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Short-interval intracortical inhibition as a function of inter-stimulus interval: Three methods compared

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ABSTRACT

Background: Short-interval intracortical inhibition (SICI), as measured by threshold-tracking as a function of inter-stimulus interval (ISI), has been proposed as a useful biomarker for amyotrophic lateral sclerosis (ALS), but its relationship to conventional amplitude measurements has not been established.
Methods: Serial tracking of SICI at increasing ISIs from 1 to 7 ms (T-SICIs) was compared in 50 healthy control subjects with the same ISIs tracked in parallel (T-SICIp), and with conventional amplitude measurements (A-SICI). For T-SICIp and A-SICI, pairs of conditioning and test stimuli with different ISIs were pseudo-randomised and interspersed with test-alone stimuli given at regular intervals. Thresholds were estimated by regression of log peak-to-peak amplitude on stimulus.
Results: T-SICIp and A-SICI were closely related: a ten-fold reduction in amplitude corresponding to an approximately 18% increase in threshold. Threshold increases were greater for T-SICIs than for T-SICIp at 3.5–5 ms ($P < 0.001$). This divergence depended on the initial settings and whether ISIs were progressively increased or decreased, and was attributed to the limitations of the serial tracking protocol. SICI variability between subjects was greatest for T-SICIs estimates and least for A-SICI, and only A-SICI estimates revealed a significant decline in inhibition with age.
Conclusions: The serial tracking protocol did not accurately show the dependence of inhibition on ISI. Randomising ISIs gives corresponding SICI measures, whether tracking thresholds or measuring amplitude measurements. SICI variability suggested that A-SICI measurements may be the most sensitive to loss of inhibition.

Introduction

Short-interval intracortical inhibition (SICI) is shown by the reduction in the response to a suprathreshold transcranial magnetic stimulation (TMS) pulse, when a subthreshold pulse is delivered 1–5 ms earlier. It was first described by Kujirai et al. [1] and following the work of Ziemann and colleagues has since become a popular non-invasive tool for investigating inhibitory circuits in the human brain that involve GABA-A receptor signalling [2–4]. Impaired SICI in patients with amyotrophic lateral sclerosis (ALS) was reported by Ziemann et al. [5], but Rothwell et al. [4] considered that, since changes in SICI have been found in very many different conditions, the method has little diagnostic value. On the other hand, Kiernan, Vucic and colleagues [6] have argued extensively for its clinical utility in diagnosis and monitoring of ALS. They have used the unconventional method of threshold-tracking, to measure inhibition by the changes in stimulus required to elicit a specified motor evoked potential (MEP) [7–10], in contrast to the conventional method of measuring changes in MEP amplitude for a constant stimulus.

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Abbreviations

ALS	amyotrophic lateral sclerosis
AN2(4)	advance ISI during T-SICIs when 2 (4) valid threshold estimates are obtained
A-SICI	short-interval intracortical inhibition obtained by amplitude measurements
A–SICI–T,	A-SICI transformed into equivalent threshold changes
EMG	electromyography
ISI	inter-stimulus interval
MEP	motor evoked potential
MSO	maximum stimulator output
RMT	resting motor threshold
RMT200	stimulus required to evoke 200 μV MEP
SD	standard deviation
SICI	short-interval intracortical inhibition
SE	standard error
T-SICI	short-interval intracortical inhibition obtained by threshold tracking
T-SICIp,	T-SICI obtained by tracking different ISIs in parallel (T-SICIp1 without and T-SICIp2 with initial thresholds set above RMT)
T-SICIs	T-SICI obtained by tracking different ISIs serially (TSICIs1 without and TSICIs2 with initial settings to improve 1 ms estimate)
TMS	transcranial magnetic stimulation
TS1mV	stimulus required to evoke 1 mV MEP

Although many studies have been published of the use of threshold-tracking SICI (T-SICI) in ALS [6], there have been few reports from outside of Sydney. One reason for this is likely to be that to perform T-SICI efficiently requires specialist threshold-tracking software, which is not widely available. Another may be that the advantages of the Sydney T-SICI protocol over conventional amplitude measurements (A-SICI) have never been clearly demonstrated. Some of us recently published a direct comparison between T-SICI and A-SICI, which examined the reproducibility of these techniques in a limited number of subjects, and found that, while both appeared to depend on similar cortical inhibitory mechanisms, T-SICI compared favourably with A-SICI with regard to reproducibility [11]. However, it is important to note that the Sydney protocol [9] was not used for T-SICI. Instead of tracking SICI as a function of increasing interstimulus interval (ISI), as done by Vucic and colleagues, SICI was determined independently for 4 different levels of conditioning stimulus, at the same ISI of 2.5 ms. We will refer to this approach, in which the different conditions were tracked in parallel, from the same starting level of stimulus, as 'T-SICI parallel', or T-SICIp. In the Sydney protocol, in contrast, the threshold is tracked from one ISI to the next in succession, so that the stimulus only has to be adjusted by the difference between the thresholds at adjacent ISIs (e.g. 2.0 and 2.5 ms). We will refer to this method as 'T-SICI serial' or T-SICIs.

T-SICIs and T-SICIp are not the only possible methods of measuring SICI as a change in stimulus for a constant response, rather than a change in response for a constant stimulus. Just as resting motor threshold (RMT) can be measured in different ways [12,13], so can the threshold for a conditioned response. For example, adaptive threshold hunting has been used by Cirillo and colleagues [14], using a 'maximum-likelihood parameter estimation by sequential testing' (PEST) [15,16]. To make an accurate comparison between the constant response and constant stimulus measures of SICI, they matched the conditioned and test stimulus intensities between the two approaches, so that the measurements were not independent. They also investigated the effects of conditioning stimulus intensity and current direction at ISIs of 2 and 3 ms.

Up to now, assessment of SICI as a function of ISI by a constant response method has only been published for T-SICIs, and there has been no study comparing T-SICIs v ISI with A-SICI v ISI or with T-SICIp v ISI. The primary aim of this study was to compare these three independent methods of assessing SICI v ISI for their ability to detect lack of intracortical inhibition, which may be relevant to their potential clinical usefulness. Instead of making repeated measurements on a limited number of subjects, to determine reproducibility, we made one set of the 3 recordings (A-SICI, T-SICIs and T-SICIp) in 50 subjects, to assess the variability of SICI measures across healthy control subjects. This knowledge is a prerequisite if SICI is to be used to detect whether lack of inhibition in a patient is significantly abnormal or merely unusual. A secondary aim of the study arose when we found that there were consistent, significant differences between T-SICIs and T-SICIp. Additional experiments were performed to determine the origin of this discrepancy. Further experiments were also added to determine the impact of the initial settings of the threshold for the T-SICIs and T-SICIp protocols.

Methods

The study was carried out in accordance with the Declaration of Helsinki. It was approved by local ethics committees, The Central Denmark Region Committees on Health Research Ethics and the local ethics committee in Ankara, and written informed consent was obtained from all participants prior to the investigations.

Subjects

Fifty healthy volunteers were recruited for this study. None had any known neurological disorder or contraindications for TMS, and none were on any regular medication. They comprised 25 men and 25 women, aged from 21 to 79 years, mean 43.2, SD 16.6 years.

