Articles in PresS. J Neurophysiol (April 19, 2017). doi:10.1152/jn.00199.2017

GABA and primary motor cortex inhibition in young and older adults: a multimodal reliability study

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Running head: Ageing effects on intracortical inhibition

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Abstract

The effects of healthy ageing on gamma-aminobutyric acid (GABA) within primary motor cortex (M1) remain poorly understood. Studies have reported contrasting results, potentially due to limitations with the common assessment technique. The aim of the present study was to investigate the effect of healthy ageing on M1 GABA concentration and neurotransmission using a multimodal approach. Fifteen young and 16 older adults participated in this study. Magnetic resonance spectroscopy (MRS) was used to measure M1 GABA concentration. Single-pulse and threshold tracking paired-pulse transcranial magnetic stimulation (TMS) protocols were used to examine cortical silent period duration, short- and long-interval intracortical inhibition (SICI, LICI) and late cortical disinhibition (LCD). The reliability of TMS measures was examined with intra-class correlation coefficient analyses. SICI at 1 ms was reduced in older adults (15.13 ± 2.59%) compared to young (25.66 ± 1.44%, $P = 0.002$). However, there was no age-related effect for cortical silent period duration, SICI at 3 ms, LICI or LCD (all $P > 0.66$). The inter-session reliability of threshold tracking measures was good-to-excellent for both young (range 0.75 – 0.96) and older adults (range 0.88 – 0.93). Our findings indicate that extrasynaptic inhibition may be reduced with advancing age, whereas GABA concentration and synaptic inhibition are maintained. Furthermore, MRS and threshold tracking TMS provide valid and reliable assessment of M1 GABA concentration and neurotransmission respectively, in young and older adults.

New and noteworthy

Gamma-aminobutyric acid (GABA) in primary motor cortex was assessed in young and older adults using magnetic resonance spectroscopy and threshold tracking paired-pulse transcranial magnetic stimulation. Older adults exhibited reduced extrasynaptic inhibition (short-interval intracortical inhibition at 1 ms) compared to young, whilst GABA concentration and synaptic inhibition were similar between age groups. We demonstrate that magnetic resonance spectroscopy and threshold tracking provide valid and reliable assessments of primary motor cortex GABA concentration and neurotransmission respectively.

Keywords: ageing, magnetic resonance spectroscopy, transcranial magnetic stimulation, gamma-aminobutyric acid, intracortical inhibition

Introduction

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter within primary motor cortex (M1), and plays an important role in optimising corticomotor output during functional tasks (Stinear and Byblow 2003; Zoghi et al. 2003). Deficits in motor performance accompany advancing age (Bedard et al. 2002; Calautti et al. 2001), which may, in part, be attributed to altered GABAergic neurotransmission (Levin et al. 2014). GABA-mediated inhibition is also important in the modulation of cortical plasticity (Cash et al. 2016; Ziemann et al. 2001) and may contribute to an age-related diminished capacity of processes important for synaptic plasticity (Zimerman and Hummel 2010). However, the effects of healthy ageing on human M1 GABAergic inhibition remain poorly understood, potentially due to limitations with common assessment techniques.

In humans, GABA concentration can be quantified non-invasively using magnetic resonance spectroscopy (Mescher et al. 1998), which uses precisely timed radio frequency pulses to excite hydrogen nuclei within various neurochemicals (Mullins et al. 2014). Frequency spectra are plotted, with individual peaks reflecting the quantity of individual chemicals within the cortical region of interest (Figure 1). There is evidence that GABA concentrations are reduced in older adults compared with young in frontal and parietal cortices (Gao et al. 2013). This finding indicates that there may be a global reduction in cortical GABA concentration with advancing age. However, whether an age-related reduction in GABA is present specifically within M1 remains unknown.

GABA has a distinct affinity for two receptor sub-types, $GABA_A$ and $GABA_B$, which can be assessed using precisely timed paired-pulse transcranial magnetic stimulation (TMS) protocols. Short-interval intracortical inhibition (SICI) is examined by delivering a sub-threshold conditioning stimulus before a supra-threshold test stimulus at short (1-5 ms) interstimulus intervals, and reflects postsynaptic $GABA_A$ receptor-mediated M1 intracortical

inhibiton (Kujirai et al. 1993). Measures of extrasynaptic (Stagg et al. 2011b) and synaptic (Ziemann et al. 1996) $GABA_A$ activity are obtained at intervals of 1 ($SICI_{1ms}$) and 3 ms ($SICI_{3ms}$) respectively, and are mechanistically distinct. Long-interval intracortical inhibition (LICI) is examined by delivering two supra-threshold stimuli at longer interstimulus intervals (100–250 ms), and is a marker of postsynaptic $GABA_B$ activity (McDonnell et al. 2006). Late cortical disinhibition (LCD) may also be evident at the end of the LICI period, providing a marker of presynaptic $GABA_B$ activity (Cash et al. 2010). Therefore, specific conditioning intensities and intervals permit investigation of $GABA_A$- and $GABA_B$–mediated networks in M1.

Conventional paired-pulse TMS uses constant stimulation parameters to probe GABAergic function. In contrast, the threshold tracking TMS (Fisher et al. 2002; Vucic et al. 2006) adjusts the stimulator intensity to maintain a target motor evoked potential (MEP) amplitude in the presence of the conditioning stimulus. Threshold tracking reduces the confound of MEP variability associated with conventional paired-pulse methods (Kiers et al. 1993). However, both conventional and threshold tracking techniques are similar in their modes of action (Cirillo and Byblow 2016; Fisher et al. 2002; Murase et al. 2015; Vucic et al. 2006). There are contrasting results about the effect of healthy ageing on GABAergic inhibition from studies using conventional paired-pulse TMS (Cirillo et al. 2011; McGinley et al. 2010; Oliviero et al. 2006; Opie et al. 2015; Opie and Semmler 2014; Peinemann et al. 2001; Rogasch et al. 2009; Sale et al. 2015; Smith et al. 2009). Motor evoked potentials are more variable in older versus younger participants (Pitcher et al. 2003). For this reason, threshold tracking may offer a preferable alternative to examine M1 inhibition in the elderly.

