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Utility of Cortical Inhibitory and Facilitatory Neuronal Circuits in Amyotrophic Lateral Sclerosis Diagnosis

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Keywords: amyotrophic lateral sclerosis | index of excitation | short interval intracortical facilitation | short interval intracortical inhibition | transcranial magnetic stimulation

ABSTRACT

Background: Cortical hyperexcitability is an early feature of amyotrophic lateral sclerosis (ALS), linked to dysfunction in inhibitory and facilitatory cortical circuits, measurable using paired-pulse transcranial magnetic stimulation (TMS). Short-interval intracortical inhibition (SICI) is a robust biomarker of inhibitory function and an ALS diagnostic marker. Short interval intracortical facilitation (SICF) serves as a biomarker of facilitatory function, while the index of excitation assesses the contribution of these circuits to hyperexcitability. This study aimed to evaluate the diagnostic effectiveness of SICF and the index of excitation in distinguishing ALS from non-ALS mimic disorders.

Methods: This cross-sectional study assessed cortical excitability in participants with suspected ALS from two Sydney centres, classified using the Gold Coast criteria. Threshold tracking TMS measured SICI, SICF, and the index of excitation. Diagnostic performance was evaluated using receiver operating characteristic (ROC) analysis, with sensitivity, specificity, and optimal cut-off values determined.

Results: Of 154 participants, 95 were diagnosed with ALS and 48 with non-ALS mimics. SICI demonstrated a marginally higher diagnostic accuracy (AUC 0.84, 95% CI:0.77–0.89) compared to SICF (AUC 0.77, 95% CI:0.68–0.84, $p = 0.028$). The index of excitation showed comparable accuracy to SICI (AUC 0.82, 95% CI: 0.75–0.88, $p = 0.328$). The optimal SICF cut-off ($\leq -13.6\%$) provided 70.5% sensitivity and 70.8% specificity, while the index of excitation cut-off ($\geq 64.5\%$) yielded 71.6% sensitivity and 70.8% specificity.

Conclusions: The present study established modest diagnostic potential of increased SICF and index of excitation in differential ALS from mimic disorders, thereby enhancing understanding of the role of inhibitory and facilitatory cortical circuits in ALS diagnosis.

1 | Introduction

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease of the human motor system, clinically characterized by the presence of upper (UMN) and lower (LMN) motor neuron signs [1]. Clinical heterogeneity is a feature of ALS, potentially resulting in diagnostic delay and preventing the institution of appropriate management and recruitment into clinical trials [2].

Cortical hyperexcitability has been established as an early feature of ALS, potentially preceding LMN dysfunction, ii [3–5], and associated with specific clinical features and patterns of disease progression [6–8]. At a pathophysiological level, dysfunction of motor cortical inhibitory interneuronal circuits, along with increased activity of facilitatory circuits, appears to underlie the development of cortical hyperexcitability in ALS [9, 10]. While important for understanding ALS pathogenesis, identification of cortical hyperexcitability has been shown to be an important biomarker in ALS, providing an objective measure for UMN dysfunction [11–13].

Cortical excitability can be measured by single and paired-pulse transcranial magnetic stimulation (TMS) techniques [9, 14]. Threshold tracking TMS has emerged as an important neurophysiological investigation in ALS, providing biomarkers for dysfunction in intracortical inhibitory and facilitatory cortical circuits [10, 15]. Reduction of short-interval intracortical inhibition (SICI), a TMS biomarker of GABAergic inhibitory interneuronal function, was shown to robustly differentiate ALS from mimicking diseases [4, 16, 17]. The diagnostic utility was evident across the ALS phenotypes, irrespective of disease onset site, duration, or presence of UMN signs [4].

Short-interval intracortical facilitation (SICF), a TMS biomarker of facilitatory interneuronal circuit activity, was shown to be increased in ALS and associated with greater functional decline [18, 19]. The increase in SICF was accompanied by a reduction of SICI, indicating that cortical hyperexcitability was mediated by overactivity of facilitatory and dysfunction of inhibitory circuits in ALS [18]. This imbalance was quantified by the index of excitation, which was increased in ALS [18, 19]. The increase in SICF was not a universal finding, with some reporting a reduction of SICF in ALS [20, 21]. As such, the value of increased SICF and index of excitation remains to be clarified, and if established, would potentially represent complementary biomarkers to SICI for aiding ALS diagnosis. Consequently, the aim of the present study was to assess the established utility of SICF and the index of excitation as diagnostic biomarkers in differentiating ALS from non-ALS mimic disorders.

2 | Methods and Materials

2.1 | Study Design and Patients

Participants with suspected ALS were prospectively recruited from two ALS clinical services (Westmead and Concord Hospitals), in accordance with pre-defined inclusion criteria which were as follows: (i) progression of motor symptoms for at least 6 months; AND (ii) presence of concomitant UMN and LMN signs in the same body regions; or (iii) pure lower motor neuron signs in two or more body regions. Exclusion criteria included: (i) use of psychotropic medications that could affect TMS parameters; (ii) use of ferromagnetic devices, including in situ pacemakers and other cardiac devices, cochlear implants, or cerebral arterial clips; (iii) acute migraine headache within 4 weeks preceding assessment as it can affect TMS parameters; (iv) history of seizures, stroke/transient ischemic attack, or brain surgery; and (v) marked wasting of the target muscle precluding recording of MEP responses.

The Gold Coast ALS diagnostic criteria were used as the reference standard [22]. All patients underwent threshold tracking TMS (index test) at recruitment, performed by examiners with neurology expertise and training in neurophysiological techniques. At the time of the index test, examiners were blinded to the final diagnosis. Data analysis was performed by separate raters with experience in the analysis of the results of the index tests and reference standard.

2.2 | Standard Protocol Approvals, Registrations, and Patient Consents

Prior to undertaking TMS, all patients were required to provide written informed consent approved by the Western and Sydney Local Health District Service Human Research Ethics Committees, in compliance with the World Medical Association Declaration of Helsinki.

