Three different short-interval intracortical inhibition methods in early diagnosis of amyotrophic lateral sclerosis

Hatice Tankisi[a,b*], Hossein Pia[a], Kristine Strunge[a,], James Howells[c], Bülent Cengiz[d], Gintaute Samusyte[e], Martin Koltzenburg[f,g], Anders Fuglsang-Frederiksen[a,b], Hugh Bostock[h]

[a] Department of Clinical Neurophysiology, Aarhus University Hospital, Aarhus, Denmark
[b]Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark
[c]Central Clinical School, Faculty of Medicine and Health, University of Sydney, Sydney, Australia
[c]Department of Neurology, Gazi University Faculty of Medicine, Beşevler, Ankara, Turkey
[d] Department of Neurology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[e]Department of Clinical Neurophysiology, National Hospital for Neurology and Neurosurgery, Queen Square, WC1N 3BG, London, United Kingdom.
[f]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, Queen Square, WC1N 3BG, London, United Kingdom

Corresponding author:
Hatice Tankisi
Department of Clinical Neurophysiology
Aarhus University Hospital
Palle Juul-Jensens Boulevard 165,
8200, Aarhus N, Denmark
E-mail address: hatitank@rm.dk

Word counts and number of figures and tables
Abstract: 233
Introduction, Methods, Results, Discussion: 3170
Number of figures: 5
Number of color figures: 3
Number of tables: 1

Three different short-interval intracortical inhibition methods in early diagnosis of amyotrophic lateral sclerosis

Abstract

Objectives: To compare the utility of conventional amplitude measurements of short-interval intracortical inhibition (A-SICI) with two threshold-tracking (T-SICI) methods, as aids to early diagnosis of amyotrophic lateral sclerosis (ALS). The new parallel threshold-tracking method (T-SICIp) was compared with the previously used serial tracking method (T-SICIs).

Methods: 112 consecutive patients referred with the suspicion of ALS and 40 healthy controls were prospectively included. Based on clinical follow-up, patients were divided into 67 patients with motor neuron disease (MND) comprising progressive muscular atrophy (PMA) as well as ALS, and 45 patient controls. SICI was recorded from first dorsal interosseus muscle using the three different protocols.

Results: MND patients had significantly reduced T-SICIp, T-SICIs and A-SICI, compared with healthy controls and patient controls, while healthy and patient controls were similar. Paradoxically, T-SICIp was least affected in MND patients with the most upper motor neuron (UMN) signs (Spearman $\rho=0.537$, $P<0.0001$) whereas there was no correlation for T-SICIs or A-SICI. T-SICIp also provided the best discrimination between patient controls and MND as determined by the receiver operating characteristic (ROC) curves. For patients with no UMN signs, area under ROC curve for 2-3ms inter-stimulus intervals was 0.931 for T-SICIp, 0.771 for T-SICIs and 0.786 for A-SICI.

Conclusions: SICI is a sensitive measure for detection of cortical involvement in ALS patients. T-SICIp has higher sensitivity and specificity than T-SICIs and A-SICI, particularly in patients without any upper motor neuron signs.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal disease with progressive degeneration of upper and lower motor neurons, and shows epidemiological and genetical heterogeneity (1-4). The disease is categorized as definite, probable or possible ALS according to the Awaji criteria (5) if there is upper motor neuron (UMN) involvement, while patients with solely lower motor neuron (LMN) involvement are defined as progressive muscular atrophy (PMA). The most recent Gold-Coast criteria proposed to set this terminology aside, and classify PMA as ALS (6). However, the specificity of these criteria is still to be determined, and more reliable methods are still needed for early and more accurate diagnosis, particularly when the clinical picture is not clear (7). Transcranial magnetic stimulation (TMS), particularly central motor conduction time measurements, are the most commonly used methods for detection of UMN involvement in ALS, but the low sensitivity of these methods is well known (8, 9). Short-interval intracortical inhibition (SICI), by use of paired-pulse threshold-tracking TMS, is an alternative and more promising method. Threshold-tracking SICI (T-SICI) has been widely investigated by one group in large cohorts of ALS patients and shown to have a high sensitivity (9-12). In these studies, a *serial tracking* method was used (T-SICIs), whereby a target MEP amplitude is tracked successively from an inter-stimulus interval (ISI) of 1 ms to longer ISIs and the changes in stimulus intensity recorded. A limitation of this method is that there is a pronounced lag between the change in ISI and the reaching of an accurate threshold estimate, so that the relationship

between SICI and ISI depends on the direction of change of ISI (13). To avoid this limitation of T-SICIs, we developed a *parallel tracking* method (T-SICIp), whereby SICI values at different ISIs are estimated independently, in parallel (13-15). We showed in a recent study the high sensitivity of T-SICIp in early diagnosis of ALS, particularly in patients with few UMN signs. T-SICIp was more sensitive than conventional SICI measurement (A-SICI) using a constant stimulus intensity and measuring the changes in the motor evoked potential (MEP) amplitude (16). However, the question of how TSICIs, which has been extensively used since 2006 by Kiernan, Vucic and colleagues, compares with T-SICIp and A-SICI has not previously been addressed.

