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Age-related differences in pre- and post-synaptic motor cortex inhibition are task dependent

George M Opie[1], Michael C Ridding[2] and John G Semmler[1]

1. Discipline of Physiology, School of Medical Sciences, The University of Adelaide, Adelaide, Australia
2. Robinson Research Institute, School of Paediatrics and Reproductive Health, The University of Adelaide, Adelaide, Australia

Running Head: Age- and task-related changes in intracortical inhibition

Correspondence: John G. Semmler, Ph.D.
School of Medical Sciences
The University of Adelaide
Adelaide, South Australia 5005
Australia
Telephone: Int + 61 8 8313 7192
FAX: Int + 61 8 8313 4398
E-mail: john.semmler@adelaide.edu.au

Abstract

Background: Previous research has shown age-related differences in short- (SICI) and long-interval intracortical inhibition (LICI) in both resting and active hand muscles, suggesting that healthy ageing influences post-synaptic motor cortex inhibition. However, it is not known how the ageing process effects the pre-synaptic interaction of SICI by LICI, and how these pre- and post-synaptic intracortical inhibitory circuits are modulated by the performance of different motor tasks in older adults.

Objective: To examine age-related differences in pre- and post-synaptic motor cortex inhibition at rest, and during index finger abduction and precision grip.

Methods: In 13 young (22.3 ± 3.8 years) and 15 old (73.7 ± 4.0 years) adults, paired-pulse transcranial magnetic stimulation (TMS) was used to measure SICI (2 ms interstimulus interval; ISI) and LICI (100 and 150 ms ISI), whereas triple-pulse TMS was used to investigate SICI when primed by LICI.

Results: We found no age-related difference in SICI at rest or during index finger abduction, but significantly greater SICI in older subjects during precision grip. Older adults showed reduced LICI in resting muscle (at an ISI of 150 ms), with no age-related differences in LICI during either task. When SICI was primed by LICI, disinhibition of motor cortex was reduced in older adults at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip.

Conclusions: Our results support age-related differences in pre- and post-synaptic motor cortex inhibition, which may contribute to impaired hand function during task performance in older adults.

Keywords: Transcranial Magnetic Stimulation, presynaptic inhibition, gamma-aminobutyric acid, task, age

Introduction

Motor deficits are a major aspect of the ageing process that can significantly impede the performance of essential activities of daily living. Subsequently, age-related deficits in motor function may lead to reduced independence, decreased quality of life and institutionalisation [1]. Despite this, our understanding of how the ageing process affects function within core components of the motor system, such as the motor areas of the brain, is somewhat rudimentary. Nonetheless, within the primary motor cortex (M1), age-related changes in inhibitory neurotransmission mediated by γ aminobutyric acid (GABA) have been increasingly investigated as a factor potentially contributing to age-related motor deficits (for review, see; [2]). This line of investigation stems from the established importance of intracortical inhibition in motor control [3-6] and has been facilitated by the use of non-invasive transcranial magnetic stimulation (TMS).

In humans, TMS allows an assessment of distinct GABAergic processes by applying pairs of magnetic stimuli to M1 (paired-pulse TMS), or an assessment of interactions between GABAergic processes by applying 3 magnetic stimuli to M1 (triple-pulse TMS). During paired-pulse TMS, application of a subthreshold conditioning stimulus at short-intervals (1 – 5 ms) prior to a suprathreshold test stimulus produces inhibition of the test motor evoked potential (MEP) via activation of post-synaptic $GABA_A$ receptors [7]. This process is known as short-interval intracortical inhibition (SICI; [8]). However, when both stimuli are suprathreshold and separated by a long inter-stimulus interval (100 – 150 ms; ISI), inhibition of the test MEP is thought to involve post-synaptic $GABA_B$ receptors [9] and is known as long-interval intracortical inhibition (LICI; [10]). During triple-pulse TMS, the interaction between LICI and SICI is assessed by preceding the conditioning and test stimuli for SICI by the conditioning stimulus for LICI [11]. This protocol results in reduced inhibition of the test MEP and is thought to involve activation of pre-synaptic $GABA_B$ receptors [9, 11-14].

In young subjects, activity-dependent changes in both SICI [3, 4, 6] and LICI [15, 16] are well established. Furthermore, the nature of this modulation is thought to be task-dependent [17, 18]. In contrast, task-related changes in inhibition in older adults have been limited to measurements made during tonic contractions [19, 20] or in the period prior to contraction [21-23]. However, some evidence suggests that the task-dependency of inhibitory tone in M1 is modified by age [24]. Interestingly, task-dependent changes in SICI and LICI have been suggested to be mediated by pre-synaptic mechanisms [18], suggesting that effects of age on the task-dependent modulation of inhibition may be influenced by changes in pre-synaptic motor cortex inhibition.

Pre-synaptic inhibition in M1 has not been previously compared between young and old adults. However, Chu and colleagues [25] investigated LICI-SICI interactions in a group of older adults (age 54-68 years) using two ISIs of 100 ms and 150 ms. While this study observed the expected reduction in inhibition using the 100 ms ISI, no change in inhibition was seen when the 150 ms interval was used [25]. However, subsequent investigations assessing the duration of pre-synaptic inhibition in young subjects have shown that effects can last > 200 ms [26]. Comparing the findings of these studies suggests that LICI-SICI interactions are reduced by the ageing process, but only at longer ISIs (i.e., 150 ms). This observation may reflect a timing-dependent reduction in presynaptic inhibition in M1.

The aim of the current study was therefore to compare the magnitude of SICI, LICI and LICI-SICI interactions between young and old subjects during relaxation, index finger abduction and precision grip (between the index finger and thumb) – tasks that have previously produced specific changes in intracortical inhibition in young subjects [18]. Also, as effects of age on pre-synaptic motor cortex inhibition were expected to be timing-dependent, LICI-SICI interactions were assessed using two ISIs of 100 ms and 150 ms. Based on previous

studies [25, 26], we expected that old subjects would show reduced pre-synaptic M1 inhibition at 150 ms. Furthermore, as the activity-dependent modulation of inhibitory tone is reduced in older adults [23], we expected that task-dependent changes in this modulation would also be influenced by advancing age.

Materials and methods

15 old (73.7 ± 4.0 years) healthy subjects were recruited to participate in the current study via advertisements placed in local media. These data were compared to those from 13 young (mean ± standard deviation; 22.3 ± 3.8) healthy subjects, the results of which have been presented previously [27]. Exclusion criteria included a history of stroke, history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory [28]. Each subject provided written, informed consent prior to participation. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki.

Experimental arrangement

For the duration of each experimental session, subjects were seated in a comfortable chair with their right arm abducted approximately 45° at the shoulder. This allowed the forearm and hand to sit comfortably on an arm support placed next to them. Surface electromyography (EMG) was used to record responses from the first dorsal interosseous (FDI) muscle of the right hand. Two Ag-AgCl electrodes (1.6 cm diameter) were attached to the skin over the muscle in a belly-tendon montage, with a strap around the wrist grounding the electrodes. EMG was amplified (300 X) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 (Cambridge Electronic Design, Cambridge, UK), and digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design), before being recorded and

stored offline for analysis. 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.

Each subject participated in two experimental sessions held on separate days, each of 2-3 hours duration. Within each session, TMS was applied during complete relaxation of FDI and while FDI was active in producing one of two low intensity (5% of maximum force) contractions, performed in random order. For one of the sessions, subjects were required to produce an isolated abduction of the index finger, whereas in the other session they were required to perform a precision grip of the index finger and thumb. As prolonged contractions were required to complete the multiple stimulation conditions needed for triple-pulse TMS (see below), assessing each active task on separate days reduced the likelihood of fatiguing the target muscle, which may have confounded measurements of intracortical inhibition [29-31]. Within each experimental session, all TMS conditions (see below) were applied twice, once with the target muscle at rest, and again with the target muscle active (either abduction or precision grip). Furthermore, paired-pulse TMS was always performed before triple-pulse TMS for all subjects, allowing the experimenter to monitor baseline levels of inhibition before applying triple-pulse TMS. During active state measurements, stimulation began after subjects had reached stable force application.

Experimental Procedures

Maximal Voluntary Contraction At the beginning of each experiment, maximum voluntary contractions (MVC) were assessed for each subject. This was performed for both index finger abduction and precision grip using the index finger and thumb. During index finger abduction, the subject's right hand was positioned with the palm facing downwards and the index finger isolated from the middle, ring and little fingers. When instructed, subjects

abducted the lateral surface of the index finger against a force transducer (LC1205-K020; A&D Mercury Pty Ltd, Australia) placed in-line with the distal interphalangeal joint. During precision grip, subjects opposed the index finger and thumb against a purpose built manipulandum that has been described previously [32]. The procedure to assess the MVC was identical for both index finger abduction and precision grip: subjects were required to produce maximum force for 3 s in several repetitions, separated by 30 s rest, until the maximal force of three trials were within a 10% margin. The largest force recorded during these trials was chosen as the subject's MVC. To optimise force production, feedback was displayed on a computer monitor placed at eye level in front of the subject, and verbal encouragement was provided by the experimenter.