The aims of the present study were two-fold. The first was to investigate the effect of healthy ageing on GABA concentration and $GABA_A$- and $GABA_B$-mediated inhibition within M1 using MRS and threshold tracking TMS respectively. We hypothesised that

overall inhibitory tone would be reduced in older adults compared to young. Secondly, we evaluated the inter-session reliability of threshold tracking in both young and older adults.

Methods

Participants

Fifteen neurologically healthy young (4 females, mean age 25 ± 1 years, range 20 – 31 years) and 16 older (7 females, mean age 70 ± 2 years, range 62 – 83 years) adults participated in this study. All participants were right-handed as assessed by the short version of the Edinburgh Handedness Inventory (Veale 2014), with a mean Laterality Quotient of 89 ± 3 (range 70 – 100) for young adults and 99 ± 1, (range 92 – 100) for older adults. Participants completed a transcranial magnetic stimulation safety screening questionnaire that was developed by our institution based on a previous report (Keel et al. 2001), which was screened by a neurologist before participation. Each participant provided written informed consent and the study was approved by the University of Auckland Human Participants Research Ethics Committee.

Experimental Design

There were three experimental sessions. In the first session, whole brain structural images and M1 metabolite concentrations were acquired using magnetic resonance imaging and MRS respectively. In sessions two and three, single- and paired-pulse TMS were used to assess measures of corticomotor excitability and the reliability of threshold tracking. The grooved pegboard task was used to assess manual dexterity in session two. Sessions one and two were separated by a mean of 9 days (range 1 - 23 days) for young adults, and 8 days (range 2 - 15 days) for the older adults. Sessions two and three were separated by a mean of 6 days (range 2 - 7 days) for the young adults, and 7 days (range 3 - 16 days) for the older adults.

Neuroimaging procedures

Magnetic resonance imaging

A Siemens Magnetom Skyra 3T scanner and 20-channel head coil (Siemens, Germany) were used for the neuroimaging session. T1-weighted whole-brain structural images were acquired using 1 x 1 x 1 mm voxels and a 256 mm field of view (TR = 1900 ms, TE = 2.07 ms).

Magnetic resonance spectroscopy

The T1-weighted structural images were used to manually place an 18 x 18 x 18 mm voxel of interest over the left precentral hand knob (Figure 1A), tangential to the cortical surface. Spectral GABA editing and simultaneous water suppression was then performed using the MEGA-PRESS sequence (TR = 1500 ms, TE = 68 ms, 96 averages) (Mescher et al. 1998). A selective double-banded 180° pulse was created from 20 ms Gaussian pulses. The frequency of the first band of this pulse was set to 4.7 ppm for suppression of water. The second band was alternated between 1.9 ppm (ON condition) and 7.5 ppm (OFF condition). The difference spectra (DIFF) between the ON and OFF conditions reveals an edited GABA spectrum without the larger overlapping creatine (Cr) resonance. Representative ON, OFF and DIFF spectra are shown in Figure 1B.

Recording and stimulation procedures

Surface electromyography

Surface electromyography (EMG) was recorded from the *first dorsal interosseous* (FDI) of the dominant right hand using 10 mm diameter Ag-AgCl recording electrodes (Ambu, Ballerup, Denmark), arranged in a belly-tendon montage. A 20 mm diameter ground surface electrode (3M, Canada Health Care) was positioned on the dorsum of the right hand. EMG signals were amplified (1000×) and band-pass filtered (10 – 1000 Hz) using a CED1902 amplifier (CED, Cambridge, UK), sampled at 2 kHz using a CED1401 interface

(CED, Cambridge, UK) and recorded onto a computer for offline analysis using Signal (Version 5.03, CED, Cambridge, UK) software.

Transcranial magnetic stimulation

A MagPro X100+option magnetic stimulator (MagVenture, Farum, Denmark) connected to a figure-of-eight coil (MC-B70, outer wing diameter 97 mm) was used to deliver TMS. The coil was held tangentially to the scalp, handle posterior, approximately 45° to the mid-sagittal line, to induce posterior-anterior current flow in the brain (Sakai et al. 1997) using a monophasic waveform (pulse width = 70 μs). The optimal site to elicit consistent MEPs in the resting right FDI muscle was marked on the scalp over the left hemisphere. TMS was delivered at 0.2 Hz, with 20% variation between trials, and optimal coil position was continually monitored throughout the experiment.

Rest motor threshold (RMT) was defined as the minimum stimulus intensity required for eliciting MEPs of at least 50 μV in amplitude, in four out of eight trials. Active motor threshold (AMT) was defined as the minimum stimulus intensity required for eliciting MEPs of at least 100 μV in amplitude, in four out of eight trials during a low-level voluntary contraction (approximately 10% of maximum voluntary contraction). Measures of cortical silent period duration were obtained while the participant maintained a 10% maximum voluntary contraction. The stimulation intensity was set to 130% RMT and 16 responses were acquired for each participant.

Protocol

Threshold tracking a target MEP amplitude of 200 μV (± 20%) was used to quantify the extent of inhibition and disinhibition in M1 in line with previous work (Cirillo and Byblow 2016; Fisher et al. 2002; Vucic et al. 2006). Similar to RMT and AMT, the threshold tracking target (TTT) was defined as the minimum stimulus intensity required for eliciting

MEPs of at least 160 μV in amplitude, in four out of eight trials (Cirillo and Byblow 2016). The TTT was determined before and after each paired-pulse protocol.

