant; dotted lines represent 2 SDs above and below the mean of the healthy control group. Baseline measures (A, B). **A**) Thresholds for eliciting a motor evoked potential in resting APB muscles with transcranial magnetic stimulation expressed as percent of stimulator output. **B**) Silent periods following an MEP in contracting APB muscles with TMS stimulation at 130% of threshold. Patients in the C9+ ALS subgroup had significantly shorter silent periods than controls and asymptomatic C9+ participants (asterisk - ANOVA $P < 0.001$; Tukey test, $P < 0.05$. Statistics were calculated on the average of the right and left side.) (C, D) Follow-up studies at 6 months show no

significant change of **C**) cortical thresholds and **D**) silent periods. (Mean and SD measures from right and left sides are combined.)

Table 1

Baseline Demographic data and physiology

			C9+ participants by diagnostic group[a]			
	Controls	**C9+ participants (all)**	**Asymptomatic**	**bvFTD**	**ALS only**	**ALS-FTD**
N	21	19	3	3	8	5
Age (years)	52.9 ± 9.3	55.4 ± 9.8	50.2 ± 10.4	57.7 ± 5.1	53.3 ± 10.1	60.2 ± 11.3
Male:Female	13:8	13:6	0:3	3:0	5:3	5:0
Disease Duration (months)	-	31.2 ± 26[b]	-	36.6 ± 26.6	27.8 ± 25.6	37.5 ± 31
Resting Threshold (% stimulator output)	49.3 ± 8.3	47.8 ± 15.4	51.6± 13.5	46.5 ± 6.8	43.5 ± 16.4	47.5 ± 10.2
Silent period duration (ms)	134 ± 36	129.9 ± 42.6	177 ± 30	138 ± 38	**102± 29	138 ± 36
Central Motor conduction time (ms)	4.5 ± 1.5	*6.1 ± 1.5	5.8 ± 0.9	5.8 ± 0.9	6.1 ± 2.0	6.6 ± 1.2
MEP/CMAP amplitude	0.62 ± 0.25	0.52 ± 0.32	0.48±0.15	0.49±0.23	0.62±0.43	0.35±0.18
ALSFRS-R	-	43.4 ± 4.5	48	43 ± 2.9	40.5 ± 4.7	43.6 ± 3.4
Dementia Rating Scale (scaled score; normal mean=10)		8.6 ± 4.2	15 ± 2.5	***3 ± 1.2	10.9 ± 2.4	***5.4 ± 3.3

[a] Revised El Escorial criteria for ALS; Rascovsky criteria for bvFTD;

[b] C9+ asymptomatic carriers excluded; MEP, motor evoked potential; CMAP, compound muscle action potential; ALSFRS-R, ALS functional rating scale-revised.

* Significantly different from control ($P < 0.05$),

** significantly different from controls and asymptomatic *C9orf72* subjects.

*** > 2 SDs below normative values

Table 2

Correlation between measures of motor function and TMS measures (Pearson r) in C9+ participants.

		Finger Tapping speed		9-Hole peg test	
		Right	**Left**	**Right**	**Left**
Right Hand	Cortical Threshold	−.254	−.339	.494*	.526*
	Silent period duration	−.131	.194	.102	−.233
	CMCT	.283	.394	−.554*	−.685*
Left Hand	Cortical threshold	−.282	−.643*	.540*	.740*
	Silent period duration	−.260	.036	.007	−.369
	CMCT	−.224	−.612*	.390	.295

*$P < 0.05$; CMCT, Central motor conduction time.