Title: Acute exercise modulates the excitability of specific interneurons in human motor cortex

Running title: Acute exercise and M1 interneuron excitability

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Abstract

Acute exercise can modulate the excitability of the non-exercised upper-limb representation in the primary motor cortex (M1). Accumulating evidence demonstrates acute exercise affects measures of M1 intracortical excitability, with some studies also showing altered corticospinal excitability. However, the influence of distinct M1 interneuron populations on the modulation of intracortical and corticospinal excitability following acute exercise is currently unknown. We assessed the impact of an acute bout of leg cycling exercise on unique M1 interneuron excitability of a non-exercised intrinsic hand muscle using transcranial magnetic stimulation (TMS) in young adults. Specifically, posterior-to-anterior (PA) and anterior-to-posterior (AP) TMS current directions were used to measure the excitability of distinct populations of interneurons before and after an acute bout of exercise or rest. Motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) were measured in the PA and AP current directions in M1 at two time points separated by 25 minutes of rest, as well as immediately and 30 minutes after a 25-minute bout of moderate-intensity cycling exercise. Thirty minutes after exercise, MEP amplitudes were significantly larger than other timepoints when measured with AP current, whereas MEP amplitudes derived from PA current did not show this effect. Similarly, SICI was significantly decreased immediately following acute exercise measured with AP but not PA current. Our findings suggest that the excitability of unique M1 interneurons are differentially modulated by acute exercise. These results indicate that M1 interneurons preferentially activated by AP current may play an important role in the exercise-induced modulation of intracortical and corticospinal excitability.

Keywords: acute exercise, interneuron circuits, AP-sensitive, M1, cortical inhibition, TMS

Introduction

A growing body of literature highlights the ability of acute exercise to alter cortical neurophysiology, facilitate neuroplasticity and enhance motor learning (Roig et al., 2012; McDonnell et al., 2013; Singh et al., 2014a, 2014b; Smith et al., 2014; Mang et al., 2014, 2016; Thomas et al., 2016; Mooney et al., 2016; Stavrinos and Coxon, 2017; Ferrer-Uris et al., 2017; Lulic et al., 2017; Neva et al., 2017, 2019; El-Sayes et al., 2019a; Yamazaki et al., 2019; Andrews et al., 2020). Transcranial magnetic stimulation (TMS) has been an important tool for understanding the effects of exercise on the human brain. For example, a single bout of exercise induces a greater response to repetitive TMS (rTMS) protocols (McDonnell et al., 2013; Mang et al., 2014, 2016; Singh et al., 2014b; Andrews et al., 2020), suggesting that exercise may prepare or 'prime' the brain for neuroplasticity. Studies have further elucidated the possible neurophysiological mechanisms underlying these effects using single- and paired-pulse TMS protocols to investigate exercise-induced changes in intra- and inter-hemispheric circuitry within the primary motor cortex (M1; Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Neva et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019; Yamazaki et al., 2019).

Various inhibitory and facilitatory circuits of the upper-limb representation in M1 are altered following acute lower limb cycling exercise. For example, acute exercise decreases short-interval intracortical inhibition (SICI; Singh et al., 2014a; Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019; Yamazaki et al., 2019) and long-interval intracortical inhibition (Mooney et al., 2016). Acute exercise also modulates intracortical facilitation (Singh et al., 2014a; Lulic et al., 2017; Yamazaki et al., 2019) and short-interval intracortical facilitation (Neva et al., 2017). Other work shows that these effects extend to

interhemispheric inhibition, as the ipsilateral silent period decreases bilaterally following acute exercise (Neva et al., 2017). Further, paired pulse and dual site TMS studies showed that inhibitory connectivity to M1 from other regions such as the somatosensory cortex (Yamazaki et al., 2019; Brown et al., 2020) and the cerebellum (Mang et al., 2016) are modulated following an acute bout of exercise. Taken together, the results to date suggest that there may be a complex interplay between cortico-cortical, M1 intracortical and corticospinal excitability modulation following an acute bout of exercise. It should be noted that not all studies reported consistent effects of acute exercise on measures of cortical circuit excitability and that different exercise intensities/types elicit differential effects. Some studies showed no modulation of SICI using moderate (Mooney et al., 2016) or light intensity (Morris et al., 2020) exercise, while others found increased (Singh et al., 2014a) or decreased (Lulic et al., 2017) intracortical facilitation using moderate intensity. Other work demonstrated a lack of consistent modulation of long-interval intracortical inhibition using moderate (Singh et al., 2014a; Mooney et al., 2016) or high-intensity interval exercise (Stavrinos and Coxon, 2017). Further, whether or not exercise, at any intensity/type (light, moderate or high-intensity interval) affects corticospinal excitability is unclear; most studies found no impact of acute exercise (McDonnell et al., 2013; Mang et al., 2014; Singh et al., 2014a, 2014b; Smith et al., 2014, 2018; Stavrinos and Coxon, 2017; Neva et al., 2017; Yamazaki et al., 2019; Andrews et al., 2020; El-Sayes et al., 2020; Morris et al., 2020), yet some recent work demonstrates evidence for facilitated corticospinal excitability (Ostadan et al., 2016; Lulic et al., 2017; El-Sayes et al., 2019b; MacDonald et al., 2019; Opie and Semmler, 2019).

To date, TMS studies have exclusively used a posterior-to-anterior (PA) current to provide insight into neurophysiological changes associated with acute exercise, despite evidence suggesting that an anterior-to-posterior (AP) current may activate a unique set of interneurons

(Hanajima et al., 1998; Ziemann and Rothwell, 2000; Di Lazzaro et al., 2001b, 2001a, 2012; Paulus et al., 2008; Hamada et al., 2013, 2014). Single-pulse TMS generates multiple descending volleys of activity via direct (D-waves) or indirect (I-waves; early and late) activation of the corticospinal neurons in M1 resulting in a motor evoked potential (MEP; Hanajima et al., 1998, 2002; Ziemann and Rothwell, 2000; Di Lazzaro et al., 2001b, 2001a, 2012; Ilić et al., 2002; Paulus et al., 2008; Hamada et al., 2013, 2014). At low intensities, PA TMS preferentially activates early I-waves, whereas an anterior-to-posterior (AP) TMS (180° relative to PA) preferentially activates late I-waves (Di Lazzaro et al., 1998b, 2001b, 2012). The I-wave patterns elicited by these two different TMS current directions may represent distinct PA-sensitive and AP-sensitive interneuron circuits within M1 (Hamada et al., 2014; Mirdamadi et al., 2017; Cirillo et al., 2018; Hannah et al., 2018; Ni et al., 2019; Spampinato, 2020). Further, the excitability of these interneuron circuits is differentially modulated with distinct motor tasks (Mirdamadi et al., 2017; Hannah et al., 2018), suggesting that there may be unique functional roles of AP- and PA-sensitive interneurons. For instance, motor tasks requiring greater attentional allocation (Mirdamadi et al., 2017), enhanced motor preparation (Hannah et al., 2018), and prolonged task practice requiring visuomotor remapping (Spampinato et al., 2020), preferentially modulate interneuron circuits sensitive to AP TMS current. However, it is currently unknown whether acute exercise differentially modulates the excitability of these distinct M1 interneurons.

Common interpretation of the evidence that acute exercise modulates TMS measures focuses on the underlying GABAergic mechanisms. Specifically, a bout of exercise modulates $GABA_A$-receptor and $GABA_B$-receptor mediated activity (Inghilleri et al., 1996; Ziemann et al., 1996b, 1998b, 1996a; Kimiskidis et al., 2006; McDonnell et al., 2006; Irlbacher et al., 2007; Chen et al., 2008; Paulus et al., 2008; Udupa et al., 2010; Neva et al., 2017; Turco et al., 2018) known

to underlie the various TMS measures used in previous studies (Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Neva et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Opie and Semmler, 2019; Yamazaki et al., 2019; Brown et al., 2020). Although GABAergic mechanisms underlie these TMS measures, each of those used in previous studies (e.g., SICI) preferentially suppress the late I-wave without affecting the early I-wave (Hanajima et al., 1998, 2002; Ilić et al., 2002; Paulus et al., 2008; Di Lazzaro et al., 2012). Taken together, these results suggest that acute exercise may preferentially modulate late I-waves, which is indicative of AP-sensitive interneuron modulation in M1. However, the role of AP interneuron modulation in the response to acute exercise has not been directly tested.

Therefore, the overall objective of the current study was to employ PA and AP TMS to understand the impact of an acute bout of cycling exercise on unique interneuron excitability within M1. As moderate-intensity exercise showed modulation of measures representing late I-wave recruitment (e.g., SICI) in the majority of previous work (Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Neva et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019a, 2020; Yamazaki et al., 2019; Brown et al., 2020), we used this type and intensity of exercise to investigate the potential changes in AP- and PA-sensitive excitability. The study had two aims. The first aim was to determine whether a single bout of lower-limb cycling exercise differentially alters corticospinal excitability in AP-sensitive as compared to PA-sensitive interneurons. Although the evidence to date suggests that acute exercise does not alter corticospinal excitability tested with PA stimulation (Mang et al., 2014, 2016; Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Neva et al., 2017; Stavrinos and Coxon, 2017; Brown et al., 2020; Morris et al., 2020), AP stimulation at low intensities may activate unique M1 interneurons (Di Lazzaro et al., 1998b, 2001b) and thus reveal M1 modulation not previously

captured. Therefore, we hypothesized that acute exercise would increase AP corticospinal excitability measured with low TMS intensity, without a significant change in corticospinal excitability measured with PA stimulation. The second aim was to investigate whether the same bout of acute exercise would differentially modulate SICI measured with AP compared to PA currents. We hypothesized that acute exercise would decrease PA SICI as found previously (Singh et al., 2014a; Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Opie and Semmler, 2019), with further decrease in AP SICI.

