Age-related changes in cortical excitability linked to decreased attentional and inhibitory control

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List of abbreviations

ANOVA: analysis of variance

EEG: electroencephalogram

ERP: event-related potential

GABA: gamma-aminobutyric acid

GMFP: global mean field power

MEPs: motor evoked potentials

NE: number of errors

N2cc: negativity central contralateral

N2pc: negativity posterior contralateral

RT: reaction times

tDCS: transcranial direct current stimulation

TEPs: transcranial magnetic stimulation evoked potentials

TMS: transcranial magnetic stimulation

η^2p: partial eta squared

ABSTRACT

Understanding age-related changes in cortical excitability and their relation to cognitive functions will help to improve interventions based on non-invasive brain stimulation that aim to support cognitive function in older adults. Here, we investigate the relationship between cortical excitability, executive function, and underlying neural activity in samples of healthy young and older adults. These participants performed a Simon task during electroencephalogram (EEG) recording. During the task, participants had to respond to the colour of a lateralized stimulus while ignoring its spatial location. We studied event-related brain potential correlates of attentional and inhibitory control [i.e., the posterior contralateral negativity (N2pc) and central contralateral negativity (N2cc), respectively] related to the Simon task performance. We also used transcranial magnetic stimulation (TMS) EEG coregistration. In detail, we applied single-pulse TMS during EEG recording in order to analyse global mean field power (GMFP) and TMS-evoked potentials (TEPs) as correlates of cortical excitability. We found lower GMFP amplitude within 101-200 ms in older compared to young adults. Moreover, older adults showed smaller N45 amplitude and slower P180 latency. These findings suggest cortical excitability alterations related to ageing. Older adults also exhibited longer reaction times and N2pc and N2cc latencies, indicating that it took them longer to allocate attention to the target stimulus and inhibit the tendency to respond to the attended location. Finally, in older adults, cortical excitability alterations correlated with longer reaction times and N2pc latencies. These results suggest that age-related alterations in cortical excitability represent a dysfunctional change associated with physiological ageing.

Keywords: ageing; cortical excitability; event-related potentials; TMS-EEG; non-invasive brain stimulation; executive functions.

Introduction

Establishing how neural changes related to physiological ageing influence cognitive functions is a fundamental challenge in contemporary cognitive neuroscience. Understanding these mechanisms will help in designing tailored interventions that maintain or enhance cognition in older adults and will allow the impact of such interventions to be monitored by assessing associated cortical excitability changes (Canu et al., 2018; Cespón et al., 2018). Although many studies have focused on how age-related changes in brain activity (e.g., Cabeza et al., 2002; Grady, 2012) and connectivity (e.g., Bagarinao et al., 2019; Varangis et al., 2019) affect cognitive functions, none have yet investigated the interplay between cortical excitability and cognitive performance in older adults. In this framework, it is crucial to determine if and how neural excitability should be modulated to maintain or even enhance cognitive functioning in this population.

Human cortical excitability can be studied non-invasively by recording the electroencephalogram (EEG) while delivering transcranial magnetic stimulation (TMS) pulses (e.g., Farzan et al., 2016; Miniussi and Thut 2010). This approach has been labelled TMS-EEG coregistration (e.g., Ilmoniemi and Kičić, 2010). Specifically, the EEG records brain activity induced by single TMS pulses delivered over a specific cortical area. These waveforms are known as TMS-evoked potentials (TEPs) and represent neurophysiological correlates of brain states (Chung et al., 2015; Hill et al., 2016). Importantly, TEPs are direct signatures of cortical excitability from "silent areas", which cannot be studied by using peripheral measures (e.g., Chung et al., 2015; Tremblay et al., 2019).

Previous studies have used peripheral measures, such as motor evoked potentials (MEPs), to investigate brain excitability in healthy older adults (Oliviero et al., 2006; Pitcher et al., 2003; Rossini et al., 1992). Overall, these studies have reported age-related hypo-excitability along the corticospinal pathway. However, as far as we know, there are only five studies that have focused on age-related changes in TEPs after applying single pulse TMS protocols (Casarotto et al., 2011; Ferreri et al., 2017; Noda et al., 2021; Opie et al., 2018; Opie et al., 2020). These studies report mixed results. Whereas Casarotto et al. (2011) did not find any differences between TEPs in healthy young and older adults after stimulating the left superior frontal cortex, Ferreri et al. (2017) described age-related hypo-excitability after applying single-pulse TMS over the left motor cortex, consistent with previous studies using MEPs (Oliviero et al., 2006; Pitcher et al., 2003; Rossini et al., 1992). Noda et al (2021) also reported reduced excitability related to ageing after stimulating the left motor cortex (with lower N45 and P180 amplitudes in older than young participants) and the left dorsolateral

prefrontal cortex (with lower N45 amplitudes in older adults than young participants). However, other studies (Opie et al., 2018; Opie et al., 2020) also stimulated the left motor cortex but found no evidence for cortical excitability changes, even though Opie et al. (2018) reported age-related changes in the topographies of the N100 and P180 TEP components. Some of the studies cited above (specifically, Ferreri et al., 2017; Opie et al., 2018) argued that changes in GABA receptors contributed to differences between young and older adults in cortical excitability. Nevertheless, as mentioned above, no study has investigated the functional implications of these age-related changes in cortical excitability. Thus, the main objective of the present study was to fill this gap in previous research by investigating changes in cortical excitability and their association with neural efficiency in older adults. We assessed neural efficiency by measuring the speed of executive control processes during the performance of a cognitive task by means of event-related brain potentials (ERP).

To measure cortical excitability, we applied TMS pulses over the left parietal lobe during TMS-EEG coregistration. The parietal region, along with frontoparietal networks, supports executive control processes linking attention and action (Cespón et al., 2020; Sebastian et al., 2013). Moreover, it has been shown that parietal regions are sensitive to physiological changes associated with ageing (Davis et al., 2008; Friedman et al., 1997). To obtain an index of cortical excitability in response to TMS pulses, we measured global mean field power (GMFP), the averaged rectified signal of activity induced by TMS pulses across the entire array of electrodes at a given point in time (Esser et al., 2006; Komssi et al., 2004). In addition, in order to deepen our understanding of the mechanisms underlying possible cortical excitability changes associated with ageing, we analysed latencies and amplitudes of the main TEP components at frontal, central, and parietal electrodes.

ERPs were used to investigate cortical activity related to specific cognitive processes. Considering that executive functions decline substantially with ageing (Diamond, 2013; Salthouse et al., 2003), in the present study we used an executive control task to investigate attentional and inhibitory control. We designed a Simon task (Simon and Small, 1969; for a review, see Cespón et al., 2020) in which participants had to respond to the colour of a lateralized stimulus by pressing one of two lateralized response buttons while ignoring the stimulus location. The target stimulus appeared either in the left or right visual hemifield at the same time as a lateralized contralateral non-target stimulus with a different colour but similar shape and size. The Simon task interference effect is produced when the required response is incongruent with the side of the stimulus location as attending to a location

triggers an ipsilateral response towards that spatial location (Hommel et al., 2001; Sheliga et al., 1997).

The Simon task requires shifting and allocating attention to the target stimulus and, at the same time, inhibiting the tendency to react towards the attended location. Therefore, we focused on ERP correlates of inhibitory control and attentional processes, namely, the central contralateral negativity (N2cc) and the posterior contralateral negativity (N2pc). The N2cc is an ERP component that reflects the level of inhibition exercised to counteract the spatial response tendency. This inhibitory response emerges from the dorsal premotor cortex about 200-300 ms after the onset of a stimulus (Cespón et al., 2012; Praamstra, 2006). It has been reported that N2cc latency is sensitive to changes in the inhibitory ability of older adults due to both physiological ageing (Amenedo et al., 2012) and clinical entities such as mild cognitive impairment related to Alzheimer's disease (Cespón et al., 2015). The N2pc is an ERP component that reflects neural activity related to shifting attention to and processing a lateralized target stimulus, as well as suppression of the non-target stimuli (Luck, 2012; Mazza et al., 2009; Van der Lubbe et al., 2012; Zyvoni et al., 2018). It emerges from parietal and occipito-temporal cortical regions (Hopf et al., 2000; Lorenzo-López et al., 2011). The longer it takes to attend to a specific stimulus, the longer the related N2pc latency (Callahan-Flintoft et al., 2018; Ruge et al., 2006). The N2pc is also sensitive to age-related changes (Cespón et al., 2013; Lorenzo-López et al., 2008).