Short-interval intracortical inhibition

To investigate $SICI_{1ms}$ and $SICI_{3ms}$, four conditioning intensities were used ranging from 50–95% AMT in 15% AMT steps. In the presence of conditioning, the test stimulus intensity was increased or decreased in 1% maximum stimulator output steps until the TTT was reached. Tracking was deemed successful when the conditioned MEP was above or within 20% of the TTT in two out of three consecutive trials (Cirillo and Byblow 2016). Due to the short ISI, a half-sine waveform (pulse width = 70 μs) was used for $SICI_{1ms}$. Both AMT and TTT were independently determined with a half-sine waveform for $SICI_{1ms}$.

Long-interval intracortical inhibition and late cortical disinhibition

Long-interval intracortical inhibition (LICI) and late cortical disinhibition (LCD) were investigated using seven interstimulus intervals (100, 160, 180, 200, 220, 240, and 260 ms). The conditioning stimulus was set to 130% RMT. Identical to the SICI protocol, the test intensity was increased or decreased by 1-2% MSO until the TTT was achieved.

Data analysis

Neuroimaging

MRS data were processed using the Java Magnetic Resonance User Interface (jMRUI) (Naressi et al. 2001). First, the free induction decay signal was corrected for any non-zero DC offset and smoothed using a 5 Hz Lorentzian filter (Blicher et al. 2015). Next, the residual water peak was filtered using the Hankel-Lanczos singular value decomposition filter. Zero-order phase correction was then manually applied to correct for peak distortion.

Spectral analysis was carried out in the time-domain using AMARES, a non-linear least square fitting optimisation algorithm (Vanhamme et al. 1997). The OFF spectrum was analysed first, with peak fitting performed using a fixed Gaussian function to obtain

linewidths for N-acetylaspartate (NAA) and Cr. For the DIFF spectrum, a single Gaussian curve was first fitted to the inverted NAA resonance, with the linewidth constrained to that of NAA in the OFF spectrum. Peak fitting the GABA resonance was then performed using two Gaussian curves, with the linewidths separately constrained to that of the Cr resonance from the OFF spectrum (Stagg et al. 2011a). Additionally, peak fitting for the co-edited Glx (glutamate + glutamine) resonance was performed in an identical manner. Total amplitude for GABA and Glx was obtained by summing the amplitudes of the two GABA and two Glx peaks respectively.

T1-weighted structural images were extracted using the Brain Extraction Tool, and segmented using FMRIB's Automated Segmentation Tool. The relative quantities of grey matter (GM), white matter (WM) and cerebrospinal fluid within the voxel were then calculated for each participant. The NAA and Cr amplitudes were corrected for the proportion of total brain tissue volume (GM + WM) within the voxel, and the GABA and Glx amplitudes corrected for the proportion of GM volume within the voxel (Stagg et al. 2011a). GABA and Glx concentrations were then calculated as ratios, using the corrected GABA and Glx amplitudes relative to the corrected Cr amplitude, and the simultaneously acquired and corrected NAA amplitude (Gao et al. 2013).

Neurophysiology

The amplitude of the first MEP from the LICI/LCD protocol was used as a measure of corticomotor excitability. Semi-automated methods were used to measure cortical silent period duration. The EMG signal was rectified and cortical silent period duration was assessed from the point of stimulation until the resumption of EMG activity levels equal to or greater than pre-trigger root mean squared (rms) EMG (pre-trigger rmsEMG window of 50 ms; 5 to 55 ms before the stimulus). Trials in which participants were not able to maintain the contraction through the perturbation of the stimulus were excluded.

For threshold tracking, trials that were contaminated by pre-stimulus EMG activity (rmsEMG >10 μV; 50 ms before stimulation) were rejected online and repeated immediately. $SICI_{1ms}$, $SICI_{3ms}$, LICI and LCD induced by the CS were quantified as the percentage increase or decrease in test stimulus intensity required to evoke the TTT (Fisher et al. 2002):

$$Threshold\ change\ (\%) = \frac{(Conditioned\ Intensity - Test\ Intensity)}{Test\ Intensity}\ x\ 100$$

where positive values indicate inhibition and negative values indicate disinhibition. For both $SICI_{1ms}$ and $SICI_{3ms}$, the largest threshold change value amongst conditioned stimulus intensities was determined as maximum inhibition for each participant. Inhibition at an ISI of 100 ms was selected for LICI, whereas the maximum disinhibition observed between the ISIs of 160 and 260 ms was used to index LCD.

Statistical analysis

Normality was assessed using the Shapiro-Wilk's test and homoscedasticity of variance using the Levene's test of equality and Mauchly's test of sphericity. Non-normal data were log transformed. Independent samples t-tests were used to analyse the effect of AGE (Young, Older) on voxel GM%, WM%, GABA and Glx concentrations and grooved pegboard task completion times.

A two-way mixed effects repeated measures ANOVA was performed to determine the effect of AGE (Young, Older) and TMS SESSION (One, Two) on RMT, AMT, TTT, MEP amplitude, cortical silent period duration, $SICI_{1ms}$, $SICI_{3ms}$, LICI, and LCD. Additional one-sample t-tests (hypothesized mean = 0) were performed for $SICI_{1ms}$, $SICI_{3ms}$, LICI, and LCD to confirm significant inhibition/disinhibition for both age groups.

Inter-session reliability of threshold tracking TMS was assessed using intra-class correlation coefficients (ICC). Reliability estimates were judged as either fair (0.40 – 0.58), good (0.59 – 0.74) or excellent (>0.75) (Cicchetti and Sparrow 1981).

Pearson correlation analyses were used to investigate the relationship between metabolite concentrations, MEP amplitude, inhibition measures and manual dexterity. The significance level was set at $P < 0.05$ and group data are presented as mean ± SEM in the text.

Results

Participants completed all three experimental sessions, with no adverse events. MEPs could not be elicited in the right FDI muscle of one older adult. Analysis of the grooved pegboard task data revealed that time to complete a single trial was slower in older adults (78.76 ± 3.27s) compared to young (60.63 ± 2.27s, $P < 0.001$).