Methods

Participants

Twenty-four healthy right-hand dominant (Edinburgh Handedness Inventory; Oldfield, 1971) individuals participated in the study (mean age ± SD: 27 ± 5.7 years, 12 females). Participants were screened for contraindications to TMS and magnetic resonance imaging (MRI) using standard screening forms. All participants were free from neurological disorders. The Clinical Research Ethics Board at the University of British Columbia approved all experimental procedures and participants provided written informed consent in accordance with the Declaration of Helsinki.

Experimental Design

Each participant completed a single experimental session to determine the differential influence of an acute bout of exercise on PA- and AP-sensitive interneurons, quantified with MEPs and SICI. Neurophysiological measurement was performed at two timepoints prior to the exercise bout (T0 and T1) separated by 25 minutes of rest, and immediately (T2) and 30 minutes after (T3) exercise completion (Figure 1). The order of testing for neurophysiological measures was

randomized across participants. Sessions were conducted at the same time of day (± 3 h) to account for fluctuations in corticospinal excitability (Tamm et al., 2009). Self-reported physical activity routines were measured via the International Physical Activity Questionnaire (IPAQ; Craig et al., 2003).

Electromyographic Recording

Electromyography (EMG) recorded from participants' right abductor pollicis brevis (APB) was used for all TMS measures. Electrodes with 1-cm diameter were arranged in a belly-tendon montage, with the ground electrode on the dorsum of the hand (Covidien, Mansfield, MA, USA). EMG data were collected using LabChart software (LabChart 7.0). EMG signals were sampled at 2 kHz, pre-amplified at 1 kHz and band-pass filtered at 1000 Hz using a PowerLab data acquisition system and a bioamplifier (AD Instruments, Colorado Springs, CO, USA). Data were recorded in a 500-ms sweep from 100 ms before to 400 ms after TMS delivery.

Transcranial Magnetic Stimulation (TMS)

During the collection of all TMS measurements, participants were seated comfortably in an adjustable chair and were at rest. A Magstim BiStim 200^2 stimulator (Magstim Co., UK) connected to a figure-of-eight shaped coil (Magstim 70 mm P/N 9790, Magstim Co., UK) was used to deliver a monophasic TMS pulse. TMS coil orientation was held to elicit current flow in either a PA or AP direction. PA coil position was held at a 45° angle to the mid-sagittal plane with the handle facing backwards; for the AP direction, the coil was rotated 180° (Figure 2A). To ensure coil positioning was accurate and consistent across the session, each individual's T1-weighted MRI image was used in conjunction with Brainsight neuronavigation software (Rogue Research Inc, Montreal, QC, Canada). With the coil held over M1 in a PA orientation, the 'hotspot' for the

APB representation was found. This same hotspot location was used for AP TMS. At this location, the resting motor threshold (RMT), defined as the lowest stimulus intensity that elicited 5 out of 10 MEPs greater than or equal to a peak-to-peak amplitude of 50 μV, was determined for both PA and AP directions. TMS pulses were delivered at a random rate between 0.15 and 0.2 Hz throughout the study.

MEP amplitudes in both PA and AP directions were used to assess potential changes in corticospinal excitability. MEPs were assessed at 110, 130, and 150% of RMT to determine if, as expected based on previous research (Day et al., 1989; Sakai et al., 1997; Di Lazzaro et al., 1998a, 2001b; Hamada et al., 2013, 2014; Cirillo et al., 2016, 2018), lower stimulation intensity (i.e., 110% RMT) was necessary to preferentially activate separate interneuron circuits targeted with PA and AP stimulation and thus identify unique exercise-related modulation within these interneuronal pools. Ten stimuli were delivered at each intensity, and the order of intensities was randomized.

SICI was measured to assess the influence of acute exercise on intracortical inhibition, measured in both the PA and AP directions. As previously described, a subthreshold conditioning stimulus (CS) was followed by a suprathreshold test stimulus (TS) over the M1 APB hotspot. The CS was delivered at 80% RMT; the TS was delivered at an intensity (% maximum stimulator output, MSO) that consistently produced an MEP with a peak-to-peak amplitude of ~ 1 mV. SICI was collected with 2-ms interstimulus interval (ISIs) in the PA TMS current direction and with 3-ms ISI for the AP TMS current direction based on previous research (Smith et al., 2014; Sale et al., 2016; Lulic et al., 2017; Cirillo et al., 2018). The 2-ms ISI for PA SICI was chosen based on the majority of past work that showed a significant reduction of inhibition following acute exercise (Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; Yamazaki et al., 2019) and other

studies that demonstrated a 3-ms ISI during PA SICI assessment may be influenced by mechanisms of facilitation (Peurala et al., 2008). Avoiding the latter confound was particularly important since our previous work showed that the same acute bout of exercise enhances the facilitatory mechanisms that may influence PA SICI assessment at 3-ms ISI (Neva et al., 2017). The 3-ms ISI for AP SICI was chosen based on recent work that showed consistent and robust inhibition compared to 2-ms ISI (Sale et al., 2016; Cirillo et al., 2018) and since there may be longer cortical transmission using AP TMS (Spampinato, 2020; Spampinato et al., 2020). To assess SICI at each timepoint, 10 paired pulses (CS+TS) and 10 TS pulses were delivered in a randomized order for PA and AP orientations.

MEP latencies were determined using the earliest MEP latency out of the block of 10 MEPs to assess I-wave recruitment and confirm unique interneuron recruitment. This was done for both PA and AP directions (at 110% RMT), as well as in the lateral-to-medial (LM) direction to provide an estimation of D-wave activation (using 150% RMT, which was determined in the PA direction).

Exercise

Participants performed a 20-minute exercise bout of lower-limb cycling conducted on a cycle ergometer (Ergoselect 200; Ergoline, Bitz, Germany) and with heart rate continuously monitored at the wrist using a wrist-mounted heart-rate monitor (Mio Alpha 53p). Participants completed a 5-minute warm-up (at 50 watts (W), at a self-selected cadence) followed by 20 minutes of continuous stationary biking at 65-70% of each individuals' age-predicted maximal heart rate while maintaining a cadence between 70-90 rotations per minute. Age-predicted maximal heart rate was determined as 220-age for males and 224-age for females. Importantly, the specific parameters used in the current study were selected to align with past work showing moderate intensity exercise induced modulation of measures related to late I-wave recruitment

(e.g., SICI), and thus may be more likely to modulate AP-sensitive interneurons. Throughout the exercise session, the modified Borg scale (1-10) was used to assess ratings of perceived exertion (RPE) (Borg, 1998), as reported every five minutes by the participants, and HR was continuously monitored by the experimenters and recorded every 5 minutes. Participants were instructed to keep their hands relaxed (not gripping the handlebars) with their arms resting on top of the handlebars during the session in order to avoid any contraction and/or fatigue of the target non-exercised intrinsic hand muscle. To confirm target muscles were relaxed, EMG was recorded from the APB.

Rest

The period of rest between the first two sets of TMS assessment consisted of sitting comfortably, semi-reclined in a chair. Participants were instructed not to perform any tasks with their upper limbs (e.g., using a mobile device). The duration of the rest period was 25 minutes to equate it to the duration of the exercise protocol.

Data Processing and Statistical Analysis

Repeated measures analyses of variance (RM-ANOVA) were used to test the effects of exercise on MEP amplitudes and SICI, measured with PA and AP coil orientations. *Post hoc* analyses were performed using Tukey's HSD where appropriate. Residual statistics, skewness and kurtosis values and plots were produced to ensure normality and homoscedasticity of data. All statistical procedures were conducted using SPSS (SPSS 25.0) software and significance was set at $p < 0.05$. Effect sizes were calculated and reported as partial eta squared (η^2_{partial}) on the strength of significant effects, and were interpreted based on previously developed guidelines (Cohen, 1988).

Neurophysiological Measures

RMT, TS %MSO and TS MEP amplitudes

RMT (%MSO) was compared across TMS current directions as determined at the beginning of the experiment using a one-way ANOVA including within-subjects factor TMS CURRENT (PA, AP). TS %MSO values during SICI assessment were compared across TMS currents to ensure stable TMS intensity before and after exercise using a two-way RM-ANOVA with within-subjects factors TIME (T0, T1, T2, T3) and TMS CURRENT (PA, AP). Similarly, TS MEP amplitudes were compared across TMS currents to ensure stable corticospinal output excitability during SICI assessment before and after exercise using a two-way RM-ANOVA with within-subjects factors TIME (T0, T1, T2, T3) and TMS CURRENT (PA, AP).

MEP latency

MEP onset latency was determined using a semi-automated system and defined as the time point where the rectified EMG signal exceeded 5-fold of the mean pre-stimulus EMG. MEP latencies elicited with each current direction (PA, AP, LM) and MEP latency differences (ΔPA-LM, ΔAP-LM) were used as indirect measures of I-wave recruitment (Ni et al., 2011; Hamada et al., 2013, 2014; Cirillo et al., 2016, 2018; Sale et al., 2016; Spampinato et al., 2020) to confirm unique interneuron activation (Di Lazzaro et al., 2001a; Cirillo et al., 2016, 2018). The earliest response MEP latency for each current direction was compared with a one-way RM-ANOVA including within-subjects factor TMS CURRENT DIRECTION (PA, AP, LM). MEP latency differences between PA-LM and AP-LM were compared using a paired samples t-test.

MEPs and SICI

MEP and SICI EMG data were inspected for voluntary muscle activity. Peak-to-peak MEP amplitudes (mV) were processed using custom MATLAB scripts. All trials with any visible voluntary pre-stimulus EMG data were discarded (0.02% of trials). SICI was expressed and analyzed as a ratio of CS+TS over TS amplitude, where smaller values represent more inhibition and larger values represent less inhibition (disinhibition).

