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Age-related changes in late synaptic inputs to corticospinal neurons and their functional significance: A paired-pulse TMS study

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ABSTRACT

Background: Recent work suggests that the function of intracortical interneurons activated by transcranial magnetic stimulation (TMS) is modified in older adults, with the circuits generating short-interval intracortical facilitation (SICF) at longer intervals appearing to be particularly affected.
Objective: To use SICF to quantify age-related changes in the excitability and recruitment of late synaptic inputs to corticospinal neurons, and investigate if changes within these circuits contribute to altered motor performance in older adults.
Methods: SICF was recorded with 3 different conditioning intensities in 23 young (23.0 ± 4.2 years) and 21 older (67.1 ± 1.1 years) adults. These measures were performed with conventional (posterior-anterior, PA) and reverse (anterior-posterior, AP) current directions using interstimulus intervals targeting late synaptic inputs to corticospinal neurons (3.5–5.3 ms).
Results: Peak SICF recorded with a PA current ($SICF_{PA}$) was reduced in older adults ($P < 0.0001$), and occurred at a longer latency ($P < 0.05$). Furthermore, there was reduced recruitment of $SICF_{PA}$ in older adults ($P < 0.0001$), but this did not interact with the age-related shift in $SICF_{PA}$ ($P = 0.2$). In addition, reduced performance on the Purdue pegboard was predicted by increased $SICF_{PA}$ ($P < 0.04$) occurring at longer latencies ($P < 0.04$) in old but not young adults. For SICF recorded with an AP current ($SICF_{AP}$), facilitation was again reduced at longer latencies in older adults ($P < 0.0001$), but recruitment was not different between groups ($P = 0.7$) and was unrelated to motor function.
Conclusion: These results suggest that there are age-related changes in late synaptic inputs to corticospinal neurons and that these changes influence fine motor performance.

Introduction

While individuals experience ageing uniquely, there are also some undeniable commonalities across the population. Arguably one of the best examples of these is the decline in motor function that is almost universally apparent amongst older individuals. While the factors contributing to this decline are numerous, studies using non-invasive brain stimulation (NIBS) techniques such as transcranial magnetic stimulation (TMS) have suggested that changes within the brain may be important. For example, the neuroplastic modulation of synaptic communication, a critical process for motor learning and memory [1], is reduced in older adults [2–6]. Furthermore, both the activity [7–9] and task-dependent modulation [10,11] of inhibitory circuits involving gamma-aminobutyric acid (GABA) are modified in the elderly. Despite this, our current understanding of how the ageing process modifies function within M1, and how neurophysiological alterations within M1 contribute to the functional deficiencies of ageing, remains limited.

When TMS is applied to M1, it results in a complex wave of activity within corticospinal neurons (CSNs) that is referred to as the descending volley. The earliest component of the descending volley is thought to derive from direct activation of corticospinal axons close to the soma, whereas subsequent waves arise due to indirect, *trans*-synaptic input on to corticospinal neurons from

Abbreviations: TMS, transcranial magnetic stimulation; SICF, short-interval intracortical facilitation; PA, posterior-anterior current direction; AP, anterior-posterior current direction; NIBS, non-invasive brain stimulation; I-wave, indirect wave; GABA, gamma-aminobutyric acid; M1, primary motor cortex; CSN, corticospinal neuron; MEP, motor evoked potential; EMG, electromyography; FDI, first dorsal interosseous; RMT, resting motor threshold; ISI, interstimulus interval; EPSP, excitatory post-synaptic potential.

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interneuronal networks (for review, see [12–14]). These indirect (I) waves are numbered according to their order of appearance (I1, I2, I3) and are generally referred to as either early (I1) or late (I2, I3) synaptic inputs on to CSNs, based on different mechanisms of action and functional relevance. In particular, the activity of the interneuronal networks generating the late synaptic inputs to CSNs has been shown to be important for plasticity induction and motor function. For example, several NIBS interventions that induce neuroplasticity within M1 specifically modify activity within the late I-wave inputs (for review, see [15]). Furthermore, late I-wave recruitment following TMS application predicts neuroplasticity induction within M1 [16,17] in addition to the acquisition of new motor skills [18,19]. Consequently, the late I-wave inputs to CSNs are likely to represent an important component of motor function.

While activation of I-wave inputs can be recorded invasively within CSNs using electrodes within the cervical epidural space [13,15,20], this approach is not practical for the majority of studies. However, the paired-pulse TMS measure of short-interval intracortical facilitation (SICF) represents a convenient approach for indirect assessment of the I-wave circuitry. SICF involves application of a suprathreshold test stimulus followed at short interstimulus intervals (ISIs) by a perithreshold conditioning stimulus, with facilitation of the associated motor evoked potential (MEP) apparent at regular intervals approximating the I-wave latency of 1.3–1.5 ms [21,22]. This facilitation is thought to arise due to the temporal summation of the I-waves produced within CSNs by conditioning and test stimuli [23]. It is therefore possible to use this measure to make inferences about the physiological and functional relevance of the I-wave generating circuitry. In particular, SICF peaks associated with the late I-waves (i.e., facilitation produced with an ISI of ~4.5 ms) are considered to be important for motor control as they predict reaction times during brief contractions [24] in addition to the recruitment of spinal motor neurons by the descending volley [25]. Importantly, the late SICF peaks are specifically modified by age [26] and some of these peaks can predict scores in tests of manual dexterity in the elderly [27], suggesting that age-related changes in late I-wave characteristics may contribute to altered motor function in older adults.