Transcranial magnetic stimulation TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with three Magstim 200 magnetic stimulators connected via two Bistim units (Magstim, Dyfed, UK). Within this setup, two stimulators were connected via the first Bistim unit, while the third stimulator and the output from the first Bistim unit were connected via the second Bistim unit. The coil was then connected to the output of the second Bistim unit. This allowed application of up to 3 stimuli at very short intervals through the same coil, but is associated with a reduction in stimulus strength of approximately 15% [11]. During testing, the coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed backwards and laterally, producing a current flow in the brain with a posterior to anterior direction. The coil was positioned on the scalp over the location producing an optimum response in the relaxed FDI muscle. This location was marked on the scalp for reference and continually checked throughout the experiment. TMS was delivered at 0.2 Hz for all conditions.

Resting and Active motor thresholds (RMT and AMT, respectively) were obtained in FDI while the TMS coil was placed at the optimal location over primary motor cortex. RMT was defined as the minimum TMS intensity producing a response amplitude ≥ 50 μV in at least three out of five trials in resting FDI muscle, and expressed relative to the maximum stimulator output (MSO). Active motor threshold (AMT) was defined as the minimum TMS intensity producing a response amplitude ≥ 300 μV in at least three out of five trials while FDI was active at 5% MVC. Force feedback was provided via an oscilloscope placed at eye level in front of the subject, with a target force set on the oscilloscope that was adjusted to 5% of each subjects MVC.

Intracortical inhibition The magnitude of post-synaptic intracortical inhibition was assessed using 4 experimental conditions (Table 1, Conditions A-D). SICI was measured with a subthreshold conditioning stimulus set at 80% AMT and an interstimulus interval (ISI) of 2 ms (Condition B; [8]) while LICI was assessed using a suprathreshold conditioning stimulus set at 120% RMT and two ISIs of 100 and 150 ms (conditions C and D; [10]). For both SICI and LICI, the intensity of the test stimulus was set at the level producing an MEP with peak-to-peak amplitude of 1 mV ($Stim_{1mV,}$ Condition A). Both paired-pulse TMS paradigms were applied in the same experimental block, allowing normalisation of all paired-pulse responses to a common test alone state. As 30 conditioned trials (10 SICI, 10 $LICI_{100}$, 10 $LICI_{150}$) and 10 control trials were included within a block, and each block was repeated with the muscle at rest and during activation, a total of 80 trials were used to assess baseline levels of intracortical inhibition.

The effect of LICI on SICI was assessed using triple-pulse TMS (Table 1, Conditions E-L). The conditioning stimulus used to activate LICI circuitry was set at 120% RMT and applied at two intervals of 100 ms ($CS_{100,}$ Condition K) and 150 ms (CS_{150}, Condition L) in separate

blocks. Within both blocks, the conditioning stimulus used to activate SICI circuitry was set at 80% AMT and applied using a 2 ms ISI (CS_2). The effect of LICI on SICI (LICI-SICI interaction) was quantified by comparing the amplitude of the test MEP generated by application of all three stimuli (CS100/CS150, CS2 and a test stimulus) to the amplitude of the test MEP generated by application of the LICI conditioning stimulus and the test stimulus. The intensity of the test stimulus was adjusted to the level producing an MEP response of 1 mV when preceded by either CS_{100} ($Stim_{100}$, Condition I) or CS_{150} ($Stim_{150}$, Condition J) and this intensity was set individually for each ISI. As increasing test TMS intensity reduces the magnitude of SICI [32, 33] the higher intensity $Stim_{100}$ and $Stim_{150}$ could account for changes in SICI observed during triple-pulse TMS. Therefore, as a control state, additional measurements of SICI were recorded using the higher intensity $Stim_{100}$ ($SICI_{adj100}$) and $Stim_{150}$ ($SICI_{adj150}$) as the test stimulus intensity (Conditions G and H, respectively). We will refer to the amplitude of the test MEP generated during $SICI_{adj100}$ and $SICI_{adj150}$ as MEP_{adj100} (Condition E) and MEP_{adj150} (Condition F), respectively. As 10 conditioned and 10 control trials were applied within four experimental blocks, and each block was repeated with the muscle at rest and during activation, a total of 160 trials were used to assess interactions between LICI and SICI.

Data Analysis

Data analysis was completed manually by visual inspection of offline EMG. Within the rest state, traces showing muscle activity > 20 μV in peak-to-peak amplitude during the 150 ms prior to the first conditioning stimulus (for conditioned trials) or the test stimulus (for unconditioned trials) were excluded from analysis. MEP amplitudes from each trial were measured peak-to-peak and expressed in mV. Paired- and triple-pulse measurements of intracortical inhibition were quantified by expressing the amplitude of individual conditioned MEPs as a percentage of the average control MEP amplitude. For measurements of adjusted

SICI and LICI-SICI interactions, the absolute change in inhibition from baseline was assessed by subtracting the amplitude of individual conditioned MEPs from the average conditioned MEP amplitude for the same condition. This value will be referred to as $SICI_{diff}$. For active trials, muscle activation was assessed by quantifying the root mean squared (rms) EMG amplitude (normalised to the maximum rmsEMG amplitude recorded during MVC) in the 100 ms leading up to application of CS150, CS100 or CS2 (depending on stimulation condition).

Statistical Analysis

RMT and the stimulus intensities used for MEP_{1mV}, MEP_{100} and MEP_{150} in resting muscle were compared between sessions using paired *t*-tests. Subject to no significant inter-session differences, these data were pooled across sessions. AMT was also compared between sessions using a paired *t*-test. Pooled RMT and handedness scores (laterality quotient) were compared between age groups using unpaired *t*-tests, while pooled AMT was compared between age groups and sessions using a two-way repeated measures analysis of variance ($ANOVA_{RM}$). A three-way $ANOVA_{RM}$ was used to investigate effects of age (young, old), test stimulus condition ($Stim_{1mV}$, $Stim_{100}$ & $Stim_{150}$) and task (rest, abduction, precision) on test stimulus intensity. Individual two-way ANOVA's were used to assess the effects of age and task on normalised EMG amplitude prior to TMS for LICI at each ISI, SICI in each stimulus condition (i.e., baseline, $SICI_{adj100}$, $SICI_{adj150}$, LICI-$SICI_{100}$, LICI-$SICI_{150}$) and the corresponding test alone MEPs. All main effects and interactions were further investigated using one-way ANOVA's with Fishers PLSD post hoc test. Mixed-model analysis was used to investigate the effects of test MEP condition (MEP_{1mV}, $MEPa_{dj100}$, MEP_{adj150}, MEP_{100} & MEP_{150}), task and age on the amplitude of the test alone MEP. Effects of task on SICI, LICI and LICI-SICI interactions were compared between young and old adults using mixed model analyses. For LICI, individual models were used for each ISI while, for LICI-SICI

interactions, individual models were used for both task and ISI. For all models, subject was included as a random effect, and significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. For assessments of adjusted SICI and LICI-SICI interactions, the absolute change in SICI relative to baseline within each stimulus condition was compared to '0' (i.e., no change in inhibition) using one-sample t-tests with Bonferroni correction. The change in SICI was also compared between groups and stimulation states using linear mixed model analysis, with separate models used for each ISI and activity state. Significant main effects and interactions were also investigated using custom contrasts with Bonferroni correction. Linear regression of individual subject data was used to further investigate age-related changes in the interactions between each measurement. As measurements in resting muscle were repeated in each session, values were averaged across sessions within each subject (subject to no significant difference between sessions, assessed using paired *t*-test's), with the resulting value used for regression analysis. Significance was set at $P \leq 0.05$ for all comparisons and data are shown as mean ± standard error of the mean (SEM), unless otherwise stated.

Results

As the results of the young cohort have been previously reported [27], and the primary interest of the current study concerns age-related effects, only findings involving main effects or interactions of age will be described in detail. All subjects completed the experiment in full and without adverse reaction. The young cohort consisted of 6 males (23.3 ± 5.4) and 7 females (21.4 ± 1.4), whereas the old cohort consisted of 8 males (75.0 ± 3.8 years) and 7 females (72.1 ± 3.8 years). Handedness was not different between age groups (average laterality quotient: young, 0.92 ± 0.04; old, 0.87 ± 0.06; $P = 0.5$). As RMT did not vary between sessions ($P = 0.5$), values were pooled and compared between age groups, with no significant difference found (young, 61.1 ± 1.5% MSO; old, 62.0 ± 2.2 % MSO; $P = 0.7$). For

AMT, values were not different between sessions (P = 0.4) or age groups (P = 0.6)(abduction - young: 47.0 ± 2.1% MSO, old: 48.7 ± 2.6 % MSO; precision - young: 47.6 ± 2.2% MSO, old: 49.6 ± 2.8% MSO). Normalised prestimulus EMG for each stimulus condition and task is compared between young and old adults in Table 2.