Magnetic resonance spectroscopy

No differences in voxel GM% and WM%, GABA:Cr, GABA:NAA, Glx:Cr or Glx:NAA were observed between young and older adults (all P > 0.10; Table 1).

Transcranial magnetic stimulation

There were no main effects of AGE or TMS SESSION, and no interaction for RMT, AMT, TTT, MEP amplitude and cortical silent period duration (all $P > 0.12$).For max $SICI_{1ms}$, data from one young participant was deemed an outlier (2 SD outside of the mean) and excluded from analysis. There was a main effect of AGE ($F_{1, 27} = 12.14$, $P = 0.002$) for $SICI_{1ms}$, revealing the extent of inhibition was reduced in older adults compared to young (Figure 3A). There was no main effect of TMS SESSION and no interaction (both $P > 0.57$). No main effects of AGE or TMS SESSION, and no interactions were observed for $SICI_{3ms}$, LICI and LCD (all $P > 0.13$; Figure 3B-D). One-sample t-tests showed that inhibition/disinhibition was present for all paired-pulse TMS protocols in both young (all $P < 0.008$) and older (all $P < 0.037$) adults.

ICC values for threshold tracking $SICI_{1ms}$, $SICI_{3ms}$, LICI and LCD are displayed in Table 2. There was good-to-excellent inter-session reliability for all paired-pulse TMS measures in both young (range 0.75 – 0.96) and older adults (range 0.88 – 0.93).

Linear regression

A negative correlation was observed between GABA concentration and max $SICI_{1ms}$ for young adults (Figure 4A and C), where individuals with higher GABA concentration exhibited lower $SICI_{1ms}$ (GABA:Cr r = -0.55, P = 0.043; GABA:NAA r = -0.65, P = 0.012). There was a positive correlation between GM quantity and GABA concentration (GABA:Cr r = 0.98, P < 0.001; GABA:NAA r = 0.93, P < 0.001) and a trend for an association between GM quantity and max $SICI_{1ms}$ (r = -0.52, P = 0.06). Partial correlation analyses with GM quantity as a controlling variable revealed no association between GABA concentration and max $SICI_{1ms}$ (GABA:Cr r = -0.17, P = 0.59; GABA:NAA r = -0.51, P = 0.08). No other correlations between GABA or Glx concentrations and single- or paired-pulse TMS measures were observed for young (all P > 0.12) or older adults (all P > 0.11). Similarly, there were no associations between manual dexterity and metabolite concentrations or paired-pulse TMS measures for either age group (all P > 0.26).

Discussion

The present study investigated the effect of healthy ageing on M1 GABA concentration and GABAergic neurotransmission, and the reliability of threshold tracking. Overall, $SICI_{1ms}$ was reduced in older adults compared to young, but GABA concentration and other measures of GABAergic neurotransmission were not significantly different between age groups. GABA concentration was negatively correlated with $SICI_{1ms}$ in young but not older adults. Threshold tracking had good-to-excellent inter-session reliability in both young and older adults. These findings indicate that M1 GABA concentration and synaptic

$GABA_A$ and $GABA_B$ activity are maintained with advancing age, whereas extrasynaptic $GABA_A$ activity is reduced.

M1 GABA concentration is maintained with advancing age

GABA concentration within M1 were similar between young and older adults. This finding is in contrast with a previous study which observed lower GABA in the frontal and parietal cortices with advancing age (Gao et al. 2013). This discrepancy between the current study and Gao et al. (2013) may highlight the non-uniform distribution of GABA concentration across the human cortex (Greenhouse et al. 2016). Alternatively, methodological differences in scanning parameters that influence the observed signal, such as the number of averages collected and voxel size (Mullins et al. 2014), may contribute to the disparate findings. A limitation of the present study is a small voxel (18 mm^3) was used to optimise the recorded signal for the hand knob region of M1. Reducing voxel size can be detrimental to the inherently low signal-to-noise ratio associated with quantifying GABA (Mullins et al. 2014). Therefore, scanning parameters and regional variations in metabolite concentrations should be carefully considered in future MRS studies investigating age-related effects.

Healthy ageing influences GABAergic neurotransmission differentially

The extent of age-related changes in M1 intracortical inhibiton with TMS are unclear. The current study showed that $SICI_{1ms}$ was reduced in older adults using threshold tracking, which supports a previous conventional TMS study (Peinemann et al. 2001). However, this finding contrasts recent studies investigating $SICI_{1ms}$ in older adults (Shibuya et al. 2016; Smith et al. 2009). Shibuya et al. (2016) assessed $SICI_{1ms}$ with threshold tracking, across a broad age spectrum (20 – 83 years), and demonstrated that extent of inhibition was not altered with advancing age. A key difference between the current study and Shibuya et al. (2016) was the intensity of the CS. Shibuya and colleagues (2016) used a single CS intensity

(70% of TTT), whereas the current study determined maximum $SICI_{1ms}$ for each participant over a range of conditioning stimulus intensities (50–95% AMT; steps of 15%). It is advantageous to use multiple conditioning intensities because the profile of the $SICI_{1ms}$ curve may differ between individuals (Smith et al. 2009). Interestingly, Smith et al. (2009) observed more $SICI_{1ms}$ in older adults with a conditioning intensity set to 5% maximum stimulator output below AMT. However, AMT was higher in older adults compared with young and when CS intensities were set relative to AMT for both groups, the age-related increase in inhibition was not evident (Smith et al. 2009). Therefore, utilising multiple conditioning intensities that are set relative to the threshold of an individual, is likely to be advantageous in detecting age-related changes in SICI.