Transcranial magnetic stimulation

Each subject was comfortably seated in an armchair and instructed to stay relaxed but alert during the recordings. All the subjects were right-handed, and the surface MEP was recorded from the right first dorsal interosseous (FDI) muscle, with Ag/AgCl electrodes placed in a belly-tendon montage, the cathode over FDI and anode on the 2nd metacarpophalangeal joint. The ground electrode was placed on the dorsum of the hand. The MEP responses were amplified (×1000 gain), filtered (3Hz-3kHz) using a D440 2-channel Amplifier (Digitimer Ltd., Hertfordshire, UK). A 50/60 Hz Humbug Noise Eliminator (Digitimer Ltd., Hertfordshire, UK) was used to remove mains frequency contamination. Amplified signals were digitized with a data acquisition system, NI-6251 (National Instruments).

TMS was carried out using two Magstim 200^2 stimulators connected in BiStim mode (Magstim Co. Ltd, Whiteland, Wales, UK) to a figure-of-eight coil (D70 Remote Coil, reference number: 3190-00, Magstim, Whitland, UK). Stimulus delivery and data acquisition were controlled by QTRACW software (©Institute of Neurology, University College London, London, UK, distributed by Digitimer Ltd. at www.digitimer.com) using QTMSG-10 recording protocols. Magnetic stimuli were delivered at 4.5 or 5 s intervals. The coil was held tangential to the scalp with the handle pointing posterolaterally at an angle of 45° to the midsagittal line to activate the

corticospinal system transsynaptically. The coil position was slightly changed over the hand area of the motor cortex at the optimum scalp position to elicit MEPs in the contralateral right FDI muscle. Stimulation was started at 35% MSO and then increased or decreased in steps of 3% MSO to induce MEPs of approximately 0.5 mV. The hotspot was defined as the site where largest MEPs could be produced at this slightly suprathreshold intensity while contractions localised to FDI could also be observed. Once the hotspot was identified, the coil outline was drawn on the swimming cap the subject was wearing, to ensure precise and consistent coil positioning throughout the examination. An automated stimulation protocol was used, allowing a single operator to carry out the whole recording without the need to reposition the coil or manually control the stimulator.

Removal of responses with incomplete relaxation

To avoid including responses when the subject was not properly relaxed, the number of EMG peaks exceeding 20 μV occurring between 30 and 330 ms before the test stimulus were counted, and if there were 5 or more, the results of that sweep were ignored. These parameters were chosen following pilot experiments to avoid unnecessary elimination of stimulus responses by occasional background noise fluctuations.

Resting motor threshold

Resting motor threshold (RMT) for a 200 μV peak-to-peak response (RMT200) and the stimulus required to elicit a 1 mV response (TS1mV) were measured by '4 → 2→1' tracking and logarithmic regression (Fig. 1). The operator determined the hotspot where a stimulus intensity regularly evoked a response. Tracking then first started at that stimulus intensity, with a step size of 4% maximum stimulator output (MSO), but this step size was reduced to 2% and then 1% when changes of step direction were required or the response was within the target error limits (20% on a logarithmic scale, i.e. from target-20% to target+25%) indicated by the dashed lines (see Fig. 1C). Tracking then continued with steps of 1% (or 0% if within target zone) until 6 valid threshold estimates had been obtained (cf [11]). A valid threshold estimate was scored

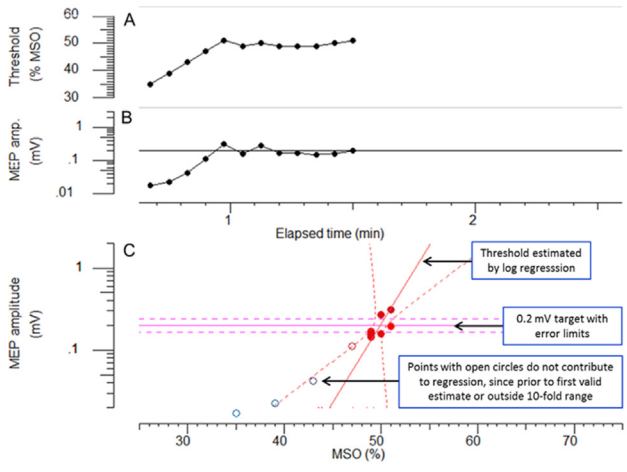

Fig. 1. Estimation of RMT200 (threshold for 0.2 mV response) by '4 → 2→1' tracking and logarithmic regression. A: Stimulus as % maximum stimulator output. B: Peak-to-peak amplitude of motor evoked potential, in mV (note logarithmic scale). C. Plot of MEP amplitude, as in B, against stimulus, as in A. Threshold is estimated by weighted logarithmic regression, where responses less than 20 μV or greater than 2 mV, and early responses before the first valid estimate, do not contribute.

every time that two responses bracket the target, or the response was within the target error limits. This method of RMT estimation was convenient for this study, since the tracking with 1% steps was simply continued during the subsequent SICI recording to follow any fluctuations in RMT. After the first valid threshold estimate, the subsequent stimuli and responses were used to estimate the RMT by log regression. This regression was also weighted, with weights reducing from 1 at the level of the target to 0 at 1/10th 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. [8], was used for all further thresholds, whether conditioned or unconditioned.

Three SICI methods

The following 3 SICI methods were applied to the 50 healthy control subjects in randomised order, either consecutively, without moving the coil (14 subjects), or within 2 min and with the coil applied to the same hotspot (36 subjects).

A-SICI (see Fig. 2A). RMT200 and TS1mV were first estimated, as described above, and then test stimuli were fixed at TS1mV and conditioning stimuli at 70% of RMT200 for the remainder of the recording. The following ISIs were selected in a pseudo-random (shuffled) order: 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 7 ms. Test-alone stimuli were given after each three paired stimuli. Each paired stimulus was delivered 10 times, making a total of 120 stimuli.

T-SICIp (see Fig. 2B). After estimating RMT200, RMT200 was tracked continuously, by decreasing the stimulus intensity by 1% MSO when responses were more than 250 μV, and increasing it by 1% when responses were less than 160 μV. In between these test-alone stimuli, paired stimuli were delivered, with pseudo-randomised ISIs as for A-SICI. The conditioning stimuli were continuously adjusted and set to 70% of the last RMT200 estimate, while the test stimuli were initially set to 106% of RMT200 (to reduce the average expected range over which tracking would be required) and then they also tracked the 200 μV target, but with different tracking steps. Proportional tracking was used, with the change in test stimulus proportional to the error (difference between last response and target), up to a maximum, for a 100% error. The maximum step size was reduced from 6% to 2% in successive complete stimulus cycles. As with the A-SICI protocol, each of the 9 paired stimuli was delivered 10 times, which with the test-alone stimuli made a total of 120 stimuli, the same as for A-SICI. The limit of 120 stimuli was chosen so that the time taken for a test did not exceed 10 min, when the inter-trial interval was 5 s.