To measure the effect of acute exercise on corticospinal excitability measured with PA and AP current, the ten MEPs were averaged for each of the three intensities and each TMS current direction. Two-way RM-ANOVAs were performed using within-subjects factors TIME (T0, T1, T2, T3) and TMS CURRENT (PA, AP) for each stimulus intensity (110, 130, 150% RMT). We performed statistical analyses on each stimulus intensity individually as the use of lower stimulus intensities (i.e., 110% RMT) increases the chance to preferentially activate unique interneuron circuits with PA and AP TMS current directions (Hamada et al., 2013, 2014; Cirillo et al., 2016, 2018; Mirdamadi et al., 2017; Ni et al., 2019).

To assess the effect of acute exercise on SICI measured with PA and AP currents, the MEPs for the 10 TS stimuli and the 10 paired CS+TS pulses were averaged for each current direction. A two-way RM-ANOVA was performed using within-subjects factors TIME (T0, T1, T2, T3) and TMS CURRENT (PA, AP).

Bivariate correlational analyses were conducted to assess relationships between MEP latency differences (AP-LM) and significant changes in neurophysiological measurements after exercise. These analyses were performed based on previous work demonstrating relationships between ΔAP-LM MEP latency and MEP differences in response to other interventions such as theta burst stimulation (Hamada et al., 2013).

Smallest Detectable Change (SDC)

In order to assess whether the significant exercise-induced change in the TMS measures exceeded values which could be attributed to measurement error (Beckerman et al., 2001), we used the data from T0 and T1 to calculate the smallest detectable change (SDC_{group}), as described previously (Schambra et al., 2015; Samusyte et al., 2018; Turco et al., 2019). Briefly, $SDC_{individual}$ was calculated first with the formula $SDC_{individual}$ = 1.96 × Standard Error of Measurement (SEMeas) x √2, where a 95% confidence interval is represented by 1.96, the variance associated with pre- and post-measurements is accounted for with √2, and the SEMeas is calculated as √Mean Squared Error (MSE) (Weir, 2005). The $SDC_{individual}$ values were then used to determine the SDC_{group} as we were interested in assessing the change that was occurring after exercise on a group level rather than an individual level. This was done with the formula: SDC_{group} = $SDC_{individual}/\sqrt{n}$., SDC_{group}.

Results

Physical activity, acute exercise and TMS values

All participants demonstrated moderate-to-high levels of physical activity according to the long-form IPAQ (Craig et al., 2003), with a mean (± SEM) metabolic equivalents-min/week of 3655 ± 495. The exercise bout was successfully completed by all participants. During the acute exercise, the average heart rate was 132.3 ± 5.8 bpm and average RPE 3.8 ± 1.2 (corresponding to between "moderate" to "somewhat hard" perceived exertion). Table 1 displays the RMT values, MEP amplitudes recorded during onset latency assessment as well as % MSO values and TS amplitudes for SICI. RMT values were significantly different across TMS CURRENT (PA mean RMT: 43 ± 7 % MSO; AP mean RMT: 55 ± 8 % MSO; $F_{1,23} = 347.45$, $p < 0.001$, $\eta^2_{partial} = 0.94$). As expected, TS % MSO values during SICI were different across TMS CURRENT ($F_{1,23}$ = 138.31, $p < 0.001$, $\eta^2_{partial} = 0.86$), but not across TIME ($F_{3,69} = 2.09$, $p = 0.11$) nor was there an

interaction between factors ($F_{3,69}$ = 0.547, p = 0.652). Importantly, TS amplitudes during SICI were not different across TMS CURRENT ($F_{1,23}$ = 2.784, p = 0.11) and TIME ($F_{3,69}$ = 1.319, p = 0.275), nor was there an interaction between factors ($F_{3,69}$ = 0.636, p = 0.594).

MEP latency

An effect of TMS CURRENT ($F_{2,46}$ = 187.02, p < 0.00001, $\eta^2_{partial}$ = 0.89; see Figure 2 for MEP onset latency data) was found, with *post-hoc* analyses showing that LM MEP latency (21.1 ± 1.4 ms) was shorter than PA (22.4 ± 1.4 ms; p < 0.001) and AP (23.9 ± 1.4 ms; p < 0.001), and PA was shorter than AP (p < 0.001). Also, MEP latency difference between PA-LM and AP-LM was significant ($t_{1,23}$ = 12.9, p < 0.000001), with ΔAP-LM (2.9 ± 0.8 ms) greater than ΔPA-LM (1.3 ± 0.8 ms). These results are similar to previous work on MEP latencies recorded at rest and are indicative of unique I-wave recruitment using different TMS currents (Ni et al., 2011; Sale et al., 2016; Spampinato et al., 2020).

The effect of acute exercise on PA and AP corticospinal excitability

For the lowest stimulation intensity (110% RMT) MEPs, a TIME x TMS CURRENT interaction was found ($F_{3,69}$ = 3.76, p = 0.015, $\eta^2_{partial}$ = 0.14), with *post hoc* analysis indicating greater AP MEP amplitude at T3 compared to T0 (p = 0.012), T1 (p = 0.002) and T2 (p = 0.023), with no statistically significant changes to PA MEPs (Figure 3A). Further, there was no statistically significant effect for TMS CURRENT ($F_{3,69}$ = 0.227, p = 0.639), whereas there was a main effect of TIME ($F_{3,69}$ = 4.34, p = 0.007, $\eta^2_{partial}$ = 0.16). For higher stimulation intensities (130% and 150% RMT), there were no significant effects due to acute exercise (Figure 3B-C). Specifically, for MEPs at 130% RMT, there was no significant effect of TMS CURRENT ($F_{1,23}$ = 1.41, p = 0.247), TIME ($F_{3,69}$ = 1.06, p = 0.371), nor an interaction between factors ($F_{3,69}$ = 1.24, p = 0.303). Finally, for MEPs at 150% RMT, there was no significant effect of TMS CURRENT ($F_{1,23}$ = 3.02,

p = 0.096), TIME ($F_{3,69}$ = 0.243, p = 0.866), nor an interaction between factors ($F_{3,69}$ = 1.134, p = 0.341).

The effect of acute exercise on PA and AP intracortical inhibition

A TIME x TMS CURRENT interaction was found ($F_{3,69}$ = 3.14, p = 0.031, $\eta^2_{partial}$ = 0.12), with *post hoc* analysis indicating less AP SICI at T2 compared to T0 (p = 0.025) and T1 (p = 0.049), with no statistically significant changes to PA SICI (Figure 4). A main effect of TMS CURRENT was also found ($F_{1,23}$ = 5.39, p = 0.029, $\eta^2_{partial}$ = 0.19) indicating greater overall inhibition elicited by AP current. Finally, the main effect of TIME was not statistically significant ($F_{3,69}$ = 2.41, p = 0.074).

Correlational analysis of MEP latency difference and changes in neurophysiological measures

No significant correlations were found between ΔAP-LM MEP latency and significant changes in neurophysiological measures following acute exercise.

Smallest Detectable Change (SDC)

The SDC_{group} for AP MEPs at 110% RMT was 0.135. The difference in AP MEP amplitudes between T3 and T0 (0.326), T3 and T1 (0.370), and T3 and T2 (0.306) exceeded this value. The SDC_{group} for AP SICI was 0.059, and the difference between T2 and T0 (0.163) as well as T2 and T1 (0.151) exceeded this value.

Discussion

This is the first study to demonstrate that a single bout of acute exercise preferentially impacts unique interneuron excitability in M1. Results revealed that AP-sensitive MEPs and SICI were modulated following exercise, whereas no evidence was found for PA-sensitive measures. Specifically, AP MEPs measured at low stimulus intensities (110% RMT) were enhanced 30

minutes following acute exercise. Also, AP SICI was disinhibited immediately following acute exercise, whereas there was no effect for PA SICI. We confirmed that these results were not due to measurement error via smallest detectable change analysis. Taken together, our results indicate that M1 interneurons that are preferentially activated by AP current may play an important role in the exercise-induced modulation of intracortical and corticospinal excitability.

AP-sensitive corticospinal excitability increased following acute exercise

As hypothesized, we found that AP-sensitive corticospinal excitability was enhanced by an acute bout of cycling exercise, whereas no evidence was found for a similar effect on PA-sensitive excitability. There is growing evidence that AP- and PA-directed TMS currents over M1 can measure and modulate different sets of interneuron input to corticospinal neurons (Day et al., 1989; Di Lazzaro et al., 2001b, 2001a; Hamada et al., 2013, 2014; Cirillo et al., 2016, 2018; Sale et al., 2016; Mirdamadi et al., 2017; Hannah et al., 2018). For example, subthreshold paired-associative stimulation (PAS) with a 25-ms ISI requires AP current to elicit a robust corticospinal excitability increase, whereas PAS with a 21.5-ms ISI requires PA TMS (Hamada et al., 2014). Additionally, cerebellar-M1 inhibition tested with a PA current over M1, but not an AP current, is modulated following PAS (21.5-ms ISI) with PA TMS (Spampinato et al., 2020). Further, motor tasks requiring greater visual attention (Mirdamadi et al., 2017) and motor preparation (Hannah et al., 2018) preferentially modulate AP-sensitive interneuron excitability, and motor task practice involving novel sensorimotor mapping alters cerebellar-M1 inhibition tested with AP current over M1 (Spampinato et al., 2020). Recent modelling evidence suggests that AP currents may activate a cortical site within the precentral gyrus that is more anterior than that activated with PA currents (Aberra et al., 2020). Similarly, it is hypothesized that AP stimulation activates more rostral areas

of M1 that receive inputs from the premotor cortex (Groppa et al., 2012; Volz et al., 2015). It has been hypothesized that AP stimulation could activate inputs from the premotor or primary somatosensory cortices (Di Lazzaro et al., 2008, 2012; Volz et al., 2015). Thus, it is possible that our findings of exercise-enhanced AP-sensitive circuitry are associated with enhanced input from such regions outside of M1 (Rajab et al., 2014; Thacker et al., 2014; Yamazaki et al., 2019; Brown et al., 2020). A recent study showed that acute exercise enhanced cortical activity contributing to movement preparation, which is likely supported by the premotor cortices (Thacker et al., 2014). Other work showed that acute exercise increased connectivity between M1 and primary somatosensory cortices (Rajab et al., 2014), and modulated measures of sensorimotor integration using a combination of peripheral nerve stimulation and TMS (Yamazaki et al., 2019; Brown et al., 2020). Contextualized with past work, our findings suggest that distinct interneuron populations activated by AP TMS may be preferentially altered by acute exercise, and that these interneuron populations could be influenced by regions outside of M1 (e.g., premotor and somatosensory cortices).