The interpretation of $SICI_{1ms}$ can be somewhat controversial. One proposition is that the inhibition reflects neuronal refractoriness due to activation of low threshold interneurons by the conditioning (Fisher et al. 2002). However, increasing the conditioning intensity reduces $SICI_{1ms}$, eventually leading to facilitation (Vucic et al. 2009). If neuronal refractoriness was solely responsible for $SICI_{1ms}$, then greater inhibition would be expected with higher conditioning intensities due to subliminal activation of a larger population of interneurons (Vucic et al. 2009). Alternatively, $SICI_{1ms}$ may reflect extrasynaptic $GABA_A$ activity (Stagg et al. 2011b; Vucic et al. 2009). Extrasynaptic $GABA_A$ receptors have high sensitivity to ambient extracellular GABA (Belelli et al. 2009), and regulate cortical excitability through tonically active inhibition (Walker and Semyanov 2008). The level of tonic inhibition is likely to be a key factor in neurorehabilitation, with animal models showing reduced inhibition in the subacute phase after stroke promotes motor recovery (Clarkson et al. 2010). Therefore, a better understanding of the mechanism(s) underlying $SICI_{1ms}$ and identifying how healthy ageing effects inhibitory tone within M1 may have key implications in older adults, typical of the age requiring neurorehabilitation after stroke.

While older adults exhibited reduced extrasynaptic $GABA_A$ activity, threshold tracking TMS of synaptic $GABA_A$ and $GABA_B$ activity were similar between young and older adults. This finding coincides with the majority of previous studies investigating age-related effects using conventional TMS (Cirillo et al. 2010; Cirillo et al. 2011; Oliviero et al. 2006; Rogasch et al. 2009; Smith et al. 2009). However, an increase (McGinley et al. 2010; Sale et al. 2015) and decrease (Heise et al. 2013; Opie and Semmler 2014; Peinemann et al. 2001) in synaptic GABAergic neurotransmission has also been reported in older adults. Interestingly, Sale et al. (2015) showed that $SICI_{3ms}$ was greater in older adults than young when using anterior-posterior current flow in M1, but not posterior-anterior. Although not utilized in the present study, threshold tracking with an antieror-posterior induced current may provide a more robust and sensitive measure of $SICI_{3ms}$ than with a posterior-anterior current (Cirillo and Byblow 2016). It has been shown previously SICI at 3 ms is more robust than 2 ms when using threshold-tracking (Murase et al. 2015). In the present study, the conditioning intensities used to assess $SICI_{3ms}$ were below the level where short-interval intracortical facilitation has been shown to interact with $SICI_{3ms}$ (Peurala et al. 2008). For these reasons, there is no reason to suspect that the absence of an age-related effect for $SICI_{3ms}$ is due to contamination from facilitatory inputs. Whether the target muscle is voluntarily activated may help differentiate age-related changes in inhibition. For example, reduced $SICI_{3ms}$ in older adults was observed during voluntary activation but not resting conditions, whereas LICI was less in older adults at rest but not during muscle contraction (Opie and Semmler 2014). Future studies are required to investigate age-related changes in intracortical inhibition using threshold tracking with different induced currents, and during voluntary activation.

Here we present evidence that LCD is maintained with healthy ageing. To our knowledge, this study is the first to examine age-related effects on LCD, a proposed marker of presynaptic $GABA_B$ activity (Cash et al. 2010). We extend findings from previous studies

assessing LCD in young adults using conventional TMS (Cash et al. 2010; Cash et al. 2011; Caux-Dedeystere et al. 2015; Caux-Dedeystere et al. 2014) by demonstrating LCD in both young and older adults using threshold tracking. The presence of LCD is not always consistent at rest, and appears to be more prominent during voluntary activation of the target muscle (Caux-Dedeystere et al. 2015; Caux-Dedeystere et al. 2014). Although LCD was not assessed during voluntary activation in the present study, LCD was observed using threshold tracking by selecting the ISI where maximum disinhibition occurred for each participant. We suggest that LCD may be examined in future studies by using threshold tracking TMS and multiple ISIs.

Assessment of $SICI_{3ms}$ with threshold tracking shows good-to-excellent intra- and inter-session reliability in young adults (Samusyte et al. 2015). We extend these findings by showing that threshold tracking $SICI_{1ms}$, $SICI_{3ms}$, LICI and LCD have good-to-excellent inter-session reliability in both young and older adults. Conventional TMS also demonstrates good inter-session reliability for both $SICI_{3ms}$ and LICI in older adults (Schambra et al. 2015). Overall, Samusyte et al. (2015) found that intra- and inter-session reliability was better with threshold tracking than conventional TMS. The two techniques are presumed to reflect activity within the same cortical networks, and differ only in the extent to which they are effected by MEP variability. Our results demonstrate that threshold tracking is a valid and reliable technique to investigate M1 GABAergic neurotransmission in young and older adults.

GABA concentration and paired-pulse TMS measures

There was a negative association between GABA concentration and $SICI_{1ms}$ in young, but not older adults (i.e. young participants with higher GABA concentration exhibited less inhibition). Interestingly, this association was not observed when controlling for the proportion of GM within the voxel and therefore this finding must be interpreted with

caution. Our finding in young adults, and a recent similar finding (Dyke et al. 2017), contrast with a previous study demonstrating a positive relationship between MRS GABA and $SICI_{1ms}$ (Stagg et al. 2011b). Different paradigms to assess $SICI_{1ms}$ between the current study (maximum inhibition using threshold tracking) and Stagg et al. (2011b; slope of inhibition curve using conventional TMS) may explain the discrepant results. While $SICI_{1ms}$ was reduced in older adults, no age-related differences in GABA concentration within M1 was found. It is possible that age-related changes in intracellular GABA levels masks a decline in extrasynaptic GABA, which may account for the lack of relationship between $SICI_{1ms}$ and GABA concentration in older adults.