Although age-related changes in the late SICF peaks have been previously reported, the neurophysiological and functional effects of ageing on late I-wave circuitry remains poorly understood. In particular, measures of SICF in young participants are sensitive to variations in the intensity of the conditioning stimulus [22,23,28,29], in addition to the direction of cortical current induced by stimulation [30], but it is currently unclear how these factors influence SICF in older adults. The aim of the current study was therefore to use SICF as an indirect assessment of the excitability and recruitment characteristics of late I-wave inputs on to CSNs, and to investigate their relationship with motor performance, in young and older adults. Late I-waves were targeted by utilising measures of SICF with long ISIs (3.7–5.3 ms), as these are known to be particularly sensitive to the effects of ageing [26] and have been used to manipulate M1 plasticity [31]. Furthermore, SICF was assessed using the conventional posterior-to-anterior (PA) current, in addition to a reversed anterior-to-posterior (AP) current that is thought to activate unique intracortical circuitry associated with different I-waves (for review, see [23–25]). Recruitment characteristics were examined using 3 different conditioning intensities, which are known to influence SICF amplitude in young subjects [21]. Based on previous findings [26], we expected that older adults would show reduced facilitation at longer ISIs, and that this would predict poorer motor performance in older individuals.

Methods

Twenty three young (23.0 ± 4.2 years, 13 females) and 21 older (67.1 ± 1.1 years, 11 females) adults were recruited from the university and wider community to participate in the current study. Exclusion criteria included a history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). A number of older adults reported chronic medical conditions common to the elderly, including osteoporosis (2), diabetes (2), high blood pressure (5), reflux (2) and high cholesterol (1). Furthermore, the older group reported a relatively high level of habitual physical activity (average IPAQ score of ~2200 MET minutes per week). All subjects reported being right handed. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki. Each subject provided written, informed consent prior to participation.

Experimental arrangement

The protocol included 2 experimental sessions that were held on separate days. For the duration of each session, participants were seated in a comfortable chair with their right arm and hand supported. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the right hand using two Ag-AgCl electrodes in a belly-tendon montage. A strap around the wrist grounded the electrodes. EMG signals were amplified (300 ×) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK), and digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design). Signal noise within the 50 Hz frequency band (associated with mains power) was also removed using a Humbug mains noise eliminator (Quest Scientific, North Vancouver, Canada). To facilitate muscle relaxation when required, real-time EMG signals were displayed under high gain (50 μV/division) on an oscilloscope placed in front of the subject.

Experimental procedures

Transcranial Magnetic Stimulation (TMS). TMS was applied to the hand area of the left M1 using a figure-of-eight branding iron coil with two monophasic Magstim 200^2 magnetic stimulators connected via a Bistim unit (Magstim, Dyfed, UK). During the first session, all stimulation was applied using a posterior-to-anterior (PA) cortical current flow, generated by directing the coil current backwards and laterally (approximately 45° to the sagittal plane). In contrast, all stimulation during the second session was applied using an anterior-to-posterior (AP) cortical current flow that was generated by rotating the coil 180° relative to the PA orientation. For both sessions, the coil was positioned tangentially to the scalp over the location producing an optimum response in the resting FDI muscle of the right hand. To maximise accuracy of coil relocalisation, a marker was used to make two reference marks on the scalp; the first traced the upper intersection of the coil windings, whereas the second traced the inner circumference of the coil wing closest to the experimenter (along the segment closest to the scalp). The position of the coil was then maintained relative to these marks throughout the duration of the experiment. Resting motor threshold (RMT), defined as the minimum stimulus intensity producing an MEP response ≥ 50 μV in at least 5 out of 10 trials during complete relaxation of the right FDI, was then assessed.

Short-interval intracortical facilitation (SICF). SICF was investigated in the resting FDI muscle using an established paired-pulse TMS protocol, which produces peaks in the surface EMG recording that are compatible with I-wave latencies recorded from

the epidural space [22]. To investigate the recruitment of SICF, the current study applied 3 conditioning stimulus intensities of 90, 100 & 110% RMT. The intensity of the test stimulus was adjusted to produce a peak-to-peak MEP amplitude of approximately 1 mV (MEP_{1mV}), when averaged over 20 trials. To focus on the late SICF peak, the test stimulus preceded the conditioning stimulus at ISIs ranging from 3.7 to 5.3 ms, in 0.2 ms steps (totalling 9 intervals). Within each session, 12 trials were recorded for each condition, resulting in a total of 336 stimuli. In order to maintain subject attention during SICF assessment, this protocol was applied as 6 blocks of 56 stimuli, with 2 repeats of each condition applied in a pseudorandomised order within each block. Within the first session, SICF was recorded using a PA current direction ($SICF_{PA}$), whereas SICF measures in the second session were recorded using an AP current direction ($SICF_{AP}$).