Although this is the first study to directly test the effects of acute exercise on AP- and PA-sensitive interneuron excitability, complimentary evidence comes from previous research investigating the response to PAS following exercise (Mang et al., 2016). Specifically, acute exercise enhanced the response to a PAS protocol (i.e., with a 25 ms ISI) shown to be dependent on AP-sensitive interneurons, whereas there was no evidence of enhanced response to a PAS protocol (i.e., with a ~21 ms ISI) shown to be dependent on PA-sensitive interneurons (Hamada et al., 2014; Mang et al., 2016). Considering these previous results, the current findings are not surprising. Thus, our work highlights the importance of AP-sensitive interneurons to the modulation of corticospinal excitability following a bout of acute exercise.

An increase of AP-sensitive corticospinal excitability was found at 30 minutes post acute cycling exercise, but this effect was not present immediately after exercise. These results are supported by and extend previous findings. Past work has shown modulations in cortical circuitry 15 to 30 minutes after exercise that was not present immediately. Specifically, previous work showed decreased SICI only 15 minutes (Smith et al., 2014), 20 minutes (Yamazaki et al., 2019), or 30 minutes (Singh et al., 2014) post acute exercise. Other work showed increased long-afferent inhibition measured only at 30 minutes post acute exercise (Brown et al., 2020). The mechanisms by which such delayed effects occur are unclear. However, it is possible that a competition between stress hormones (e.g., cortisol), neurotrophic factors (e.g., brain derived neurotrophic factor) and neurotransmitters (e.g., GABA) released during and after acute exercise (Mang et al., 2013; Skriver et al., 2014) contribute to a delayed enhancement of cortical circuit excitability. The release of neurotrophic factors and neurotransmitters may accumulate with time after exercise and eventually offset the detrimental effects of cortisol (Sale et al., 2008; Mang et al., 2013) on cortical excitability as measured using TMS. As we did not measure these factors this idea remains speculative. It is important to note that several measures of enhanced cortical excitability occur immediately following exercise, including measures of SICI (Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019), intracortical facilitation (Singh et al., 2014a), short-interval intracortical facilitation (Neva et al., 2017), long-interval intracortical inhibition (Mooney et al., 2016), transcallosal inhibition (Neva et al., 2017) and cerebellar inhibition (Mang et al., 2016), as well as some studies that showed an immediate increase of corticospinal excitability following exercise (Lulic et al., 2017; El-Sayes et al., 2019; Opie and Semmler, 2019). Therefore, future work should comprehensively measure the timing of cortical excitability

modulation in parallel with hormone and neurotrophic factor release following exercise to further understand the nature of immediate and delayed effects of acute exercise.

AP-sensitive intracortical inhibition decreased following acute exercise

Partially supporting our hypothesis, we found that AP-sensitive SICI decreased following acute exercise, whereas no evidence for a similar effect was found for PA-sensitive SICI. Our combined findings of SICI disinhibition and increased MEP amplitude, both tested with AP current, may provide evidence that distinct AP-sensitive interneuron populations are preferentially modulated by acute exercise. It is important to note that the exercise-induced change in SICI was not confounded by the increased AP MEP amplitudes following acute exercise. This is because the test stimulus MEP amplitude (i.e., corticospinal output excitability) was controlled to remain constant before and after exercise. We discuss the potential explanations for the current findings below.

Several studies now indicate that distinct interneuron excitability modulation can be tested using paired pulse paradigms similar to the current SICI measures, such as the combination of a conditioning peripheral nerve stimulation (Mirdamadi et al., 2017), or TMS pulse over the cerebellum (Spampinato et al., 2020), followed by AP TMS over M1. Specifically, Mirdamadi et al. (2017) showed greater disinhibition of short-afferent inhibition tested with AP current while performing a task that required high visual attention allocation, whereas short-afferent inhibition tested with PA current was unaltered (Mirdamadi et al., 2017). Similarly, Spampinato et al. (2020) showed decreased cerebellar-to-M1 inhibition tested with PA TMS over M1 following PAS with a 21.5 ms ISI, whereas this measure tested with AP TMS was unaltered. Spampinato et al. (2020) also showed that cerebellar-to-M1 inhibition tested with AP TMS over M1 decreased following prolonged practice of a sequential motor task involving visuomotor remapping compared to the

same measure with PA TMS over M1 (Spampinato et al., 2020). Since the paired pulse measures collected in these previous studies used both AP and PA TMS over M1, findings were interpreted as indicative of unique AP-sensitive interneuron modulation. In combination with our data, these previous studies suggest that modulation of AP-sensitive interneurons contributed to the disinhibition of AP SICI following acute exercise. However, as discussed below, there are other potential explanations for the current findings.

Previous work has postulated that modulation of $GABA_A$-receptor mediated activity contributes to the exercise-induced disinhibition of SICI (Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Neva et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Opie and Semmler, 2019; Brown et al., 2020). The role of $GABA_A$-receptor mediated activity underlying the phenomenon of SICI has been demonstrated by previous pharmacological studies (Ziemann et al., 1996a, 1996b; Hanajima et al., 1998; Di Lazzaro et al., 2012). It is possible that modulation of GABAergic receptor activity may underpin the current findings, as several studies confirm that acute exercise modulates response to various single and paired pulse TMS measures (Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Neva et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Yamazaki et al., 2019), all of which likely involve certain levels of $GABA_A$-receptor and/or $GABA_B$-receptor mediated activity (Inghilleri et al., 1996; Ziemann et al., 1996a, 1996b, 1998b; Kimiskidis et al., 2006; Chen et al., 2008; Paulus et al., 2008). The influence of $GABA_A$-receptor mediated activity would presumably be common in SICI measured with AP and PA TMS. Since the current study showed exercise-induced disinhibition in AP, but not PA SICI, it seems that AP-sensitive interneuron populations play an important role in exercise-induced disinhibition of SICI. However, we cannot discount the

possibility that our findings stem from a combination of the two mechanisms (i.e., GABAergic receptor activity and AP-sensitive interneurons).

The use of AP current is thought to provide a more robust and reliable measure of SICI compared to a PA current (Amassian et al., 1987; Di Lazzaro et al., 1998b, 2001b; Hanajima et al., 1998; Hamada et al., 2014; Cirillo et al., 2016, 2018; Sale et al., 2016). For example, one study found that AP SICI showed age related differences in healthy people whereas SICI measured with PA current did not (Sale et al., 2016). SICI measured in the AP direction elicits consistently greater inhibition (with a 3 ms ISI) compared to PA SICI (with either 2 or 3 ms ISI) in young healthy individuals (Cirillo et al., 2018). It is possible that the current findings of exercise-induced SICI disinhibition measured with an AP current may simply reflect the greater sensitivity of this measure. However, previous work demonstrated that late I-wave suppression during SICI collection occurred regardless of PA or AP TMS current direction (Hanajima et al., 1998), which suggests that exercise likely modulated these unique interneuron populations differently.

Lack of significant PA-sensitive intracortical inhibition change following acute exercise

Contrary to our hypothesis and the majority of previous findings (Singh et al., 2014a; Smith et al., 2014; Lulic et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Opie and Semmler, 2019; Yamazaki et al., 2019), we did not find evidence that acute exercise changed SICI measured with PA current. This finding underscores a degree of inconsistency in the effects of acute exercise on measures of cortical excitability (Nicolini et al., 2020). For instance, most past work found a reduction in PA SICI following acute exercise, yet there are two studies that found no evidence of a change, similar to the current results (Mooney et al., 2016; Morris et al., 2020). While the reason for the discrepancy is unclear, it may be due to the possible lack of sensitivity of PA current during SICI collection (Cirillo et al., 2016, 2018; Sale et al., 2016). First, although

research has shown that PA TMS preferentially activates early I-waves, there is also evidence that PA current recruits both early and late I-waves (Di Lazzaro et al., 1998a, 2001a; Hanajima et al., 1998; Di Lazzaro and Rothwell, 2014). By activating interneurons responsible for the generation of both early and late I-waves, it is possible that measurement of SICI with PA current is less robust as compared with AP current (Amassian et al., 1987; Di Lazzaro et al., 1998b, 2001b; Hanajima et al., 1998; Hamada et al., 2014). This difference could have led to the discrepant findings (Mooney et al., 2016; Morris et al., 2020). Relatedly, previous work showed that greater suppression of the late I-wave occurred with higher conditioning stimulus intensities when measuring SICI with both PA and AP current (Hanajima et al., 1998). It is possible that employing a higher conditioning stimulus intensity than that used in the current study (80% RMT) may have increased the possibility to detect a change in PA SICI following exercise. Although beyond the scope of the current study, future work should comprehensively examine the stimulus parameters of SICI with both PA and AP currents to further understand the effects of acute exercise. Our results could be explained by specific EMG and TMS parameters employed to measure the effects of exercise on SICI, such as recording from different upper-limb muscles (e.g., APB rather than flexor pollicis brevis, first dorsal interosseous, extensor carpi radialis) or using various conditioning and test stimulus intensities as well as ISIs (Singh et al., 2014a; Smith et al., 2014; Mooney et al., 2016; Lulic et al., 2017; Stavrinos and Coxon, 2017; El-Sayes et al., 2019b; Opie and Semmler, 2019; Morris et al., 2020). Future work could explore the effects of acute exercise on multiple non-exercised muscle representations in M1 and employ various TMS parameters (e.g., conditioning and test stimulus intensities, ISIs, etc.) to further understand the effects of acute exercise on SICI.