In the present study, we compared the sensitivity and specificity of T-SICIs, T-SICIp and A-SICI measurements in patients referred with the suspicion of ALS, after the diagnoses were confirmed or excluded by clinical follow-up. Furthermore, we correlated T-SICIs, TSICIp and A-SICI with the clinical scores.

2. Materials and methods

2.1. Participants

We included consecutive patients referred with the suspicion of ALS, and compared with 40 healthy controls similar in age and sex. The exclusion criteria for patients were (a) the use of drugs that could affect TMS variables, (b) having contraindications for TMS application, and (c) a history of known neurological or psychiatric disorder, other than the symptoms that determined referral. None of the patients was receiving riluzole at recruitment. Healthy controls were

required to not have any complaints of neurological disease, in addition to the exclusion criteria for the patients. Only right-handed participants were included.

In total, 153 patients were eligible for inclusion and accepted to participate in the study between January 2019 and February 2022 (Fig. 1). Of these, 30 patients were excluded because either A-SICI (n=11) or both A-SICI and T-SICI (n=19) could not be performed due to high motor threshold, or a MEP amplitude of less than 1mV.

The index tests and the reference standard were applied to all patients at the time of recruitment. The index tests were the TMS measures and the reference standard was the categorisation of the patients according to the Awaji criteria (5) that was done at the time of inclusion. The patients who did not fulfil the ALS criteria were categorized as PMA, when they had LMN signs in at least two regions, or as unclassified MND if neither ALS nor PMA diagnosis could be made based on the clinical and conventional electrophysiological test results on the examination day. However, the ALS or PMA diagnosis had to be confirmed by follow-up and a progression was required as well as exclusion of ALS mimics. We had to exclude 11 patients due to uncertain diagnosis at follow-up.

All participants gave written informed consent in accordance with the Declaration of Helsinki II. The project was approved by The Central Denmark Region Committees on Health Research Ethics (Case #:1-10-72-201-17).

2.2. Clinical Scores

The disease duration in months from time of symptom onset and the disease onset as bulbar, upper or lower extremity spinal, were noted for all patients. All patients received a detailed clinical examination. The UMN involvement was graded using a modified Penn UMN score (UMNS), which ranged from 0 to 27, with higher scores corresponding to greater disease burden

(17). For this study, single points were given for an abnormal jaw-jerk reflex, palmomental sign, and central nervous system lability scale, and in the extremities, the deep tendon reflexes, pathological reflexes (Hoffman´s and Babinski´s sign and clonus) and spasticity were evaluated. Muscle strength was assessed using the Medical Research Council (MRC) score from 0 to 5. Muscle groups that were evaluated bilaterally were shoulder abduction, elbow flexion, wrist dorsiflexion, finger abduction, thumb abduction, hip flexion, knee extension and ankle dorsiflexion, yielding a maximum total score of 80. Disease severity was staged in all patients using the revised ALS Functional Rating Score (ALSFRS-R) (18), which has a maximum score of 48.

2.3. Transcranial Magnetic Stimulation (TMS)

T-SICIs, TSICIp and A-SICI measurements were performed in a randomized order. The subjects were seated comfortably in an armchair and asked to relax and keep awake. Stimulation started using a Magstim® D70 figure-of-8 coil placed approximately 4 cm left of the vertex on the binauricular line, with the handle pointing 45° to the parasagittal plane. When the hotspot was located, the outline of the coil was drawn on a swimming cap to enable constant coil positioning. One of the three automated stimulation protocols was then initiated, with stimulus delivery and data acquisition controlled by the QtracS component of QtracW software (©UCL, distributed by Digitimer Ltd.) using QTMSG-12 recording protocols (QTMS Science).

The MEPs were recorded from the FDI muscle of the right hand, using disposable pre-gelled surface electrodes placed in a belly-tendon fashion. The MEP was amplified (1000× gain) and filtered (3 Hz to 3 kHz) using a D440-2 Isolated Amplifier (Digitimer Ltd). A Humbug Noise Eliminator (Digitimer Ltd.) was used to remove 50-Hz noise, and the amplified signals were

digitized at 10 kHz with a 16-bit data acquisition system (NI USB-6251, National Instruments.).