The main goal of the present study was to investigate age-related changes in cortical excitability and their functional implications; that is, to investigate whether cortical excitability modulations represent dysfunctional changes associated with physiological ageing. We predict that this will be revealed by relationships between cortical excitability, task performance, the neural correlates of attention to the stimulus location, and inhibition of the response spatial tendency (i.e., the key neural processes deployed during the performance of the Simon task). Specifically, the existence of negative correlations between cortical excitability changes and cognitive processing efficiency would be consistent with the interpretation that cortical excitability changes are dysfunctional alterations associated with ageing. In order to achieve this issue, we recruited samples of healthy young and older adults, who performed a Simon task while we recorded EEG. In line with previous research, we expected to find age-related decreases in cortical excitability, as measured by GMFP, in response to TMS pulses. In addition, we expected to find age-related behavioural and EEG changes during performance of the Simon task, specifically slower reaction times (RT) and longer N2pc and N2cc peak latencies in older compared to young adults.

We hypothesized that neural efficiency would be lower in older adults, with a slowed allocation of attention to the target stimulus and reduced inhibition of the spatial response tendency, as shown by longer N2pc and N2cc latencies. If cortical excitability changes (measured by GMFP) represent a dysfunctional alteration linked to ageing, then they should correlate with impaired performance (i.e., longer RT and stronger interference effects) within the older group. Moreover, we expected to find correlations between cortical excitability changes in the older group and loss of efficiency in neural processing, which would be indicated by correlations between age-related changes in the GMFP and longer N2cc and N2pc latencies.

Experimental Procedures

Participants

Twenty-one healthy young (16 females; mean age = 22.6 SD = 2.59) and twenty older (10 females; mean age = 67.0, SD = 3.11) adults took part in the study after being screened to exclude any with contraindications for TMS (Rossi et al., 2009). Participants were right-handed, as assessed by the Edinburgh handedness inventory test (Oldfield, 1971), and had normal or corrected to normal visual acuity. In addition, participants reported no previous history of neurological or psychiatric disorders.

Experimental protocols for TMS were performed in accordance with the suggested checklist for a routine clinical TMS examination recommended by Rossini et al. (2015). This research was performed in compliance with the ethical guidelines delineated by the Declaration of Helsinki and received prior approval from the local Ethics Committee. Before taking part in the experiment, all participants were informed about the procedures of the study and provided written informed consent.

Procedures

The whole experiment was conducted in a dimly lit and shielded room. At the beginning of the experimental session, the individual resting motor threshold was calculated as the intensity at which a MEP of 50μV was observed in the electromyogram (montage in first dorsal interosseous of the right hand) 50% of the times for at least 10 TMS pulses delivered through a standard 70 mm figure-of-eight coil (Magstim Super Rapid Whitland, UK). Individual motor threshold was determined for each participant by stimulating the left primary motor cortex with the EEG cap in place. This was done to ensure the right intensity given the spacing of the EEG cap. Subsequently, participants performed the Simon task (see below and

Figure 1) during EEG recording. After performing the Simon task, which lasted for about 15 minutes, participants received 80 TMS pulses over CP5 (angular gyrus / supramarginal gyrus according to estimations by Koessler et al., 2009). TMS pulses were delivered during a resting-state condition, while participants were sitting in a comfortable position and looking ahead at a fixation point. The coil was placed tangentially on the scalp, with the handle pointing backward at an angle of 45º from the mid-sagittal axis of the participant's head. TMS pulses were delivered at a frequency jittered between 0.25 and 0.5 Hz at 80% of the intensity of the individual motor threshold. Subjects wore protective headphones to minimize the auditory response produced by the TMS coil click. A neuronavigation system (SofTaxic Optic EMS, Bologna, Italy) allowed for constant control of the stability and spatial consistency of the coil position and its orientation throughout the stimulation period. The average motor threshold, evaluated in relation to the maximum intensity of the stimulator output, was higher in older (61%) than young (51%) participants. This difference was statistically significant, as revealed by independent t-tests ($t = -4.69$, $p < 0.001$).

Task

During the Simon task (Simon and Small, 1969; Cespón et al., 2020), participants had to direct their gaze to the centre of the screen. A grey fixation cross appeared in the centre of the screen for 500 ms against a black background. After that, a bilateral array of stimuli appeared for 100 ms. Participants, who were sitting 100 cm in front of the screen, were instructed to respond as fast as possible to the colour of the stimulus (red or blue) by pressing one of the two response buttons arranged horizontally. The target and a non-target stimulus (a contralateral stimulus with similar morphology and eccentric position) were presented 5 cm to the left or right of the central fixation cross so that the entire display was within the foveal region (i.e., around 3 degrees of visual angle from the centre of the foveal region) (Bargh and Chartrand, 2000). The presence of a contralateral non-target stimulus prevents any influence from an asymmetrical ERP response, called the N1, located in central regions (Oostenveld et al., 2001), without altering spatial stimulus-response compatibility effects (O'Leary and Barber, 1993). After the bilateral stimuli presentation, the screen remained blank for a random time period of 2000 ± 250 ms. Then, the new trial started with the appearance of the central fixation cross. The task consisted of 120 trials per condition, which resulted in 240 trials (120 congruent and 120 incongruent trials) presented in random order. The task was divided into two blocks of 120 trials per block. At the end of the first block, the participant rested for about

one minute. Before starting the task, participants performed a practice block of 10 trials. The task is graphically represented in Figure 1.

Figure 1 about here

EEG recording

TMS-compatible EEG equipment (BrainAmp 32MRplus, BrainProducts GmbH, Munich, Germany) was used to record the EEG from 57 sintered, Ag/AgCl passive electrodes (Oz, FCz, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T7, T8, P7, P8, Fz, Cz, Pz, FC1, FC2, CP1, CP2, FC5, FC6, CP5, CP6, F1, F2, C1, C2, P1, P2, AF3, AF4, FC3, FC4, CP3, CP4, PO3, PO4, F5, F6, C5, C6, P5, P6, AF7, AF8, FT7, FT8, TP7, TP8, PO7, PO8, CPz, POz), which were embedded directly in the fabric of the cap (EasyCap, Brain Products GmbH, Germany). The ground electrode was placed at Fpz. The right mastoid served as an online reference for all electrodes, whereas the left mastoid electrode was used offline to re-reference the scalp recordings to the average of the left and the right mastoid, i.e., including the implicit reference (right mastoid) in the calculation of the new reference. A continuous recording mode, without the use of any sample-and-hold circuits, was adopted. The EEG signal was acquired with a bandpass filter of 0.01-1000 Hz and digitized at a sampling rate of 5000 Hz (Veniero et al., 2009). Vertical and horizontal electrooculogram signals were recorded by two electrodes located above and beneath the right eye and two electrodes located lateral to the outer canthi of both eyes. The impedance between the skin and EEG electrodes was maintained below 5 kΩ.

Data analysis

Behavioural performance was evaluated by analysing RTs and accuracy (Number of Errors – NE). We considered it important to analyse the NE in order to exclude a possible speed-accuracy trade-off. The magnitude of interference produced by the stimulus location was evaluated by the subtraction "RT incongruent trials - RT congruent trials". We used z-transformed scores to test whether age-related increases in the size of the interference effect were proportional to the age-related slowing in RT. Thus, before applying the mentioned subtraction, we obtained the z-scores for RT in each group of participants.