Limitations

There are limitations to this study to consider when interpreting the current results. First, the test stimulus intensity used during SICI collection was slightly higher (~1.4 mV) than that used in previous studies (~1-1.3 mV) using AP TMS current (Ni et al., 2011; Hamada et al., 2013, 2014; Mirdamadi et al., 2017; Cirillo et al., 2018; Hannah et al., 2018), which may have influenced the current SICI results. However, since the test stimulus MEP amplitude and the %MSO remained consistent before and after rest and acute exercise, and previous results showed that TS MEP amplitudes up to 4 mV (with PA TMS) elicited significant inhibition (Roshan et al., 2003), it is unlikely that the test stimulus alone influenced the current results. Second, the conditioning stimulus intensity was determined at rest using 80% RMT, which is different from several other studies that used a percentage of active motor threshold during slight isometric muscle contraction (Hamada et al., 2013, 2014; Cirillo et al., 2018, 2020a; Hannah et al., 2018; Mooney et al., 2019a). It is possible that the higher conditioning stimulus intensity used in the current study influenced the results, since the majority of previous studies used 70 to 90% active motor threshold when assessing AP SICI (Cirillo et al., 2018, 2020b; Hannah et al., 2018; Mooney et al., 2019b) and it is not currently known how different %RMT conditioning stimulus intensities may influence AP SICI. Third, mechanisms of short-interval intracortical facilitation may have influenced the current AP SICI findings, as previous work has shown a relationship between these measures (Ziemann et al., 1998a; Ortu et al., 2008; Peurala et al., 2008). Although previous work showed that conditioning and test stimulus intensities similar to the current study are likely below threshold for eliciting short-interval intracortical facilitatory mechanisms using PA TMS (Ortu et al., 2008), it is possible that such facilitatory mechanisms may influence SICI using AP TMS. Fourth, the MEP latencies may have been underestimated in two ways: 1) the collection of MEPs at rest and 2) the use of 150% RMT determined in the PA direction for LM stimulation. We assessed MEP latencies

elicited using different TMS currents (LM, PA and AP) at rest using %RMT, which is not common in past studies using a small pre-activation and percentage of active motor threshold (Hamada et al., 2013; Cirillo et al., 2016, 2018; Hannah et al., 2018). Still, our results indicate significant differences between raw MEP onset latencies (LM, PA and AP) and MEP latency differences (ΔPA-LM and ΔAP-LM), which is indicative of unique I-wave recruitment and consistent with studies using a similar method at rest (Ni et al., 2011; Sale et al., 2016; Spampinato et al., 2020). Finally, RMT was not assessed in the LM TMS direction. Instead, 150% RMT in the PA direction was used to assess LM MEP onset latency. Therefore, the LM MEP latency could have been underestimated. However, previous work used a TMS intensity to elicit a ~1 mV MEP to assess MEP onset latencies with LM, PA and AP currents (Ni et al., 2011) and we confirmed that the average (± SD) MEP amplitude collected during LM onset latency was 1.7 ± 0.8 mV, indicating that 150% RMT assessed in PA direction was likely a sufficient intensity to elicit corticospinal excitability indicative of D-wave generation.

Future Directions

The results from the current work provide an important first step in investigating the impact of exercise on AP- and PA-sensitive interneurons within M1; however, there are a number of questions that remain to be answered to further our understanding. The current work used a very precise methodology for measuring M1 neurophysiology; expanding this to include a number of additional parameters will be important for enhanced applicability of results. First, we performed TMS measures at rest to control for the potential combination of effects from performing a task during measures (i.e., 10% of maximum voluntary contraction using visual feedback) and to control for potential fatigue that may combine with lower-limb exercise. Future studies should

consider performing single-pulse TMS measures with light pre-activation, since previous studies have shown this may further isolate the AP and PA circuits (Hamada et al., 2013; Cirillo et al., 2016, 2018; Hannah et al., 2018). Second, increasing the range of CS and TS intensities and the number of interstimulus intervals may extend the current findings of exercise-induced modulation of SICI. Third, in line with previous literature, we found that AP SICI showed greater baseline inhibition to PA SICI (Cirillo et al., 2016, 2018). Although pre-exercise PA SICI was similar to previous research that found a decrease in SICI following acute exercise (Singh et al., 2014a; Smith et al., 2014; Stavrinos and Coxon, 2017), future work could consider controlling the level of pre-exercise inhibition by adjusting the CS intensity to elicit ~50% inhibition (Mooney et al., 2016) with AP and PA TMS to confirm the current findings. Fourth, assessing different types (continuous vs. interval), intensities (low, moderate, high) and durations is warranted as past work has indicated that such parameters can uniquely impact cortical excitability and plasticity. Understanding the differential neurophysiological impact of these parameters will inform targeted exercise prescription for specific populations of interest (e.g., older adults, individuals with stroke). Fifth, the individuals in the current study were highly active (> 3000 METs/week), which may lead to physiological changes that impact the response to exercise, such as increased efficiency of BDNF uptake (Nofuji et al., 2012). Given past research suggesting that there might be differences in exercise-induced changes in corticospinal excitability based on activity levels (Lulic et al., 2017), we cannot rule out the possibility that a sample with less active individuals would have responded differently to our exercise protocol. Therefore, since it is beyond the scope of the current study, future studies should compare between groups of individuals with different activity levels to identify the limits of acute exercise to enhance M1 excitability. Finally, there is some evidence to indicate that response to neuroplasticity-inducing protocols (e.g., PAS) may be greater in the

afternoon (Sale et al., 2007). While this has not been tested for exercise specifically, this avenue of inquiry is worth pursuing as the results could have important implications for future research on the effects of acute exercise on cortical excitability. Taken together, understanding the parameters and timing of exercise, and how responses may vary in different populations will be critical to enhancing knowledge and applicability of this field.

Conclusion

This study revealed that the excitability of unique M1 interneurons is preferentially modulated by acute exercise. Taken together, our results of increased corticospinal excitability and decreased intracortical inhibition measured with AP current indicate that AP-sensitive interneuron modulation may play an important role in the exercise-induced alterations of M1 excitability. Our findings may have important implications for the development of adjunct interventions in healthy and clinical populations involving bouts of acute exercise and for the application of TMS current direction for the assessment and modulation of M1 excitability.

Author Contributions

JLN and KEB co-conceived of the study, analysed and interpreted the data and wrote the manuscript. JLN contributed to data collection. SP contributed to data collection and analysis, and edited the manuscript. SJF and NM contributed to data collection and edited the manuscript. MPB and LAB contributed to the interpretation of data and to editing the manuscript.

Conflict of Interest Declaration

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Figure Captions

Figure 1. Experimental Design. TMS measures were assessed prior to (T0) and following (T1) 25 minutes of rest. Exercise was then performed and TMS measures were reassessed immediately (T2) and 30 minutes after the exercise was completed.

Figure 2. Motor evoked potential (MEP) latency (I-wave recruitment) results. A. TMS coil orientations over the left (dominant) M1 APB representation. Lateral-medial (LM), depicted in black, was used to preferentially elicit D-waves. Posterior-anterior (PA), represented by dark grey lines, was used to preferentially elicit early I-waves. Anterior-posterior (AP), shown with light grey lines, was used to preferentially elicit late I-waves. Electromyographic (EMG) traces are displayed from a representative participant, recorded from the right (dominant) APB. Vertical dotted lines represent MEP onset latency elicited by each TMS current direction (LM, PA, AP). B-C. Box whisker plots for MEP latency data elicited by LM (black), PA (dark grey), and AP (light grey) current directions are shown in B. MEP latency differences are shown in C, in which the box depicts the median, 25th and 75th percentiles, and the whiskers represent the 5th and 95th percentile. Individual data is overlayed in both B and C. Individual data in C are connected with lines and are slightly staggered for ease of viewing. Statistical findings are displayed within the graph. ** $p < 0.05$, *** $p < 0.001$.

Figure 3. Corticospinal excitability (MEP) results. A-C. Displays average peak-to-peak MEP amplitudes elicited with AP (black) and PA (white) current directions for 110% RMT (A), 130% RMT (B), and 150% RMT (C) at each timepoint (T0, before rest; T1, after rest; T2, immediately after exercise; T3, 30 minutes after exercise). D-F. Individual data is displayed for each corresponding stimulus intensities of 110% RMT (D), 130% RMT (E), and 150% RMT (F) at each timepoint for MEP data elicited with AP (dark grey) and PA (light grey) current directions. G-I. Box and whisker plots for MEP amplitudes at 110% RMT (G), 130% RMT (H), 150% RMT (I), in which the box depicts the median, 25^{th} and 75^{th} percentiles, and the whiskers represent the 5^{th} and 95^{th} percentile. Individual data is overlayed. AP is displayed in black and PA is shown in light grey. Bars represent standard error of the mean. * $p < 0.05$, ** $p < 0.01$.

Figure 4. SICI results. A. Displays conditioned/unconditioned ratios, where greater values represent less inhibition, for AP (black) and PA (white) current directions at each timepoint (T0, before rest; T1, after rest; T2, immediately after exercise; T3, 30 minutes after exercise). B. Individual data is displayed at each timepoint for SICI data elicited with AP (dark grey) and PA (light grey) current directions. C. Box and whisker plots for AP (black) and PA (light grey) SICI. The median, 25^{th} percentile and 75^{th} percentile are represented in the box, while the 5^{th} and 95^{th} percentiles are depicted by the whiskers. Bars represent standard error of the mean. * $p < 0.05$.