The coil in use was connected to two Magstim® 2002 stimulators in a BiStim configuration.

Resting motor threshold

A '4→2→1' tracking rule and logarithmic regression were used to determine resting motor

thresholds (RMT) for a 200µV (RMT200) or for a 1000µV (TS1mV) peak-to-peak response

(13). This regression is also weighted, with weights reducing from 1 at the level of the target to

0 at $1/10^{th}$ and 10× target (i.e. points outside the plotted area are ignored). This method of

threshold estimation, which was first described by Fisher et al. (19), was used for all further

thresholds, whether conditioned or unconditioned.

T-SICIs protocol

RMT200 was tracked continuously, by decreasing stimulus by 1% MSO if response was more

than the target, and increasing it by 1% if the response was less than the target response. The

conditioning stimulus was set to 70% of RMT200, and paired stimuli were delivered followed by

test-alone stimuli. The recording was started with 1ms ISI and the thresholds were tracked

successively from 1 ISI to the next over 9 ISIs, (1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 7ms).

T-SICIp protocol

In this protocol, SICI at different ISIs are estimated independently, in parallel in pseudo-

randomised order as described in detail elsewhere (13). Paired stimuli were delivered, with the

same 9 ISIs as for T-SICIs. Each of the 9 paired stimuli was delivered 10 times, and test-alone

stimuli were given after each three paired stimuli, making in total 120 stimuli.

A-SICI protocol

After estimating the RMT for 200µV and 1000µV, test stimuli were fixed at TS1mV and

conditioning stimuli were set to 70% of RMT200. Similar to the T-SICIp, the paired stimuli were

delivered at 9 ISIs in a pseudo-random order, and each paired stimulus was given 10 times, together with test alone stimuli, making in total 120 stimuli.

2.4. Data analysis

The QtracP component of the QtracW software was used for the analysis of the data and creating the figures. T-SICI thresholds were estimated by log regression, as described above, and A-SICI amplitudes were averaged as geometric means. A-SICI amplitudes were normalized, to overcome the 'floor' effect, by log conversion, and scaled to become comparable with the T-SICI thresholds, using the relationship found in the healthy controls (13). SICI was compared between healthy subjects and patients as well as between patients and patient controls using independent samples t-tests. Receiver operating characteristic (ROC) curves were used for the analysis of sensitivity and specificity. Correlation analysis between SICI values and clinical scores were done using parametric or non-parametric tests depending on the normality.

3. Results

3.1. Patients demographics

Of the 112 patients included in the study (Fig. 1), 67 patients had disease progression at the clinical follow-up and were classified as MND. Of these, 42 patients fulfilled the criteria for ALS (definite= 2, probable= 17 and possible= 23). In 25 patients the MND diagnosis could only be confirmed at clinical follow-up. Forty-five patients received other diagnoses, according to up to 2 years of clinical follow-up, and were classified as patient controls (Fig. 1).

Of these 112 patients, 61 patients (39 MND and 25 patient controls) and 39 healthy controls were the same as in our previous study (16).

There was no significant difference in age between the patient groups or healthy controls. The mean disease duration for the MND patients was 11.3±5.6 months, and their mean ALSFRS-R score was 41.9±3.9.

3.2. T-SICIs, T-SICIp and A-SICI in healthy controls

Fig. 2A shows the pronounced 'floor effect' for mean A-SICI values between ISIs of 2 and 3 ms. The normalized A-SICI measurements are compared with T-SICIs and T-SICIp in Fig. 2B, where it can be seen that, as for the younger healthy subjects in the previous study (13), the A-SICI distribution is effectively normalized and the A-SICI-T values have the least variation between subjects, while the T-SICIs values show the largest variation.

3.3. T-SICIs, T-SICIp and A-SICI in healthy controls, patients and patient controls

Mean SICI was lower in patients compared with healthy controls and patient controls for T-SICIs, T-SICIp and A-SICI, whereas there was no significant difference between patient and healthy controls (Fig. 3). The difference between patients and controls was less for A-SICI than T-SICIs and T-SICIp, but the variation in A-SICI values was less than for T-SICIs and T-SICIp, as indicated by the standard deviations in Fig. 3D-F.