Test MEP characteristics

The amplitude of the test alone MEP in each test MEP condition and task is compared between young and old subjects in Table 3. For this comparison, all main effects and interactions were significant (all P-values < 0.0001). Age-related differences were found only for MEP_{adj100} and MEP_{adj150}, (i.e., the test MEPs recorded using the increased test stimulus intensity applied during triple-pulse TMS) and these varied between activity states (see Table 3). Furthermore, no differences in MEP_{1mV}, MEP_{100} or MEP_{150} were found between age-groups in any task (P-values ranging from 0.1 – 0.9). These comparisons demonstrate that, for those test MEP conditions which aimed to produce a response of 1 mV (i.e., MEP_{1mv}, MEP_{100} and MEP_{150}), the amplitude was well matched between conditions and groups. However, for those conditions which did not adjust MEP amplitude, but instead adjusted the intensity of the test stimulus in order to produce a descending volley comparable to that which could be expected during triple-pulse TMS (i.e., MEP_{adj100} and MEP_{adj150}; see Discussion for explanation of methodology), the test MEP amplitude was significantly increased. The intensity of the test stimulus for each stimulus condition is shown in Table 4. All main effects and interactions of age failed to reach significance for these data (all P-values > 0.3).

Short-interval intracortical inhibition

Measurements of SICI in young and old subjects during each task are shown in Figure 1. The magnitude of inhibition was affected by age (P = 0.007) and there was a significant

interaction between age and task ($P = 0.001$). Age-related comparisons within each task showed no difference in SICI during rest ($P = 0.2$) and index finger abduction ($P = 0.2$), but significantly greater inhibition in old subjects during precision grip ($P < 0.0001$).

Long-interval intracortical inhibition

Effects of age and task on LICI are shown in Figure 2. $LICI_{100}$ was not significantly affected by age ($P = 0.9$) and there was no interaction between age and task ($P = 0.1$, Figure 2A). For $LICI_{150}$, inhibition was again unaffected by age ($P = 0.4$), but the interaction between age and task reached significance ($P = 0.02$, Figure 2B). Age-related comparisons in each task showed that older subjects had significantly reduced $LICI_{150}$ in resting muscle ($P = 0.01$), but no difference in LICI between groups during index finger abduction ($P = 0.1$) or precision grip ($P = 0.4$).

SICI in the presence of LICI

The effect of LICI on SICI in resting muscle is compared between young and old subjects in Figure 3. When using the 100 ms ISI, the magnitude of inhibition was not affected by age ($P = 0.1$), but there was an interaction between age and stimulation state ($P = 0.02$, Fig. 3A). Age-related comparisons showed that $SICI_{adj100}$ did not differ between groups ($P = 0.08$), whereas older subjects had a significantly smaller reduction in SICI during LICI-$SICI_{100}$ than young subjects ($P = 0.05$). Within each stimulation state, there was a significant change in SICI from baseline ($SICI_{diff}$) for both $SICI_{adj100}$ ($P < 0.0001$) and LICI-$SICI_{100}$ ($P < 0.0001$) in young subjects, but only LICI-$SICI_{100}$ ($P < 0.0001$) in old subjects (Fig. 3B). Furthermore, comparing $SICI_{diff}$ between groups showed significant effects of age ($P < 0.0001$) and stimulation state ($P < 0.0001$) but no interaction between factors ($P = 0.9$). When SICI in the presence of LICI was assessed using the 150 ms ISI, inhibition was not affected by age ($P = 0.5$) and there was no interaction between age and stimulation state ($P = 0.5$, Fig. 3C). For

$SICI_{diff}$ at 150 ms, values were significant for all stimulation states in both groups (all *P*-values < 0.001; Fig. 3D). However, although $SICI_{diff}$ showed a significant effect of stimulation state ($P < 0.0001$), there was no effect of age ($P = 0.2$) and no interaction between factors ($P = 0.5$).

LICI-SICI interactions during index finger abduction are shown in Figure 4. For LICI-$SICI_{100}$, effects of age failed to reach a conventional significance level ($P = 0.06$). Furthermore, there was no interaction between stimulation state and age ($P = 0.1$; Fig 4A). For both age groups, $SICI_{diff}$ was significant for $SICI_{adj100}$ (young, $P < 0.0001$; old, P = 0.002) but failed to reach significance for LICI-$SICI_{100}$ (young, $P = 0.05$; old, $P = 0.3$; Fig. 4B). $SICI_{diff}$ failed to show effects of stimulation state ($P = 0.1$) or age ($P = 0.3$) and there was no interaction between factors ($P = 0.1$). For measurements using the 150 ms ISI, inhibition was significantly greater in older subjects ($P = 0.04$), but the interaction between factors was not significant ($P = 0.2$; Fig 4C). $SICI_{diff}$ was not significant for either group during $SICI_{adj150}$ (young, $P = 0.5$; old, $P = 0.5$), or for old subjects during LICI-$SICI_{150}$ ($P = 0.2$), but did reach significance for young subjects during LICI-$SICI_{150}$ ($P = 0.003$; Fig. 4D). $SICI_{diff}$ at 150 ms failed to show an effect of age ($P = 0.08$), whereas an effect of stimulation state ($P = 0.002$) and an interaction between factors ($P = 0.04$) was found. Age-related comparisons within each stimulation state showed no differences between age groups for $SICI_{adj150}$ ($P = 0.7$) whereas $SICI_{diff}$ was significantly greater in young subjects during LICI-$SICI_{150}$ ($P = 0.04$).

LICI-SICI interactions during precision grip are shown in Figure 5. For measurements using the 100 ms ISI, a significant effect of age ($P = 0.009$) and interaction between age and stimulation state was found ($P = 0.0002$; Fig 5A). However, age-related comparisons showed no differences between groups for $SICI_{adj100}$ ($P = 0.1$) and LICI-$SICI_{100}$ ($P = 0.1$). For $SICI_{adj100}$, $SICI_{diff}$ was significant for young ($P < 0.0001$) but not old ($P = 0.9$) subjects,

whereas $SICI_{diff}$ was not significant for either group during LICI-$SICI_{100}$ (young, $P = 0.2$; old, $P = 0.6$; Fig. 5B). $SICI_{diff}$ at 100 ms showed a significant effect of age ($P = 0.001$), but no effect of stimulation state ($P = 0.9$) or interaction between factors ($P = 0.07$). For measurements using the 150 ms ISI, inhibition was significantly reduced in younger subjects ($P < 0.0001$) but there was no interaction between factors ($P = 0.5$; Fig 5C). $SICI_{diff}$ was significant during $SICI_{adj150}$ for old ($P < 0.0001$) but not young subjects ($P = 0.6$). Furthermore, $SICI_{diff}$ failed to reach significance for either group during LICI-$SICI_{150}$ (young, $P = 0.1$; old, $P = 0.3$; Fig. 5D). $SICI_{diff}$ showed a significant stimulation state effect ($P = 0.002$) but no effect of age ($P = 0.2$) or interaction between factors ($P = 0.5$).

Linear regression

Linear regression of individual subject data was used to investigate if task- and age-related changes in the activity of one inhibitory circuit was related to altered activity in either of the other inhibitory circuits. Subsequently, all inhibitory measurements (i.e., SICI, LICI and LICI-SICI interactions) in each task (rest, abduction, precision grip) were regressed against each other. In young but not old subjects, significant interactions were found between measurements of $LICI_{150}$ at rest and during index finger abduction (young: $r = 0.73$, $F_{1,12} = 12.24$, $P = 0.005$; old: $r = 0.39$, $F_{1,14} = 2.38$, $P = 0.15$; Fig 6A & 6C), between $LICI_{150}$ at rest and during precision grip (young: $r = 0.58$, $F_{1,12} = 5.57$, $P = 0.04$; old: $r = 0.31$, $F_{1,14} = 1.34$, $P = 0.3$; Fig 6B & 6E) and between LICI-$SICI_{100}$ and LICI-$SICI_{150}$ at rest (young: $r = 0.60$, $F_{1,12} = 6.11$, $P = 0.03$; old: $r = 0.38$, $F_{1,14} = 2.22$, $P = 0.16$). For old but not young subjects, significant interactions were found between measurements of $LICI_{100}$ at rest and during precision grip (young: $r = -0.16$, $F_{1,12} = 0.29$, $P = 0.6$; old: $r = 0.76$, $F_{1,14} = 17.65$ $P = 0.001$), and between $LICI_{100}$ and $LICI_{150}$ at rest (young: $r = 0.52$, $F_{1,12} = 4.05$, $P = 0.07$; old: $r = 0.84$, $F_{1,14} = 32.04$, $P < 0.0001$). In both groups, interactions between $LICI_{150}$ during abduction and

precision grip were significant (young: $r = 0.72$, $F_{1,12} = 11.55$, $P = 0.006$; old: $r = 0.56$, $F_{1,14} = 6.06$, $P = 0.03$; Fig 6C & 6F). All other regressions failed to reach significance.