After EEG signal storage, continuous data were linearly interpolated between -1 to 19 ms from each TMS pulse. The EEG was segmented in epochs between -1500 and 1500 ms relative to the TMS pulse (for TEPs) and the task stimuli (for ERPs) and subsequently down

sampled from 5000 to 1024 Hz. The EEG signal was filtered with a 0.1-80 Hz digital bandpass and a 50 Hz notch filter. Ocular and muscular artefacts were removed by using independent component analysis [algorithm Infomax (Gradient) restricted to the whole dataset implemented in Brain Vision Analyzer]. Epochs exceeding ±100 μV were automatically rejected. All remaining epochs were individually inspected to identify any remaining artefacts at any electrode and any horizontal electrooculogram movements that exceeded 5 μV during the period from 100 to 400 ms after the onset of the stimulus. These epochs were also eliminated from subsequent analyses. Epochs around the TMS pulse were extracted from -200 to 300 ms post-stimulus for TEPs and from -200 to 800 ms post-stimulus for ERPs, then baseline-corrected on the interval from -200 to -2 ms.

To obtain an index of global cortical excitability from TEPs, GMFP was computed on the averaged activity of the 57 cortical electrodes within the three time windows using Brain Vision Analyzer 2.2 software. GMFP was obtained at each time point as the mean-squared value of data in the available electrodes (Lehmann and Skrandies, 1980; Tremblay et al., 2019) by applying the following formula:

$$\mathrm{GMFP} = \frac{\sqrt{[\Sigma_{\mathrm{i}}^{\mathrm{k}}(\mathrm{Vi(t)} - \mathrm{V\ mean\ (t)})^2]}}{\mathrm{K}}$$

where t is time, K is the number of channels, Vi is the voltage in channel i averaged across participants, and Vmean is the mean of the voltages measured across all channels (Lehmann and Skrandies, 1980).

In order to obtain temporal indices of global cortical excitability evoked by delivering TMS, GMFP values were cumulated within each of the three time windows following TMS-pulses: 20-100 ms (the first 20 ms were excluded because the time range between -1 and 19 ms was interpolated and, thus, did not represent physiological activity), 101-200 ms, and 201-300 ms. This allowed us to obtain temporal indices of the cortical excitability evoked by delivering a TMS pulse. Such indices were correlated with behavioural and ERP outcomes.

In order to obtain temporal indices of local cortical excitability, we analysed TEP latencies and amplitudes (i.e., P30, N45, P60, N110 and P180 latencies) over the stimulated area by pooling C5, CP5, P5, CP3 and TP7 electrodes within a region of interest. Also, TEP latencies and amplitudes were analysed in three representative locations of the scalp (Fz, Cz, and Pz electrodes) (in the Supplementary Material we provide methods and results regarding the age-related changes in TEP amplitudes in the whole array of electrodes). After inspection

of the grand averages, we identified, for each subject, peak latencies and amplitudes of P30, P60, and P180 as the maximum positive peak observed between 25-45 ms, 50-70 ms, and 150-220 ms, respectively, after the TMS pulse. In addition, we identified, for each subject, latencies, and amplitudes of N45 and N110 as the maximum negative peak observed between 35-55 ms and 80-130 ms, respectively, after the TMS pulse.

ERPs were calculated only for the correct responses. Following previous studies (e.g., Woodman and Luck, 2003), the N2pc and N2cc were obtained based on the hemifield of the target stimulus location. For N2pc, we applied the following formula: [P8 - P7 (left hemifield stimuli) + P7 - P8 (right hemifield stimuli)] / 2]; for N2cc, the following formula was applied: [C4 - C3 (left hemifield stimuli) + C3 - C4 (right hemifield stimuli)] / 2]. In line with previous studies investigating differences between two different groups in the N2pc and N2cc components (Amenedo et al., 2012; Cespón et al., 2015), we obtained N2pc and N2cc regardless of whether the stimulus location was congruent or incongruent with the required response. This procedure did not allow for the comparison of conditions but had the advantage of removing residual motor activity from the N2cc and N2pc waveforms. Specifically, half of the arrows located in the left hemifield required a left-handed response, whereas the other half required a right-handed response. So, by averaging across these two types of trials, motor activity was removed. Importantly, as the target was always located in the left hemifield, the target-related activity (i.e., N2cc and N2pc) remained in these waveforms. The same reasoning applied to averages for right-hemifield stimuli. The N2pc and N2cc peak latencies were identified as the largest negative peaks between 200–400 ms after stimulus presentation. The N2pc and N2cc amplitudes were calculated, for each participant, in a time window of ±25 ms around their peak latencies.

Statistical analysis

To calculate RTs and NEs, we carried out repeated measures ANOVAs with Condition as a within-subject factor (two levels: Congruent, Incongruent) and Age as a between-subject factor (two levels: Young, Older adults). For behavioural interference effects as well as N2pc and N2cc ERP latencies and amplitudes, one-way ANOVAs were carried out with Age as a between-subjects factor (two levels: Young, Older adults). For GMFP on each time window (i.e., 20-100 ms, 101-200 ms, and 201-300 ms), we carried out a one-way ANOVA using Age as a between-subjects factor (two levels: Young, Older adults). For latencies and amplitudes of P30, N45, P60, N110, and P180, we carried out a one-way ANOVA using Age as a between-subject factor (two levels: Young, Older adults) in order to analyse local TEPs and,

in addition, we carried out a repeated measures ANOVA with Electrode as a within-subject factor (three levels: Fz, Cz, and Pz) and Age as a between-subject factor (two levels: Young, Older adults). When these ANOVAs showed significant effects related to the main factors and/or their interactions, Bonferroni correction was applied to post hoc comparisons. The normal distribution of the obtained behavioural, GMFP, and ERP data was tested by means of Kolmogorov-Smirnov tests. If the condition of sphericity was not met, then the Greenhouse-Geisser correction for degrees of freedom was applied. Also, partial eta squared (η2p)—a measure of effect size—was calculated for significant results.

Pearson correlation analyses were carried out to correlate GMFP with RT, interference, and the N2pc and N2cc latencies obtained during the Simon task. This allowed us to test whether and how cortical excitability was associated with cognitive performance and/or underlying neural activity. As the relationships between cortical excitability (GMFP) and correlates of cognitive functioning (RT, interference, and ERP latencies) might differ between young and older adults, correlations were carried out for each group separately. We focused on ERP latencies, which can be easily interpreted, unlike ERP amplitudes (Cespón and Carreiras, 2020).

Results

Behavioural results

The repeated measures ANOVA for RT (Age x Condition) showed a Condition effect [$F(1, 39) = 84.9$, $p < 0.001$, $\eta^2p = 0.685$], as RT was longer in incongruent (481 ms) than congruent (441 ms) trials. These analyses also revealed an Age effect [$F(1, 39) = 47.41$, $p < 0.001$, $\eta^2p = 0.549$], as the RT was longer in older (527 ms) than young (398 ms) participants ($p < 0.001$). Moreover, the Condition x Age interaction reached significance [$F(1, 39) = 6.83$, $p = 0.010$, $\eta^2p = 0.149$]. RT was longer in older than young adults in the congruent (502 ms vs. 384 ms) ($p < 0.001$) and in the incongruent (553 ms vs. 413 ms) ($p < 0.001$) condition ($p < 0.001$). Additionally, RT was longer in incongruent than congruent trials for both young (413 vs. 384 ms) ($p < 0.001$) and older (553 vs. 502 ms) ($p < 0.001$) subjects. For NE, we observed an effect of Condition [$F(1, 39) = 30.9$, $p < 0.001$, $\eta^2p = 0.442$], as the NE was higher in incongruent (8.7) than congruent (1.9) trials ($p < 0.001$). Age and Condition x Age interactions did not reach significance (the average number of errors for young and older adults was 5.6 and 5.0, respectively). Finally, the one-way ANOVA used to determine the size of the interference effect (in z-scores) did not reveal any differences between young and

older participants (z-scores were -0.4 vs. 0.4, respectively; t (39) = 1.83, p = 0.184). The behavioural results are graphically summarized in Figure 2.

Figure 2 about here

Global mean field power

The one-way ANOVA for GMFP between 101 and 200 ms revealed a significant effect of Age [F (1, 39) = 4.13, p = 0.049, η^2p = 0.096], as GMFP was larger in young than older adults (see Figure 3). A significant effect at the statistical level was not observed in the analyses carried out on the 20-100 ms [F (1, 39) = 0.15, p = 0.904, η^2p = 0.000] and 201-300 ms [F (1, 39) = 0.588, p = 0.448, η^2p = 0.015] time windows.