T-SICIs (see Fig. 2C). After estimating RMT200, this was tracked continuously as in T-SICIp, and conditioning stimuli were set to 70% of this test-alone stimulus. Paired stimuli were started with an ISI of 1 ms and a stimulus of 110% of RMT200, but the test stimulus then tracked the 200 μV target continuously, with 1% tracking steps as for RMT200. (The initial value was based on preliminary data from controls and patients to minimize the range over which tracking was needed to reach threshold. See below for further details.) Following Vucic et al. [9], the ISI was increased when two valid threshold estimates were registered, where valid threshold estimates were scored as for the RMT determination. The ISIs were increased over the same 9 values as for A-SICI and T-SICIp. The number of stimuli required for a T-SICIs assessment was variable. It was usually less than the 120 required for A-SICI and T-SICIp, as in Fig. 2, but it could be more in subjects with high thresholds (see Results).

Initial stimulus settings for T-SICIs and T-SICIp. In the T-SICIs recordings by Vucic et al. [9], the 1 ms stimulus was initialised at RMT200, and the 1 ms threshold was tracked only once. We refer to this protocol as T-SICIs1. To improve the estimation of the 1 ms

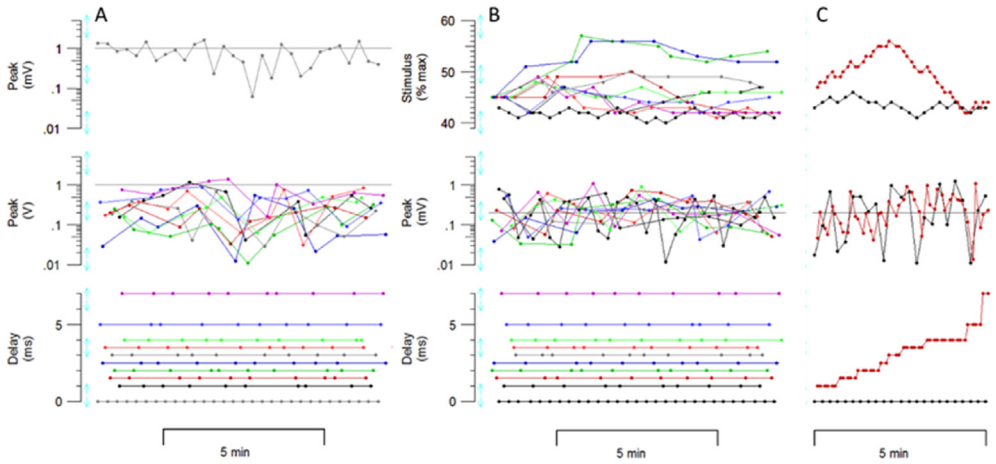

Fig. 2. Time courses of three SICI measurements on same subject. **A.** A-SICI: after determination of RMT200 and TS1mV, conditioning stimuli at 70% RMT200 were followed by test stimuli at TS1mV. *Top*: responses to test-alone stimuli. *Middle*: responses to conditioning + test stimuli. *Bottom*: pseudorandom distribution of 9 ISIs from 1 to 7 ms, each delivered 10 times, with test-alone stimuli delivered as every fourth stimulus. **B.** T-SICI parallel recording after determination of RMT200. *Top*: test-alone stimuli track RMT200 (black) while 9 channels track conditioned thresholds, starting at 6% above RMT200. *Middle*: peak responses: *Bottom:* as in A. **C**. T-SICI parallel recording after determination of RMT200. The three plots are as in B, but only one conditioned threshold is tracked (red), while ISI is increased progressively from 1 to 7 ms, and test-alone stimuli are delivered as every third stimulus. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

threshold, for most recordings we initialised the stimulus at 110% of RMT200 (approximately the middle of the expected range of 1 ms threshold in patients and controls) and also tracked the 1 ms threshold twice, so that the ISI was only incremented to 1.5 ms after 4 valid threshold estimates were registered. We refer to this protocol as T-SICIs2. To check for a difference between the T-SICIs1 and T-SICIs2 protocols, in 25 subjects (mean age 41.5 years, SD 15.0 years) we made recordings with both protocols. Similarly, for most T-SICIp recordings we initialised the stimulus for all ISIs at 106% of RMT200, to reduce the ranges over which thresholds had to be tracked, and we refer to this protocol as T-SICIp2. To check for the impact of this initialisation on the threshold estimates, in 25 subjects (mean age 42.7 years, SD 14.5 years) we also initialised all stimuli to RMT200, as done in the earlier T-SICIp recordings [11], and we refer to this protocol as T-SICIp1.

'Forwards/Backwards' T-SICIs recordings. When it became clear that there were substantial differences between the serial and parallel T-SICI recordings, we performed additional experiments in which the direction of ISI change was reversed. In 25 subjects (mean age 37.1 years, SD 12.3 years), recordings were made in the same session in which ISI increased from 1 to 7 ms, and in which ISI was decreased from 7 to 1 ms. When the substantial difference between these 'forwards' and 'backwards' recordings became evident, in 20 of these subjects we also increased the tracking accuracy by only changing the ISI when 4 rather than 2 valid threshold estimates had been recorded (Qtrac command AN4 rather than AN2).

Analysis

A-SICI. Fig. 3 illustrates part of an analysis of an A-SICI recording. Fig. 3A shows just the peaks recorded for the test-alone channel (grey) and the channel recording peaks for an ISI of 3 ms (black). As is clear in Fig. 3B, the test-alone stimuli were delivered at regular intervals, while the 3 ms intervals were shuffled with the other 8 conditioning-test intervals (not shown). Fig. 3A also shows that the drop towards the end in the average response to the test-alone stimuli (which was originally set to 1 mV) roughly matches a drop in the conditioned responses. Because the responses tend to be normally distributed on a logarithmic scale, the geometric means are calculated, and in this case A-SICI at 3 ms is given by 64/537 or 11.9%. For the same reason, geometric means were used when averaging A-SICI across subjects.

For *T-SICIp* and *T-SICIs*, thresholds were estimated by log regression, as for RMTs (Fig. 1).

QtracP, part of the QTRACW software package, was used for the analysis, including statistical tests, and for generating the plots. When comparing thresholds of the 50 subjects, in nearly every case the distributions passed the Lilliefors test of normality, so that paired *t*-tests were used. For the subset of 25 subjects used in the 'Forwards/Backwards' studies, however, the 'Forwards' distributions failed the Lilliefors test, so that the thresholds were compared with the Wilcoxon signed ranks test.

Results

RMT200 and TS1mV

The RMT200 values averaged 55.2% MSO with a standard deviation of 8.5% MSO, while the TS1mV values averaged 65.6% MSO, with a standard deviation of 13.0% MSO. The RMT200 and TS1mV

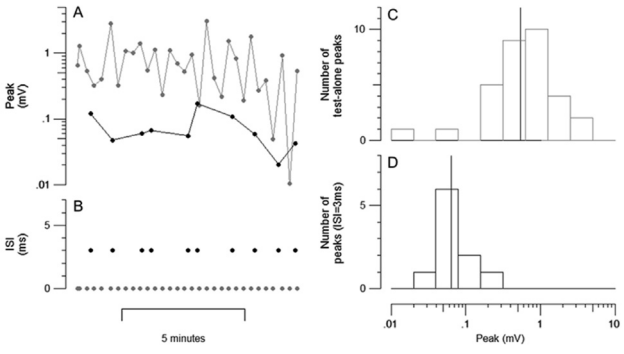

Fig. 3. Part of the analysis of an A-SICI recording, showing only 2 of the 10 channels recorded. The 30 test-alone responses are shown in grey, and the 10 conditioning + test responses with an ISI of 3 ms are shown in black. A. Peak amplitudes (shown on logarithmic scale). B. Inter-stimulus intervals. C. Histogram of test-alone peaks. D Histogram of peaks at ISI of 3 ms. Vertical lines in C and D show geometric means of peak amplitudes: 537 μV for test-alone, and 64 μV at ISI of 3 ms.

values were well correlated ($R = 0.907$), but neither were significantly correlated with age (R = 0.043 and 0.165 respectively).