Table Caption

Table 1. Test Stimulus and resting motor threshold (RMT) data. Displayed are the test stimulus intensities (shown in % of maximum stimulator output, % MSO) and corresponding MEP amplitudes (shown in mV) during collection of short-interval intracortical inhibition (SICI) at all timepoints (T0, before rest; T1, after rest; T2, immediately after exercise; T3, 30 minutes after

exercise) for each current direction (posterior-anterior, PA; anterior-posterior, AP). At the bottom of the table, RMT for each current direction (PA, AP) is shown in % MSO as well as MEP amplitudes elicited during assessment of MEP onset latencies in each current direction (LM, PA and AP). Group average values are shown with standard deviation.

References

Aberra AS, Wang B, Grill WM, Peterchev A V. (2020) Simulation of transcranial magnetic stimulation in head model with morphologically-realistic cortical neurons. Brain Stimul 13:175–189

Amassian V, Stewart M, Quirk G, Rosenthal J (1987) Physiological basis of motor effects of a transient stimulus to cerebral cortex. Neurosurgery 20:74–93.

Andrews SC, Curtin D, Hawi Z, Wongtrakun J, Stout JC, Coxon JP (2020) Intensity Matters: High-intensity Interval Exercise Enhances Motor Cortex Plasticity More Than Moderate Exercise. Cereb Cortex 30:101–112

Beckerman H, Roebroeck ME, Lankhorst GJ, Becher JG, Bezemer PD, Verbeek AL (2001) Smallest real difference, a link between reproducibility and responsiveness. Qual life Res an Int J Qual life Asp Treat care Rehabil 10:571–578.

Borg G (1998) Borg's perceived exertion and pain scales. Champaign, IL, USA.

Brown K, Neva J, Mang C, Chau B, Chiu L, Francisco B, Staines W, Boyd L (2020) The influence of an acute bout of moderate intensity cycling exercise on sensorimotor integration. Eur J Neurosci 52:4779–4790.

Chen R, Cros D, Curra A, Di Lazzaro V, Lefaucheur J-P, Magistris MR, Mills K, Rösler KM, Triggs WJ, Ugawa Y, Ziemann U (2008) The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. Clin Neurophysiol 119:504–532

Cirillo J, Byblow WD, Thut G (2016) Threshold tracking primary motor cortex inhibition: the influence of current direction. Eur J Neurosci 44:2614–2621.

Cirillo J, Semmler JG, Mooney RA, Byblow WD (2018) Conventional or threshold-hunting TMS? A tale of two SICIs. Brain Stimul 11:1296–1305

Cirillo J, Semmler JG, Mooney RA, Byblow WD (2020a) Primary motor cortex function and motor skill acquisition : insights from threshold ‐ hunting TMS. Exp Brain Res 238:1745–1757

Cirillo J, Semmler JG, Mooney RA, Byblow WD (2020b) Primary motor cortex function and motor skill acquisition : insights from threshold ‐ hunting TMS. Exp Brain Res 238:1745–1757.

Cohen J (1988) Statistical power anlaysis for the behavioural sciences, 2nd ed. Hillsdale, NJ: Lawrence Eribaum Associates.

Craig C, Marshall A, Sjostrom M, Bauman A, Booth M, Ainsworth B, Pratt M, Ekelund U, Yngve A, Sallis J, Oja P (2003) International physical activity questionnaire: 12-country reliability and validity. Med Sci Sport Exerc 35:1381–1395.

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 412:449–473

Di Lazzaro V, Oliviero a, Mazzone P, Insola a, Pilato F, Saturno E, Accurso a, Tonali P, Rothwell JC (2001a) Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. Exp brain Res 141:121–127

Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, Mazzone P, Tonali P, Rothwell JC (1998a) Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol 109:397–401

Di Lazzaro V, Oliviero A, Saturno E, Pilato F, Insola A, Mazzone P, Profice P, Tonali P,

Rothwell JC (2001b) The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. Exp Brain Res 138:268–273.

Di Lazzaro V, Profice P, Ranieri F, Capone F, Dileone M, Oliviero a, Pilato F (2012) I-wave origin and modulation. Brain Stimul 5:512–525

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC (1998b) Magnetic transcranial stimulation at intensities below active motor threshold activates intracortical inhibitory circuits. Exp Brain Res 119:265–268

Di Lazzaro V, Rothwell JC (2014) Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. J Physiol 592:4115–4128.

El-Sayes J, Turco C, Skelly L, Locke M, Gibala M, Nelson A (2020) Acute high-intensity and moderate-intensity interval exercise do not change corticospinal excitability in low fit, young adults. PLoS One 15:e0227581

El-Sayes J, Turco C V., Skelly LE, Nicolini C, Fahnestock M, Gibala MJ, Nelson AJ (2019a) The Effects of Biological Sex and Ovarian Hormones on Exercise-Induced Neuroplasticity. Neuroscience 410:29–40

El-Sayes J, Turco C V., Skelly LE, Nicolini C, Fahnestock M, Gibala MJ, Nelson AJ (2019b) The Effects of Biological Sex and Ovarian Hormones on Exercise-Induced Neuroplasticity. Neuroscience 410:29–40.

Ferrer-Uris B, Busquets A, Lopez-Alonso V, Fernandez-Del-Olmo M, Angulo-Barroso R (2017) Enhancing consolidation of a rotational visuomotor adaptation task through acute exercise. PLoS One 12:3–9.

Groppa S, Werner-Petroll N, Munchau A, Deuschl G, Ruschworth MF, Siebner HR (2012) A

novel dual-site transcranial magnetic stimulation paradigm to probe fast facilitatory inputs from ipsilateral dorsal premotor cortex to primary motor cortex. Neuroimage 62:500–509.

Hamada M, Galea JM, Di Lazzaro V, Mazzone P, Ziemann U, Rothwell JC (2014) Two Distinct Interneuron Circuits in Human Motor Cortex Are Linked to Different Subsets of Physiological and Behavioral Plasticity. J Neurosci 34:12837–12849

Hamada M, Murase N, Hasan A, Balaratnam M, Rothwell JC (2013) The role of interneuron networks in driving human motor cortical plasticity. Cereb Cortex 23:1593–1605

Hanajima R, Ugawa Y, Terao Y, Enomoto H, Shiio Y, Mochizuki H, Furubayashi T, Uesugi H, Iwata NK, Kanazawa I (2002) Mechanisms of intracortical I-wave facilitation elicited with paired-pulse magnetic stimulation in humans. J Physiol 538:253–261.

Hanajima R, Ugawa Y, Terao Y, Sakai K, Furubayashi T, Machii K, Kanazawa I (1998) Paired-pulse magnetic stimulation of the human motor cortex: differences among I waves. J Physiol 509:607–618.

Hannah R, Cavanagh SE, Tremblay S, Simeoni S, Rothwell JC (2018) Selective suppression of local interneuron circuits in human motor cortex contributes to movement preparation. J Neurosci 38:2869–17.

Ilić T V., Meintzschel F, Cleff U, Ruge D, Kessler KR, Ziemann U (2002) Short-interval paired-pulse inhibition and facilitation of human motor cortex: the dimension of stimulus intensity. J Physiol 545:153–167

Inghilleri M, Berardelli A, Marchetti P, Manfredi M (1996) Effects of dizepam, baclofen and thiopental on the silent period evoked by transcranial magnetic stimulation in humans. Exp brain Res 109:467–472.

Irlbacher K, Brocke J, Mechow J V, Brandt SA (2007) Effects of GABA A and GABA B

agonists on interhemispheric inhibition in man. Clin Neurophysiol 118:308–316.

Kimiskidis VK, Papagiannopoulos S, Kazis D a., Sotirakoglou K, Vasiliadis G, Zara F, Kazis a., Mills KR (2006) Lorazepam-induced effects on silent period and corticomotor excitability. Exp Brain Res 173:603–611.

Lulic T, El-Sayes J, Fassett HJ, Nelson AJ (2017) Physical activity levels determine exercise-induced changes in brain excitability. PLoS One 12:e0173672

MacDonald MA, Khan H, Kraeutner SN, Usai F, Rogers EA, Kimmerly DS, Dechman G, Boe SG (2019) Intensity of acute aerobic exercise but not aerobic fitness impacts on corticospinal excitability. Appl Physiol Nutr Metab 44:869–878.

Mang C, Brown K, Neva J, Snow N, Campbell K, Boyd L (2016) Promoting Motor Cortical Plasticity with Acute Aerobic Exercise: A Role for Cerebellar Circuits. Neural Plast 2016:6797928.

Mang C, Snow N, Campbell K, Ross CC, Boyd L (2014) A single bout of aerobic exercise facilitates response to paired associative stimulation and promotes sequence-specific implicit motor learning. J Appl Physiol 117:1325–1336

McDonnell MN, Buckley JD, Opie GM, Ridding MC, Semmler JG (2013) A single bout of aerobic exercise promotes motor cortical neuroplasticity. J Appl Physiol 114:1174–1182

McDonnell MN, Orekhov Y, Ziemann U (2006) The role of GABA B receptors in intracortical inhibition in the human motor cortex. Exp brain Res 173:86–93

Mirdamadi JL, Suzuki LY, Meehan SK (2017) Attention modulates specific motor cortical circuits recruited by transcranial magnetic stimulation. Neuroscience 359:151–158

Mooney RA, Cirillo J, Byblow WD (2019a) Neurophysiological mechanisms underlying motor skill learning in young and older adults. Exp Brain Res 237:2331–2344

Mooney RA, Cirillo J, Byblow WD (2019b) Neurophysiological mechanisms underlying motor skill learning in young and older adults. Exp Brain Res 237:2331–2344.

Mooney RA, Coxon JP, Cirillo J, Glenny H, Gant N, Byblow WD (2016) Acute aerobic exercise modulates primary motor cortex inhibition. Exp Brain Res 234:1–8.