Figures 3 about here

TMS evoked potentials (TEPs)

For TEPs (see Figure 4, which shows the waveforms in all the electrodes for young and older adults separately, in addition to the current density maps corresponding to the time windows in which the GMFP was analysed), the repeated measures ANOVA revealed an effect of Age for the P180 latency [F (1, 39) = 9.72, p = 0.003, η^2p = 0.200], with shorter latencies in young (180 ms) than older (195 ms) adults (see Figure 5, which represents the studied TEPs –i.e., P30, N45, P60, N110, and P180- for young and older adults in the analysed electrodes, in addition to the current density maps corresponding to each TEP). For N45 amplitude, the repeated measures ANOVA revealed an effect of Age [F (1, 39) = 4.096, p = 0.050, η^2p = 0.095], with larger N45 amplitude in young (-1.29 µV) than older (0.43 µV) adults. The remaining Age or Age x Electrode interactions did not show any other significant effect for latencies or amplitudes of the studied TEPs. For TEPs in the stimulated area (Figure 5), the one-way ANOVA revealed an effect of Age for P180 latency [F (1, 41) = 6.51, p = 0.015, η^2p = 0.143], with shorter P180 latencies in young (179 ms) compared to older adults (194 ms).

Figures 4 and 5 about here

Event-related potentials

The one-way ANOVA revealed an effect of Age [F (1, 40) = 23.7, p < 0.001, η^2p = 0.379], with shorter N2cc latencies in young (217 ms) than older (266 ms) adults (p < 0.001). The

analysis did not reveal any significant difference in Age for N2cc amplitudes [F (1, 40) = 2.47, p = 0.124, η^2p = 0.060]. The one-way ANOVA showed an effect of Age [F (1, 40) = 24.6, p < 0.001, η^2p = 0.388], with shorter N2pc latencies in young (205 ms) than older (256 ms) adults (p < 0.001). The one-way ANOVA for N2pc amplitude did not show a significant effect of Age [F (1, 40) = 0.645, p = 0.427, η^2p = 0.016]. The obtained ERP waveforms for young and older adults are represented in Figure 6.

Figure 6 about here

Correlation analyses

No significant correlations were observed between GMFP and indices of behavioural performance (RT, NE, and interference effect). Even so, it is worth mentioning the marginally significant correlations between RT and GMFP found in the 20-100 ms time window (rxy = 0.401, p = 0.071) in young adults and between RT and GMFP in the 201-300 ms time window (rxy = -0.395, p = 0.085) in older adults. N2pc latency did not correlate with GMFP in the young, but in older adults, significant correlations were observed between N2pc latency and GMFP in the 101-200 ms time window (rxy = -0.487, p = 0.029), with marginal correlations in the 201-300 ms time window (rxy = -0.438, p = 0.053). On the other hand, correlation analyses of GMFP and N2cc latencies did not reveal any significant effects in either young or older adults (a higher correlation between N2cc latency and GMFP in older adults was obtained in the 201-300 ms time window but it was not significant: rxy = -0.248, p = 0.291). Figure 7 graphically represents the correlations specified above.

Figure 7 about here

Discussion

The results of the present study suggest age-related changes in cortical excitability, as indexed by lower GMFP amplitudes in older than young adults in the time window between 101 and 200 ms. Analyses from TEPs showed smaller N45 amplitude in older compared to young adults. Moreover, the P180 latency was slower in older than young adults. Regarding the executive control functions, we found slower RT and peak latencies for the N2pc and N2cc during performance of the Simon task. Both RTs and ERPs provide converging evidence for age-related slowing in the allocation of attention to the target stimulus and inhibitory control to prevent the spatial response tendency. Nevertheless, behavioural analyses showed that the

size of the interference measured in z-scores was not greater in older than young adults. In older adults, correlation analyses suggested that cortical excitability changes revealed by GMFP represent a dysfunctional change; decreased GMFP amplitude correlated with longer RT and N2pc latencies. In the following paragraphs, we discuss these results, their relation to previous studies, and their practical implications.

Our results showed lower GMFP amplitudes in older than young adults, which might point to decreased cortical excitability in physiological ageing. These results align with previous TMS-EEG studies that applied TMS pulses over the motor cortex (Ferreri et al., 2017; Noda et al., 2021) and dorsolateral prefrontal cortex (Noda et al., 2021) as well as findings from studies using MEPs (Oliviero et al., 2006; Pitcher et al., 2003; Rossini et al., 1992). Importantly, we found increased motor thresholds in older compared to young adults. This result is consistent with the decreased cortical excitability of motor areas in older adults reported by earlier studies using MEPs. However, while differences in motor threshold could result from increased scalp-cortex distance in older adults, this is unlikely to be the case with the TEPs we measured because we adjusted the intensity of TMS pulses to 80% of each individual's motor threshold. Since older adults had increased motor thresholds, they received higher intensity of stimulation over the left parietal area than young adults. Even so, older adults exhibited lower GMFP than young adults. It should be noted that two previous studies stimulating the left superior frontal cortex (Casarotto et al., 2011) and the left motor cortex (Opie et al., 2020) did not find any differences in cortical excitability between young and older adults. In detail, Casarotto et al. (2011) applied TMS pulses over the left superior frontal cortex but did not find any differences in cortical excitability between young and older adults. Methodological differences (that is, differences in the intensity of the stimulation, the stimulated brain area, or the fact that Casarotto and colleagues only analysed early TEPs) may explain these inconsistent results. It should also be noted that Casarotto and colleagues (2011) analysed only nine subjects per group, which possibly explains their null effects. Likewise, Opie et al (2020) reported no differences between young and older adults after applying TMS pulses over the left motor cortex. A major methodological difference between the current study and Opie et al (2020) was that we applied TMS at an intensity of 80% of the resting motor threshold (rMT) whereas Opie et al. (2020) used an intensity of 120% of the rMT. Regardless of TMS intensity, Opie et al (2020) did not use a neuronavigation system, which likely lowered the precision with which they could stimulate the left motor cortex throughout the stimulation period.

We also found differences between young and older adults in specific TEP components. Namely, older adults showed smaller N45 amplitude than young adults. This finding is consistent with some previous studies that reported smaller N45 in older than young adults after stimulating the left dorsolateral prefrontal cortex (Noda et al., 2021) and the left motor cortex (Ferreri et al., 2017; Noda et al., 2021). Although the neurophysiological mechanisms related to N45 are not completely understood, previous studies linked N45 amplitude modulations to GABA-A receptors (Ferreri et al., 2017; Premoli et al., 2014) as well as GABA-Aergic inhibition and NMDA receptor-mediated excitation balance (Bellardinelli et al., 2021). Also, the results of the present study showed longer P180 latencies in older compared to young adults. These results are consistent with previous research on age-related changes in TEPs after stimulating the left motor cortex (Opie et al., 2018). The absence of differences in N110 peak latency and amplitude, in addition to the faster rising of P180 in young than older adults, could be related to the observed differences between both groups in GMFP at the 101-200 ms time window. Overall, results from GMFP and TEPs suggest an altered excitatory-inhibitory balance with ageing.

The behavioural results and concomitant neural activity we observed during performance of the Simon task revealed longer RTs as well as N2pc and N2cc latencies in older than young adults. This cognitive slowing is consistent with previous studies using the Simon task (Proctor et al., 2005) and other types of cognitive tasks (Harada et al., 2013; Salthouse, 1996). In general, during the performance of executive control tasks, ERP latencies are delayed with ageing (Zurrón et al., 2018). This aligns with the well-established observation of age-related slowing in cognitive processing (Salthouse, 1996). In fact, previous studies investigating the allocation of attentional processes showed age-related slowing of N2pc latencies (Lorenzo-López et al., 2008; Cespón et al., 2013), indicating it took longer for older participants to allocate attention to the target stimulus and suppress the non-target stimulus. Similarly, N2cc latencies are also slowed with ageing (Amenedo et al., 2012), indicating that neural activity related to preventing prepotent responses based on stimulus location is delayed in older adults compared to young participants. However, we did not find that the extent of interference increased in older with respect to young participants after we controlled for age-related slowing, thus, our results do not provide support for the inhibitory deficit hypothesis of ageing (Butler and Zacks, 2006; Hasher et al., 1999). In this context, the results of the present study are consistent with recent meta-analytical evidence suggesting that the existence of inhibitory deficits with ageing is not a general finding but depends on the specific experimental paradigm employed (Rey-Mermet and Gade, 2018).