Purdue Pegboard. In order to assess motor function, participants completed each subtest of the Purdue pegboard [32]. These involve either placing small pegs in corresponding holes (peg test) or assembling a series of items (peg, washer & collar) in a specific order (construction test). The peg test is repeated separately with the left and right hand, and then again using both hands simultaneously. The construction test requires alternating use of both hands. Participants were given 30 s to complete each peg test and 60 s to complete the construction test. Performance was scored based on the number of pegs successfully placed, or number of items constructed.

Data analysis

Analysis of EMG data was completed manually via visual inspection of offline recordings. For all recordings, traces showing muscle activity >20 μV peak-to-peak amplitude in the 100 ms prior to TMS application were excluded from the analysis. MEP amplitudes were measured peak-to-peak and expressed in mV. For SICF measures within each subject, individual conditioned MEP trials within each ISI were expressed as a percentage of the mean test alone MEP amplitude.

Statistical analysis

Unpaired student *t*-tests were used to compare age, RMT and score on each subtest of the pegboard between groups (GRP effect: young, older). Linear mixed model analysis was used to compare the amplitude of the test alone MEP between groups, whereas linear mixed model analysis with repeated measures was used to investigate the fixed effects of ISI (ISI effect: 3.7–5.3 ms), conditioning intensity (CI effect: 90, 100 & 110% RMT) and group on measures of SICF. Measures recorded with different coil orientations were analysed using separate models. For all models, subject was included as a random effect, and significant main effects and interactions were further investigated with Bonferroni corrected custom contrasts. Within each group, linear regression analysis was used to explore relationships between pegboard performance and SICF data (peak SICF, ISI of peak SICF). Unless otherwise stated, data are presented as mean ± SEM and significance was set at $P < 0.05$.

Results

While all participants completed the PA stimulation session in full, it was not possible to generate sufficiently sized responses to AP stimulation in 7 individuals (4 young, 3 older). Consequently, results for the AP condition include 19 young (23.0 ± 4.0 years, 10 females) and 18 older (67.4 ± 5.3 years, 9 females) adults. For both TMS current directions, no differences were found between age groups for RMT, the stimulus intensity required to produce MEP_{1mV} or the amplitude of MEP_{1mv} (Table 1). In contrast, young participants performed significantly better than the older group on all pegboard subtests (Table 2).

Table 1
Baseline parameters.

	Young	Older
RMT (%MSO)		
PA	48.0 ± 1.4	50.2 ± 1.9
AP	60.6 ± 2.4	63.3 ± 1.8
MEP_{1mV} – intensity (%MSO)		
PA	60.2 ± 2.4	64.1 ± 2.9
AP	74.2 ± 2.9	81.2 ± 2.7
MEP_{1mV} – amplitude (mV)		
PA	1.1 ± 0.07	1.0 ± 0.06
AP	1.0 ± 0.07	0.9 ± 0.06

Fig. 1 shows data from representative participants, matched for MEP_{1mV} amplitude, following application of $SICF_{PA}$ (Fig. 1A) and $SICF_{AP}$ (Fig. 1B) using ISIs of 4.1, 4.5 and 5.1 ms and a conditioning intensity of 110% RMT. Following $SICF_{PA}$, facilitation in the young participant was maximal at 4.5 ms. In contrast, the older participant showed maximal facilitation that was reduced in magnitude and temporally delayed to the 5.1 ms ISI. Following $SICF_{AP}$, maximal facilitation occurred at 4.1 ms in the young and older participant.

Fig. 2 shows group data comparing effects of age, ISI and conditioning intensity on $SICF_{PA}$ in young and older adults. For $SICF_{PA}$, the magnitude of facilitation was reduced in older adults (GRP effect, $P < 0.0001$; Fig. 2A), varied between ISIs (ISI effect, $P < 0.0001$) and increased with increasing conditioning intensity (CI effect, $P < 0.0001$). Furthermore, there was a significant interaction between group and conditioning intensity (GRP × CI interaction, $P < 0.0001$; Fig. 2C), with post hoc comparisons showing that facilitation with a 90% RMT conditioning intensity was not different between groups ($P = 0.08$), whereas facilitation was reduced in older adults at 100% RMT ($P < 0.0001$) and 110% RMT ($P < 0.0001$) conditioning intensities. For both groups, $SICF_{PA}$ with a 100% RMT conditioning intensity was greater than with a 90% RMT conditioning intensity (young, $P < 0.0001$; older, $P = 0.04$), whereas $SICF_{PA}$ with a 110% RMT conditioning intensity was greater than with both 90% RMT (young, $P < 0.0001$; older, $P < 0.0001$) and 100% RMT (young, $P < 0.0001$; older, $P = 0.0007$) conditioning intensities. The interaction between age group and ISI was also significant (GRP × ISI interaction, $P < 0.0001$; Fig. 2E), with post hoc comparisons between groups showing that $SICF_{PA}$ was reduced in older adults for ISIs of 3.7–4.9 ms (*P*-values ranging from 0.007 to < 0.0001). For young participants, $SICF_{PA}$ increased from 3.7 to 4.1 ms (all *P*-values < 0.009), was not different from 4.1 to 4.9 ms (all *P*-values > 0.05) and decreased from peak facilitation after 5.1 ms (all *P*-values < 0.01). For older participants, facilitation increased from 3.7 to 4.3 ms ($P = 0.005$) and from 4.3 to 5.3 ms ($P = 0.005$). A significant interaction between conditioning intensity and ISI was also found (CI × ISI interaction, $P = 0.02$), with post hoc comparisons showing that increasing conditioning intensities were associated with greater facilitation at a broader range of ISIs. The three-way interaction between conditioning

Table 2
Purdue pegboard performance.