Morris TP, Fried PJ, Macone J, Stillman A, Gomes-Osman J, Costa-Miserachs D, Tormos Muñoz JM, Santarnecchi E, Pascual-Leone A (2020) Light aerobic exercise modulates executive function and cortical excitability. Eur J Neurosci 51:1723–1734.

Neva J, Ma J, Orsholits D, Boisgontier M, Boyd L (2019) The effects of acute exercise on visuomotor adaptation, learning, and inter-limb transfer. Exp brain Res 237:1109–1127.

Neva JL, Brown KE, Mang CS, Francisco BA, Boyd LA (2017) An acute bout of exercise modulates both intracortical and interhemispheric excitability. Eur J Neurosci 45:1343–1355.

Ni Z, Cash RFH, Gunraj C, Bercovici E, Hallett M, Chen R (2019) Involvement of different neuronal components in the induction of cortical plasticity with associative stimulation. Brain Stimul 12:84–86.

Ni Z, Charab S, Gunraj C, Nelson AJ, Udupa K, Yeh I-J, Chen R (2011) Transcranial magnetic stimulation in different current directions activates separate cortical circuits. J Neurophysiol 105:749–756

Nicolini C, Michalski B, Toepp SL, Turco C V, D'Hoine T, Harasym D, Gibala MJ, Fahnestock M, Nelson AJ (2020) A single bout of high-intensity interval exercise increases corticospinal excitability, brain-derived neurotrophic factor and uncarboxylated osteolcalcin in sedentary, healthy males. Neuroscience 437:242–255

Nofuji Y, Suwa M, Sasaki H, Ichimiya A, Nishichi R (2012) Different circulating brain-derived

neurotrophic factor responses to acute exercise between physically active and sedentary subjects. J Sport Sci Med 11:83–88.

Oldfield R (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia Mar 9:97–113.

Opie GM, Semmler JG (2019) Acute Exercise at Different Intensities Influences Corticomotor Excitability and Performance of a Ballistic Thumb Training Task. Neuroscience 412:29–39

Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC (2008) Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol 586:5147–5159

Ostadan F, Centeno C, Daloze J, Frenn M, Lundbye-jensen J, Roig M (2016) Changes in corticospinal excitability during consolidation predict acute exercise-induced off-line gains in procedural memory. Neurobiol Learn Mem 136:196–203

Paulus W, Classen J, Cohen LG, Large CH, Di Lazzaro V, Nitsche M, Pascual-Leone A, Rosenow F, Rothwell JC, Ziemann U (2008) State of the art: Pharmacologic effects on cortical excitability measures tested by transcranial magnetic stimulation. Brain Stimul 1:151–163.

Peurala SH, M. Müller-Dahlhaus JF, Arai N, Ziemann U (2008) Interference of short-interval intracortical inhibition (SICI) and short-interval intracortical facilitation (SICF). Clin Neurophysiol 119:2291–2297.

Roig M, Skriver K, Lundbye-Jensen J, Kiens B, Nielsen JB (2012) A single bout of exercise improves motor memory. PLoS One 7:e44594

Roshan L, Paradiso GO, Chen R (2003) Two phases of short-interval intracortical inhibition. Exp Brain Res 151:330–337.

Sakai K, Ugawa Y, Terao Y, Hanajima R, Furubayashi T, Kanazawa I (1997) Preferential

activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. Exp Brain Res 113:24–32.

Sale M V., Lavender AP, Opie GM, Nordstrom MA, Semmler JG (2016) Increased intracortical inhibition in elderly adults with anterior-posterior current flow: A TMS study. Clin Neurophysiol 127:635–640

Sale M V, Ridding MC, Nordstrom M a (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. Exp Brain Res 181:615–626

Samusyte G, Bostock H, Rothwell J, Koltzenburg M (2018) Short-interval intracortical inhibition: Comparison between conventional and threshold-tracking techniques. Brain Stimul 11:806–817.

Schambra HM, Ogden RT, Martínez-Hernández IE, Lin X, Chang YB, Rahman A, Edwards DJ, Krakauer JW (2015) The reliability of repeated TMS measures in older adults and in patients with subacute and chronic stroke. Front Cell Neurosci 9:335

Singh AM, Duncan RE, Neva JL, Staines WR (2014a) Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. BMC Sports Sci Med Rehabil 6:23

Singh AM, Neva JL, Staines WR (2014b) Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex. Exp Brain Res 232:3675–3685

Smith A, Goldsworth M, Wood F, Olds T, Garside T, Ridding M (2018) High-intensity Aerobic Exercise Blocks the Facilitation of iTBS-induced Plasticity in the Human Motor Cortex. Neuroscience 373:1–6.

Smith AE, Goldsworthy MR, Garside T, Wood FM, Ridding MC (2014) The influence of a single bout of aerobic exercise on short-interval intracortical excitability. Exp brain Res 232:1875–1882

Spampinato D (2020) Dissecting two distinct interneuronal networks in M1 with transcranial magnetic stimulation. Exp Brain Res 238:1693–1700

Spampinato DA, Celnik PA, Rothwell JC (2020) Cerebellar-Motor Cortex Connectivity: One or Two Different Networks? J Neurosci 40.

Stavrinos E, Coxon J (2017) High-intensity interval exercise promotes motor cortex disinhibition and early motor skill consolidation. J Cogn Neurosci 29:593–604.

Tamm AS, Lagerquist O, Ley AL, Collins DF (2009) Chronotype Influences Diurnal Variations in the Excitability of the Human Motor Cortex and the Ability to Generate Torque during a Maximum Voluntary Contraction. J Biol Rythm 24:211–224.

Thomas R, Beck MM, Lind RR, Korsgaard Johnsen L, Geertsen SS, Christiansen L, Ritz C, Roig M, Lundbye-Jensen J (2016) Acute Exercise and Motor Memory Consolidation: The Role of Exercise Intensity. PLoS One 2016:1–11

Turco C V, El-sayes J, Locke MB, Chen R, Baker S, Nelson AJ, Forsythe I, Taylor J (2018) Effects of lorazepam and baclofen on short- and long-latency afferent inhibition. J Physiol 21:5267–5280.

Turco C V, Pesevski A, McNicholas PD, Beaulieu L-D, Nelson AJ (2019) Reliability of transcranial magnetic stimulation measures of afferent inhibition. Brain Res 1723:146394.

Udupa K, Ni Z, Gunraj C, Chen R (2010) Effect of long interval interhemispheric inhibition on intracortical inhibitory and facilitatory circuits. J Physiol 588:2633–2641

Volz LJ, Hamada M, Rothwell JC, Grefkes C (2015) What Makes the Muscle Twitch: Motor

System Connectivity and TMS-Induced Activity. Cereb Cortex 25:2346–2353.

Weir JP (2005) Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. J strength Cond Res 19:231–240.

Yamazaki Y, Sato D, Yamashiro K, Nakano S, Onishi H, Maruyama A (2019) Acute Low-Intensity Aerobic Exercise Modulates Intracortical Inhibitory and Excitatory Circuits in an Exercised and a Non-exercised Muscle in the Primary Motor Cortex. Front Physiol 10:1361.

Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W (1996a) Effects of antiepileptic drugs on motor cortex excitability in humans: a transcranial magnetic stimulation study. Ann Neurol 40:367–378

Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W (1996b) The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res 109:127–135

Ziemann U, Rothwell JC (2000) I-waves in motor cortex. J Clin Neurophysiol 17:397–405.

Ziemann U, Tergau F, Wassermann EM, Wischer S, Hildebrandt J, Paulus W (1998a) Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. J Physiol 511 (Pt 1):181–190.

Ziemann U, Tergau F, Wischer S, Hildebrandt J, Paulus W (1998b) Pharmacological control of facilitatory I-wave interaction in the human motor cortex. A paired transcranial magnetic stimulation study. Electroencephalogr Clin Neurophysiol - Electromyogr Mot Control 109:321–330.

Figure 1

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Figure 2

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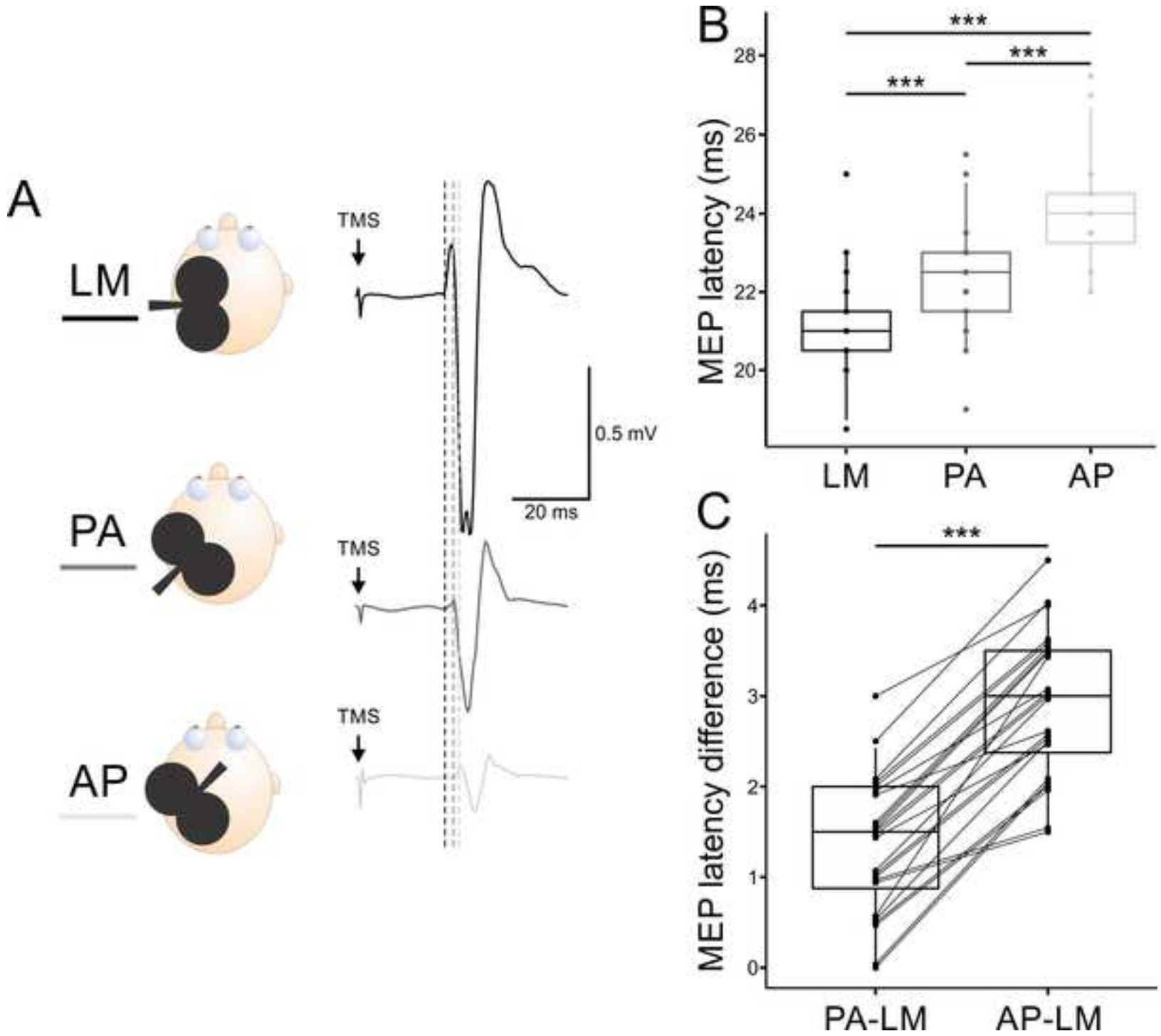