Discussion

The current study assessed age-related differences in pre- and post-synaptic M1 inhibition during relaxation, index finger abduction and precision grip. This was achieved by using paired- and triple pulse TMS to assess SICI, LICI and the interaction between LICI and SICI in young and old adults. At least 3 new findings were obtained from this novel experimental approach. First, we found age-related differences in SICI during the precision grip task, but not at rest or during index finger abduction. Second, age-related differences in LICI were only evident in resting muscle (at an ISI of 150 ms), with no age-related differences during task performance. Third, we found age-related differences in LICI-SICI interactions at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip. Taken together, these findings suggest that there are subtle differences in pre- and post-synaptic M1 inhibition with advancing age, which may contribute to deficits in motor performance during some tasks in older adults.

Effects of age on short-interval intracortical inhibition

Although age-related changes in SICI have been extensively investigated, the majority of studies have focussed on measurements in resting muscle, and have produced conflicting findings. Within the current study, SICI in resting muscle was not different between young and old adults, suggesting that resting post-synaptic $GABA_A$ mediated inhibitory tone in motor cortex is maintained with age [7]. This supports previous investigations from within our lab [20, 34-36], and from elsewhere [37-39], but is in contrast to reports of reduced [23, 40, 41] or increased [19, 42] SICI with age. The reasons for these inconsistencies are

currently unclear, but likely relate to variations in subject characteristics and methodological approach [20].

In contrast to measurements in resting muscle, only two previous studies have investigated age-related changes in SICI during muscle activation, reporting no effect of age [19] or reduced SICI in old subjects [20]. These inconsistent findings have been previously suggested to stem from variations in test MEP characteristics [20]. In the current study, we expanded this investigation by including different motor tasks, as the disinhibition of SICI during muscle activation has been shown to also be task-dependent in young subjects [17, 18]. For example, greater reductions in SICI were observed during a synergistic precision grip between the index finger and thumb than during an isolated index finger abduction [18]. This greater disinhibition of motor cortex during synergistic contractions has been suggested to contribute to the functional coactivation of cortical areas innervating task-related muscles [18].

Consistent with these previous findings, the current study observed greater reductions in SICI during precision grip in young subjects. In contrast, although inhibition was reduced in both tasks relative to rest in old subjects, the magnitude of this reduction did not differ between abduction and precision grip states, suggesting a lack of task-dependency with advancing age. Small (8%) differences in pre-stimulus EMG that we observed between age groups is unlikely to contribute to this effect, as the increased EMG in older adults was consistent for both tasks, but a difference in SICI was only observed for precision grip. Therefore, despite maintaining the ability to reduce post-synaptic $GABA_A$-mediated inhibition within motor cortex during tonic muscle activation, old adults demonstrate a reduced modulation of inhibitory tone for tasks requiring more complex activation of primary motor cortex. This may contribute to age-related impairments in motor performance during this task, as has been shown previously [43, 44]. Alternatively, a recent study by Fujiyama and colleagues [21]

showed that greater SICI during the foreperiod of a warned reaction task was associated with faster reaction times in old but not young subjects. This could suggest that the increased inhibition observed during precision grip is a compensatory mechanism to maintain performance. This remains to be explored by future research.

Effects of age on long-interval intracortical inhibition

In resting muscle, we found that $LICI_{100}$ was unaffected by age, whereas $LICI_{150}$ was significantly reduced in older adults. These observations suggest that the strength of post-synaptic $GABA_B$ mediated inhibitory tone in motor cortex [9] at rest may be reduced in older adults in a timing dependent manner. Age-related changes in the magnitude of LICI have only been investigated by two previous studies. The first reported increased inhibition with age [19], whereas the second, from our group, reported reduced inhibition with age [20]. As McGinley and colleagues used a 100 ms ISI, whereas our previous study used a 150 ms ISI, the current results contradict previous findings for the shorter interval, but support previous findings for the longer interval.

For $LICI_{100}$ and $LICI_{150}$ in active muscle, we found that the magnitude of inhibition was not significantly different between age groups for both tasks, supporting a previous study in active muscle at the 100 ms ISI [19]. However, we also found that the activity-dependent modulation of $LICI_{150}$ was different in older adults. Specifically, young subjects showed a progressive reduction in $LICI_{150}$ from rest, to index finger abduction, to precision grip (Figure 2B), and these changes in inhibition were all significantly related to each other (Figure 6A - C). However, in old subjects, there was no modulation of $LICI_{150}$ from rest to index finger abduction, and no significant relationship between $LICI_{150}$ in resting and active muscle (irrespective of task; Figure 6D & 6E). These observations suggest that the ageing process changes the way in which $LICI_{150}$ is modulated during the transition from resting to active

muscle, but that once the muscle is active, old adults maintain the ability to modulate $LICI_{150}$ according to task demands. However, as the functional role of LICI is still not clear, the ramifications of this altered inhibitory modulation require further investigation.

Effects of age and task on the interaction between LICI and SICI

All previous investigations of age-related changes in intracortical inhibition have focussed on the conventional paired-pulse TMS measurements of SICI and LICI. The current study is the first to compare the difference in the interaction between these paradigms in young and old adults. The LICI-SICI interaction is seen as reduced SICI when assessed in the presence of LICI [11], and several lines of evidence suggest that this is due to the activation of pre-synaptic $GABA_B$ receptors on the terminal of SICI neurons by collateral branches of LICI neurons [9, 11-13, 45], providing a measure of pre-synaptic motor cortex inhibition. LICI-SICI interactions may be altered in active muscle [14, 27] and are reduced in individuals with Parkinson's disease [46], suggesting a role for pre-synaptic motor cortex inhibition in motor function.

In the current study, LICI-SICI interactions were investigated using two intervals (between LICI's conditioning stimulus and the test stimulus) of 100 ms and 150 ms, as inconsistent findings from previous studies suggested that pre-synaptic inhibition in older adults may be timing-dependent [25, 26]. In resting muscle, although both groups showed reduced SICI in the presence of LICI at each ISI, the magnitude of this effect was reduced in old subjects at 100 ms, but not different between groups at 150 ms. Although we expected to see a timing-dependent effect of age on the LICI-SICI interaction, the findings of Chu and colleagues suggested that it would occur at 150 ms, not 100 ms. The reasons for this discrepancy are currently unclear, but seem unlikely to stem from the minor methodological differences between studies. Despite this, these findings suggest that the ageing process may cause a

timing specific reduction in pre-synaptic $GABA_B$-mediated intracortical inhibition within motor cortex. Interestingly, results of our linear regression analysis further supports a timing-dependent dissociation of pre-synaptic inhibition in old adults, with young but not old subjects showing a significant interaction between resting LICI-$SICI_{100}$ and LICI-$SICI_{150}$.

In active muscle, LICI-$SICI_{100}$ measurements during both abduction and precision grip failed to show any effects of age. Furthermore, while a consistent age effect was observed across stimulus conditions for LICI-$SICI_{150}$ during precision grip, it seems likely that this effect was driven by the age-related increase in baseline SICI observed during precision grip (Figure 1). Despite this, for LICI-$SICI_{150}$ during index finger abduction, the absolute change in inhibition from baseline (i.e., $SICI_{diff}$; Figure 4D) was significant for young but not old subjects, and the magnitude of $SICI_{diff}$ was significantly greater in young subjects. These observations show an age-related reduction in the interaction between SICI and LICI during index finger abduction at 150 ms, reflecting reduced presynaptic motor cortex inhibition in older adults under these conditions.

One possible reason for these observed changes in LICI-SICI interactions could be due to age-related differences in CSP duration [19, 24, 38]. However, the majority of studies reporting age-related changes in the CSP suggest a reduced duration with age [24, 38], whereas our observed changes in LICI-SICI interactions in older adults could only be explained by an increased CSP duration with advancing age. It therefore seems unlikely that these changes in LICI-SICI interactions were confounded by CSP duration, but instead reflect an age-related reduction in the activation of pre-synaptic $GABA_B$ receptors when performing isolated index finger abductions. As pre-synaptic $GABA_B$ receptors limit the release of GABA from inhibitory neurons (for review, see; [47]), this reduced presynaptic inhibition may reflect a less specific cortical activation that impairs task performance. For example, both TMS and neuroimaging studies have shown that old adults demonstrate more non-

specific patterns of cortical activation during performance of a motor task [48, 49], and that this increased activation may be detrimental to task performance [48]. Therefore, these age-related changes in inhibitory tone may represent a compensatory mechanism to regain more specific patterns of cortical activation.