	Young	Older
Left hand	13.3 ± 0.3	11.6 ± 0.4*
Right hand	15.9 ± 0.4	13.0 ± 0.4*
Both hands	12.0 ± 0.7	10.1 ± 0.4*
Construction	34.8 ± 1.4	27.1 ± 1.1*

*$P < 0.05$ compared to young participants.

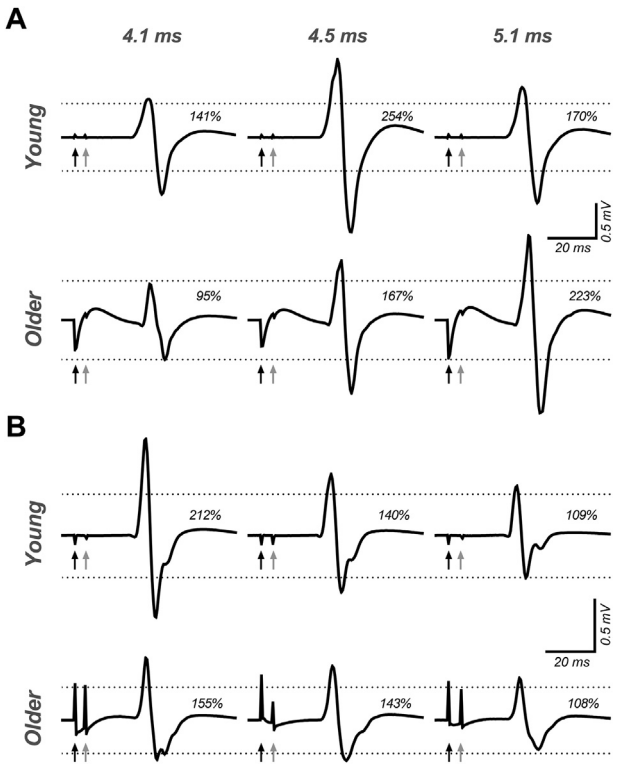

Fig. 1. Measures of SICF in representative young and older adults. Traces show data from individual participants following $SICF_{PA}$ ***(A)*** and $SICF_{AP}$ ***(B)*** using ISIs of 4.1 *(left column)*, 4.5 *(middle column)* and 5.1 *(right column)* ms. All traces were recorded using a 110% RMT conditioning intensity. Black arrows show timing of the test stimulus, grey arrows show timing of the conditioning stimulus. Italicised inset numbers show the magnitude of SICF, relative to the test alone MEP (shown by the dashed horizontal line).

intensity, ISI and group was not significant (CI x ISI × GRP interaction, $P = 0.2$).

For $SICF_{AP}$, facilitation was not different between groups (GRP effect, $P = 0.3$; Fig. 2B) and there was no interaction between conditioning intensity and group (CI × GRP interaction, $P = 0.7$; Fig. 2D), conditioning intensity and ISI (CI × ISI interaction, $P = 0.1$) or conditioning intensity, ISI and group (CI x ISI × GRP interaction, $P = 0.1$). In contrast, $SICF_{AP}$ varied between ISIs (ISI effect, $P < 0.0001$) and increased in response to increasing conditioning intensity (CI effect, $P < 0.0001$). Furthermore, there was a significant interaction between group and ISI (GRP × ISI interaction, $P < 0.0001$; Fig. 2F), with post hoc comparisons between groups showing that $SICF_{AP}$ was reduced in older adults for ISIs of 3.9–4.3 ms (*P*-values ranging from 0.01 to < 0.0001), but increased at 4.7 ($P = 0.04$), 4.9 ($P = 0.04$) and 5.3 ms ($P = 0.04$). For young participants, $SICF_{AP}$ increased from 3.7 to 4.1 ms (all *P*-values < 0.02), decreased from 4.1 to 4.9 ms ($P < 0.0001$) and was not different between subsequent ISIs ($P > 0.9$). For older participants, $SICF_{AP}$ at 4.3 ms was greater than at 5.3 ms ($P = 0.04$), but no other significant differences were found.