3.3. Discrimination of MND patients from controls by T-SICIs, TSICI-p and A-SICI

The sensitivity and specificity of T-SICIs, T-SICIp and A-SICI as diagnostic tools is best compared by the areas under the ROC curves (Table 1). T-SICIp provided better discrimination, especially between patients without UMN symptoms (UMNS=0) and patient controls than T-SICIs and A-SICI (Fig. 4A). In Figure 4B, the mean thresholds from 2-3 ms were shown as dot plots for T-SICIs, TSICI-p and A-SICI showing the degree of discrimination between MND patients and patient controls without UMN signs. T-SICIp showed higher sensitivity and

specificity than T-SICIs and A-SICI, with a cut-off very close to 100% RMT200, indicating that the majority of MND patients exhibited facilitation, rather than inhibition.

3.4. Correlation between SICI measurements and UMN findings in MND patients

Figure 5 shows the association between UMN scores and SICI and RMT in MND patients divided into 4 subgroups on the basis of their UMN scores. Mean T-SICIp at 2-3ms ISIs shows a progressive change with UMN score that is paradoxical, in the sense that SICI is normal in the MND patients with the highest UMN scores, and gets 'worse', i.e. is reduced more, as UMN score gets better, i.e. smaller. The correlation between these two variables was very strong (Spearman's $\rho = 0.537$, $P <0.0001$) (Fig. 5a). For T-SICIs (Fig. 5b) and A-SICI (Fig. 5c), on the other hand, there was no significant correlation, although there was a trend in the same direction. For RMTs, no correlation with UMN score was seen for any of the SICI methods (Fig. 5D-F).

Discussion

This is the first study that has compared 3 SICI methods as potential biomarkers for early diagnosis of ALS. We showed in a previous study that the new parallel threshold-tracking SICI method (T-SICIp) is more sensitive than conventional amplitude measurements (A-SICI), particularly in MND patients with fewer clinical signs of UMN (16). However, all previous studies using threshold-tracking TMS in ALS by Kiernan, Vucic and co-workers were done using a serial approach (T-SICIs) (9-12). Therefore, a head-to-head comparison of T-SICIs and T-SICIp was called for. The present study has shown that T-SICIs measurements on patients are more variable than T-SICIp ones, as shown for healthy controls in a previous study (13). The sensitivity and specificity of T-SICIs was lower than T-SICIp and A-SICI, and the only correlation between UMNS and SICI parameters was for T-SICIp.

Why do T-SICI and A-SICI differ in MND?

T-SICIp and A-SICI are very much alike in healthy subjects (13) but not in MND patients (16). In a previous study, we showed substantial differences between T-SICIp and A-SICI, and that MND patients with little clinical evidence of UMN damage showed the greatest loss of T-SICIp, whereas patients with abundant UMN signs had relatively normal SICI. A-SICI showed a much weaker correlation with UMNS. In the present study, T-SICIp showed a similarly strong correlation with UMNS. Thus, T-SICI and A-SICI showed different susceptibilities for cortical degeneration in MND patients. This difference is not only important for diagnostic purposes but also because it can provide new insights into the pathophysiological mechanisms of ALS. Although the conditioning stimuli are the same for A-SICI and T-SICIp, T-SICIp uses weaker test stimuli when there is sufficient facilitation in a subset of neurons to generate the 200 µV target response. The stronger test stimuli used for A-SICI are less selective, exciting a mixture of neurons experiencing inhibition as well as facilitation. The normalisation of T-SICIp with increasing UMN damage may indicate that the neurons experiencing facilitation are progressively degenerating, leaving ones with relatively normal inhibition. The differential susceptibility of the two sets of cortical neurons may relate to the suggestion that there is a preferential involvement of the fast-conducting direct corticospinal tracts in ALS, sparing the slower or polysynaptic projections (20).

Interestingly, T-SICIs did not show a significant correlation with the UMNS, or as much facilitation in patients without UMN signs as T-SICIp. Also, the classic peak in SICI at 2-3ms was clear in the MND recordings for T-SICIp and A-SICI, but not for T-SICIs (Fig. 3). These differences were probably because the strong dependency of each threshold estimate on the

previous one in serial tracking had the effect of blurring SICI measurements across ISIs, as previously shown in healthy subjects (13).

We found that the profile of SICI vs ISI was strongly dependent on the initial settings and on the direction of change of ISI with serial tracking (13). This limitation of serial threshold-tracking is avoided in the parallel protocol, since thresholds at each ISI are tracked independently and in parallel.

T-SICIs, T-SICIp and A-SICI as diagnostic biomarkers for ALS

T-SICIs has been shown to be reduced in ALS in several studies (10, 11, 21, 22), and proposed as a diagnostic biomarker for ALS (22-24). We showed also in the present study that T-SICIs is reduced in MND. The sensitivity of T-SICIs and areas under the ROC curves were comparable with the previous studies (9). However, T-SICIp as well as the conventional A-SICI showed higher sensitivity and specificity than T-SICIs.