Figure 3

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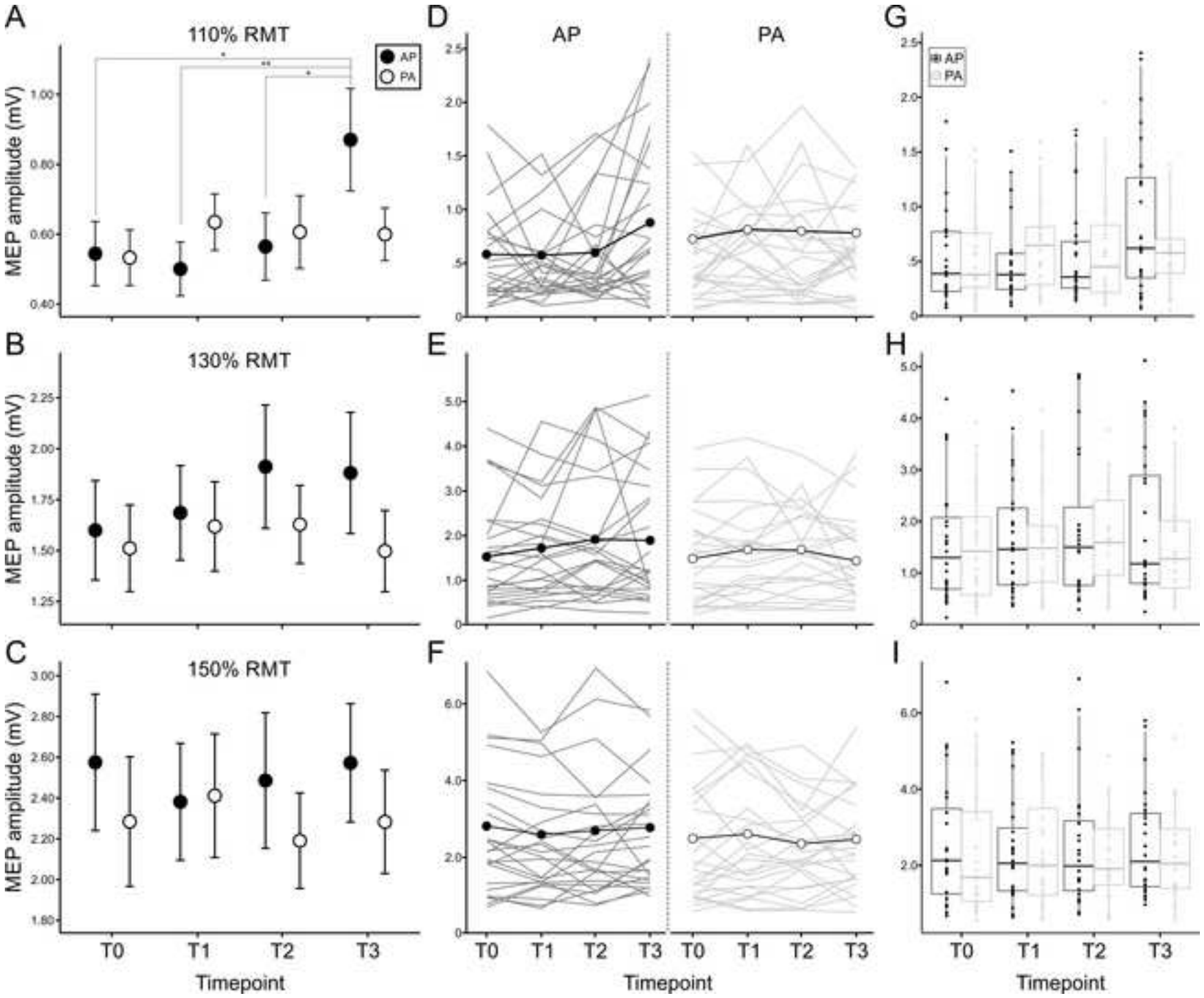

Figure 4

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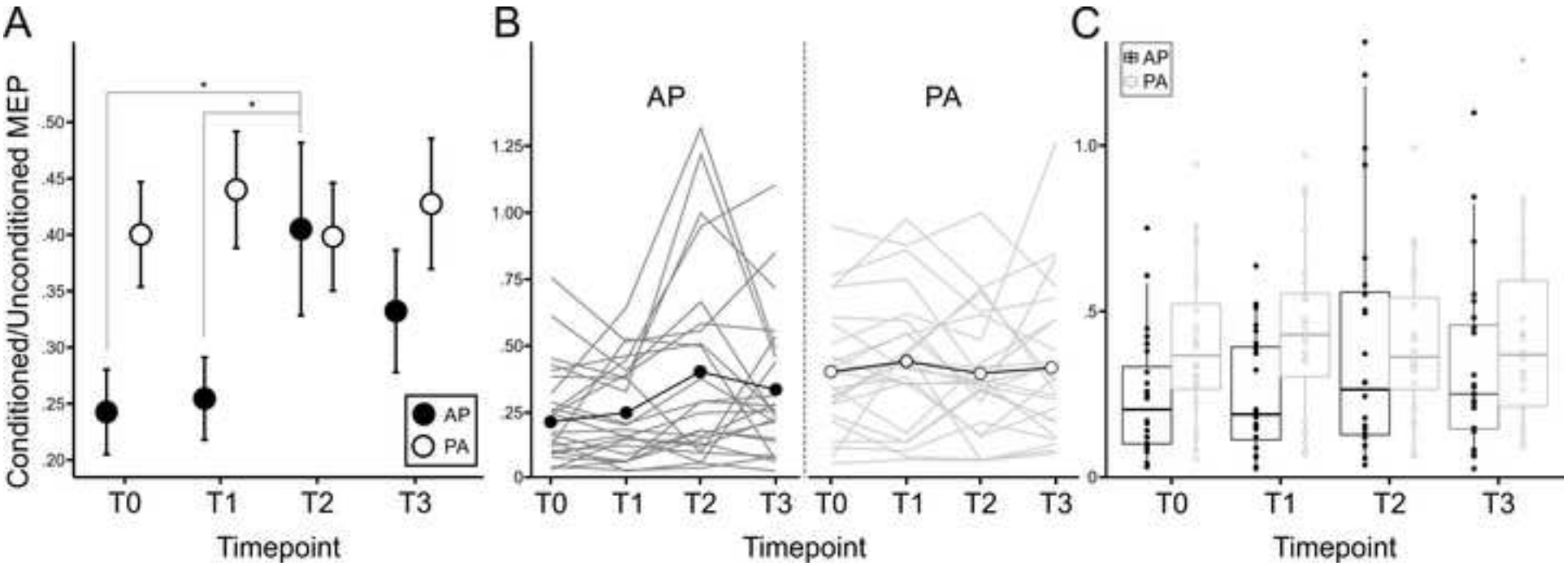

Test Stimulus (% MSO)	**Pre-T0**	**Pre-T1**	**Post-T2**	**Post-T3**
Posterior-to-anterior current (PA)	59 ± 15	59 ± 15	59 ± 15	58 ± 14
Anterior-to-posterior current (AP)	74 ± 17	74 ± 16	74 ± 16	73 ± 16
Test Stimulus MEP (mV)	**Pre-T0**	**Pre-T1**	**Post-T2**	**Post-T3**
Posterior-to-anterior current (PA)	1.3 ± 0.3	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4
Anterior-to-posterior current (AP)	1.4 ± 0.4	1.5 ± 0.5	1.4 ± 0.4	1.4 ± 0.5
Resting Motor Threshold (% MSO)	**Pre-T0**			
Posterior-to-anterior current (PA)	43 ± 7			
Anterior-to-posterior current (AP)	55 ± 8			
MEP amplitude (mV) - onset latency assessment	**Pre-T0**			
Lateral-to-medial current (LM)	1.7 ± 0.8			
Posterior-to-anterior current (PA)	0.53 ± 0.4			
Anterior-to-posterior current (AP)	0.54 ± 0.4			

Table 1