Results of linear regression analysis demonstrated that older participants with greater levels of $SICF_{PA}$ at 90% RMT placed fewer pegs with both left ($P = 0.002$, $r^2 = 0.4$) and right ($P = 0.009$, $r^2 = 0.3$) hands (Fig. 3A and B). A similar negative association was observed for $SICF_{PA}$ at 110% RMT and number of pegs placed for the left hand in older adults ($r^2 = 0.2$, $P = 0.04$; data not shown). Furthermore, older participants that demonstrated peak $SICF_{PA}$ (90% RMT) at longer latencies (i.e., had a later ISI of peak SICF) placed fewer pegs with both left ($P = 0.02$, $r^2 = 0.25$) and right ($P = 0.04$, $r^2 = 0.2$) hands (Fig. 3C and D). In contrast, no significant relationships were found for $SICF_{AP}$ in older adults, or for either coil orientation for young adults.

Discussion

The current study investigated the neurophysiological and functional effects of age on the excitability of late synaptic inputs on to CSNs. This was achieved by comparing measures of SICF, recorded with increasing conditioning stimulus intensities using ISIs associated with the late MEP peaks, between young and older adults, and correlating the observed facilitation with manual performance of a pegboard task. Our results show that the gain of PA-sensitive facilitation is reduced in older adults. In contrast, although older adults showed reduced AP-sensitive facilitation at longer latencies, interneuronal recruitment within these circuits was maintained. Finally, pegboard performance was reduced in older individuals, and this was predicted by the magnitude and timing of facilitation produced by PA-sensitive circuits.

Effects of age and coil orientation on late I-wave circuits

Application of TMS to M1 results in the generation of a multiphasic descending volley within CSNs that usually includes early and late I wave components, which result from indirect activation of M1 interneuronal networks. Although the specific mechanisms by which the descending volley is generated remain unclear, growing evidence suggests that the early and late I waves result from activity in independent motor networks that have unique synaptic input pathways to corticospinal neurons (for review, see [33]) and that these have particular importance for certain types of plasticity induction [16,17] and motor behaviour [18]. In the current study we have used SICF as an indirect marker of I-wave activity, as the facilitatory effects of this paradigm are generally assumed to reflect activity within cortical circuits responsible for I-wave generation. This is based on several lines of evidence, including the close temporal relationship between SICF peaks and I-wave periodicity, the observation that SICF application produces a descending volley within epidural recordings that is cumulatively larger than would be expected from addition of the response to single stimuli [34], and pairs of transcranial electrical stimuli that do not produce intracortical activation fail to produce SICF [21]. Based on these lines of evidence, MEP facilitation following SICF has been suggested to derive from temporal summation (within CSNs) of the post-synaptic potentials produced by repeated activation of the I-wave generating circuitry within M1 [23]. However, recent work has reported that SICF peaks are modified in spinal cord injury patients [24], suggesting that the observed facilitation may also have a spinal component.

Within the current study, the applied ISIs were chosen to characterise the peak of MEP facilitation associated with late synaptic inputs on to CSNs, which have been implicated in plasticity induction [16,17] and skill acquisition [18]. In young participants, MEP facilitation following $SICF_{PA}$ (collapsed over conditioning intensity) was maximal at 4.5 ms, which is consistent with the expected values [22,35]. In contrast, maximum facilitation in older participants was observed with an ISI of 5.3 ms, suggesting that the timing of facilitatory interactions involving the late synaptic inputs is delayed in older adults. Furthermore, the magnitude of facilitation was also reduced in the older group. These findings closely replicate age-related changes in the response to $SICF_{PA}$ that were recently reported by our research group [26].

In an attempt to further investigate age-related changes within the interneuronal circuitry involved with these late synaptic inputs to CSNs, facilitation associated with application of $SICF_{AP}$ was also