A-SICI-T showed less variability than both T-SICI methods. On the other hand, T-SICIp showed better discrimination between MND patients and either healthy or patient controls, whether for the whole group, or for those patients with least UMN signs. Taking into account that these patients are the most challenging ones in daily clinical routine, a new diagnostic biomarker of UMN pathology would be most useful for this group.

We propose that T-SICIp may be accepted as strong supporting evidence of UMN pathology, to allow earlier diagnosis and increased recruitment into therapeutic trials, especially in the early stages of the disease when treatments are likely to be most effective (9).

One difference in our study compared to previous studies with T-SICIs is the type of coils used. Previous studies have used a circular coil while we used a figure-of-eight coil (9-12). However, in a recent study, we compared these two types of coils on healthy subjects and showed that the

difference in coils does not influence the T-SICIs parameters while the circular coil is more unpleasant for the subjects (25).

Limitations

Our study has a number of limitations. First, a substantial number of patients had to be excluded because of high motor threshold. We cannot exclude the possibility that these patients could have had strong SICI, and that our results could be biased by excluding these patients. Secondly, we used for A-SICI a test stimulus intensity that produced a 1 mV MEP, whereas T-SICI tracked a 200μV target. If we had used the RMT200μV test stimulus intensity for A-SICI, T-SICI and A-SICI would have been more comparable in some respects, and fewer patients would have been excluded due to their inability to produce MEPs with a 1mV amplitude. However, the use of 200μV control MEP might not be optimal to demonstrate inhibition as it has been shown to result in facilitation in a subset of healthy subjects (26).

Conclusion

We have found that T-SICIs, T-SICIp and A-SICI are all sensitive early indicators of UMN dysfunction in patients referred for suspicion of ALS. However, T-SICIp is most diagnostic, especially before UMN signs have developed. We therefore strongly support the arguments that SICI should be accepted as an aid to the early diagnosis of ALS, with the recommendation that a parallel threshold-tracking protocol, such as T-SICIp, be used.

Table 1. The discrimination of short-interval intracortical inhibition (SICI) between healthy controls, patients and patient controls

ISI (ms)	Healthy controls (40) v MND Patients (67)			Patient controls (45) v MND Patients (67)			No UMN signs: Patient controls (30) v MND patient (19)		
	T-SICI parallel	T-SICI serial	A-SICI	T-SICI parallel	T-SICI serial	A-SICI	T-SICI parallel	T-SICI serial	A-SICI
1.0	0.759	0.624	0.702	0.735	0.662	0.645	0.826	0.641	0.740
1.5	0.832	0.707	0.807	0.696	0.662	0.748	0.770	0.662	0.710
2.0	0.802	0.697	0.757	0.751	0.649	**0.777**	0.889	0.711	0.747
2.5	0.847	0.785	0.774	0.771	0.691	0.770	0.902	**0.774**	**0.791**
3.0	0.827	0.786	0.808	0.761	0.679	0.702	0.904	0.749	0.716
3.5	0.822	0.783	0.788	0.743	0.665	0.697	0.875	0.746	0.734
4	0.749	0.781	0.745	0.660	0.675	0.661	0.801	0.768	0.718
5	0.711	0.706	0.643	0.605	0.629	0.604	0.718	0.751	0.645
7	0.666	0.641	0.578	0.513	0.585	0.545	0.633	0.687	0.534
1 - 7	0.840	0.779	0.765	0.754	0.687	0.686	0.899	0.763	0.714
1 – 3.5	**0.864**	0.778	**0.831**	**0.788**	**0.693**	0.764	0.926	0.750	0.775
2.5 – 3.5	0.852	**0.792**	0.812	0.774	0.686	0.731	0.925	0.761	0.766
2 - 3	0.851	0.779	0.808	0.782	0.689	0.775	**0.931**	0.771	0.786

Areas under receiver operator characteristic curves comparing healthy controls with all motor neuron disease (MND) patients and patient controls, and comparing MND patients with patient controls without any signs of upper motor neuron. T-SICI = SICI measured by threshold

tracking. A-SICI = SICI measured by amplitude changes. ISI = inter-stimulus interval. N.B. Bold is highest of all ISIs and ISI combinations tested.