In line with previous studies [11, 13, 25], the current study was able to match the test MEPs under all conditions by adjusting the test TMS intensity. This process assumes that a similar test MEP reflects a comparable descending volley involving a similar contribution of early (I_1) and late (I_3) I-waves, even when preceded by the activation of inhibitory circuits. This interpretation has been supported by experimental evidence from epidural recordings recorded during relaxation of target muscles [45], although opposing results may be found in some subjects [50]. Nonetheless, the contribution of early and late I waves to the MEP are known to be altered when the target muscle is active [51], which may complicate the interpretation of the interactions between inhibitory circuits in active muscle. Furthermore, it is not known whether the composition of the descending volley is different in older adults, although recent evidence comparing MEP latencies between antero-posterior (preferential I_3 wave activation) and posterior-anterior (preferential I_1 wave activation) TMS showed similar recruitment of I waves in older adults [52]. Further research is therefore needed to confirm whether the descending volley (that produces similar MEP amplitudes) is comparable in young and old adults, particularly when the muscle is active.

In conclusion, the current study has demonstrated complex effects of age on the task-dependent modulation of intracortical inhibition. In resting muscle, SICI was unaffected by age, whereas LICI and LICI-SICI interactions showed timing-dependent reductions in old adults. During muscle activation, older adults showed a reduced modulation of both SICI and LICI, resulting in reduced SICI during precision grip in older adults, and no age-related difference in LICI. When SICI was primed by LICI, disinhibition of motor cortex was

reduced in older adults at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip. These findings suggest that there are age-related differences in pre- and post-synaptic motor cortex inhibition that are dependent on the task performed, which may occur due to a reduced ability to modulate inhibitory circuits in the ageing motor cortex.

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Disclosures

The authors declare no conflict of interest

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Figure 1. Age-dependent changes in the effect of task on SICI. Measurements of SICI were compared between young *(black bars)* and old *(white bars)* subjects during rest, index finger abduction and precision grip of the index finger and thumb. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. $^{\#}P < 0.05$ when compared to values in resting muscle; $^{\dagger}P < 0.05$ when compared to values in resting muscle and during index finger abduction. $*P < 0.05$ between young and old adults.

Figure 2. Task-related changes in LICI compared between young and old adults. The magnitude of LICI was compared between young *(black bars)* and old *(white bars)* subjects during rest, index finger abduction and precision grip tasks using interstimulus intervals of 100 ms *(A)* and 150 ms *(B)*. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. $^{\#}P < 0.05$ when compared to values in resting muscle; $^{\dagger}P < 0.05$ when compared to values in resting muscle and during index finger abduction. $*P < 0.05$ between young and old adults.

Figure 3. Effects of age on the interaction between SICI and LICI in resting muscle. Triple-pulse TMS measures were compared between young *(black bars)* and old *(white bars)* adults at two interstimulus intervals of 100 ms *(A)* and 150 ms *(C)* during complete relaxation of FDI. For all panels, $SICI_{adj100/150}$ refers to conditions G/H, and LICI-$SICI_{100/150}$ refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., $SICI_{diff}$) for the 100 ms and 150 ms intervals is also quantified in panels *B* and *D*. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. $^{\#}P < 0.05$ when compared to baseline SICI and adjusted SICI; †magnitude of change from baseline is significant ($P < 0.01$); $*P < 0.05$ between young and old adults.

Figure 4. Effects of age on the interaction between SICI and LICI during index finger abduction. Triple-pulse TMS measures were compared between young *(black bars)* and old

(white bars) adults at two interstimulus intervals of 100 ms *(A)* and 150 ms *(C)* during isolated abduction of the index finger. For all panels, $SICI_{adj100/150}$ refers to conditions G/H, and LICI-$SICI_{100/150}$ refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., $SICI_{diff}$) for the 100 ms and 150 ms intervals is also quantified in panels *B* and *D*. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. $^{\#}P < 0.05$ when compared to baseline SICI and adjusted SICI; †magnitude of change from baseline is significant ($P < 0.01$); $^{*}P < 0.05$ between young and old adults.

Figure 5. Effects of age on the interaction between SICI and LICI during precision grip. Triple-pulse TMS measures were compared between young *(black bars)* and old *(white bars)* adults at two interstimulus intervals of 100 ms *(A)* and 150 ms *(C)* during precision grip between the index finger and thumb. For all panels, $SICI_{adj100/150}$ refers to conditions G/H, and LICI-$SICI_{100/150}$ refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., $SICI_{diff}$) for the 100 ms and 150 ms intervals is also quantified in panels *B* and *D*. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. $^{\#}P < 0.05$ when compared to baseline SICI and adjusted SICI; † magnitude of change from baseline is significant ($P < 0.01$); $^{*}P < 0.05$ between young and old adults.

Figure 6. Interactions between $LICI_{150}$ during different tasks in young and old subjects. In young *(black circles)* but not old *(white circles)* subjects, $LICI_{150}$ in resting muscle was significantly related to $LICI_{150}$ during both index finger abduction *(A/D)* and precision grip *(B/E)*. For both groups, significant interactions were found between $LICI_{150}$ during index finger abduction and precision grip *(C/F)*.

Table 1. TMS protocol

	Condition	CS150	CS100	CS2	Test Stimulus
A	Test MEP	—	—	—	$Stim_{1mV}$
B	SICI	—	—	80% AMT	$Stim_{1mV}$
C	$LICI_{100}$	—	120% RMT	—	$Stim_{1mV}$
D	$LICI_{150}$	120% RMT	—	—	$Stim_{1mV}$
E	MEP_{adj100}	—	—	—	$Stim_{100}$
F	MEP_{adj150}	—	—	—	$Stim_{150}$
G	$SICI_{adj100}$	—	—	80% AMT	$Stim_{100}$
H	$SICI_{adj150}$	—	—	80% AMT	$Stim_{150}$
I	Test MEP_{100}	—	120% RMT	—	$Stim_{100}$
J	Test MEP_{150}	120% RMT	—	—	$Stim_{150}$
K	LICI-$SICI_{100}$	—	120% RMT	80% AMT	$Stim_{100}$
L	LICI-$SICI_{150}$	120% RMT	—	80% AMT	$Stim_{150}$

Abbreviations: CS150, conditioning stimulus applied 150ms prior to the test stimulus; CS100, conditioning stimulus applied 100ms prior to the test stimulus; CS2, conditioning stimulus applied 2ms prior to the test stimulus; MEP_{1mV}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV; MEP_{100}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS_{100}; MEP_{150}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS_{150}; RMT, resting motor threshold; AMT active motor threshold.

Table 2. Normalised prestimulus EMG (%MVC EMG)

	Young		Old		Main effects (*P*-value)	
Condition	Abduction	Precision	Abduction	Precision	Age	Task
Baseline ICI						
SICI	9.6 ± 1.4	16.3 ± 1.7	20.7 ± 1.9	21.6 ± 3.2	0.0007	0.1
$LICI_{100}$	9.4 ± 1.4	16.0 ± 1.7	20.9 ± 2.1	20.3 ± 2.7	0.0005	0.2
$LICI_{150}$	9.1 ± 1.3	15.2 ± 1.7	21.5 ± 2.1	21.3 ± 2.9	0.0001	0.2
Test MEP	7.7 ± 0.9	15.7 ± 1.7	21.0 ± 2.1	20.9 ± 2.9	< 0.0001	0.08
$SICI_{adj100}$						
Conditioned	10.9 ± 1.4[a]	21.3 ± 3.1	24.5 ± 2.9	21.9 ± 2.3	0.008	0.1
Test MEP	9.3 ± 1.2[a,b]	22.0 ± 3.5	23.7 ± 2.5	22.8 ± 2.4	0.004	0.02
$SICI_{adj150}$						
Conditioned	10.7 ± 1.3	23.0 ± 4.7	21.2 ± 1.7	25.9 ± 3.4	0.03	0.008
Test MEP	9.2 ± 1.4	21.8 ± 4.1	22.0 ± 1.8	26.1 ± 3.6	0.006	0.007
LICI-$SICI_{100}$						
Conditioned	10.6 ± 1.4	21.3 ± 2.9	23.5 ± 2.4	25.1 ± 2.9	0.002	0.02
Test MEP	9.3 ± 1.3	21.1 ± 3.0	22.7 ± 2.0	25.7 ± 2.9	0.0005	0.004
LICI-$SICI_{150}$						
Conditioned	10.4 ± 1.3	17.9 ± 2.2	20.4 ± 2.2	23.5 ± 2.6	0.02	0.0008
Test MEP	8.8 ± 1.2	18.7 ± 2.7	21.3 ± 2.2	23.5 ± 2.9	0.0006	0.01

[a]$P < 0.05$ compared to the same task in old subjects; [b]$P < 0.05$ compared to precision grip

Table 3. Test MEP amplitude

	Young			Old		
	Rest	*Abduction*	*Precision*	*Rest*	*Abduction*	*Precision*
MEP_{1mV}	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.4 ± 0.1	1.0 ± 0.1
MEP_{adj100}	2.0 ± 0.1[a]	7.2 ± 0.2[a,b]	7.3 ± 0.1[a,b]	2.8 ± 0.1	5.9 ± 0.1[b]	4.1 ± 0.1[b]
MEP_{adj150}	2.4 ± 0.1[a]	3.2 ± 0.2[a,b]	3.1 ± 0.1[a,b]	2.1 ± 0.1	2.7 ± 0.1[b]	2.7 ± 0.1[b]
MEP_{100}	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.2
MEP_{150}	1.1 ± 0.1	1.2 ± 0.2	1.4 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1

See text for a definition of MEP amplitude conditions. [a]$P < 0.05$ compared to the same MEP condition in old subjects; [b]$P < 0.05$ compared to rest.