There were no associations between GABA concentration and TMS surrogate measures of synaptic $GABA_A$ ($SICI_{3ms}$) or $GABA_B$ (LICI, LCD and CSP) in young and old adults. These findings are consistent with previous studies focussing on healthy young cohorts (Stagg et al. 2011b; Tremblay et al. 2013). Limited sensitivity of MRS to synaptic GABA may account for the lack of a relationship between GABA concentration and paired-pulse TMS measures of synaptic GABA activity. GABA stores within the presynaptic bouton of inhibitory interneurons comprise approximately 30% of cortical GABA concentration (Petroff 2002), with the amount of GABA directly related to vesicular release (Golan et al. 1996). Improving the specificity of MRS assessments of GABA concentration will aid interpretation of data from combined MRS and paired-pulse TMS studies.

In summary, threshold tracking demonstrated that extrasynaptic $GABA_A$ activity may be reduced as a consequence of ageing. Conversely, GABA concentration and synaptic GABAergic activity may be maintained with ageing. Furthermore, threshold tracking with paired-pulse TMS is a reliable technique for assessing M1 GABAergic function. These findings may have implications for age-related conditions, such as stroke, where tonic inhibition plays an important role in motor recovery.

Acknowledgements: The authors would like to thank the Centre for Advanced MRI for assistance with data acquisition and Mr. Terry Corrin for technical assistance.

Disclosures: The authors declare no conflict of interest

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Tables

Table 1. Participant characteristics, MRS and single-pulse TMS data

	Age group		*P*-value
	Young	Older	
Number of participants	15 (4F)	16 (7F)	
Age (yrs)	24.6 (1.1)	70.3 (1.7)	<0.001
Magnetic resonance spectroscopy			
Voxel GM%	35.36 (2.00)	32.66 (1.12)	0.25
Voxel WM%	55.65 (2.85)	49.34 (2.32)	0.10
GABA:Cr	0.102 (0.008)	0.114 (0.008)	0.29
GABA:NAA	0.050 (0.005)	0.061 (0.005)	0.10
Glx:Cr	0.094 (0.008)	0.088 (0.007)	0.59
Glx:NAA	0.045 (0.004)	0.048 (0.005)	0.68
Transcranial magnetic stimulation			
RMT (%MSO)	49.2 (2.4)	50.7 (2.9)	0.70
AMT (%MSO)	38.8 (2.1)	39.9 (2.3)	0.73
TTT (%MSO)	53.1 (2.7)	54.7 (3.5)	0.72
MEP amplitude ($\log_{10mV}$)	0.19 (0.07)	0.15 (0.11)	0.77
Cortical silent period duration (ms)	180.1 (6.0)	176.2 (7.9)	0.70

Note: Values are mean ± SEM. *GM* – grey matter, *WM* – white matter, *GABA* – gamma-aminobutyric acid, *Cr* – creatine, *NAA* – N-acetylaspartate, *RMT* – rest motor threshold, *AMT* – active motor threshold, *TTT* – threshold tracking target.

Table 2. Intraclass correlation coefficients

	Age group	
Protocol	Young	Older
$SICI_{1ms}$	0.75	0.89
$SICI_{3ms}$	0.93	0.92
LICI	0.96	0.88
LCD	0.89	0.93

Note: *SICI* – short interval intracortical inhibition, *LICI* – long interval intracortical inhibition, *LCD* – late cortical disinhibition

558

Figure Captions

Figure 1. T1-weighted anatomical images were acquired to manually place an 18x18x18 mm voxel over the hand-knob region of left primary motor cortex (A). (B) Representative ON, OFF and edited (DIFF) spectra from a young participant showing respective creatine (Cr), N-acetylaspartate (NAA), gamma-aminobutyric acid (GABA) and glutamate + glutamine (Glx) peaks.

Figure 2. Example EMG traces depict motor evoked potentials (MEP) from an individual young participant. (A) TMS intensity required to elicit a fixed MEP amplitude (200 μV) to the single-pulse test stimulus (TS; threshold tracking target, TTT). (B and C) Short-interval intracortical inhibition (SICI; conditioning stimulus [CS] = 50–95 % AMT, 15% steps) at 1 and 3 ms respectively. (D) Long-interval intracortical inhibition (LICI; CS = 130% RMT, ISI = 100 ms). (E) Late cortical disinhibition (LCD; CS = 130 % RMT, ISI = 160–260 ms, 20 ms steps). Threshold tracking requires an increase or decrease in the TS intensity to evoke the target response in the presence of conditioning (grey traces in B, C, D and E).

Figure 3. Threshold tracking values obtained from each paired-pulse protocol. (A) Short-interval intracortical inhibition (SICI) at 1 ms was reduced in older adults compared to young in both TMS sessions. No differences in SICI at 3 ms (B), long-interval intracortical inhibition (C) or late cortical disinhibition (D) were observed between young and older adults in either session. In panels A-C greater inhibition is indicated upward. In panel D greater disinhibition is indicated downward. Data are presented as mean + SEM. N = 15 young and 15 older adults.

Figure 4. Correlation analyses between maximal short-interval intracortical inhibition at 1 ms ($SICI_{1ms}$) and magnetic resonance spectrometry GABA concentration relative to creatine (Cr) and N-acetylaspartate (NAA) in young (A and C) and older (B and D) adults. There was a

583 negative relationship in young adults, with higher GABA concentration associated with less

584 $SICI_{1ms}$. Greater inhibition is indicated upward. No relationship was observed in older adults.