Three SICI methods compared

As expected, the serial tracking protocol T-SICIs2 was in most cases faster than the other two methods, taking an average of 87.4 stimuli (range 59–140), against the constant 120 for T-SICIp2 and A-SICI. There was a strong linear correlation between the number of T-SICIs2 stimuli and the peak inhibition reached ($R = 0.708$) as more steps were required to track to the target.

The three types of SICI measurement are compared as functions of ISI in Fig. 4. There are significant differences between the serial and parallel T-SICI estimates, at all ISIs except 1 ms, with P values from paired t-tests as low as 1.6×10^{-7} at 1.5 ms (see Fig. 5).

Relationship between A-SICI and T-SICI

In Fig. 4 there appears to be a near mirror-image relationship between the plots for T-SICIp2 and A-SICI, and this relationship is explored further in Fig. 6A. This shows that A-SICI and T-SICIp are closely related by the straight line through the control condition, which has the equation T-SICIp $\approx$ 100–17.85 $\times$ Log_{10}(A-SICI/100). The coefficient of determination R^2, for the relationship between mean T-SICIp2 and geometric mean A-SICI = 0.968. On the other hand, in Fig. 6B, T-SICIs2 is not quite so well explained by A-SICI or by the straight line T-SICIs $\approx$ 100–29.58 $\times$ Log_{10}(A-SICI/100) ($R^2 = 0.870$).

Transformation of A-SICI to resemble T-SICIp (A–SICI–T)

Whereas the variability of T-SICIp2 and T-SICIs2 measures of inhibition can be compared directly, as in Fig. 5 above, the A-SICI amplitude measures of inhibition cannot be compared without transformation. For example, with T-SICIp and A-SICI there is a peak in inhibition at 2.5 ms. T-SICI thresholds at 2.5 ms are plotted in Fig. 7A, together with the corresponding A-SICI amplitudes,

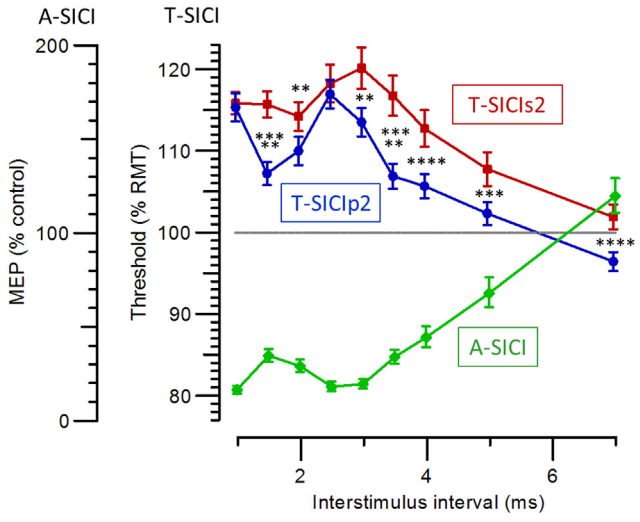

Fig. 4. Comparison between SICI recordings by the three methods made on the same 50 healthy control subjects. TSICIp2 (blue circles) and TSICIs2 (red squares) thresholds are means ± SE, expressed as % of RMT, while the ASICI MEP amplitudes (green diamonds) are geometric means × /÷ geometric SE, expressed as % of the unconditioned amplitude. The serial and parallel recorded TSICI thresholds differed significantly at several (** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$, ***** = $P < 0.00001$ by paired t-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

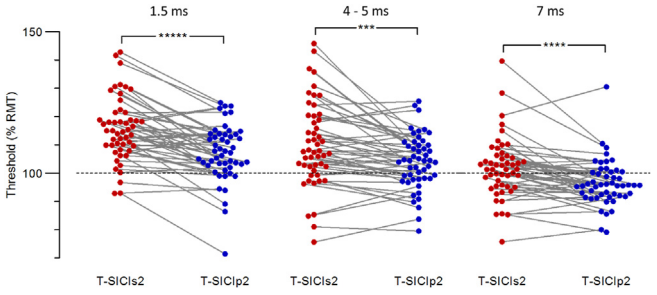

Fig. 5. T-SICIs and T-SICIp values for the 50 healthy control subjects compared at ISIs of 1.5, average of 4 and 5, and 7 ms. (*** = $P < 0.001$, **** = $P < 0.0001$, ***** = $P < 0.00001$).

transformed to equivalent thresholds A–SICI–T (according to the straight line in Fig. 6A) by the relationship:

$$\text{A–SICI–T} = 100 - 17.85 \times \text{Log10(A-SICI/100)}$$

The original A-SICI amplitudes are plotted for comparison in Fig. 7B, and show a pronounced 'floor' effect, since amplitudes cannot fall below zero.

Two things are notable about the transformed A-SICI data in Fig. 7A: first, the transformation has normalised the data (so that unlike the original A-SICI data it passes the Lilliefors test of normality), and secondly the variability of the data is noticeably less than for the T-SICIs2 and T-SICIp2 data.

Applying this transformation over all ISIs, we can compare not only the mean thresholds (Fig. 8A), but threshold variability (Fig. 8B) and the numerical values for the individual ISIs and some average ISIs are listed in Table 1. The last row indicates that the average SD of A–SICI–T estimates is less than half that for the T-SICIs2 values. From the means and standard deviations of threshold, we can estimate the probability of a healthy subject having no inhibition at any ISI (or averaged over several ISI) from the area under one tail of the normal curve, and these figures are also given in Table 1. For example, from the thresholds and SDs at an ISI of 2.5 ms, and the area under one tail of a normal distribution, the expected percentage of healthy subjects without any inhibition (i.e. conditioned threshold less than or equal to RMT) is 13.2% for T-SICIs2, 8.3% for T-SICIp2, and 4.2% for A–SICI–T. Table 1 also shows that the actual percentage of subjects without inhibition (12, 8 and 4 respectively) were close to these figures, only 2 subjects (4%) having A–SICI–T threshold $\leq$ RMT (or A-SICI amplitude $\geq$ 1 mV, since a 1 mV conditioned MEP translates into a conditioned threshold of RMT). This confirms that the threshold distributions were all close to normal. Table 1 shows that, just as threshold variability is lowest for A–SICI–T, so the probability of a healthy subject having no inhibition is appreciably lower for amplitude than for threshold measurements.