2.3 | Demographical and Clinical Data

All enrolled participants underwent phenotyping, peripheral neurophysiology, and threshold tracking TMS at recruitment. Disease duration from symptom onset (months), site, and age of disease onset were recorded. Functional status was evaluated using the ALS Functional Rating Scale-Revised (ALSFRS-R) [23] while the rate of disease progression (ΔFs) was calculated according to a previously published formula [24, 25]. Muscle strength was assessed by the Medical Research Council (MRC) score (0–5) with the following groups assessed: (i) *upper limb*: shoulder abduction, elbow flexion and extension, wrist extension, finger abduction (first dorsal interosseous) and thumb abduction on both sides, yielding a maximal upper limb MRC score of 60 (normal strength); (ii) *lower limb*: hip flexion, knee extension, and ankle dorsiflexion on both sides, yielding a maximal lower limb score of 30 (normal strength). Upper motor neuron (UMN) function was clinically assessed using a dedicated UMN score, which resulted from the sum of the presence (1) or absence (0) of pathologically brisk deep tendon reflexes of the jaw, triceps, biceps, supinator, patella, ankle, and extensor plantar response, ranging from 0 (absence of UMN signs) to 15 (severe UMN signs) [19]. The Edinburgh Handedness Inventory score was used to determine handedness [26].

2.4 | Procedures

Cortical excitability was assessed from the primary motor cortex on the dominant side using either a circular (90 mm)

or figure-of-eight coil (internal wing diameter of 70 mm) connected to a BiStim2 device (Magstim Co., Whitlands, South-West Wales, UK), using purpose-built software (MagXite, Australia). While TMS data was collected prospectively, analysis was performed retrospectively. Recording of SICI and SICF was performed by the circular coil between 2017-to-2020. In 2020, the laboratory transitioned to recording SICI and SICF with the figure-of-eight coil. Both coils were oriented to induce posterior-to-anterior directed currents in the motor cortex. The motor evoked potential (MEP) response was recorded over the *abductor pollicis brevis* (APB) muscle. All MEP and CMAP responses were recorded using Ag/AgCl disk gel electrodes (3 M Corp, Maplewood, MN) arranged in a belly-tendon montage.

Initially, resting motor threshold (RMT) was determined and defined as the stimulus intensity required to elicit and maintain the target MEP response of 0.2 mV (±20%) [14]. SICI and SICF were determined in accordance with established methodology [14]. Briefly, SICI was assessed by using the paired-pulse threshold tracking paradigm with a conditioning stimulus (CS) set to 70% RMT and determined over the following ISIs: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 7 ms [14]. SICF was determined by increasing the CS to 95% RMT and delivering after the test stimulus over ISIs of 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 ms [18]. A sequential ordering of ISI was performed with TMS intensity changed in 1% increments or decrements of maximum stimulator output. SICI and SICF were determined using the following formula [18]:

$$\text{Inhibition (or Facilitation)} = \frac{\text{Conditioned test stimulus intensity} - \text{RMT}}{\text{RMT}} * 100$$

To measure the degree of inhibitory and facilitatory circuit function, the index of excitation was determined according to the previously reported formula [18].

$$\text{Index of Excitation} = \frac{\text{SICF Average}}{\text{SICF Average} - \text{SICI Average}} * 100$$

Both averaged SICI and averaged SICF were calculated for each patient. The degree of lower motor neuron dysfunction was assessed in the same sitting. The compound muscle action potential (CMAP) peak-to-peak amplitude (mV), distal motor and F-wave latencies (ms) were recorded from the APB, *abductor digiti minimi* (ADM), and *first dorsal interosseous* (FDI) muscles. Additionally, the split hand index (SI) and neurophysiological index (NI) were calculated by previously reported formulas [27, 28].

2.5 | Outcomes

The primary outcome measure was the effectiveness of mean SICF (ISI 1 to 5 ms) and the index of excitation in differentiating ALS from ALS mimic disorders. Secondary outcome measures included comparison of accuracy between mean SICF (ISI 1 to 5 ms), index of excitation, and SICI (ISI 1 to 7 ms), as well as diagnostic utility of SICF/index of excitation across different subgroups, defined by site of disease onset (bulbar vs. limb), phenotype (UMN score ≤6 vs. >6), functional disability (ALSFRS-$R \leq 41$ vs. > 41), symptom duration (≤13.5 months vs. >13.5 months), and CMAP amplitude of the dominant hand recorded over the APB (≤6.2 vs. >6.2 mV).

2.6 | Statistical Analysis

Normality was assessed using the Shapiro–Wilk test. Categorical variables are summarized as frequency (percentage). Student *t*-test, Pearson's Chi Square, or Mann–Whitney *U* test tests were used to compare differences between groups. A two-way repeated measures ANOVA was used to assess the effect of different coil types (circular and figure-of-eight) at consecutive ISI for SICI (ISI 1-7 ms) and SICF (ISI 1-5 ms) in both ALS and non-ALS groups. Receiver operator curve (ROC) analysis was utilized to assess the diagnostic utility of SICF and the index of excitation. The DeLong method was used to assess statistical differences in AUC values for each TMS biomarker [29]. The sensitivity, specificity, positive and negative likelihood (LR) ratios, as well as the diagnostic odds ratio (DOR) for SICF and the index of excitation were assessed, along with their respective 95% confidence interval (CI). The optimal diagnostic cut-off value for SICF and the index of excitation was selected as the point of *minimum* difference between sensitivity and specificity values, in order to balance the frequency of false positives and false negatives. For the comparison of sensitivities and specificities between SICI and SICF/index of excitation, the previously reported mean SICI (ISI 1–7 ms) value of ≤5.5% was used [4]. The median split method was applied to derive the parameters for the ALSFRS-R, UMN score, symptom duration, and APB CMAP amplitude subgroups. Correlation between SICF and the index of excitation and ALSFRS-R, age at assessment, UMN score, and symptom duration was analyzed using the Pearson correlation coefficient. For all statistical analyses, a p-value < 0.05 was considered significant. Results are presented as mean ± standard error of the mean (SEM) for normally distributed data and median (interquartile range, IQR) for non-normally distributed data. While no formal a priori power calculation was performed, the sample size was justified based on prior studies demonstrating significant differences in cortical excitability measures between ALS and mimic disorders [18]. Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) software (version 29.0.1), and figures were generated using Python (version 3.12.6) with Matplotlib (version 3.6.3).