585 N = 14 young and 15 older adults.

Figure 1

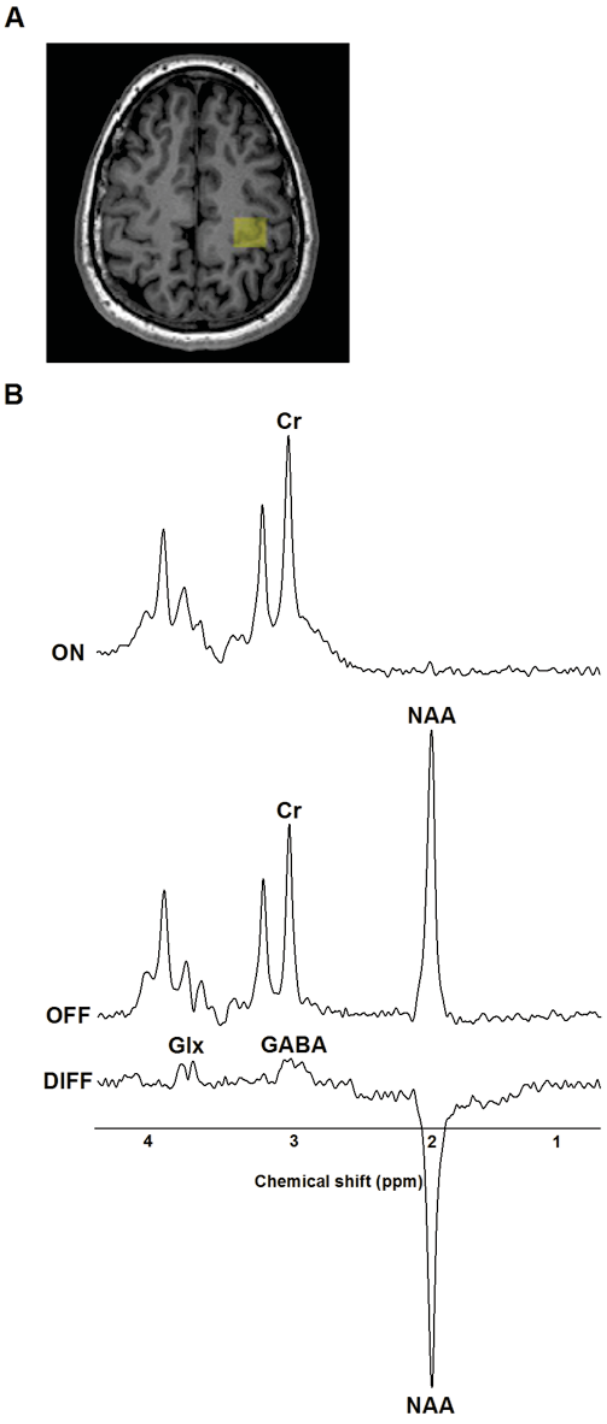


Figure 2

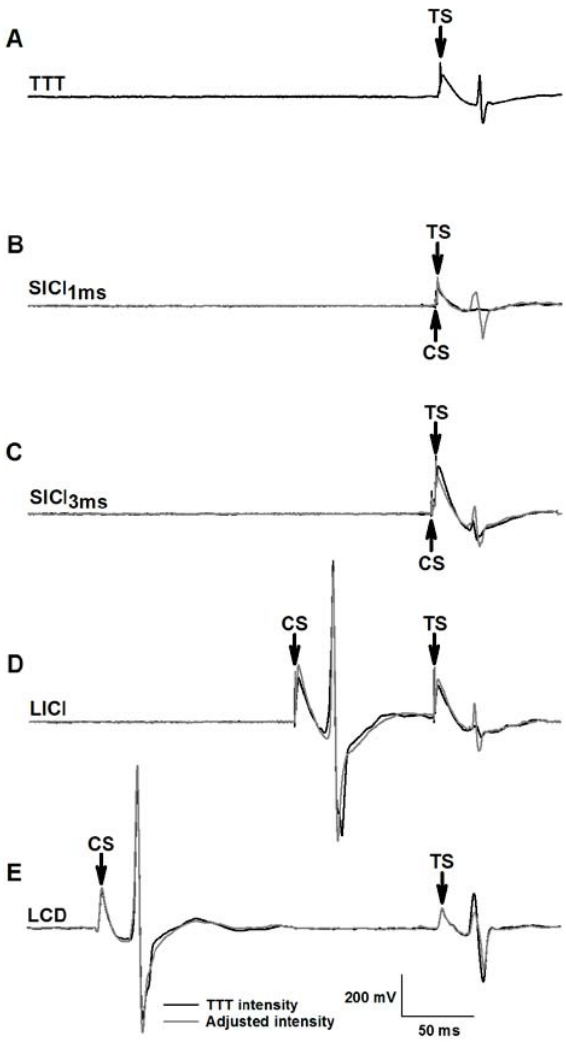

A
TTT
TS
B
SICI1ms
TS
CS
C
SICI3ms
TS
CS
D
LICI
CS
TS
E
LCD
CS
TS
TTT intensity
Adjusted intensity
200 mV
50 ms