Age-dependence of the three SICI measures

Since a reduction in SICI in older adults has been reported previously [17,18], we have checked for a correlation between each A-SICI parameter and age (by Spearman rank correlation) and each T-SICIs2 and T-SICIp2 parameter and age (by Pearson product moment correlation) and the correlation coefficients and P values for these arising by chance are listed in Table 2. It can be seen that none of the T-SICIs2 or T-SICIp2 parameters reveal a dependence on age, but A–SICI–T declines significantly with age at ISIs from 1 to 2.5 ms, as do the averages. The last columns show that this is not because the transformation from A-SICI to A–SICI–T has somehow

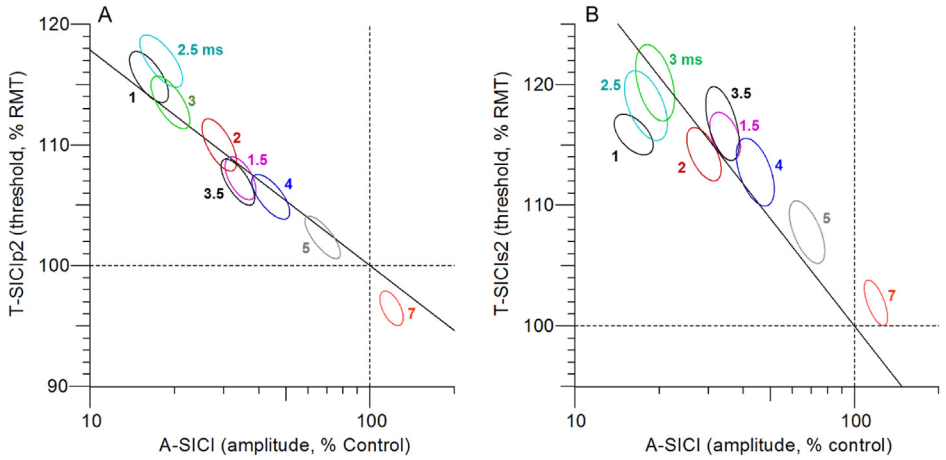

Fig. 6. A: Relationship between A-SICI amplitudes and T-SICIp threshold values for the 9 different ISIs recorded. For each ISI, the 50 data points are represented by an ellipse, one standard error from mean T-SICIp2 and geometric mean A-SICI. The straight line is the best fit through control condition and 450 data points. B: Relationship between A-SICI and T-SICIs2 values plotted similarly.

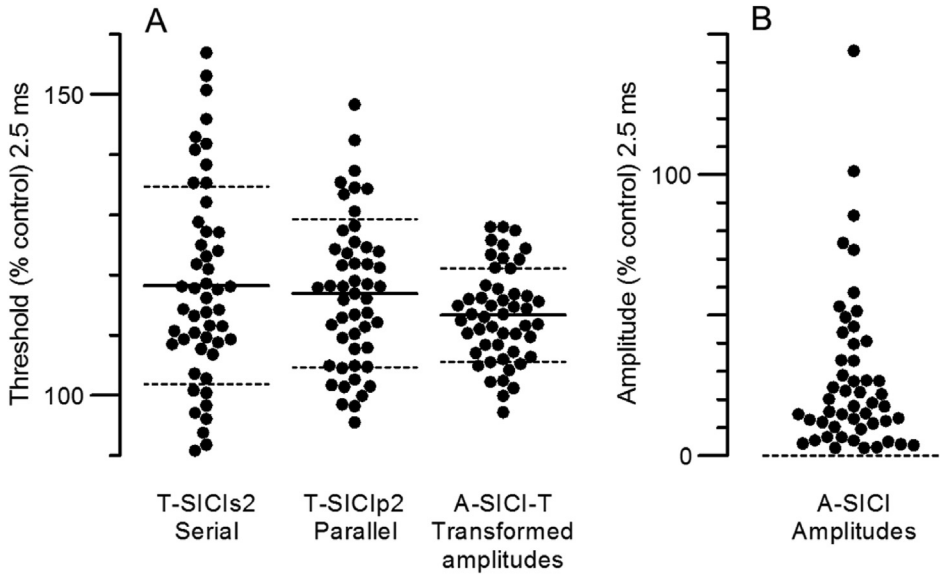

Fig. 7. A: T-SICIs2 serial, T-SICIp2 parallel and A–SICI–T data for peak inhibition at 2.5 ms, where A–SICI–T data is A-SICI amplitudes transformed to estimated thresholds. Horizontal lines and dashed lines are means and means±standard deviations. B. Original A-SICI amplitudes, showing pronounced 'floor effect'.

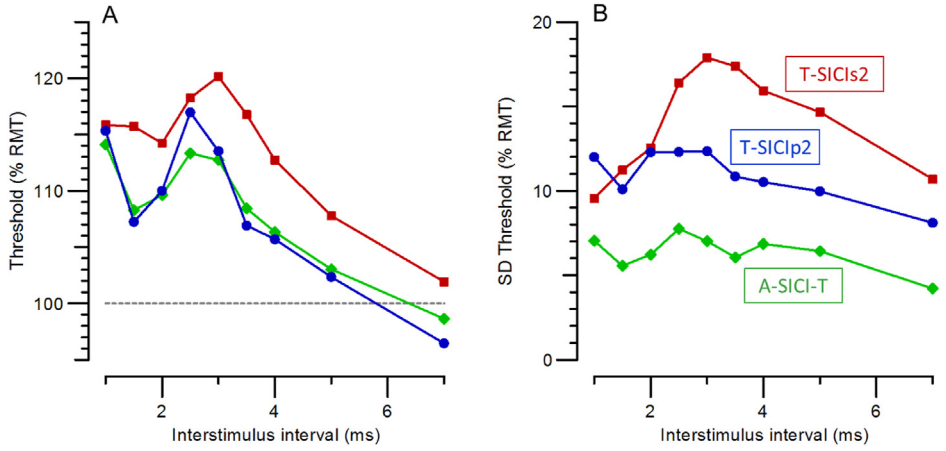

Fig. 8. Comparison between 3 SICI measurements averaged over 50 healthy subjects. A: Mean threshold (% RMT) as a function of ISI. B: Standard deviation of threshold estimates. Red squares: T-SICI serial, Blue circles: T-SICI parallel, Green diamonds: T-SICI estimated by transformation of A-SICI amplitudes. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Mean thresholds, threshold variation as standard deviations, and percentage of subjects without inhibition, as a function of interstimulus interval. In the last group, percentages are given both as estimated from the means and SDs, and (in parentheses and grey) the actual percentages of the 50 subjects observed. The asterisks indicate conditions for which the estimated probability of a subject having no inhibition (or the actual percentage of subjects without inhibition) is less than 0.05.