The goal of the present study was to determine whether age-related changes in cortical excitability play a functional or dysfunctional role by examining their association with attentional and inhibitory control processes. We found decreased GMFP values in older adults correlated with increases in RT and N2pc latencies, suggesting that cortical excitability changes with ageing are a dysfunctional characteristic of the aged brain. Longer N2pc latencies pointed to an age-related decline in the efficiency of attentional selection processes. We found GMFP differences between 101 and 200 ms. This time window matches the rising of P180, which was delayed in older compared to young adults. Thus, cortical age-related changes detected in the GMFP can be, at least partially, explained by differences between young and older adults in P180 latencies. Previous studies argued that age-related differences in P180 could be linked to age-related changes in GABA-B receptors (Ferreri et al., 2017; Opie et al., 2018). A resonance magnetic spectroscopy study revealed that increased excitability of corticospinal pathways was associated with higher levels of GABA in the motor cortex (Greenhouse and King, 2017). Thus, GABA was suggested to indicate a homeostatic mechanism in the excitatory-inhibitory balance, as higher levels of an inhibitory neurotransmitter were related to more highly excitable pathways (Greenhouse and King, 2017). It is worth mentioning that several studies have shown that age-related alteration of intrinsic neuronal excitability is associated with decreases in soma size and with the loss of dendrites, spines, channels, and synaptic efficiency (see Rizzo et al., 2015). These changes in neuronal structure and physiology might correspond to a functionally responsive stage in the down-regulation of synaptic function. Each cognitive function is the expression of neural network activity and connectivity efficiency changes over time (McIntosh, 2000; Milner et al., 1998). So, dysregulation of network excitability may be one of the causal mechanisms leading to cognitive changes. Actually, P180 has also been related to the reverberant activity of cortico-cortical and cortico-subcortical circuits (Ferreri et al., 2011). Thus, slowed attentional control and modulated cortical excitability may be related to GABAergic neurotransmission, in line with statements from Ferreri et al. (2017) and Opie et al. (2018), and/or altered brain connectivity. Additional research is still needed on the specific mechanisms underlying age-related differences revealed by neural correlates of cortical excitability.

Interpreting decreased GMFP as diminished cortical excitability related to ageing would be consistent with previous investigations that found neural enhancement—as determined by increased ERP amplitudes—after delivering excitatory currents (Cespón et al., 2017; Cespón et al., 2019). In detail, these studies showed correlations between increased

ERP amplitudes and improved cognitive performance in healthy older adults after applying anodal transcranial direct current stimulation (tDCS), a protocol that should increase cortical excitability (Cespón et al., 2017; Cespón et al., 2019). Even so, it is important to note that increasing cortical excitability may not be an efficacious strategy to improve cognition in young subjects, as suggested by the tendency towards a correlation between increased cortical excitability and longer RTs in the young group. This result aligns with a recent study showing that anodal tDCS delivered over parietal areas impaired cognitive functioning in a visuospatial learning task (Grasso et al., 2021). Crucially, future research should shed light on the neurophysiological mechanisms underlying changes related to ageing in cortical excitability and their association with cognitive functioning. Such research would provide an empirical basis to guide the design of non-invasive brain stimulation protocols to maintain executive and other cognitive functions in healthy older adults (Cespón et al., 2018; Brisochi et al., 2021).

The present study is not exempt from limitations. We cannot rule out brain atrophy related to ageing as a factor that contributes to the higher motor threshold found in older adults. Indeed, such age-related brain atrophy (Kozel et al., 2000; List et al., 2013) could increase the distance between the TMS coil and the neural tissue. Future experimental protocols that include magnetic resonance imaging to control for the degree of brain atrophy would help shed light on this issue. Also, future research should aim to establish causal relationships between cortical excitability and neural and cognitive function by assessing how the application of excitatory and/or inhibitory non-invasive brain stimulation protocols affects neural activity and associated cognitive performance. Finally, it is important to highlight that TMS pulses cause auditory and somatosensory evoked potentials (Biabani et al., 2019; Guerra et al., 2021; Gordon et al., 2018; Rocchi et al., 2021), which could overlap with activity induced by magnetic discharge (mainly from 80 ms onward). Thus, although participants wore earplugs and topographical maps did not show any evidence for a negative central source linked to the auditory component (Rocchi et al., 2021), this issue represents a methodological limitation of the present study.

In summary, the results of the present study support the existence of cortical excitability changes associated with physiological ageing, as assessed by stimulating the left parietal cortex. We showed that cortical excitability changes in older adults were related to longer RT and ERP latencies during the performance of an executive control task. Our results strongly suggest that cortical excitability modulation in older adults is a dysfunctional modification of the aged brain. Future studies should more closely investigate the

neurophysiological mechanisms underlying age-related changes in cortical excitability and the consequences of stimulating other cortical regions and using other task paradigms. Such work would help to establish a sound foundation for non-invasive brain stimulation protocols that modulate cortical excitability to preserve or even restore cognitive function in healthy older adults.

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JC: designed the study, collected the data, analysed the data, and wrote the manuscript.
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Figure 1. Task and experimental procedures