3 | Results

In total, 154 underwent cortical excitability testing (Figure 1). The included participants exhibited a mean age of 64.7 ± 13.6 years, and 101 (65.6%) were males, while 53 (34.4%) were females. A total of 95 participants (61.7%) were diagnosed with ALS as per the Gold Coast criteria. After extensive clinical assessment and follow-up, 48 (31.2%) participants were diagnosed with a non-ALS mimic disorder and served as pathological controls. In 11 (7.1%) participants, the final diagnosis was inconclusive, and these were not included in further analysis.

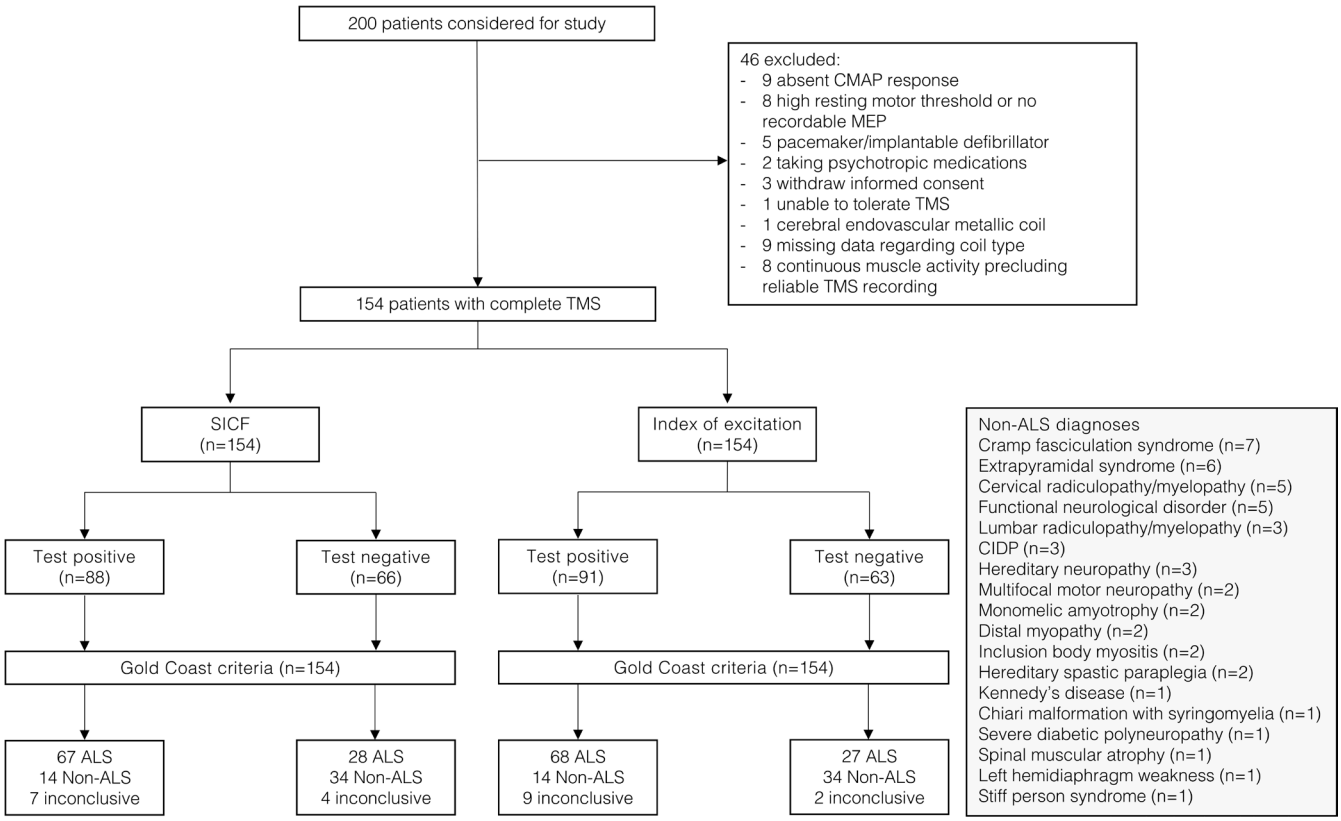

FIGURE 1 | Flow diagram of study participants. ALS, amyotrophic lateral sclerosis; CIDP, Chronic inflammatory demyelinating polyradiculoneuropathy; CMAP, compound motor action potential; compound muscle action potential; MEP, motor evoked potential; SICF, short interval intracortical facilitation; TMS, transcranial magnetic stimulation.

The ALS participants were older, exhibited a greater degree of muscle weakness, and had more prominent UMN signs when compared to non-ALS mimic participants (Table 1). The ALS participants exhibited a moderate rate of disease progression (median 0.5, IQR 0.7) at the time of TMS testing, and 22 (23.2%) ALS participants were taking riluzole. The CMAP amplitude, SI, and NI were significantly lower in ALS compared to non-ALS mimic participants, indicating a greater degree of lower motor neuron dysfunction in ALS, with values outlined in Table 2.

3.1 | Cortical Function

Prior to assessing the diagnostic utility of SICF and the index of excitation, we investigated whether there were significant differences in SICF and SICI values recorded with the circular and figure-of-eight coils within each participant cohort. There was no difference in SICF and SICI values recorded with circular versus figure-of-eight coils within the ALS and non-ALS mimic cohorts (Figure 2). Subsequently, SICF and SICI values recorded with circular and figure-of-eight TMS coils were grouped to increase power and were used in further analysis.

As previously established, averaged SICF was significantly increased in ALS participants (mean $-19.8\% \pm 1.0\%$) when compared to pathological controls ($-11.2\% \pm 1.0\%$, $p < 0.001$, see Table 2). The increase in averaged SICF was accompanied by a significant reduction in averaged SICI (see Table 2). Of further relevance, the index of excitation was significantly increased in ALS participants (mean $80.8\% \pm 15.6\%$) when compared to non-ALS participants ($48.4\% \pm 3.8\%$, $p < 0.001$), in keeping with the previous study [18]. Riluzole therapy did not influence the findings, given that SICF, SICI, and the index of excitation were comparable between participants on (mean SICI $0.5\% \pm 2.0\%$; mean SICF $-20.1\% \pm 2.0\%$; mean index of excitation 101.5 ± 11.4) and off riluzole (mean SICI $1.9\% \pm 1.2\%$, $p = 0.527$; mean SICF $-19.1\% \pm 1.4\%$, $p = 0.709$; mean index of excitation $62.2\% \pm 29.6\%$; $p = 0.384$) at the time of TMS assessment. Consequently, riluzole-treated patients were included in subsequent analysis.