	Mean threshold (% RMT)			SD threshold (% RMT)			% of thresholds ≤ RMT Estimated (actual)		
ISI(ms)	T-SICIs2	T-SICIp2	A–SICI–T	T-SICIs2	T-SICIp2	A–SICI–T	T-SICIs2	T-SICIp2	A-SICI
1	115.9	115.3	114.1	9.5	12.0	7.0	4.7(4*)	10.1(10)	2.2*(2*)
1.5	115.7	107.2	108.3	11.2	10.1	5.6	8.0(6)	23.8(18)	6.9(4*)
2	114.2	110.0	109.6	12.5	12.3	6.2	12.8(12)	20.8(18)	6.1(2*)
2.5	118.3	117.0	113.3	16.4	12.3	7.7	13.2(12)	8.3(8)	4.2*(4*)
3	120.1	113.5	112.7	17.9	12.3	7.0	13.0(16)	13.7(14)	3.5*(4*)
3.5	116.8	106.9	108.4	17.4	10.8	6.1	16.7(12)	26.2(20)	8.2(6)
4	112.7	105.7	106.3	15.9	10.5	6.9	21.2(12)	29.4(32)	17.8(15)
5	107.8	102.3	103.0	14.7	10.0	6.4	30.1(26)	40.7(40)	31.9(26)
7	101.9	96.5	98.6	10.7	8.1	4.2	42.9(44)	66.9(72)	62.8(72)
1.0–7.0	113.7	108.3	108.3	12.7	8.8	5.0	13.9(10)	17.3(16)	4.9*(4*)
1.0–3.5	116.8	111.7	111.1	13.2	9.8	5.3	10.2(8)	11.6(10)	1.9*(2*)
2.5–3.0	119.2	115.2	113.0	17.0	11.3	7.0	12.9(14)	8.8(10)	3.1*(2*)
Mean of 9 SDs, 1–7 ms				14.0	10.9	6.3			

Table 2
Age-dependence of SICI parameters, indicated by correlation coefficients (Pearson product moment R for T-SICI parameters, and Spearman rank correlation Rho for A-SICI parameters) and probabilities of these being due to chance.

ISI(ms)	Coefficients of correlation between age and SICI parameters							
	T-SICIs2		T-SICIp2		A–SICI–T		A-SICI	
	R	*p*	R	*p*	R	*p*	Rho	*p*
1	−0.171	0.232	−0.253	0.073	−0.332	0.018*	0.312	0.026*
1.5	−0.193	0.175	−0.054	0.707	−0.304	0.030*	0.229	0.105
2	−0.250	0.077	−0.160	0.266	−0.412	0.0030**	0.416	0.0028**
2.5	−0.159	0.271	−0.188	0.187	−0.306	0.029*	0.242	0.087
3	−0.150	0.299	−0.088	0.551	−0.209	0.142	0.245	0.083
3.5	−0.063	0.699	0.016	0.877	−0.186	0.194	0.221	0.120
4	−0.037	0.786	0.003	0.932	−0.270	0.056	0.228	0.107
5	−0.140	0.334	0.045	0.752	−0.151	0.295	0.182	0.204
7	−0.052	0.719	0.155	0.281	−0.023	0.850	−0.026	0.835
1.0–7.0	−0.146	0.313	−0.089	0.545	−0.321	0.022*	0.284	0.043*
1.0–3.5	−0.167	0.244	−0.149	0.303	−0.361	0.0098**	0.418	0.0026**
2.0–3.0	−0.186	0.192	−0.163	0.256	−0.354	0.011*	0.365	0.0089**

altered the age-dependence, since the original A-SICI values exhibit similarly significant increases in amplitude with age by rank correlation.

To help understand why the A-SICI values should show an age-dependence while the T-SICI values do not, the data points for average SICIs from 1 to 3.5 ms are plotted against age on the same scales in Fig. 9. It is interesting that in each case regression of SICI on age shows an average decline in inhibition with age (T-SICIs2: 0.134%/year, T-SICIp2: 0.088%/year, A–SICI–T: 0.119%/year) but this decline is only significant for A–SICI–T because the inter-subject variability is much lower. There is also, however, a possibility that differences in MEP amplitude between the A-SICI and T-SICI recordings contributed to the difference in age-dependence [19].

Dependence of T-SICI measurements on initial settings

As explained in Methods, the T-SICIs measurements were initialised in two different ways: T-SICIs1 recordings followed the practice of Vucic and colleagues [9] in which tracking the threshold at 1 ms started at RMT, and the 1 ms threshold was determined by the first two valid threshold estimates, whereas in our preferred method T-SICIs2, tracking at 1 ms was started at 110% of RMT, and the 1 ms threshold only was determined by the first 4 valid threshold estimates. By testing 25 of the subjects with both protocols, the effects of these different initial settings are compared in Fig. 10A and B. It can be seen that not only is the threshold at an ISI of 1 ms much higher with T-SICIs2, but also that this difference is not eliminated until an ISI of 4 or 5 ms is reached. This shows clearly that the tracking protocol used, in which stimuli were changed by no more than 1% MSO at a time, is quite unable to follow the changes of threshold with ISI accurately.

In the case of the T-SICIp recordings, thresholds at all ISIs were initialised to the same value, which for T-SICIp1 recordings was RMT (as used by Samusyte and colleagues [11]), while in our preferred protocol T-SICIp2 the thresholds were initialised to 106% of RMT. With this protocol the initial setting was not so critical, and in 25 subjects there was no significant difference between T-SICIp1 and T-SICIp2 at any ISI (Fig. 10C). There was however a trend for T-SICIp2 thresholds to be higher than T-SICIp1 ones over the 1–3.5 ms range, which would have been significant if maintained over another 25 subjects.

Dependence of T-SICIs on direction of ISI change: 'forwards/backwards' recordings

It is clear from Fig. 10A that the T-SICIs estimates depend strongly on the previous threshold, so that it is not surprising to see in Fig. 11A and B that the T-SICIs estimates also depend very strongly on the direction of ISI change. These recordings from 25

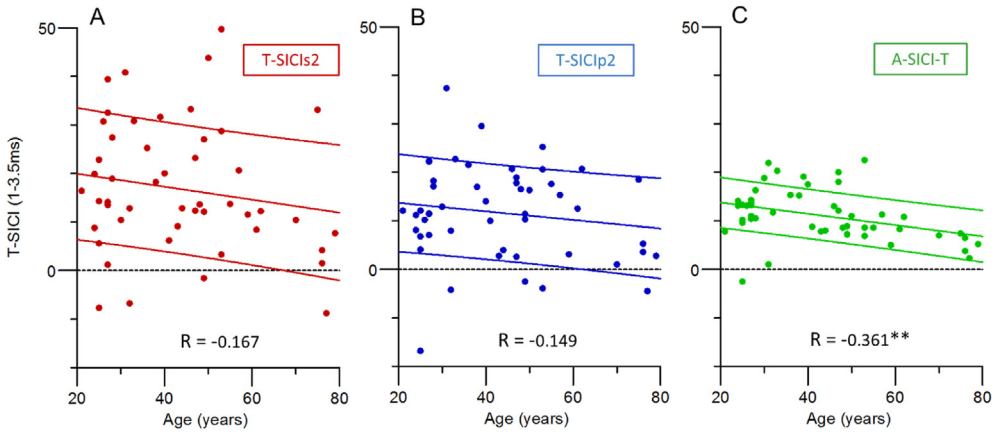

Fig. 9. Age-dependence of mean SICI from 1 to 3.5 ms derived from the 3 SICI methods. All show a trend for SICI reducing with increasing age, but this is only significant for the transformed A-SICI values. Lines are for linear regression of SICI on age, ± SD of threshold estimate. R = correlation coefficient. ** = $P < 0.01$.