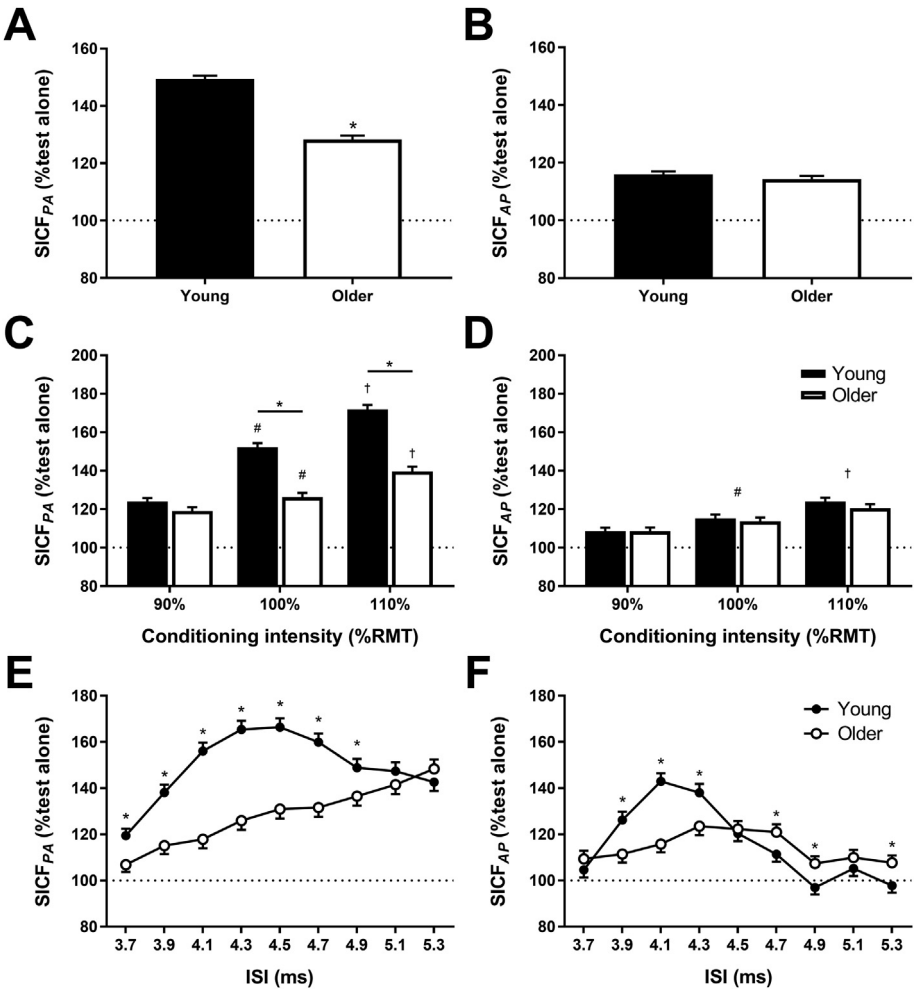

Fig. 2. Age-related changes in SICF. Group data comparing the response to SICF$_{PA}$ *(left column)* and SICF$_{AP}$ *(right column)* in young and older adults collapsed over all factors ***(A, B)***, as a function of conditioning intensity ***(C, D)*** and as a function of ISI ***(E, F)***. Dashed horizontal line shows the amplitude of the test alone MEP, with values above 100% representing MEP facilitation. $^{\#}P < 0.05$ compared to 90% RMT; $^{\dagger}P < 0.05$ compared to 90% and 100% RMT; $^{*}P < 0.05$ between groups.

recorded in young and older adults. In young participants, facilitation with an AP current was maximal at 4.1 ms, which is consistent with previous findings [30]. In contrast, facilitation in the older group was reduced in amplitude and demonstrated a broadened, delayed peak (Fig. 2). These results suggest that the ageing process results in reduced excitability and delayed temporal dynamics within the late synaptic inputs activated by SICF$_{AP}$, an outcome that is comparable to the effects of age on the response to SICF$_{PA}$. While the specific circuits activated during SICF$_{AP}$ are not known, it is well established that the composition of the descending volley is strongly influenced by the direction of the cortical current induced by stimulation (for review, see [12,13,20]). For example, PA stimulation produces a highly synchronised volley consisting of regularly spaced I-waves, with the I1 wave being preferentially activated. In contrast, the response to AP stimulation is less synchronised, preferentially generating late I-wave peaks that tend to be more temporally variable, and to only partially overlap the timing of responses produced by PA stimulation [36]. These observations have led to speculation that unique interneuronal circuits contribute to the descending volleys generated with different coil orientations [12,20], and this hypothesis is being increasingly substantiated by a growing body of experimental evidence [16,18,19,37–41]. Within the current study, it is therefore likely that the facilitation observed following SICF$_{AP}$ and SICF$_{PA}$ involved contributions from different I-wave generating circuitry. This raises the question of whether the similar effects of age on MEP facilitation observed with both SICF$_{PA}$ and SICF$_{AP}$ reflect comparable (yet unique) age-related changes within specific interneuronal circuits, or is indicative of a more generalised effect of age on interneuronal characteristics within motor cortex. However, one limitation of the current study that may be potentially relevant here is that the test stimulus intensity used during SICF was likely high enough to produce a descending volley consisting of multiple I-waves [42,43]. Consequently, it is possible that changes to components of the descending volley that are commonly activated in both coil orientations (when using higher stimulus intensities) contributed to the reduced facilitation and temporal shift observed in older adults. Future studies utilising more specific activation of different late I-wave circuits will therefore be an important extension of the current study.

Effects of age and TMS conditioning intensity on late I-wave recruitment

Given that facilitation produced by SICF$_{PA}$ is dependent on the intensity of the conditioning stimulus [23,28,29], it could be suggested that age-related changes in the recruitment of intracortical facilitation, as opposed to alterations to intrinsic interneuronal properties, could contribute to age-related changes in SICF. To