3.2 | Assessment of Diagnostic Utility

Receiver operator curve was utilized to assess the diagnostic effectiveness of TMS biomarkers (Figure 3). The AUC for SICF was 0.77 (95% CI 0.68–0.84), indicating a "good" utility in differentiating ALS from non-ALS mimic disorders. In keeping with previous studies [7] the AUC for SICI was 0.84 (95% CI 0.77–0.89), underscoring a "very good" diagnostic potential for differentiating ALS from non-ALS mimic disorders. The AUC for the index of excitation also exhibited "very good" diagnostic utility as evidenced by an AUC of 0.82 (95% CI 0.75–0.88). The AUC value for SICI was significantly higher when compared to SICF (AUC difference, 0.068, 95% CI 0.007–0.128, $p = 0.028$). Similarly, the AUC for index of excitation (AUC difference

TABLE 1 | Demographic, clinical characteristics of ALS and non-ALS participants.[a]

	ALS $n=95$	Non-ALS $n=48$	p
Demographic characteristics			
Male, *n* (%)	61 (64.2)	30 (62.5)	0.841
Age at assessment (years), mean ± SE (range)	64.3 ± 1.1 (32–83)	53.6 ± 2.2 (27–88)	**< 0.001**
Clinical characteristics			
Disease duration at assessment (months), median (IQR)	11.9 (15.9)	24.3 (65.0)	**0.001**
Onset region, *n* (%)			0.070
Limb	64 (67.4)	38 (79.2)	—
Bulbar	27 (28.4)	4 (8.3)	—
Generalized	1 (1.1)	1 (2.1)	—
Respiratory	1 (1.1)	1 (2.1)	—
UMN score, median (IQR)	8.0 (6.0)	0.0 (6.0)	**< 0.001**
Total MRC score, median (IQR)	81.5 (13.0)	88.0 (8.0)	**< 0.001**
Upper limb MRC score, median (IQR)	56.0 (8.5)	60.0 (3.0)	**< 0.001**
Lower limb MRC score, median (IQR)	28.0 (5.3)	30.0 (3.8)	**0.010**
ALSFRS-R at assessment, median (IQR)	41.0 (6.0)	—	—
Δ*F*, median (IQR)	0.5 (0.7)	—	—
Riluzole, *n* (%)	22 (23.2)	—	—
Riluzole treatment duration at assessment (months), median (IQR)	2.0 (5.0)	—	—

Abbreviations: ALS, amyotrophic lateral sclerosis; IQR, interquartile range; Δ*F*, disease progression rate until diagnosis.
[a]11 patients were not included as the diagnosis was not established at census date. Significant values ($p<0.05$) are shown in bold.

of 0.050, 95% CI 0.013–0.088, $p=0.008$) was also slightly but significantly higher when compared to SICF but was comparable to SICI (AUC difference 0.017, 95% CI −0.017 to 0.051, $p=0.328$).

The optimal diagnostic cut-off value for mean SICF was ≤ −13.6% with a sensitivity of 70.5% and specificity of 70.8%, while for mean index of excitation, the optimal cut-off value was ≥ 64.5% with a sensitivity of 71.6% and specificity of 70.8% (Table 3). The positive likelihood ratios (LR) for increased mean SICF (2.4) and index of excitation (2.5) indicated a moderate utility for increasing the probability of ALS (Table 3). Similarly, the negative LRs suggested that "normal" SICF and index of excitation values (negative test) moderately reduced the probability of ALS. Lastly, the DORs for SICF (5.8) and the index of excitation (6.1) suggested a moderate effectiveness in diagnosing ALS (Table 3).

The diagnostic utility of the data collected by the figure-of-eight coil used for stimulation was undertaken to assess the performance of SICF and the index of excitation. The AUC for SICF was 0.77 (95% CI 0.66–0.87), whereas the AUC for the index of excitation was 0.84 (95% CI 0.75–0.92). These values were comparable to those obtained when combining circular coil and figure-of-eight data, suggesting that the coil type does not significantly influence diagnostic performance.

To further assess the utility of increased SICF and index of excitation, validation was undertaken on a new cohort of 45 participants (ALS, 28; ALS mimics, 17). Using the established diagnostic cutoff for averaged SICF (≤ −13.6%), the sensitivity was 75%, with a specificity of 71%. The sensitivity for increased index of excitation (cutoff ≥ 64.5%) demonstrated a sensitivity of 71.4% and a specificity of 70.6%. Taken together, the present findings suggest moderate diagnostic utility of SICF and the index of excitation in distinguishing ALS from ALS-mimic disorders.

3.3 | Subgroup Analysis

Subgroup analysis disclosed a similar area under the curve in ALS participants with bulbar and limb onset disease from SICF ($AUC_{difference}=0.04$, 95% CI −0.15 to 0.22, $p=0.709$) and index of excitation ($AUC_{difference}=-0.02$, 95% CI −0.21 to 0.16, $p=0.779$). Additionally, the AUC values were similar for SICI and index of excitation across the ALS cohort, irrespective of symptom duration, level of functional decline (ALSFRS-R), and LMN dysfunction (CMAP amplitude) or degree of UMN signs (Table 4).