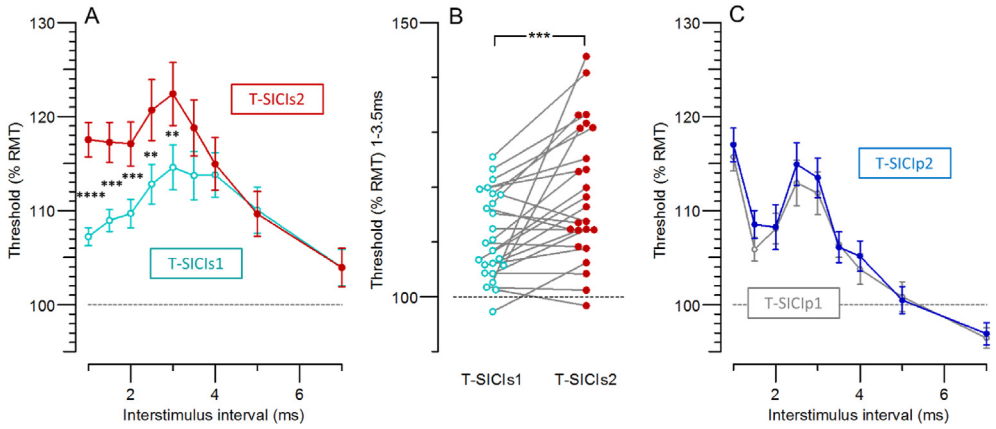

Fig. 10. Dependence of T-SICI on initial settings. A. Comparison between mean ± SE estimates of T-SICIs1 (Cyan open circles) and T-SICIs2 (Red filled circles) from 25 healthy controls. Significance of differences by paired *t*-test: ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. B. Mean TSICIs1 and T-SICIs2 values between 1 and 3.5 ms. C. Comparison between mean ± SE estimates of T-SICIp1 (Grey open circles) and T-SICIp2 (Blue filled circles) from 25 healthy controls. No differences in C were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

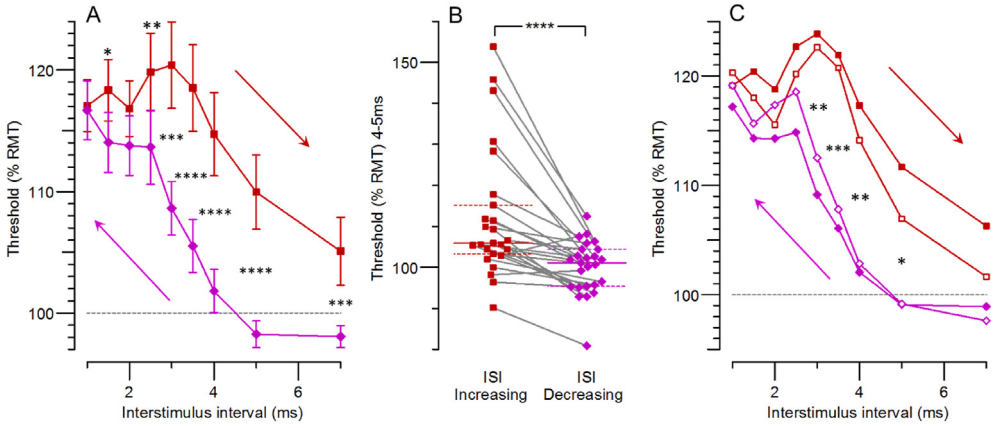

Fig. 11. Dependence of T-SICIs2 on direction of ISI change. A. Comparison between mean ± SE estimates of T-SICIs2 with ISI increasing from 1 to 7 ms (Red filled squares) and with ISI decreasing from 7 to 1 ms (Magenta filled diamonds) from 25 healthy controls. Significance of differences by paired *t*-test: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. If no * difference was not significant. B. T-SICIs2 values for the 25 subjects at 4–5 ms. C. Filled red squares and magenta diamonds: T-SICIs2 means as in A but from 20 healthy controls. Open red squares and magenta diamonds: same, but with points determined by 4 valid threshold estimates (AN4) rather than 2 (AN2). *P* values indicated as in A but for AN4 differences only. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

control subjects used the conventional serial tracking strategy, in which ISI was changed after 2 valid estimates were obtained (AN2).

Fig. 11C shows that improving the accuracy of the tracking, by increasing the number of valid threshold estimates to AN4 before changing the ISI, only slightly reduced the dependence of T-SICIs2 on the direction of ISI change. It is worth noting that whereas the 20 AN2 T-SICIs2 'forwards' recordings in Fig. 11C were quicker than the T-SICIp ones, requiring an average of 81.2 stimuli as against a constant 120 for T-SICIp, the AN4 T-SICIs2 'forwards' recordings were slower than the T-SICIp ones, taking an average of 142.9 stimuli and as many as 188. It is evident that the time advantage of T-SICIs over T-SICIp would be entirely lost if attempts were made to eliminate the forwards/backwards difference by further increasing the number of valid threshold estimates to improve the tracking accuracy.

Discussion

The most striking finding of this study is that the frequently used method of assessing SICI, by serial threshold-tracking as a function of increasing ISI, i.e. T-SICIs, has severe limitations, at least when applied with a figure of eight coil to activation of FDI. This is clearly shown both in Fig. 10A, which shows its extreme sensitivity to how the tracking is initialised, and by Fig. 11A, which shows a very different relationship between SICI and ISI when threshold was tracked with increasing ISI values from that when ISI values were decreased. Even increasing the accuracy of this serial tracking method by doubling the number of valid threshold estimates required for changing the ISI (Fig. 11C), did not eliminate this major discrepancy. We attribute the difference between T-SICIs1 and T-SICIs2 in Fig. 10A, and the dependence on direction of ISI change in Fig. 11, to the fact that when thresholds are very variable, a simple serial tracking technique tends to underestimate the difference between successive threshold estimates. 'Valid threshold estimates' can be scored well before the stimulus has reached the true mean threshold level. It is clear that the parallel tracking strategy, in which threshold was estimated independently at each ISI, produced a more credible relationship between SICI and ISI, which was less dependent on how the tracking was initialised (Fig. 10C), and also a relationship that was more closely related to the conventional method of assessing SICI by amplitudes measurements (Fig. 6A).

We note that a conspicuous weakness of the T-SICIs1 method is that it seriously underestimates inhibition at 1 ms. In agreement with previous work [8], our A-SICI and T-SICIp methods show that SICI has two phases, peaking at 1 and 2–3 ms (Fig. 4). These are thought to reflect different underlying physiology, specifically extrasynaptic and synaptic GABA-A activity respectively [20,21], and may therefore be differentially affected in diseases and modulated by drugs. Only the A-SICI and T-SICIp methods can be relied upon to adequately reveal such differences.

Relationship between A-SICI and T-SICI

The relationship shown in Fig. 6A between SICI assessed by threshold-tracking (T-SICI) and that assessed by conventional amplitude measurements (A-SICI), was closer than might have been expected, with almost 97% of the variance in mean T-SICIp values accounted for by the logarithmic relationship with the A-SICI geometric means. We were comparing independent SICI methods, so that no attempt was made to match the MEP amplitudes, as was done in a previous A-SICI/T-SICI comparison [14]: T-SICI used a target response of 0.2 mV, while A-SICI started with a target response of 1 mV. The present finding of a good correlation between A-SICI and T-SICIp across a range of ISIs extends a previous finding that a correlation is maintained across conditions with both comparable and non-comparable test stimulus intensities [11].