Table 4. Test TMS intensity

	Young			Old		
	Rest	*Abduction*	*Precision*	*Rest*	*Abduction*	*Precision*
$Stim_{1mV}$	65.3 ± 1.8	45.5 ± 2.0[a]	44.7 ± 2.3[a]	67.1 ± 2.9	46.9 ± 2.4[a]	48.7 ± 3.1[a]
$Stim_{100}$	72.5 ± 2.2	64.2 ± 2.6	60.4 ± 3.2[a]	75.5 ± 2.7	63.4 ± 3.9[a]	63.1 ± 4.8[a]
$Stim_{150}$	73.5 ± 2.3	50.0 ± 3.1[a]	47.4 ± 3.7[a]	72.6 ± 3.0	52.3 ± 3.4[a]	50.8 ± 4.3[a]

See text for a definition of MEP amplitude conditions. Differences between age groups were not significant.
[a]$P < 0.05$ compared to rest.

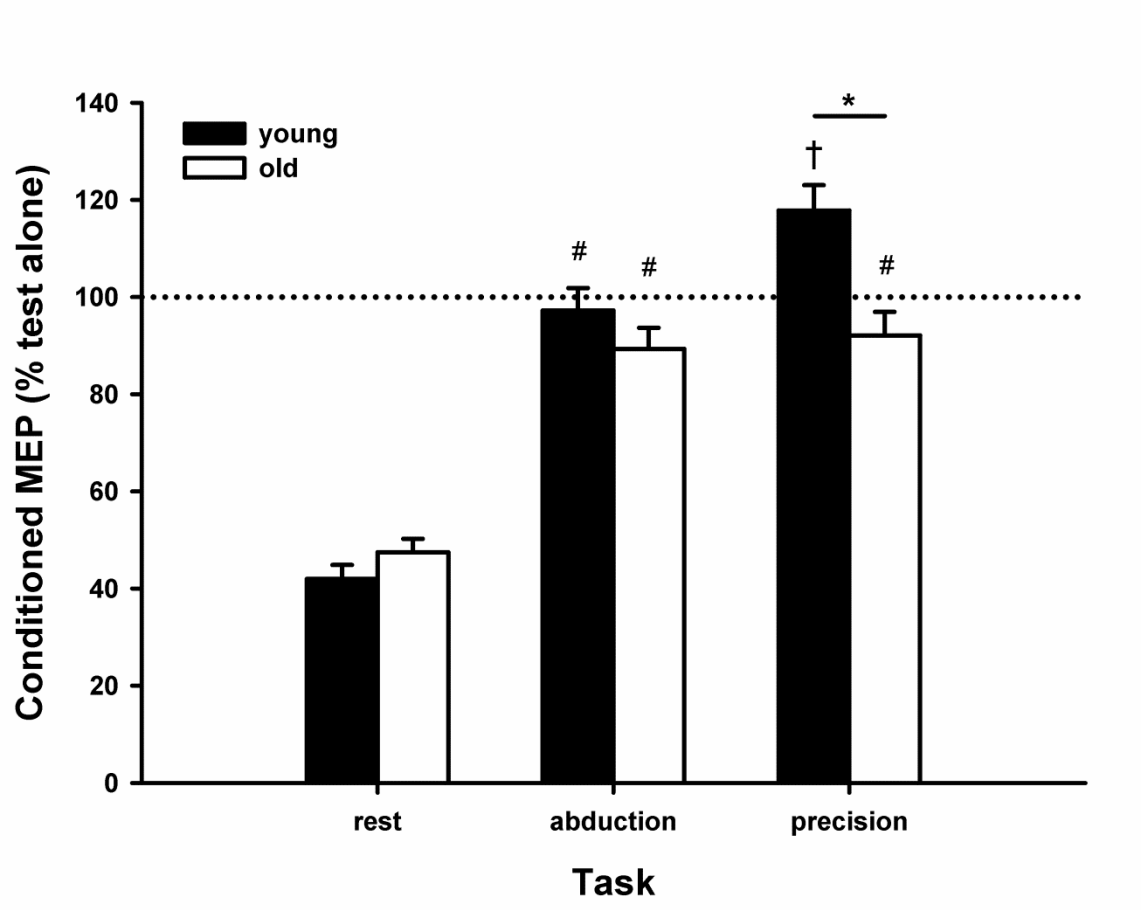
young
old
Conditioned MEP (% test alone)
140
120
100
80
60
40
20
0
*
†
#
#
#
rest
abduction
precision
Task

Figure 1.

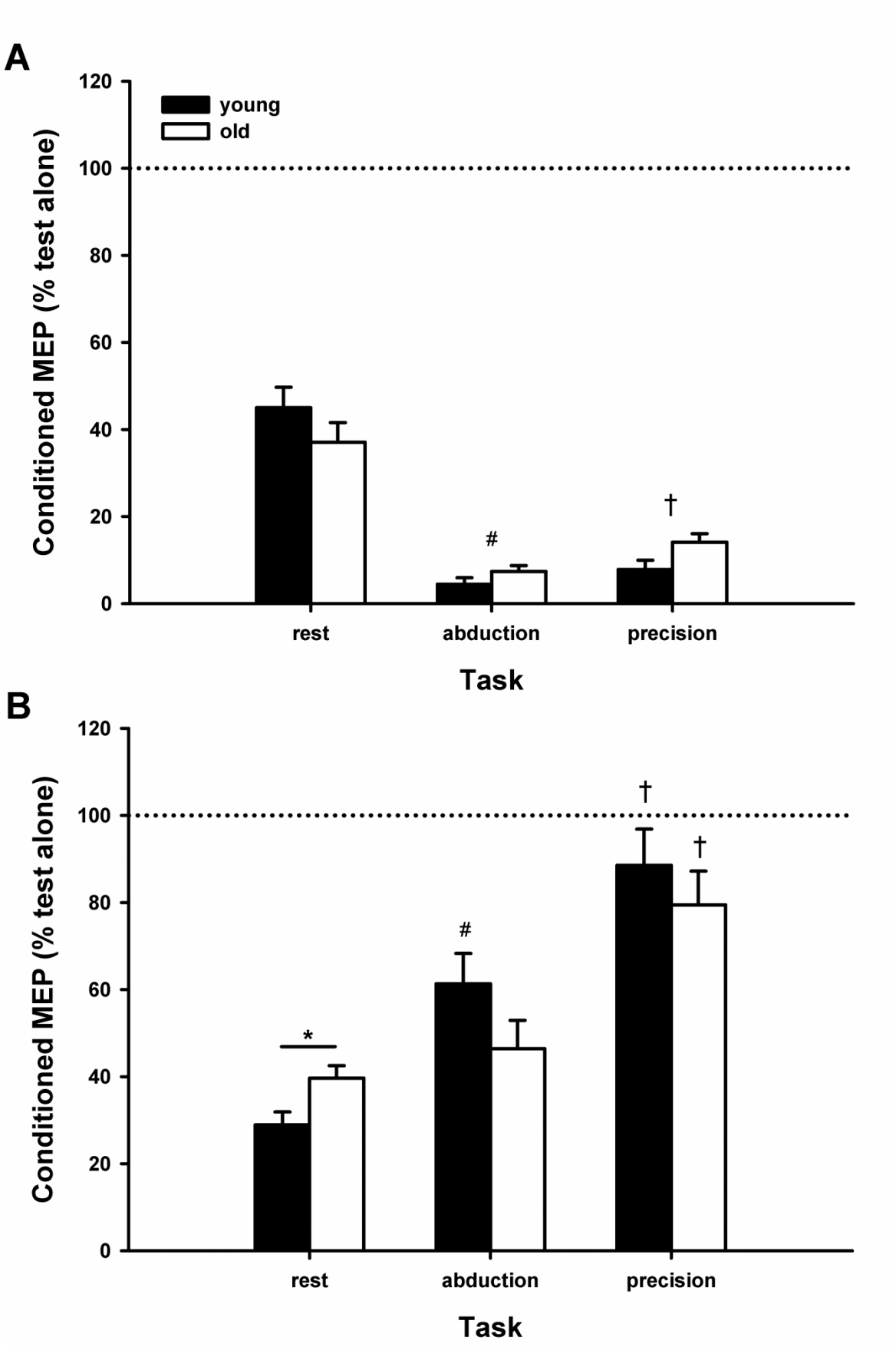
A
young
old
Conditioned MEP (% test alone)
120
100
80
60
40
20
0
#
†
rest
abduction
precision
Task
B
Conditioned MEP (% test alone)
120
100
80
60
40
20
0
†
†
#
*
rest
abduction
precision
Task

Figure 2.