TABLE 2 | Neurophysiological results of ALS and non-ALS participants.[a]

	ALS ($n=95$)	ALS mimics ($n=48$)	p
CMAP amplitude, median (IQR)			
Right side	5.1 (4.2)	7.8 (3.0)	**<0.001**
Left side	5.3 (3.9)	7.4 (3.7)	**<0.001**
SI, median (IQR)			
Right side	4.6 (5.7)	7.1 (4.7)	**<0.001**
Left side	4.0 (3.8)	7.4 (6.3)	**<0.001**
NI, median (IQR)			
Right-side	1.0 (1.2)	1.8 (2.2)	**0.017**
Left side	3.2 (1.6)	1.8 (0.2)	**0.032**
Averaged SICI, mean ± SEM	1.0 ± 0.8	12.0 ± 0.8	**<0.001**
Averaged SICF, mean ± SEM	−19.8 ± 1.0	−11.2 ± 1.0	**<0.001**
Index of Excitation, mean ± SEM	80.8 ± 15.6	48.4 ± 3.8	**<0.001**

Abbreviations: CMAP, compound muscle action potential; NI, neurophysiological index; SE, standard error; SI, split hand index; SICF, short interval intracortical facilitation; SICI, short interval intracortical inhibition.
[a]11 patients were not included as the diagnosis was not established at census date. Significant values ($p<0.05$) are shown in bold.

When considering a combination of TMS parameter abnormalities, namely the previously established reduced averaged SICI (≤5.5%) [18] and/or an increased averaged SICF (≤−13.6%), the sensitivity for diagnosing ALS significantly increased from 66.3% (when using reduced SICI in isolation) to 81.1% (95% 73.2%–89.0%, $p<0.001$). The increase in sensitivity was accompanied by a reduction in specificity from 81.3% to 64.6% (95% CI 51.1%–78.1%, $p<0.001$). A diagnostic tree model outlining the utility of TMS biomarkers in ALS is provided in Figure 4.

3.4 | Clinical Correlations

None of the clinical variables exhibited a significant correlation with SICF or the index of excitation. This included ALSFRS-R (SICF: $p=0.239$, index of excitation: $p=0.280$), age at assessment (SICF: $p=0.069$; index of excitation: $p=0.597$), MRC score (SICF: $p=0.703$; index of excitation: $p=0.755$), UMN score (SICF: $p=0.190$; index of excitation: $p=0.056$) and symptom duration (SICF: $p=0.106$; index of excitation: $p=0.787$).

4 | Discussion

The findings in the present study demonstrate that increased SICF and index of excitability reliably differentiated ALS from ALS-mimic disorders, with a modest level of diagnostic certainty that was marginally but significantly higher for the latter TMS measure. Additionally, the diagnostic utility of SICF and index of excitation was similar across different ALS subgroups, including patients with bulbar- and limb-onset disease, "on" or "off" riluzole therapy, and was unaffected by the degree of functional disability, lower motor neuron dysfunction, and UMN signs. It should also be stressed that the diagnostic usefulness of SICI was marginally but significantly higher when compared to SICF and probably accounts for the greater efficacy of the index of excitation. The study underscores the importance of threshold tracking TMS as an objective neurophysiological investigation in ALS, with measures reflecting cortical inhibitory circuit dysfunction and an imbalance between facilitatory and inhibitory circuit activity, providing objective evidence for UMN dysfunction in ALS, and thereby potentially aiding diagnosis.

4.1 | Functional Biomarkers of ALS

The diagnosis of ALS relies on the identification of concomitant UMN and LMN dysfunction along with disease progression, with multiple clinical criteria emerging [22, 30, 31]. While objective assessment of LMN dysfunction has been incorporated into the recent diagnostic criteria, as reflected by conventional neurophysiological studies, UMN dysfunction remains clinically based. The clinical reliance on identification of UMN dysfunction in ALS can be limited by complex physiological factors, including the degree of muscle wasting along with dysfunction of descending motor pathways and local spinal circuits, thereby limiting the sensitivity of diagnostic criteria and resulting in diagnostic delays [32, 33]. Reduction of SICI, as measured by the threshold tracking TMS technique, has proved to be an objective diagnostic biomarker of UMN dysfunction in ALS, enhancing the diagnosis by ~34% and reducing diagnostic delay [4, 16].

SICF assesses the activity of high-threshold facilitatory circuits [34, 35], which are distinct from inhibitory neuronal populations assessed by SICI [15], thereby potentially providing an additional TMS biomarker for UMN dysfunction. The present study established a significant increase in SICF in ALS, in keeping with previous studies [18, 19, 36], which differentiated ALS from ALS mimics with an optimal diagnostic cut-off value of ≤ −13.6%. The value of SICF as a biomarker was less pronounced than SICI, and while combining SICF and SICI increased the sensitivity for diagnosing ALS, the specificity was reduced, thereby limiting the usefulness of simultaneously assessing inhibitory and facilitatory cortical neuronal populations for the diagnosis of ALS. This reduction in specificity may lead to the misclassification of non-ALS mimic disorders as ALS, particularly in conditions that share overlapping neurophysiological features, such as multifocal motor neuropathy, cervical spondylitic myelopathy, hereditary spastic paraplegia, or Kennedy's disease. Such misclassification risks highlight the importance of interpreting TMS biomarkers in the context of the full clinical presentation, including conventional neurophysiology, imaging, and, where appropriate, fluid biomarkers.

Of relevance, the index of excitation was significantly increased in the current ALS cohort, a finding consistent with previous studies [18, 25, 36] and implying an imbalance in facilitatory and