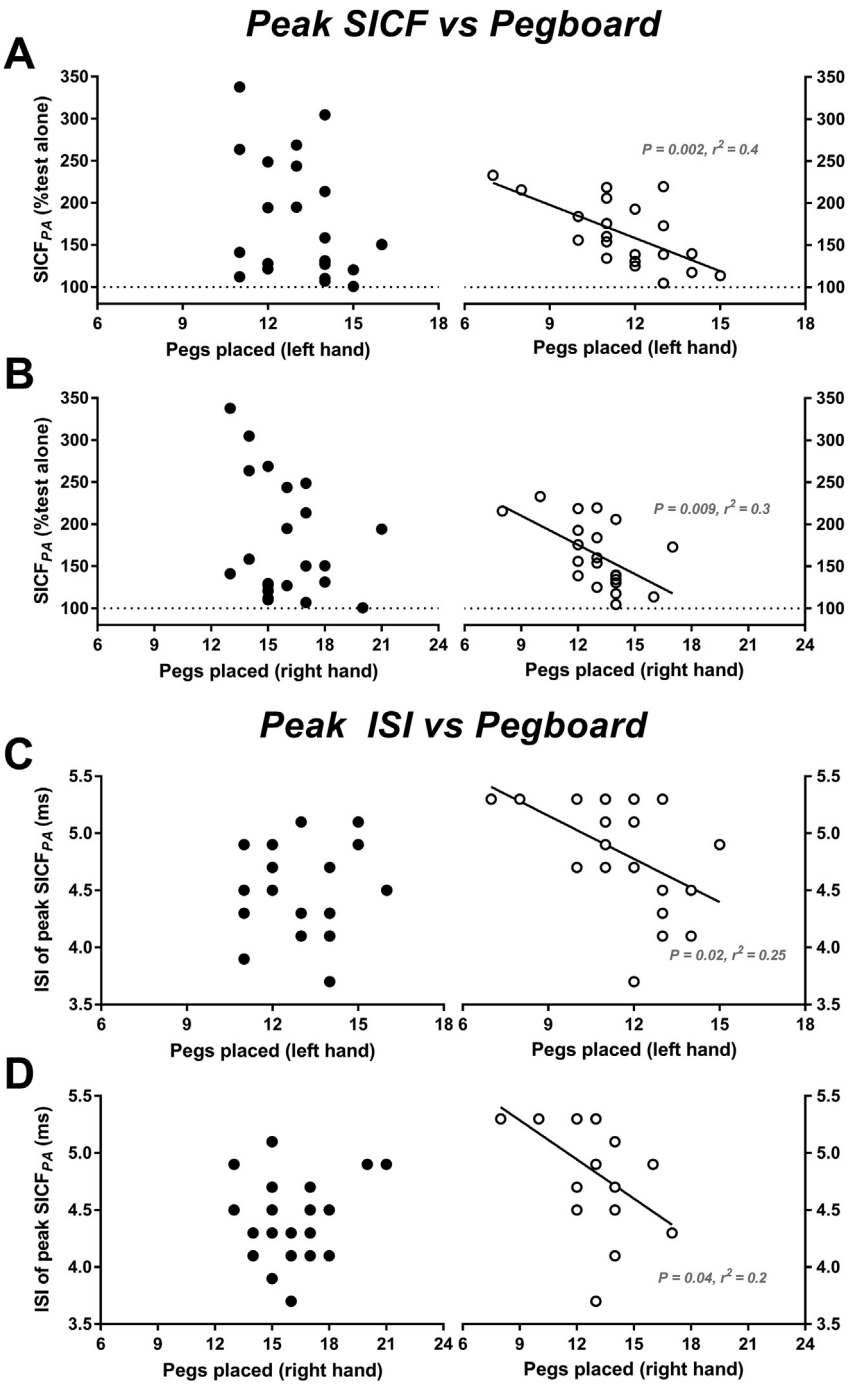

Fig. 3. Age-related changes in SICF predict reduced motor performance in older adults. Data shows relationship between peak SICF$_{PA}$ assessed with CI of 90% RMT (***A, B***) and the ISI at which peak SICF$_{PA}$ was observed (***C***, ***D***), and number of pegs placed during Purdue pegboard performance (*A & C, left hand; B & D, right hand*) in young (*black circles, left column*) and older (*white circles, right column*) adults. Dashed horizontal line in *A, B* shows amplitude of the test alone MEP, with values above 100% showing MEP facilitation. Regression line is included for all significant interactions.

investigate this possibility, the current study characterised the recruitment of SICF by modifying the intensity of conditioning stimulation. In support of the previous literature, both groups demonstrated greater SICF$_{PA}$ as the intensity of the conditioning stimulus increased. However, the magnitude of this effect was influenced by age: while facilitation with a 90% RMT conditioning stimulus was comparable between groups, the response was significantly reduced in older adults at higher conditioning intensities. Subsequently, the recruitment of PA-sensitive intracortical circuits that are associated with late I-wave circuitry appears to be reduced in older adults. While we cannot exclude the possibility that this reduced recruitment gain influenced the temporal delay observed in the older group, the lack of interaction between all factors suggests that any influence was minor. One limitation to this interpretation is that it is unclear if SICF recruitment included maximal facilitation, and it may consequently be possible that differences between groups may be reduced at higher levels of interneuronal activation. In addition, we are unable to exclude the possibility that age-related changes within the spinal

cord may have contributed to the altered facilitation observed in older adults [24,35].