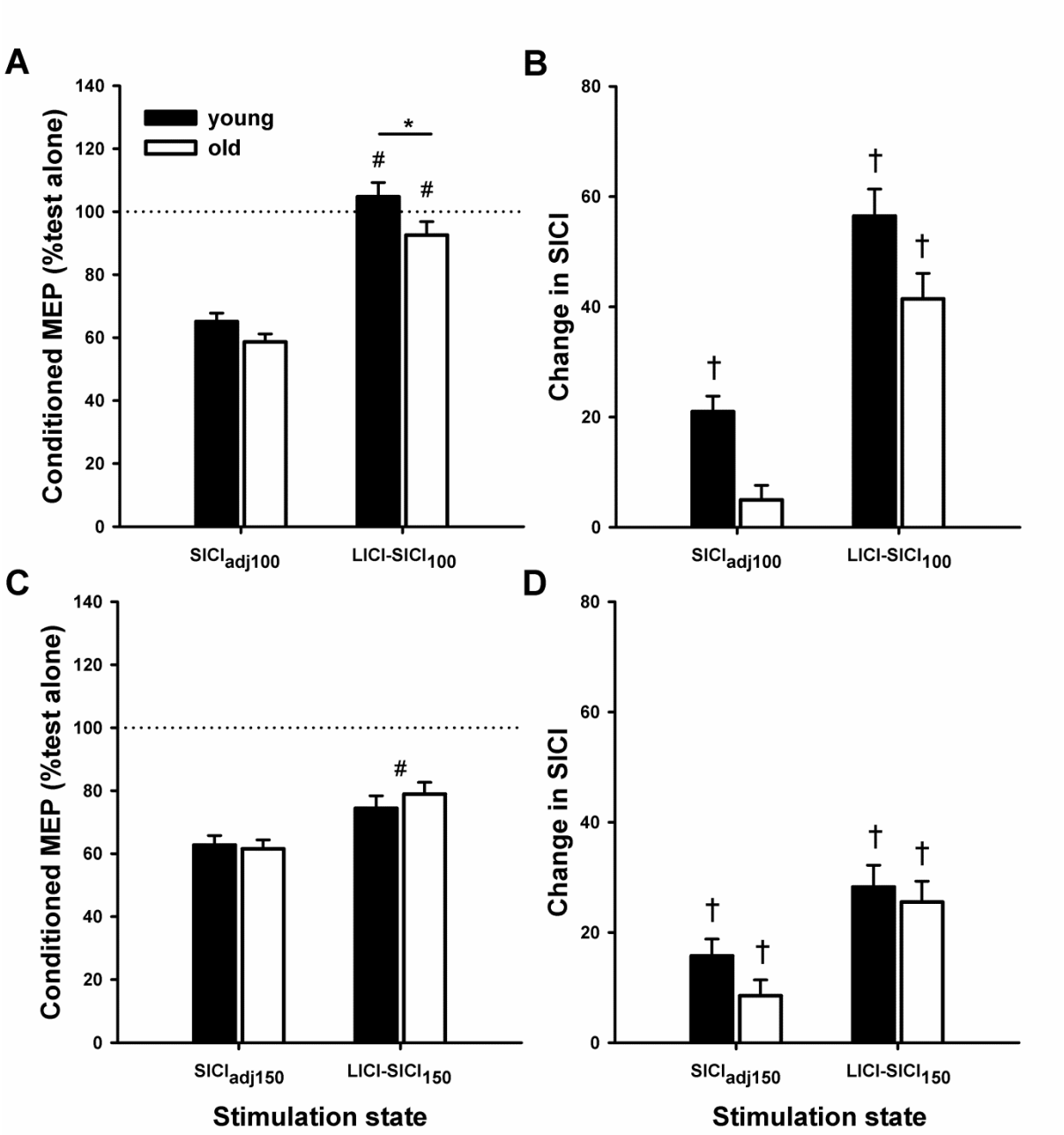
A
B
C
D
young
old
*
#
†
Conditioned MEP (%test alone)
Change in SICI
SICIadj100
LICI-SICI100
SICIadj150
LICI-SICI150
Stimulation state
0
20
40
60
80
100
120
140

Figure 3.

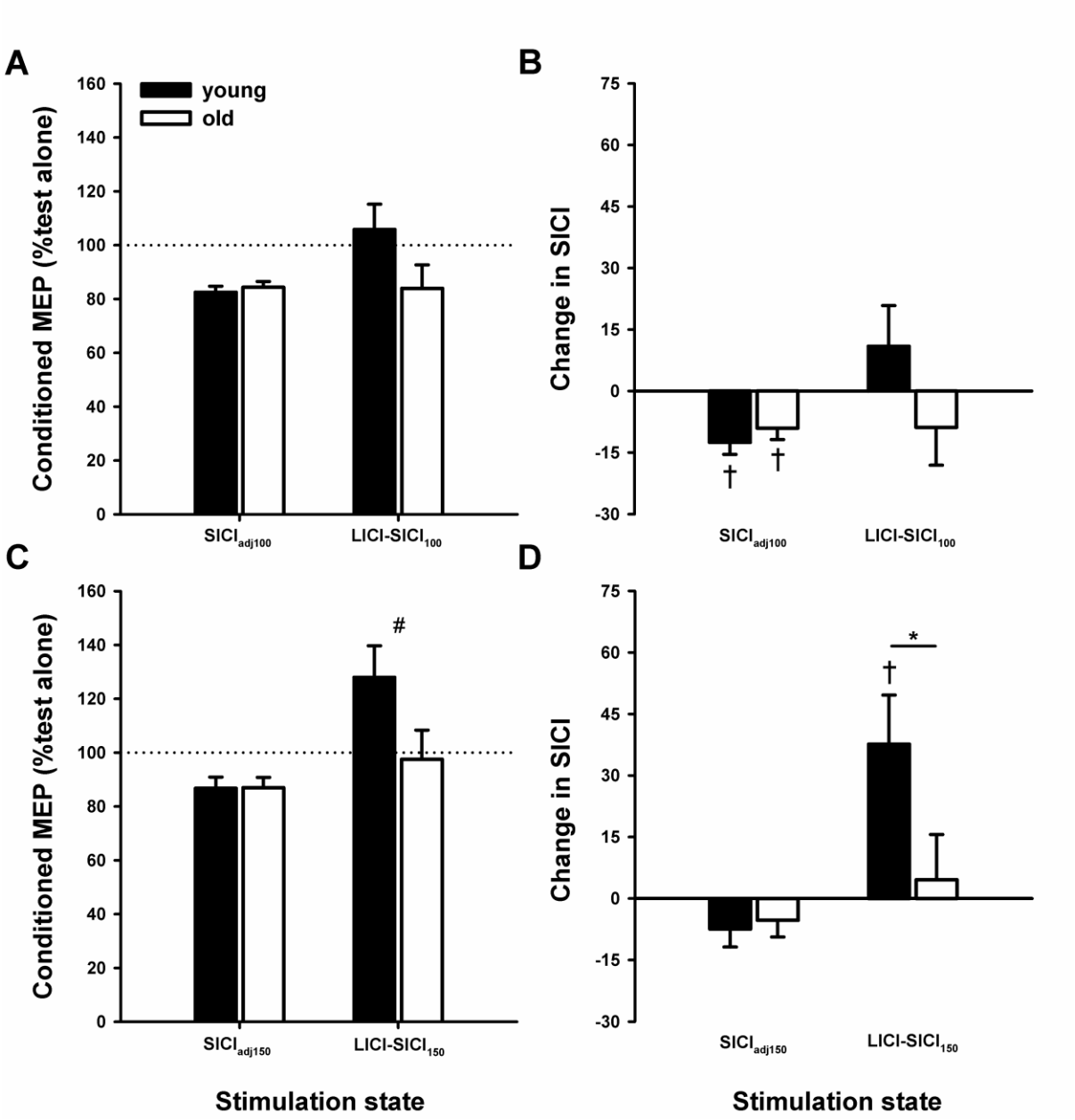
A
B
C
D
young
old
Conditioned MEP (%test alone)
Change in SICI
SICI adj100
LICI-SICI 100
SICI adj150
LICI-SICI 150
Stimulation state
#
*
†