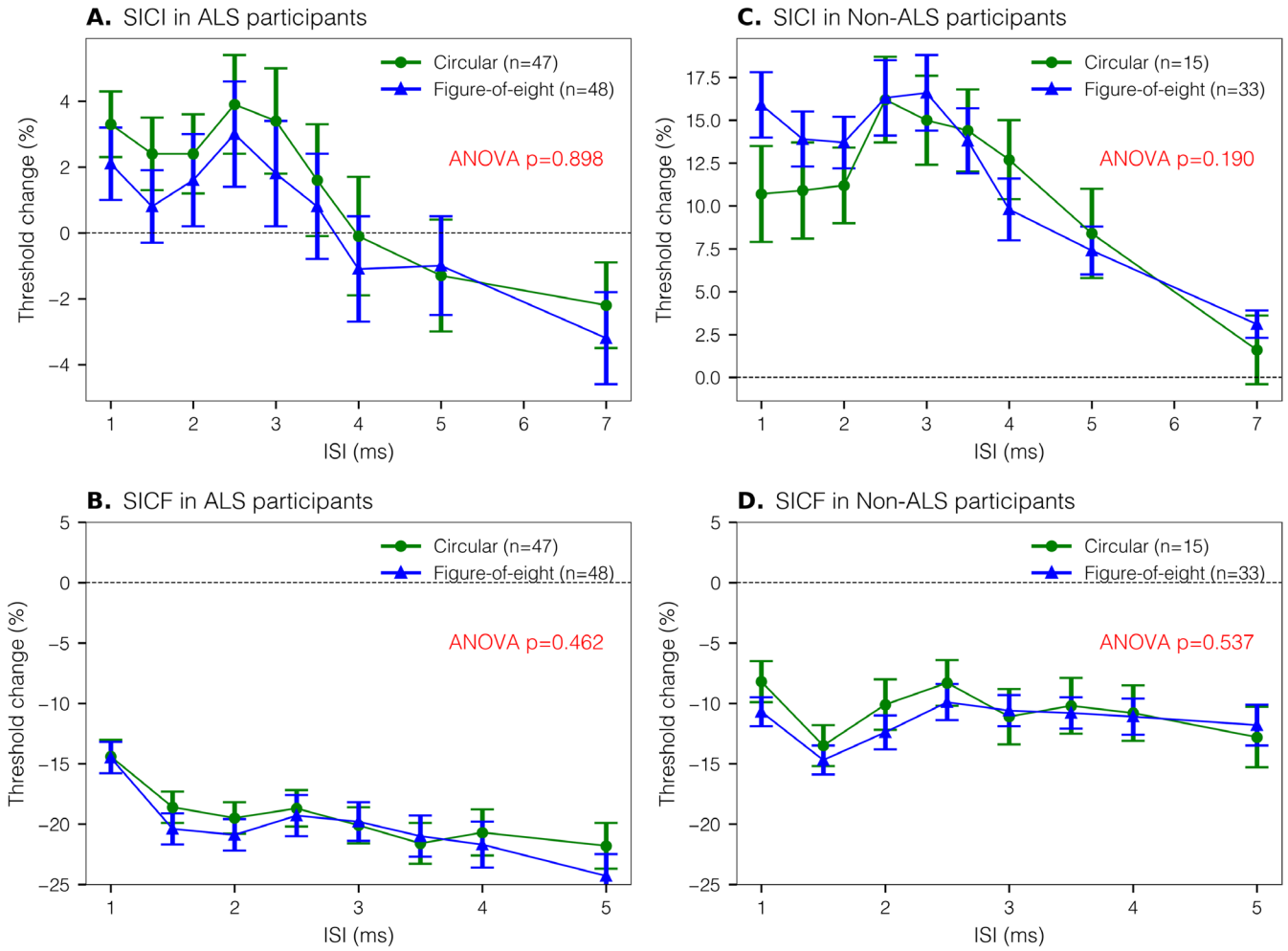

FIGURE 2 | Coil type comparison for SICI and SICF in ALS and non-ALS participants. Short interval intracortical inhibition (SICI) was comparable between circular and figure-of-eight coils when recorded in (A) amyotrophic lateral sclerosis (ALS) and (C) non-ALS mimic participants between interstimulus intervals (ISI) of 1-to-7 ms. Short interval intracortical facilitation (SICF) was also comparable between circular and figure-of-eight coils when recorded in (B) ALS and (D) non-ALS mimic participants.

inhibitory neuronal population activity in ALS. At a diagnostic level, an increase in the index of excitation exhibited a comparable area under the curve to that evident for reduced SICI, suggesting comparable diagnostic utility. This potential diagnostic utility was maintained across different ALS phenotypes and disease stages. Given, however, that the specificity is ~70.8%, interpretation of the increased index of excitation should be made in an appropriate clinical context. Interestingly, there was no correlation between the index of excitation and functional decline (as reflected by ALSFRS-R) in the current ALS cohort, contrasting with a previous study [18] a finding that may be related to disease heterogeneity.

A reduction in SICF has been reported in some ALS cohorts [20, 21], contrasting with the current and previous studies presented [18, 27, 36]. A study using peristimulus time histogram techniques also reported enhanced excitability of corticomotoneuronal projections onto spinal motor neurons [37], a finding consistent with the present study. It was suggested that methodological differences could account for the discordant findings, particularly differences in conditioning stimulus intensity and ISI steps (0.5 ms present study vs. 0.2 ms). This seems unlikely given that our threshold tracking paradigm consistently elicited a robust SICF over all the ISI values, and that previous studies have reported SICF with comparable or lower conditioning stimulus intensity values [34]. It could also be argued that the pathophysiological heterogeneity of ALS cohorts could explain the discrepancy in findings, particularly given the greater functional disability of enrolled patients reported in a previous study [20]. Synaptic remodeling of pyramidal neurons has been reported in ALS models with disease progression, leading to a reduction in excitatory inputs [38]. While the findings in the present study do not support such a mechanism, since the utility of SICF was evident in ALS patients with more advanced disease, it could account for the variability of SICF findings between studies. Longitudinal TMS studies, combined with sophisticated neuroimaging, genetic, and molecular techniques, could further clarify the reasons for the discordant SICF findings.

A potential limitation of the present study pertains to combining data from two different TMS coil types (circular and figure-of-eight). It could be argued that such an approach may introduce variability and bias due to distinct stimulation and biophysical