Figure Legends

<u>**Figure 1**</u>. **Schematic diagram of patient classification**

ALS = Amyotrophic lateral sclerosis, SICI = short interval intracortical inhibition, MEP = Motor evoked potential, TSICIs = Threshold tracking SICI serial, TSICIp = Threshold tracking SICI parallel, ASICI = Conventional amplitude SICI, MND = Motor neuron disease, PMA = Progressive muscular atrophy, MND-U = MND-unclassified. PLS = Primary lateral sclerosis,

Figure 2.   T-SICIs, T-SICIp and A-SICI in healthy controls

Mean A-SICI values between inter-stimulus intervals (ISIs) of 2 and 3 ms show pronounced 'floor effect' (A). Normalized A-SICI measurements (A-SICI-T) show the least variation between subjects, while the T-SICIs values show the largest variation (B). SICI = short interval intracortical inhibition, T-SICIs = Threshold tracking SICI serial, T-SICI = Threshold tracking SICI parallel, A-SICI = Conventional amplitude SICI. RMT= Resting motor threshold, ms= milli seconds.

Figure 3. T-SICIs, T-SICIp and A-SICI in healthy controls, patients and patient controls

Mean SICI is lower in patients compared with healthy controls and patient controls for T-SICIs, T-SICIp and A-SICI, whereas there is no significant difference between patient and healthy controls (A-C). The variation in A-SICI values was less than for T-SICIs and T-SICIp, as indicated by the standard deviations (D-F).

Healthy control subjects (black squares), patient controls (gray open triangles) and MND patients (closed circles).

SICI = short interval intracortical inhibition, T-SICIs = Threshold tracking SICI serial, T-SICI = Threshold tracking SICI parallel, A-SICI = Conventional amplitude SICI, RMT = Resting motor threshold, ms = milli seconds

Figure 4. Discrimination of MND patients from controls by T-SICIs, TSICI-p and A-SICI

 T-SICIp provided better discrimination, especially between patients without upper motor neuron symptoms and patient controls than T-SICIs and A-SICI (A). T-SICIp showed higher sensitivity and specificities than T-SICIs and A-SICI when the mean thresholds from 2-3 ms were compared as dot plots (B).

SICI = short interval intracortical inhibition, T-SICIs = Threshold tracking SICI serial, T-SICI = Threshold tracking SICI parallel, A-SICI = Conventional amplitude SICI. RMT = Resting motor threshold, ms= milli seconds, PC = Patients control, MND = Motor neuron disease, UMNS= Upper motor neuron score.

Figure 5. Association between UMN scores and SICI and RMT in MND patients

Mean SICI from between 2-3ms comparing healthy controls with progressive changes with UMNS in subgroups of MND patients (A-C). Mean T-SICIp at 2-3ms ISIs shows the most abnormal SICI in patients with the fewest UMN signs (A) whereas there is no significant correlation for T-SICIs (B) or A-SICI-T (c). For RMTs, no correlation is seen for any of the SICI methods (D-F).

Asterisks indicate significant difference from healthy controls by Mann-Whitney U-test: * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001, ***** = P<0.00001. Spearman's rho values also shown for correlations within MND patients as a whole.

Declaration Competing of Interest

Acknowledgements

Data sharing Statement

We agree to make data and materials supporting the results or analyses presented in this paper available upon reasonable request.

References

1. Erazo D, Luna J, Preux PM, Boumediene F, Couratier P. Epidemiological and genetic features of amyotrophic lateral sclerosis in Latin America and the Caribbean: a systematic review. Amyotroph Lateral Scler Frontotemporal Degener. 2022;23(1-2):4-15.
2. Borghero G, Pierri V, Vasta R, Ercoli T, Primicerio G, Pili F, et al. Incidence of amyotrophic lateral sclerosis in Sardinia, Italy: age-sex interaction and spatial-temporal variability. Amyotroph Lateral Scler Frontotemporal Degener. 2022:1-7.
3. Hardiman O, Heverin M, Rooney J, Lillo P, Godoy G, Saez D, et al. The Latin American Epidemiology Network for ALS (Laenals). Amyotroph Lateral Scler Frontotemporal Degener. 2022:1-6.

4.   Marques Couto C, de Melo Queiroz E, Nogueira R, Pires Duarte Kusel AP, O JMN. A Brazilian multicentre study on the clinical and epidemiological profiles of 1116 patients with amyotrophic lateral sclerosis and its phenotypic variants. Amyotroph Lateral Scler Frontotemporal Degener. 2021:1-10.

5.   de Carvalho M, Dengler R, Eisen A, England JD, Kaji R, Kimura J, et al. Electrodiagnostic criteria for diagnosis of ALS. Clin Neurophysiol. 2008;119(3):497-503.

6.   Shefner JM, Al-Chalabi A, Baker MR, Cui LY, de Carvalho M, Eisen A, et al. A proposal for new diagnostic criteria for ALS. Clin Neurophysiol. 2020;131(8):1975-8.