While increasing conditioning intensities resulted in the recruitment of greater $SICF_{AP}$, this response was not different between age groups. This outcome is in contrast to our findings with $SICF_{PA}$, suggesting that it is unlikely to stem from changes within circuitry that is commonly activated by both coil orientations. Consequently, the gain of recruitment within AP-sensitive intracortical circuits associated with late I-waves appears to be maintained in the elderly, whereas recruitment gain in PA-sensitive circuits is reduced. The factors contributing to these differential effects of age on interneuronal recruitment are currently unclear. However, previous work suggests that the magnitude of facilitation produced by $SICF_{AP}$ is greater than with $SICF_{PA}$, whereas the opposite effect was observed in the current study. One factor that could have contributed to this was that the current study used a higher test TMS intensity compared with previous studies; this may have introduced a ceiling-effect on the observable facilitation or tested a part of the recruitment curve that was less sensitive to facilitation [28]. It therefore remains possible that age-related differences in $SICF_{AP}$ may be apparent when using lower intensity test stimuli.

Age-related changes in SICF and manual dexterity

Linear regression analysis revealed that older adults with greater peak $SICF_{PA}$, and with peak $SICF_{PA}$ occurring at longer latencies, tended to show reduced pegboard performance (Fig. 3). In contrast, the timing and magnitude of $SICF_{AP}$ in older adults, and both $SICF_{PA}$ and $SICF_{AP}$ in young adults, failed to predict performance. This is contrast to findings from a previous study by Clark and colleagues [27] which suggested that the second but not third $SICF_{PA}$ peak significantly predicts pegboard performance in both young and older adults. While the cause of this discrepancy is currently unclear, assessment of the third SICF peak within the previous study was limited to a single ISI that is usually associated with peak facilitation in young subjects (i.e., 4.5 ms). It may therefore be possible that the broader range of ISIs included within the current study allowed greater sensitivity for detecting functionally relevant neurophysiological alterations in the older group, resulting in the significant correlations with pegboard performance.

An interesting observation within the correlations of the current study is that, although better function was observed in older adults having a 'young' temporal profile of facilitation (i.e., peak facilitation occurring at shorter latencies), motor function was actually worse in older adults with a 'young' magnitude of facilitation (i.e., greater peak facilitation). Given that previous work shows that the timing of interneuronal activation determines the magnitude of SICF [22], and that the magnitude of SICF consequently predicts motor function [27], these interactions may suggest that primary effects of ageing on the temporal dynamics of these circuits result in poorer motor function, with secondary changes in the magnitude of facilitation. However, the current study was unable to separate the effects of SICF peak timing and amplitude on motor performance, as a delay in peak timing was often associated with a reduction in peak amplitude, so this will require substantiation in future studies.

The results of our correlational analysis show that age-related changes to PA-sensitive late synaptic inputs are specifically important to performance of a pegboard task, whereas AP-sensitive circuits do not appear to be relevant. In support of this functional divergence between interneuronal populations, growing evidence suggests that I-wave circuits recruited with different current directions influence motor function in task-specific ways [18,19,40,41]. Interestingly, different I-wave circuits are also amenable to targeted manipulation using stimulation paradigms with specific parameters [18,19]. Taken together, the findings of the current and previous studies therefore highlight the potential of different I-wave generating circuits as targets to more specifically influence motor behaviour in the elderly. However, designing interventions to realise this goal will require further identification of how different late I-wave circuits influence different kinds of motor function. Furthermore, it will also be necessary to investigate how age-related changes within late I-wave circuits influence the ability of older adults to learn new motor skills. As the existing literature appears to indicate that the excitability of interneuronal circuits mediating I-wave facilitation relates to motor execution [27,40,41], whereas the temporal characteristics of these circuits may be more important during neuroplasticity induction [16,18,19], it may be that the dissociable effects of age on late I-wave circuits (i.e., changes in magnitude *vs.* timing of facilitation) contribute to different domains of motor function/deficit in the elderly. If so, strategically modifying these facets of function within late I-wave circuitry may provide a means of adding specificity to an intervention.

The current study included several limitations that need to be acknowledged. First, neuronavigation was not used to guide placement of the stimulating coil, and small variations in the consistency of re-localisation may therefore have contributed to the variability of our data. Second, many of the older adults included within the current study reported chronic medical conditions common to the elderly, in addition to high levels of habitual physical activity, both of which may influence the response to TMS in older adults [44]. It is therefore possible that SICF characteristics may be different in elderly populations that are less active, or do not report chronic health conditions. Third, it remains possible that the peri- and suprathreshold conditioning stimuli may have resulted in descending activity that could have influenced the magnitude of facilitation. While the current study is unable to quantify if this occurred, this could be achieved in future studies by subtracting the response to the conditioning stimulus (applied in isolation) from the response to paired stimulation.

In conclusion, the current study used paired-pulse TMS with different conditioning intensities and current directions to investigate if the recruitment of late synaptic inputs on to corticospinal neurons are modified in older adults, and whether this is correlated with manual performance. We found that older adults showed reduced facilitation at longer latencies, and this was comparable for both PA- and AP-sensitive circuits. However, in PA-sensitive circuits only, the gain of interneuronal recruitment was reduced in older adults. Furthermore, both the magnitude and timing of facilitation produced by PA-sensitive circuits was negatively correlated with pegboard performance. In addition to providing further evidence that interneuronal circuits responsible for late I-wave generation are modified in older adults, our findings also demonstrate that these modifications are functionally relevant.

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Declaration of interest

The authors declare no conflict of interest.

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