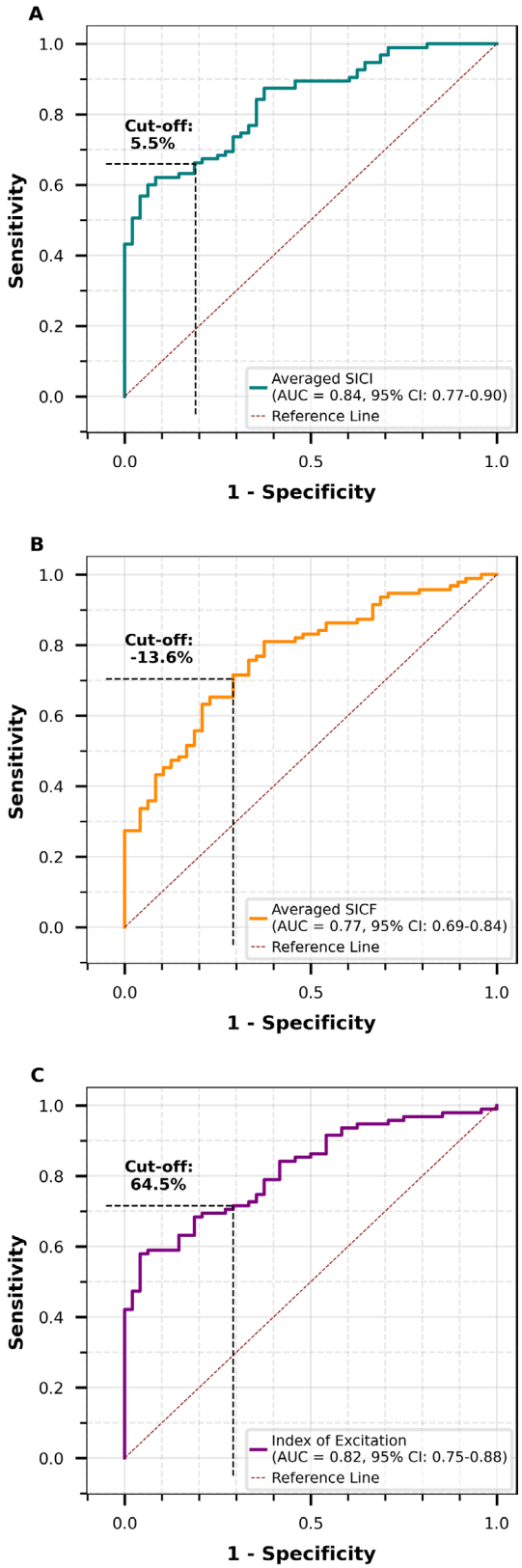

FIGURE 3 | Receiver operator curves for (A) averaged short interval intracortical inhibition (SICI), (B) averaged short interval intracortical facilitation (SICF), and (C) index of excitation. The area under the curve (AUC) was higher for SICI and the index of excitation compared to SICF. Diagnostic cut-off values are depicted for each parameter.

properties of different coil types [39]. Given that there were no significant differences in SICI and SICF in ALS and non-ALS mimic disorder patients recorded by the two coil types (Figure 2), it seems unlikely that combining data would exert a meaningful impact on the current findings; however, the potential for variability and bias introduced by their distinct stimulation properties cannot be completely excluded. Lack of inter-centre variability and external validation represent additional limitations, although with the recent integration of the threshold tracking TMS technique into a commercially available neurophysiological device (MagXcite) [40], these limitations could be readily addressed in future studies. While our findings suggest that disease progression does not impact the diagnostic performance of SICF and index of excitation, longitudinal studies are required to further evaluate the utility of these TMS biomarkers with disease progression. Exploratory analysis correlating SICF and the IE with genetic ALS subtypes could also offer novel insights into the pathophysiological basis of cortical hyperexcitability in ALS. Unfortunately, genetic data was not available on the current ALS cohort, and future studies should assess the clinical utility of SICI and IE in these genetic phenotypes.

4.2 | Implications for Clinical Studies

The presence of cortical hyperexcitability has been identified as an important diagnostic, pathophysiological, and prognostic biomarker for ALS mediated by dysfunction of inhibitory circuits and overactivity of facilitatory cortical circuits [4–6, 12, 18, 36, 41–43]. The Gold Coast criteria, which represent the diagnostic standard for ALS, continue to rely on clinical assessment for the detection of UMN signs [22]. Given that identification of UMN signs may be difficult in ALS for a variety of reasons [32], objective biomarkers of UMN dysfunction could aid in ALS diagnosis. The findings in the present study suggest that an increase in SICF and index of excitation, along with reduced SICI, are potential biomarkers of UMN dysfunction in ALS, providing moderate diagnostic effectiveness. Combining TMS abnormalities with genetic, molecular (neurofilament light chain, NfL), and neuroimaging data may provide further utility for identifying UMN dysfunction. Along these lines, a recent study reported an increase in diagnostic utility when combining SICI and NfL levels in differentiating ALS from ALS mimics [44]. Magnetic Resonance Imaging (MRI) studies have established abnormalities of motor and extra-motor cortical regions in ALS, with diffusion-weighted MRI (dMRI) techniques demonstrating the most consistent signature of UMN dysfunction [9]. Specifically, abnormalities in DTI metrics in the core white matter tracts, namely the corticospinal tract and corpus callosum, differentiate ALS from mimicking diseases with high accuracy (>87% accuracy) [45]. Consequently, a multimodal approach encompassing TMS measures (SICI, SICF, and index of excitation) along with molecular (NfL) and neuroimaging biomarkers may enhance ALS diagnosis in a clinical setting and exhibit applicability in a therapeutic clinical trial setting (Figure 5) [2]. It should also be stressed that the diagnostic utility of SICF and the index of excitation is moderate. Consequently, TMS data should be interpreted in the correct clinical context and combined with conventional diagnostic techniques such as electromyography for optimal clinical utility.

TABLE 3 | Diagnostic performance of SICF and index of excitation in ALS.

	Cut-off	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)	DOR (95% CI)
Averaged SICF	−13.6	70.5 (61.4–79.7)	70.8 (58.0–83.7)	2.4 (1.5–3.8)	0.4 (0.3, 0.6)	5.8 (2.7–12.5)
Index of excitation	64.5	71.6 (62.5–80.7)	70.8 (56.0–83.7)	2.5 (1.6–3.9)	0.4 (0.3, 0.6)	6.1 (2.8–13.2)

Abbreviations: CI, confidence interval; DOR, diagnostic odds ratio; LR, likelihood ratio; SICF, short interval intracortical facilitation.

TABLE 4 | Comparison of diagnostic accuracy of SICF and index of excitation in different subgroups.