Threshold-tracking versus conventional SICI

It has been suggested that threshold-tracking was introduced into paired-pulse TMS testing to help overcome the variability in MEP amplitude with consecutive stimuli [22]. This was not, however, one of the rationales for the original use of threshold-tracking [7,8]. The first reason was that threshold-tracking enabled inhibition and facilitation to be measured over a wide range, avoiding the 'floor effect' that renders conventional measurements insensitive when inhibition approaches 100%.(e.g. Fig. 7B). The second reason was that by keeping the output of the nervous system constant it was proposed that threshold-tracking helps limit the contribution of spinal and peripheral elements to the measurements, and ensures that any abnormalities in inhibition and facilitation reflect intracortical changes. In this study we have found that in healthy subjects the A-SICI 'floor effect' can be eliminated by taking the geometric mean of MEP amplitudes, and that a log transformation effectively normalizes the amplitude distributions (e.g. Fig. 7A). The argument that threshold-tracking should help limit any abnormalities detected to intracortical ones remains valid, although there is currently no direct evidence to prove this point.

As to whether threshold-tracking helps overcome the limitation of MEP variability, so far as we are aware this question has not previously been addressed. While it is clear that variability between consecutive stimuli while threshold tracking is much less than MEP variability to a constant stimulus (especially when tracked thresholds are only allowed to change by 1% MSO between stimuli), this has little bearing on the variability of estimates of inhibition by the two methods. In this study we have attempted to provide a fair comparison between the estimates of inhibition by the 3 methods by log-transforming the A-SICI amplitudes and scaling to match the T-SICI values, as in Fig. 7A. We found that while the A–SICI–T transformed threshold estimates were similar to the T-SICIp ones (Fig. 8A), their variability between subjects was consistently lower than the T-SICI ones (Fig. 8B). As far as SICI variability between subjects is concerned, therefore, our results do not support the idea that threshold tracking helps overcome the limitation of MEP variability.

Variability between subjects is not, however, the only important source of variability for a clinical test, and a comparison with the only previous head-to-head comparison of A-SICI and T-SICI [11] is instructive. That study measured SICI at the single ISI of 2.5 ms, but with conditioning stimuli from 50 to 80% of RMT, rather than the single level of 70% in this study. It also found a close relationship between A-SICI and T-SICIp, indicating that both techniques reflect similar inhibitory mechanisms, but by making multiple tests on the same subjects, it focused on intra-subject reproducibility, rather than inter-subject variability, and revealed some advantages of threshold measurements. As a measure of relative reliability, or reproducibility, they used intraclass correlation coefficients, which assess the degree to which subjects maintain their position within a group over repeated measurements. With this measure they found that A-SICI had poor intra- and poor-to-adequate inter-day reproducibility, whereas T-SICI showed adequate-to-excellent intra- and inter-day reproducibility, implying that T-SICI may have better discriminative power within a group [11]. Another benefit of T-SICI over A-SICI according to that study was the inference that threshold-tracking may be able to shorten acquisition time and reduce sample sizes for interventional studies. Uncertainty remains, however, whether the findings in healthy young individuals would apply to patient populations.

The variabilities of both A-SICI and T-SICIp estimates depend of course on the numbers of stimuli used. In this study we used 10 stimuli per ISI for both methods, so that recordings at 9 ISIs with the BiStim could be completed within 10 min. Further studies will be required to determine optimal numbers of ISIs and stimuli per ISI, which may depend on the purpose for which the test is being used and the equipment available.

Detection of abnormal lack of SICI

An important aim of this study was to compare the abilities of A-SICI and T-SICI to detect an abnormal lack of inhibition, which has been reported to be useful in early diagnosis of ALS [6]. Since this study has been restricted to healthy control subjects, it cannot directly answer any question about diagnostic usefulness, but it can compare methods for their ability to state how abnormal a recording is in which there is no SICI. Surprisingly, perhaps, only A-SICI measurements were predicted from threshold variabilities to register an absence of inhibition as abnormal, in that it would occur in less than 5% of healthy subjects (Table 1). From these considerations, it seems unlikely that either threshold-tracking protocol can provide more diagnostic information regarding SICI than A-SICI, but it will take a similar head-to-head comparison between the methods in patients to resolve this. A further complicating factor which will need investigation is coil orientation. In common with the majority of TMS studies, our SICI measurements were obtained exclusively using the posterior-anterior current direction, whereas it has been reported that the anterior-posterior direction may result in more robust inhibition [23,24].

Why is serial T-SICI so different from parallel T-SICI?

We have seen in Figs. 10 and 11 that T-SICIs is very strongly dependent on the initial setting of the stimulus, and on the direction of change of ISI, even when the condition for advancing is increased from 2 acceptable threshold estimates to 4 (where a threshold estimate is considered acceptable if the response is within 20% of the target, or successive responses bracket the target). The main difference from the parallel tracking strategy is that in T-SICIs the stimulus is only changed by 1% MSO at a time, whereas in T-SICIp, the stimulus was changed by varying amounts, proportional to the error, starting with a maximum of 6% MSO and reducing to a maximum of 2% MSO. This strategy always resulted in some overshooting of the target, whereas in T-SICIs there was clearly an excessive lag before the stimulus reached the true mean threshold. This was presumably because the thresholds fluctuated so widely that when there was an appreciable difference between one mean threshold and the next, the condition of 2 'valid threshold estimates' could be met well before the stimulus had actually reached the true mean threshold. We therefore cannot recommend the serial threshold-tracking strategy used in T-SICIs.

In conclusion, we have found that conventional amplitude measurements of SICI as a function of ISI and threshold-tracking measurements give almost interconvertible results when ISIs are pseudorandomised. Significantly different results were obtained with the serial threshold-tracking protocol, which were strongly dependent on initial stimulus settings and on whether ISIs were increased progressively from 1 to 7 ms or decreased from 7 to 1 ms. Threshold-tracking has been reported to have the advantage of generating more reproducible SICI results more quickly, but our findings have directly determined in a head-to-head comparison that A-SICI measurements are less variable between healthy subjects and may therefore be more sensitive at detecting a pathological absence of SICI.

CRediT authorship contribution statement

Hatice Tankisi: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing, Writing, Supervision, Project administration, Funding acquisition. **Bülent Cengiz:** Investigation, Writing - review & editing, Writing – Review &Editing. **James Howells:** Software, Writing - review & editing, Writing – Review &Editing. **Gintaute Samusyte:** Conceptualization, Conceptualization, Software, Writing - review & editing. **Martin Koltzenburg:** Conceptualization, Methodology, Software, Writing - review & editing, Writing – Review &Editing. **Hugh Bostock:** Conceptualization, Methodology, Software, Formal analysis, Writing - original draft, Writing - review & editing, Writing, Visualization.

Declaration of competing interest

Hugh Bostock receives from UCL a share of the royalties for sales of his Qtrac software used in this study. Other authors have no conflicts of interest to declare.

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