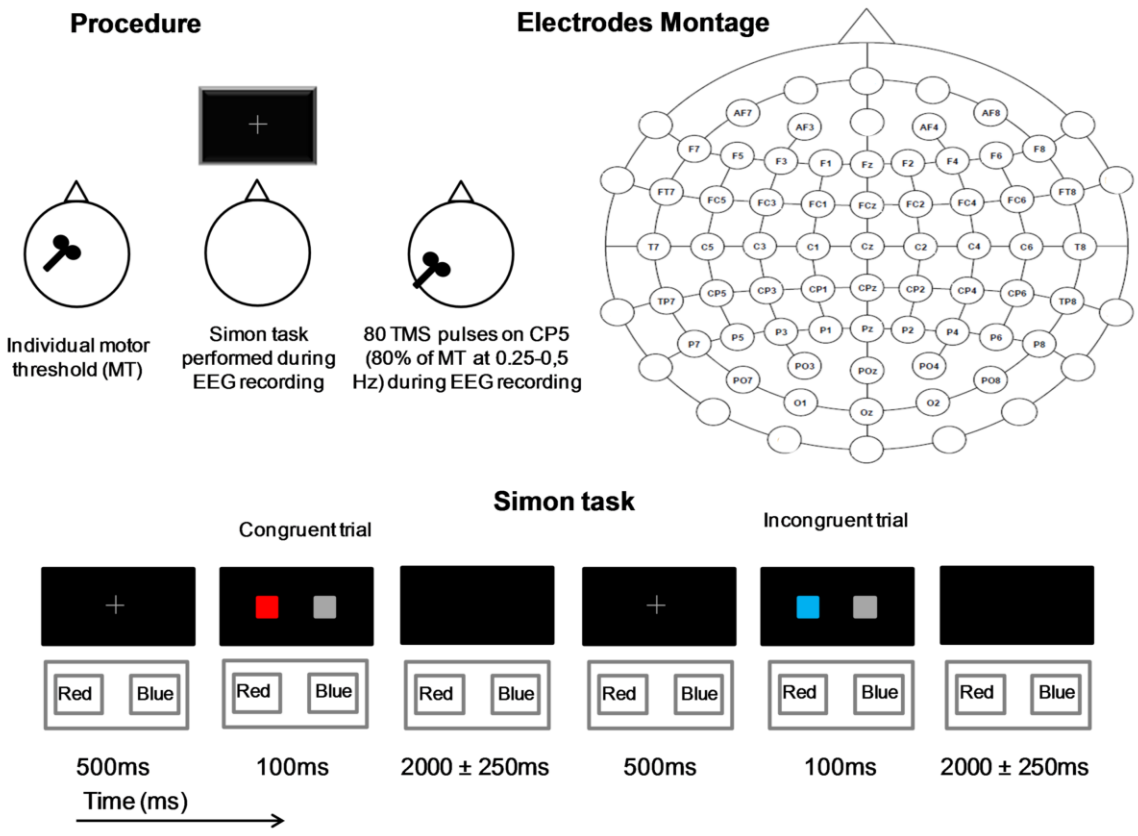


Figure 2. Behavioural performance during the Simon task

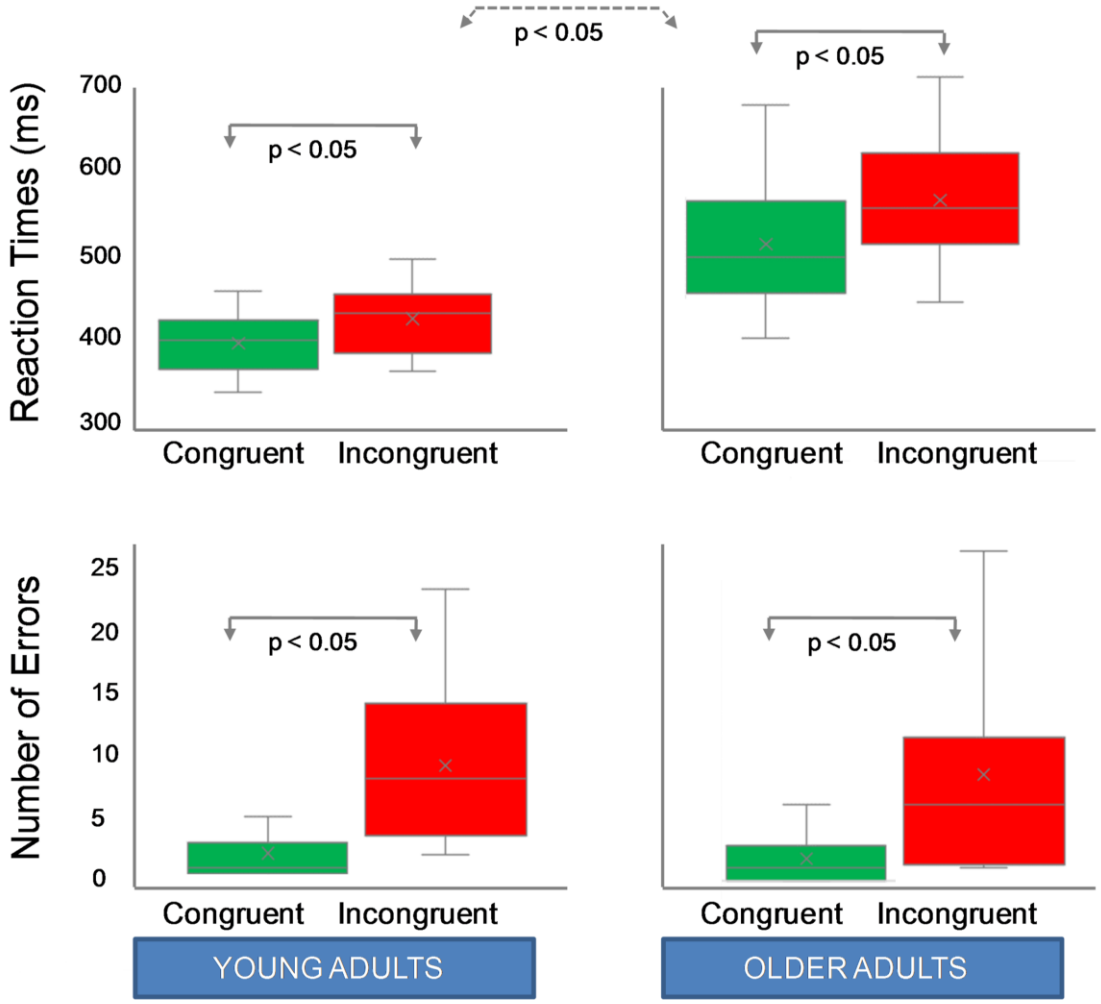


Figure 3. Global Mean Field Power

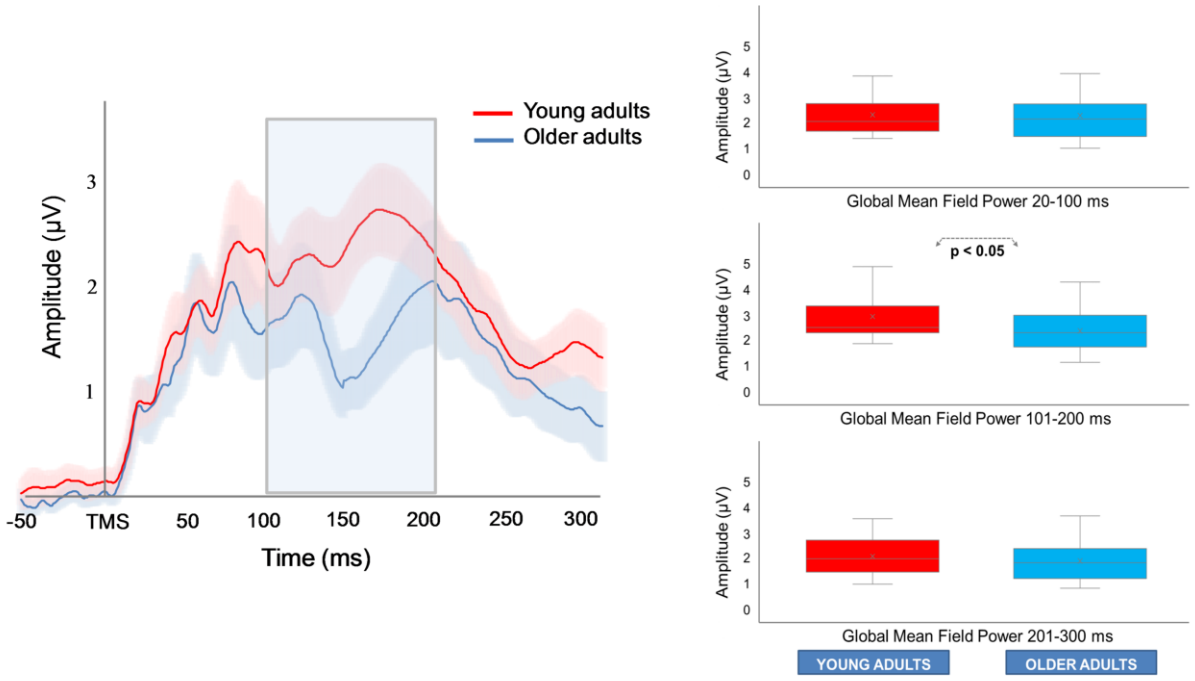


Figure 4. TMS evoked potentials