7.   Sennfalt S, Klappe U, Thams S, Samuelsson K, Press R, Fang F, et al. The path to diagnosis in ALS: delay, referrals, alternate diagnoses, and clinical progression. Amyotroph Lateral Scler Frontotemporal Degener. 2022:1-9.

8.   de CM, Swash M. Sensitivity of electrophysiological tests for upper and lower motor neuron dysfunction in ALS: a six-month longitudinal study. Muscle Nerve. 2010;41(2):208-11.

9.   Menon P, Geevasinga N, Yiannikas C, Howells J, Kiernan MC, Vucic S. Sensitivity and specificity of threshold tracking transcranial magnetic stimulation for diagnosis of amyotrophic lateral sclerosis: a prospective study. 2015;14(5):478.

10.   Vucic S, Kiernan MC. Novel threshold tracking techniques suggest that cortical hyperexcitability is an early feature of motor neuron disease. Brain. 2006;129(Pt 9):2436-46.

11.   Vucic S, Cheah BC, Yiannikas C, Kiernan MC. Cortical excitability distinguishes ALS from mimic disorders. Clin Neurophysiol. 2011;122(9):1860-6.

12.   Vucic S, Howells J, Trevillion L, Kiernan MC. Assessment of cortical excitability using threshold tracking techniques. Muscle Nerve. 2006;33(4):477-86.

13.   Tankisi H, Cengiz B, Howells J, Samusyte G, Koltzenburg M, Bostock H. Short-interval intracortical inhibition as a function of inter-stimulus interval: Three methods compared. Brain Stimul. 2021;14(1):22-32.

14.   Tankisi H, Howells J, Cengiz B, Samusyte G, Koltzenburg M, Bostock H. Conventional and Threshold-Tracking Transcranial Magnetic Stimulation Tests for Single-handed Operation. J Vis Exp. 2021(174).

15.   Tankisi H, Cengiz B, Samusyte G, Howells J, Koltzenburg M, Bostock H. Short interval intracortical inhibition: Variability of amplitude and threshold-tracking measurements with 6 or 10 stimuli per point. Neurophysiol Clin. 2022.

16.   Tankisi H, Nielsen CS, Howells J, Cengiz B, Samusyte G, Koltzenburg M, et al. Early diagnosis of amyotrophic lateral sclerosis by threshold tracking and conventional transcranial magnetic stimulation. Eur J Neurol. 2021;28(9):3030-9.

17.   Woo JH, Wang S, Melhem ER, Gee JC, Cucchiara A, McCluskey L, et al. Linear associations between clinically assessed upper motor neuron disease and diffusion tensor imaging metrics in amyotrophic lateral sclerosis. PLoS One. 2014;9(8):e105753.

18.   Cedarbaum JM, Stambler N, Malta E, Fuller C, Hilt D, Thurmond B, et al. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). J Neurol Sci. 1999;169(1-2):13-21.

19.   Fisher RJ, Nakamura Y, Bestmann S, Rothwell JC, Bostock H. Two phases of intracortical inhibition revealed by transcranial magnetic threshold tracking. Exp Brain Res. 2002;143(2):240-8.

20.   Kohara N, Kaji R, Kojima Y, Kimura J. An electrophysiological study of the corticospinal projections in amyotrophic lateral sclerosis. Clin Neurophysiol. 1999;110(6):1123-32.

21.   Vucic S, Kiernan MC. Utility of transcranial magnetic stimulation in delineating amyotrophic lateral sclerosis pathophysiology. Handb Clin Neurol. 2013;116:561-75.

22.   Vucic S, Kiernan MC. Transcranial Magnetic Stimulation for the Assessment of Neurodegenerative Disease. Neurotherapeutics. 2017;14(1):91-106.

23.   Vucic S, Rutkove SB. Neurophysiological biomarkers in amyotrophic lateral sclerosis. Curr Opin Neurol. 2018;31(5):640-7.

24.   Vucic S, van den Bos M, Menon P, Howells J, Dharmadasa T, Kiernan MC. Utility of threshold tracking transcranial magnetic stimulation in ALS. Clin Neurophysiol Pract. 2018;3:164-72.

25.   Orskov S, Bostock H, Howells J, Pugdahl K, Fuglsang-Frederiksen A, Nielsen CS, et al. Comparison of figure-of-8 and circular coils for threshold tracking transcranial magnetic stimulation measurements. Neurophysiol Clin. 2021;51(2):153-160.26.