Figure 3

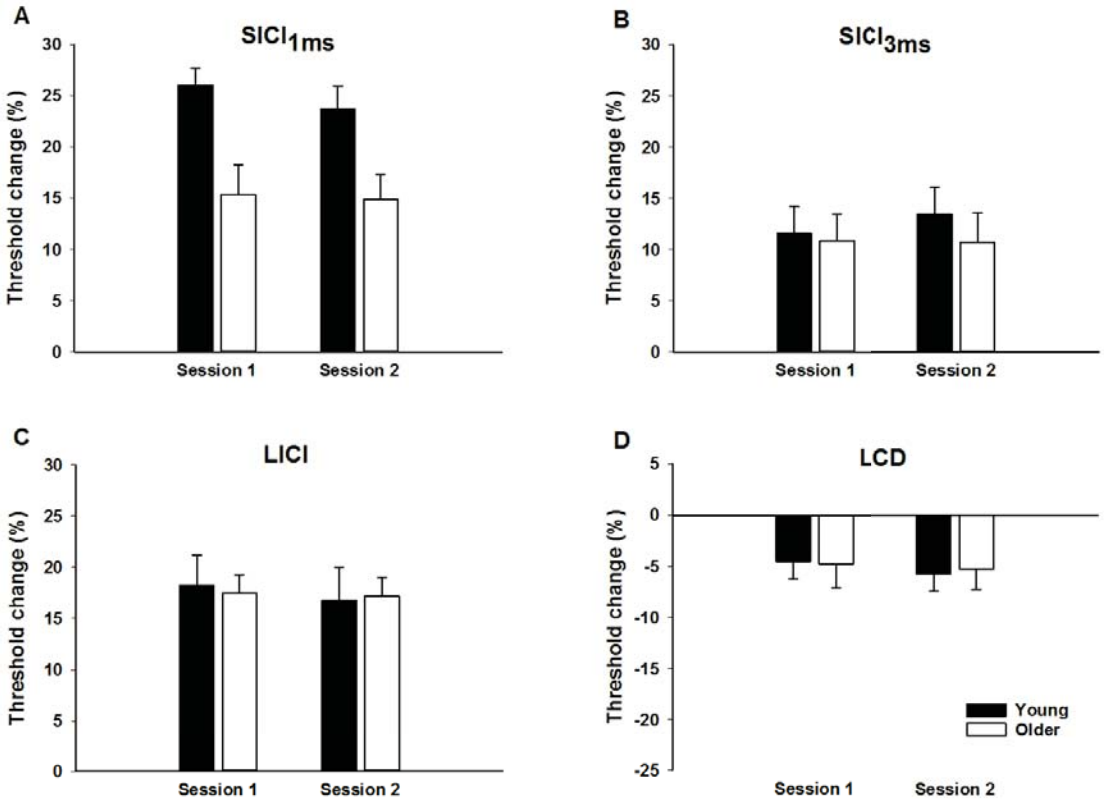
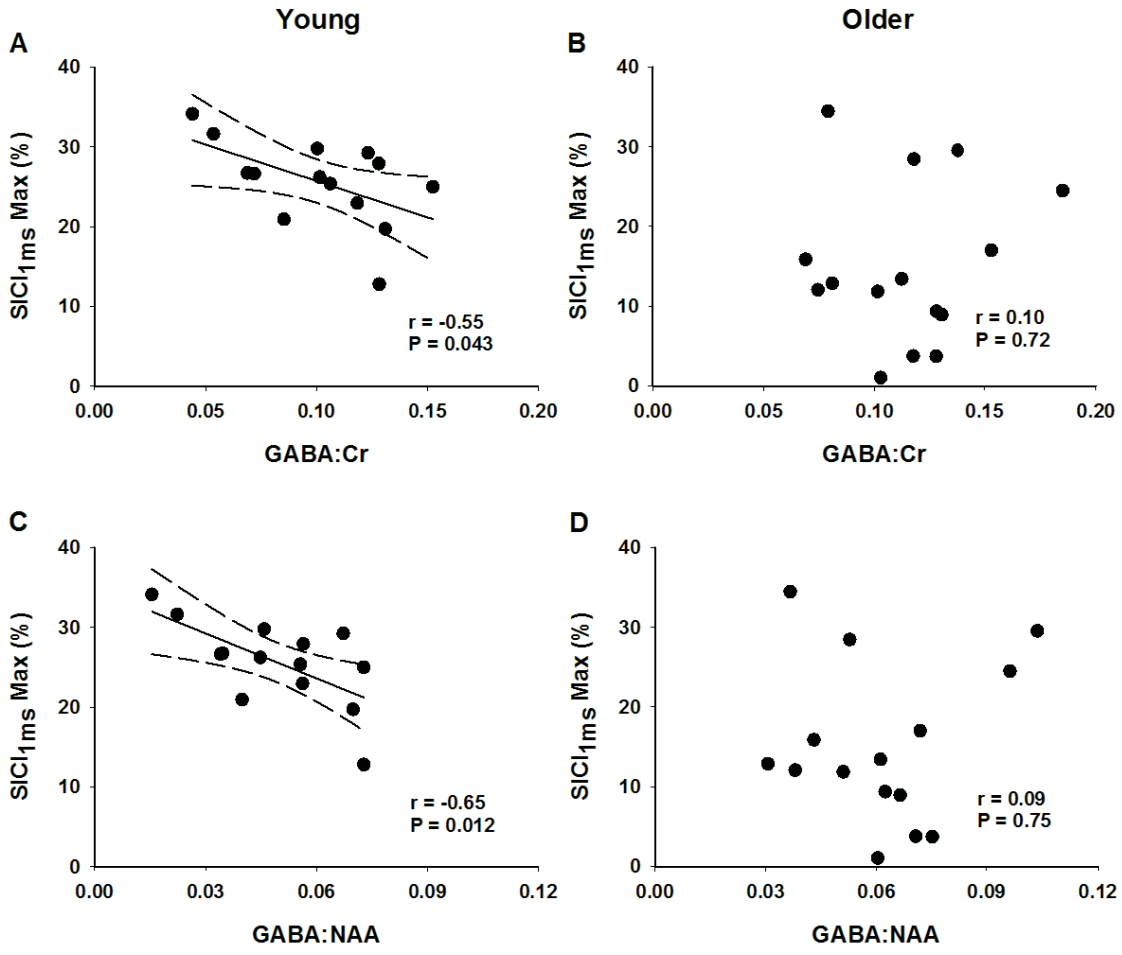

A
SICI1ms
Threshold change (%)
30
25
20
15
10
5
0
Session 1
Session 2
B
SICI3ms
Threshold change (%)
30
25
20
15
10
5
0
Session 1
Session 2
C
LICI
Threshold change (%)
30
25
20
15
10
5
0
Session 1
Session 2
D
LCD
Threshold change (%)
5
0
-5
-10
-15
-20
-25
Young
Older
Session 1
Session 2

Figure 4


Young
Older
A
B
C
D
SICI1ms Max (%)
GABA:Cr
GABA:NAA
r = -0.55
P = 0.043
r = 0.10
P = 0.72
r = -0.65
P = 0.012
r = 0.09
P = 0.75