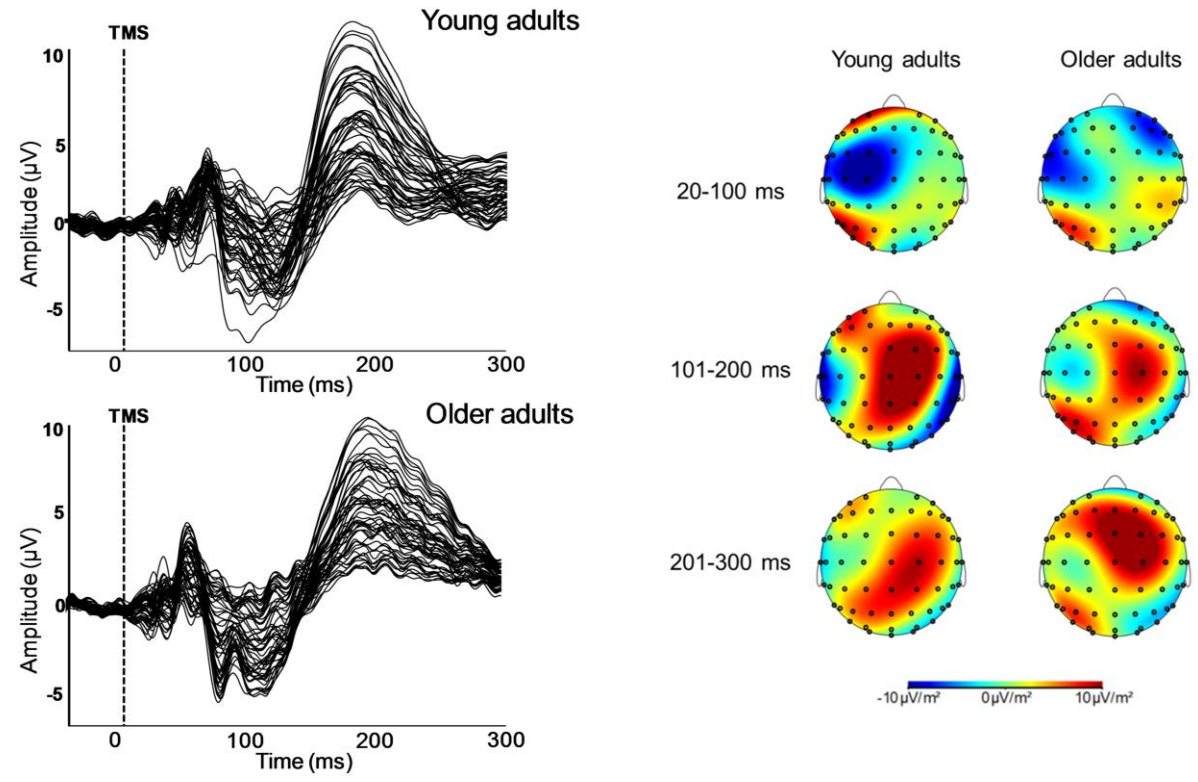


Figure 5. TMS evoked potentials in electrodes/regions of interest

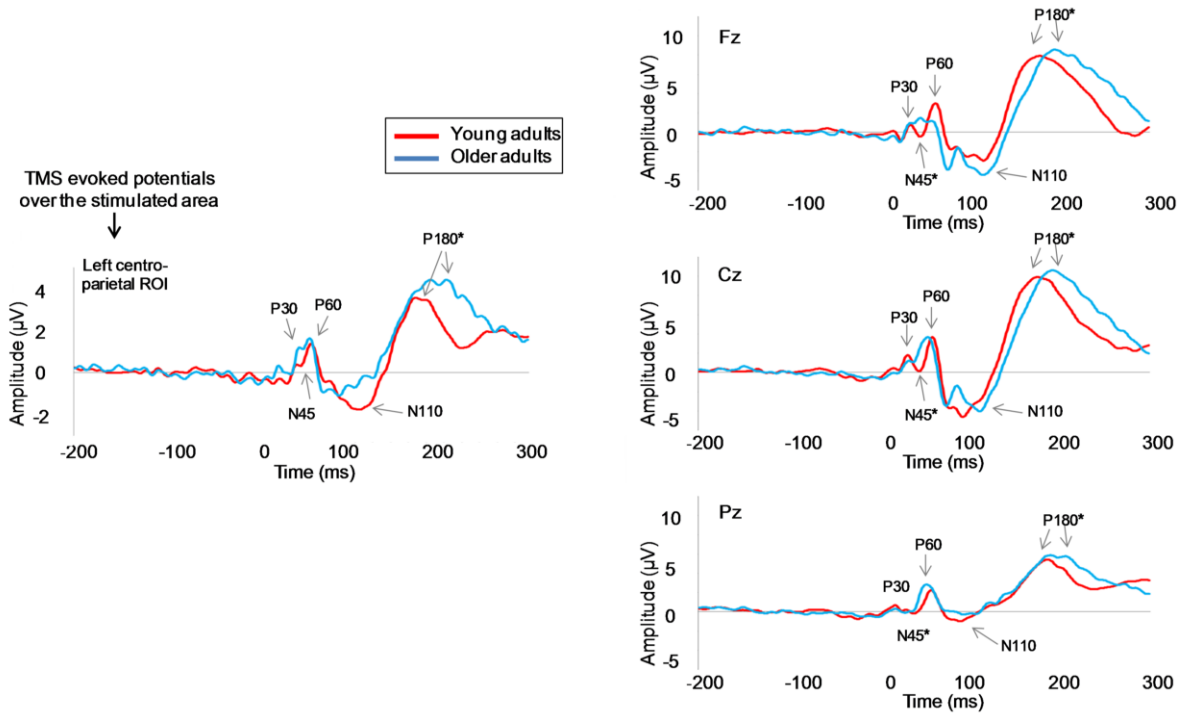


Figure 6. Lateralized Event-Related Potentials

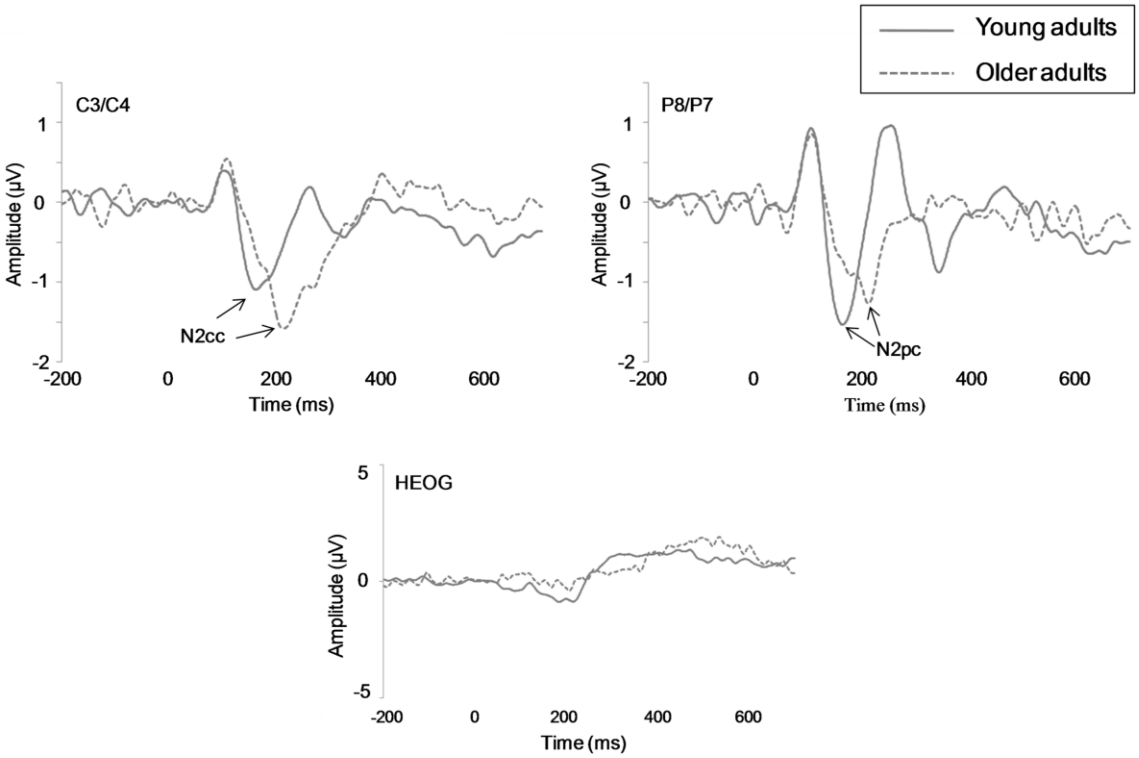
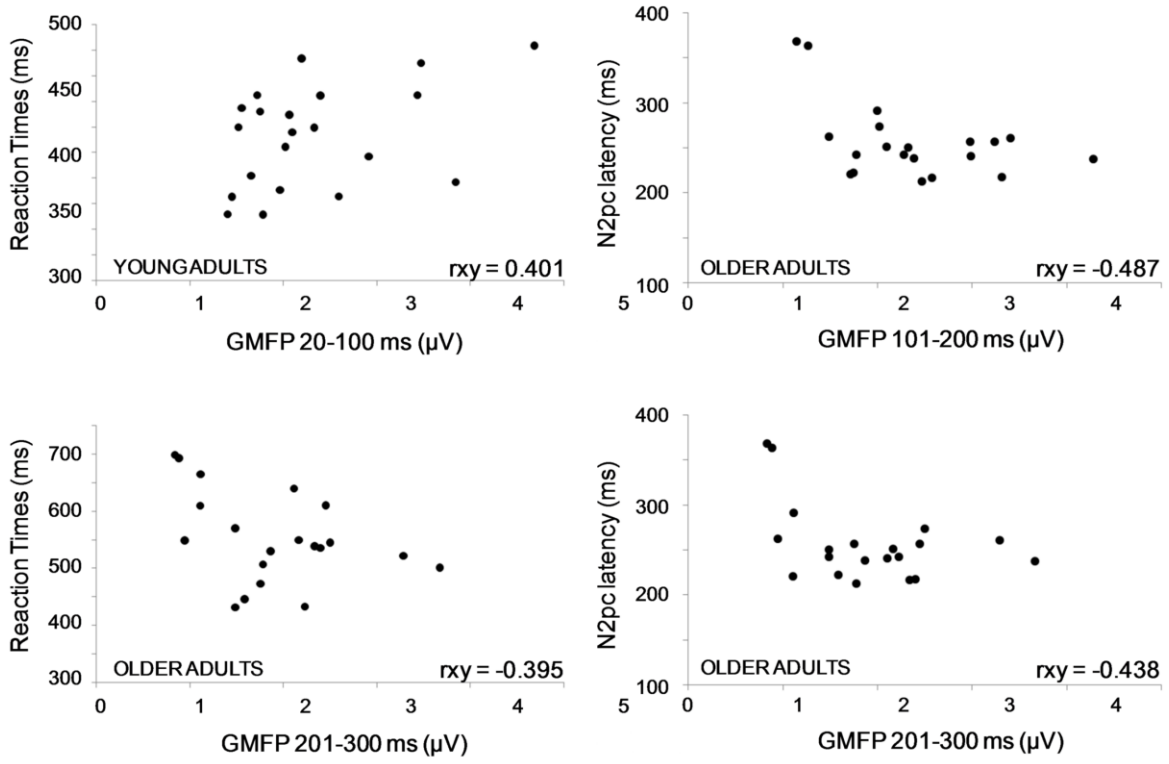