Figure 4

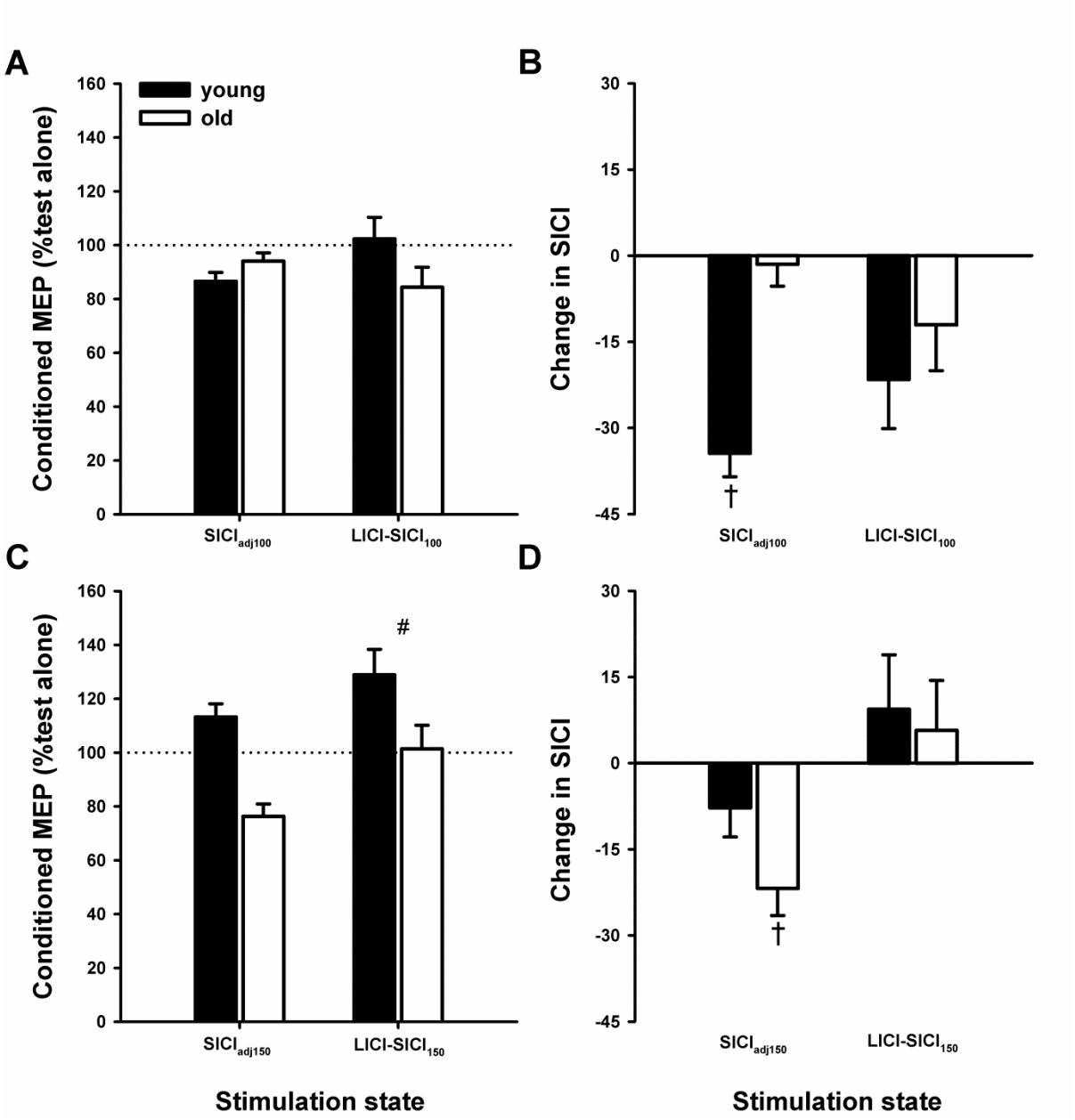
A
B
C
D
young
old
Conditioned MEP (%test alone)
Change in SICI
SICIadj100
LICI-SICI100
SICIadj150
LICI-SICI150
#
†
Stimulation state

Figure 5

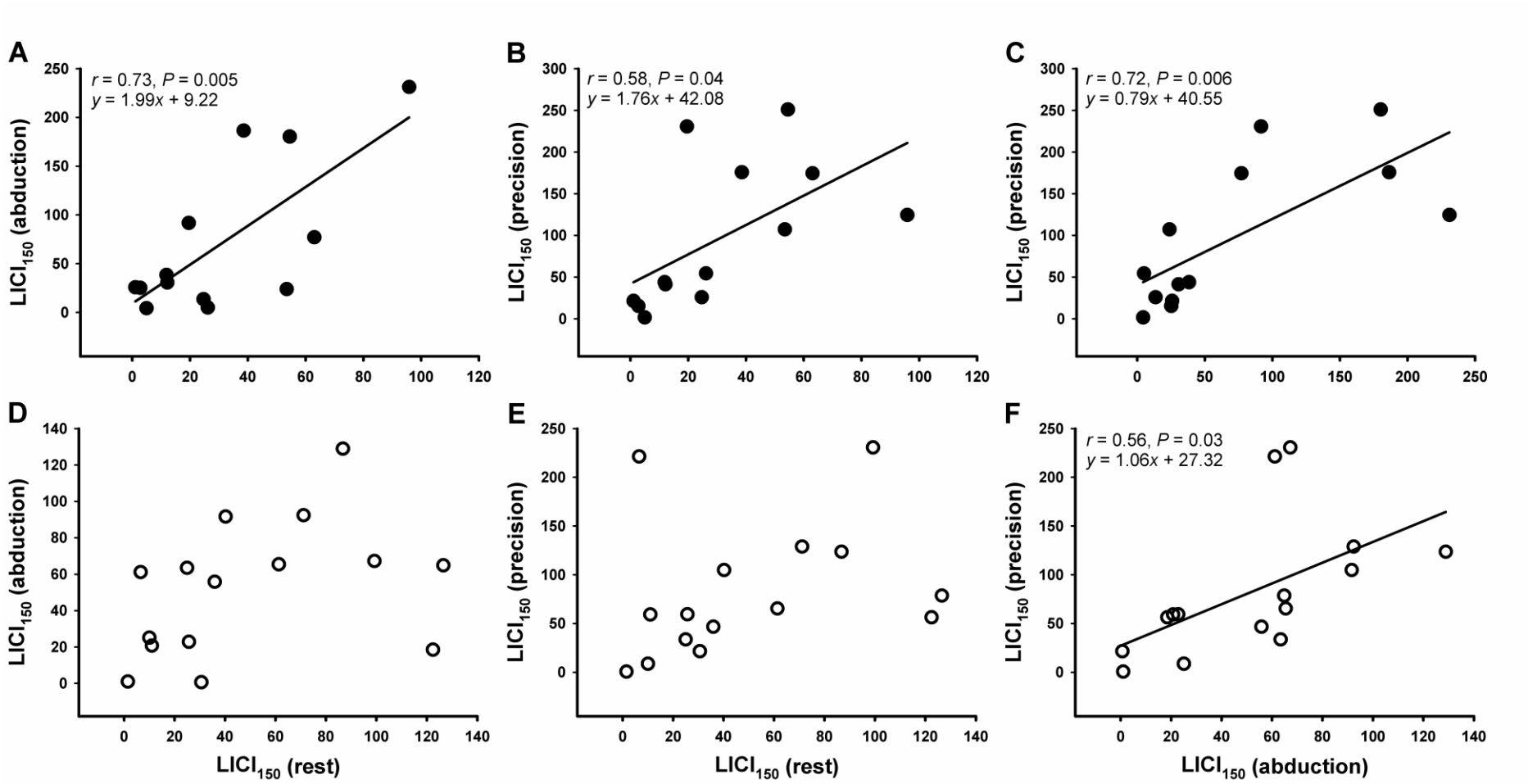
A
r = 0.73, P = 0.005
y = 1.99x + 9.22
LICI150 (abduction)
B
r = 0.58, P = 0.04
y = 1.76x + 42.08
LICI150 (precision)
C
r = 0.72, P = 0.006
y = 0.79x + 40.55
LICI150 (precision)
D
LICI150 (abduction)
LICI150 (rest)
E
LICI150 (precision)
LICI150 (rest)
F
r = 0.56, P = 0.03
y = 1.06x + 27.32
LICI150 (precision)
LICI150 (abduction)

Figure 6