26.   Roshan L, Paradiso GO, Chen R. Two phases of short-interval intracortical inhibition. Exp Brain Res. 2003;151(3):330-7.
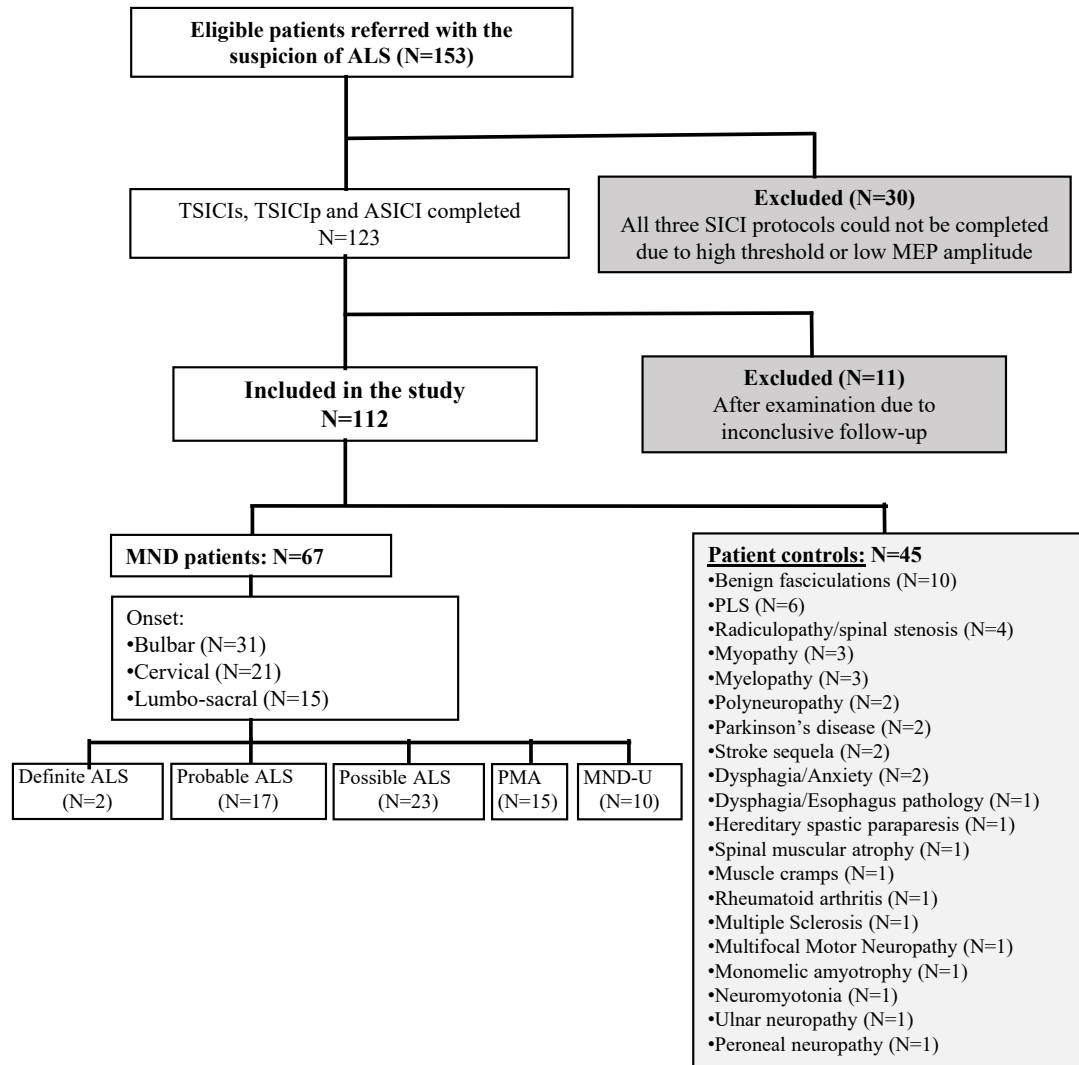
Figure 1. Schematic diagram of patient classification

ALS = Amyotrophic lateral sclerosis, SICI = short interval intracortical inhibition, MEP = Motor evoked potential, TSICIs = Threshold tracking SICI serial, TSICIp = Threshold tracking SICI parallel, ASICI = Conventional amplitude SICI, MND = Motor neuron disease, PMA = Progressive muscular atrophy, MND-U = MND-unclassified. PLS = Primary lateral sclerosis,

A T-SICIp
B TSICIs
C A-SICI-T

Panel A: Rho = 0.537****
Panel B: Rho = 0.149^{NS}
Panel C: Rho = 0.116^{NS}

Y-axis (A–C): Threshold (%RMT200) 2-3ms
Y-axis (D–F): RMT200 (%MSO)

X-axis labels (D, E, F):
Healthy controls (40)
Patient controls (45)
Patient UMN scores
0 (19)
1-5 (15)
6-8 (19)
9-22 (14)