	Subgroups	AUC difference (95% CI)	*p*
SICF	Bulbar vs. non-bulbar onset	0.04 (−0.15 to 0.22)	0.709
	Symptom duration (months) ≤13.5 vs. >13.5	0.01 (−0.16 to 0.18)	0.899
	ALSFRS-R ≤41 vs. >41	0.10 (−0.07 to 0.27)	0.242
	$CMAP_{amp}$ (mV) ≤6.2 vs. >6.2	−0.06 (−0.24 to 0.11)	0.463
	UMN score ≤6 vs. >6	−0.05 (−0.22 to 0.12)	0.554
Index of excitation	Bulbar vs. non-bulbar onset	−0.02 (−0.21 to 0.16)	0.779
	Symptom duration (months) ≤13.5 vs. >13.5	−0.003 (−0.16 to 0.15)	0.967
	ALSFRS-R ≤41 vs. >41	0.061 (−0.09 to 0.22)	0.434
	$CMAP_{amp}$ (mV) ≤6.2 vs. >6.2	−0.045 (−0.20 to 0.11)	0.575
	UMN score ≤6 vs. >6	−0.08 (−0.24 to 0.07)	0.299

Note: The subgroup cohort size was as follows: (i) bulbar ($n=31$), limb ($n=106$), 6 missing site of onset data; (ii) symptom duration ≤13.5 months ($n=68$), >13.5 months ($n=75$); (iii) ALSFRS-*R* ≤41 ($n=74$), >41 ($n=69$); (iv) CMAP amplitude (mV) ≤6.2 ($n=65$), >6.2 ($n=78$); and (v) UMN score ≤6 ($n=75$), >6 ($n=68$). The subgroup sizes include ALS and non-ALS mimics. For the subgroup with paucity of UMN signs (UMN score ≤6), there were a total of 40 ALS patients, and included the flail arm variant ($n=7$, 17.5%), flail leg variant ($n=8$, 20%) and progressive muscular atrophy ($n=25$, 62.5%).
Abbreviations: ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; amp, amplitude; AUC, area under the curve; $CMAP_{amp}$, amplitude of compound muscle action potential; SICF, short-interval intracortical facilitation; UMN, upper motor neuron.

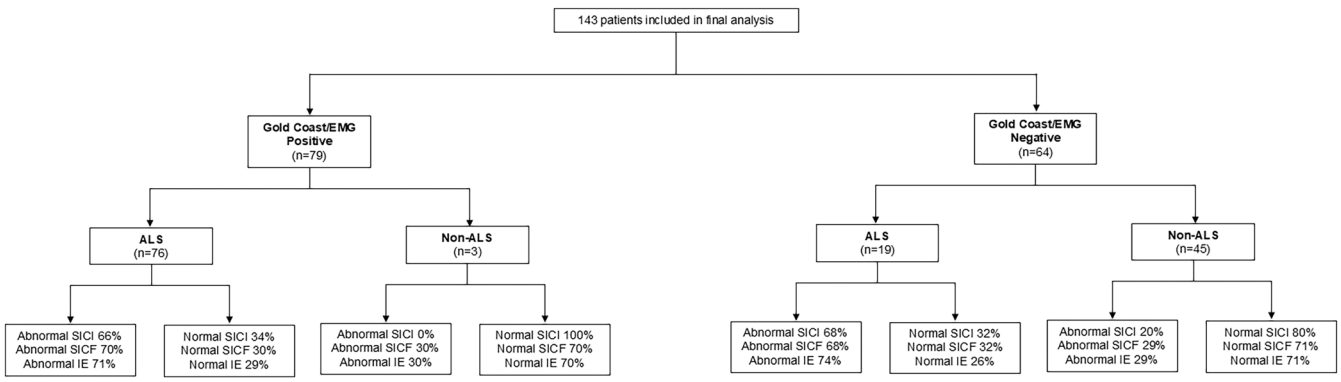

FIGURE 4 | Decision tree model quantifying the diagnostic utility of short interval intracortical facilitation (SICF), short interval intracortical inhibition (SICI), and index of excitation (IE) in ALS.

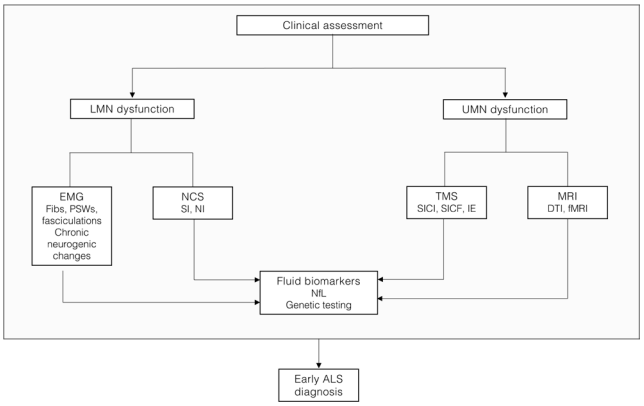

FIGURE 5 | Multimodal diagnostic workflow for amyotrophic lateral sclerosis (ALS). DTI, diffusion tensor imaging; EMG, electromyography; Fibs, fibrillations; fMRI, functional magnetic resonance imaging; IE, index of excitation; LMN, lower motor neuron; NCS, nerve conduction studies; Nfl, neurofilaments; NI, neurophysiological index; PSW, positive sharp waves; SICI, short interval intracortical inhibition; SICF, short interval intracortical facilitation; TMS, transcranial magnetic stimulation; UMN, upper motor neuron.

Author Contributions

Cláudia Santos Silva: methodology, data curation, writing – original draft, investigation, formal analysis, writing – review and editing. **Nathan Pavey:** conceptualization, investigation, funding acquisition, writing – review and editing, data curation, supervision, formal analysis, methodology, project administration. **Aicee Dawn Calma:** investigation, writing – review and editing, data curation. **Matthew C. Kiernan:** investigation, project administration, conceptualization, writing – review and editing, methodology. **Parvathi Menon:** investigation, writing – review and editing, supervision, data curation, formal analysis, funding acquisition, conceptualization. **Mehdi van den Bos:** conceptualization, investigation, methodology, writing – review and editing, formal analysis, data curation, supervision. **Steve Vucic:** conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, methodology, formal analysis, project administration, data curation, supervision.

Conflicts of Interest

Cláudia Santos Silva, Nathan Pavey, Aicee Dawn Calma, Parvathi Menon, and Mehdi van den Bos report no disclosures relevant to the manuscript. Matthew C. Kiernan and Steve Vucic developed MagXite software utilized in this study.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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