Figure 7. Correlations between GMFP, RT and N2pc



Supplementary Figure 1

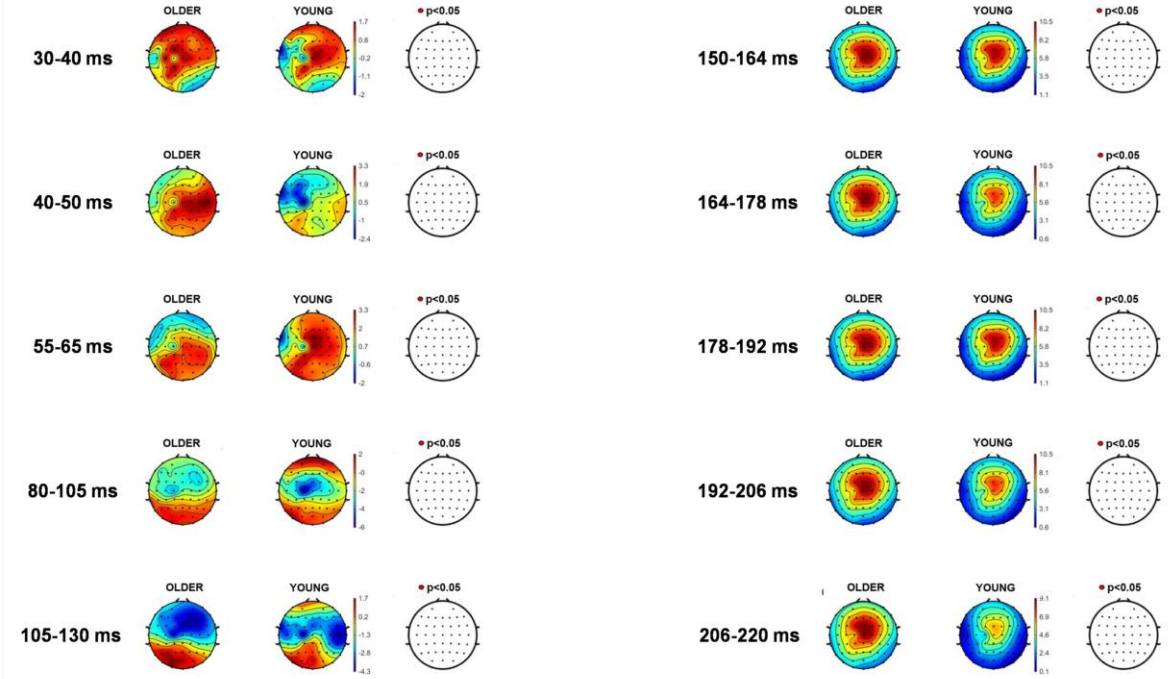


Figure captions

Figure 1. Experimental procedures. This figure shows the experimental procedures used in the present research. The Simon task is represented in the bottom panel. The hand assigned to each colour was counterbalanced across participants.

Figure 2. Behavioural results. This figure represents the reaction times and number of errors obtained in the incongruent compared to the congruent condition in both groups of participants by using box-and-whiskers plots. The "X" represents the mean whereas the horizontal bar across the rectangle represents the median. The bottom and top parts of the rectangles are the 25^{th} and 75^{th} percentiles of the distribution, respectively. The bottom and top whiskers represent the 5^{th} and 95^{th} percentiles of the distribution, respectively. The number of errors did not differ significantly between the two groups of participants, excluding a speed-accuracy trade-off.

Figure 3. Global Mean Field Power (GMFP). The GMFP was analysed in 20-100 ms, 101-200 ms, and 201-300 ms time windows. The amplitude of GMFP was significantly larger in young compared to older adults in the 101-200 ms time window. The right panel of the figure shows, in the three analysed time windows, the GMFP values by means of box-and-whiskers plots, where the bottom and top parts of the rectangles represent the 25^{th} and 75^{th} percentiles of the distribution, respectively, whereas the bottom and top whiskers represent the 5^{th} and 95^{th} percentiles of the distribution, respectively.

Figure 4. Transcranial magnetic stimulation evoked potentials (TEPs). In the left panel, this figure shows the TEPs in the available set of electrodes for young and older adults. The right panel represents current source density maps for the three time windows in which GFMP was analysed.

Figure 5. Transcranial magnetic stimulation evoked potentials (TEPs) in electrodes/regions of interest (ROIs)**.** This figure shows the TEP components (i.e., P30, N45, P60, N110, and P180) in Fz, Cz, Pz and in the left centro-parietal ROI. The asterisks denote significant differences found between young and older adults (this was the case for N45 amplitude and P180 latency in midline electrodes and P180 latency in the left centro-parietal ROI). Although P30 and N45 were not evident in the grand averages at the Pz

electrode, they were also analysed because both peaks were clearly identified in the individual subjects.

Figure 6. Event-related brain potentials obtained during the performance of the Simon task. The top left panel shows slower contralateral central negativity (N2cc) peak latency in older than young adults, indicating slower inhibition of the tendency to make a spatial response. The top right panel shows slower negativity posterior contralateral (N2pc) in older than young adults, indicating slowed processes related to attentional allocation to the target stimulus and suppression of the non-target stimulus. The bottom panel shows the absence of differences between young and older adults in the horizontal electrooculogram (HEOG) movements.

Figure 7. Relationships between GMFP and neural activity (ERP) in young and older adults. This figure represents the correlations between GMFP and correlates of cognitive functioning (RTs and ERPs). In older adults, decreased GMFP values are related to slower RT and N2pc latencies. These results suggest that age-related modulations in cortical excitability (i.e., reduced GMFP in older compared to young adults) represent a dysfunctional change.

Supplementary Figure 1. TEP amplitudes at each electrode. This figure represents the TEP amplitudes comparison between young and older adults at each electrode. The left panel shows the mean activity within 30-40 ms (P30 time window), 40-50 ms (N45 time window), 55-65 ms (P60 time window), 80-105 ms (N110 time window) and 105-130 ms (N110 time window). The right panel shows results for 5 time windows between 150 and 220 ms matching the P180 component. After correcting for multiple comparisons, group-related differences did not reach statistical significance.