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INTENSITY OF ACUTE AEROBIC EXERCISE BUT NOT AEROBIC FITNESS IMPACTS ON CORTICOSPINAL EXCITABILITY

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ABSTRACT

Aerobic exercise (AE) modulates cortical excitability. It can alter both corticospinal excitability and intra-cortical networks, which has implications for its use as a tool to facilitate processes such as motor learning, where increased levels of excitability are conducive to the induction of neural plasticity. Little is known about how different intensities of AE modulate cortical excitability or how individual-level characteristics impact on it. Therefore, we investigated whether AE intensities, lower than those previously employed, would be effective in increasing cortical excitability. We also examined whether the aerobic fitness of individual participants was related to the magnitude of change in AE-induced cortical excitability. In both experiments we employed transcranial magnetic stimulation to probe corticospinal excitability before and after AE. We show that 20 min of continuous moderate- (40 and 50% of heart rate reserve, HRR), but not low- (30% HRR) intensity AE was effective at increasing corticospinal excitability. We also found that while we observed increased corticospinal excitability following 20 min of continuous moderate-intensity (50% HRR) AE, aerobic fitness was not related to the magnitude of change. Our results suggest that there is a lower bound intensity of AE that is effective at driving changes in cortical excitability, and that while individual-level characteristics are important predictors of response to AE, aerobic fitness is not. Overall these findings have implication for the way that AE is used to facilitate processes such as motor learning, where increased levels of cortical excitability and plasticity are favourable.

Keywords: aerobic exercise; aerobic fitness; corticospinal excitability; physical activity; plasticity; transcranial magnetic stimulation

INTRODUCTION

Beyond its well-established cardiovascular effects, evidence indicates that aerobic exercise (AE) has positive effects on brain function (Cotman et al. 2007; Erickson et al. 2011; Fernandes et al. 2017; Gomez-Pinilla and Hillman 2013; Ploughman and Kelly 2016; Thomas et al. 2012). Even a single session of moderate-intensity AE, defined as 45-60% of an individual's heart rate reserve (HRR) or 60-70% of an individual's maximum heart rate (HR_{max}), results in measurable brain plasticity in the primary motor cortex (M1) (McDonnell et al. 2013; Singh et al. 2014a; Singh et al. 2014b; Smith et al. 2014). That AE drives plasticity in the motor cortex is of particular interest in applications where an environment that is conducive to plasticity is advantageous, such as learning new motor skills, or re-learning skills lost to brain injury such as stroke (Mang et al. 2013; Murdoch et al. 2016; Ploughman and Kelly 2016; Quaney et al. 2009). As such, attention has recently focused on the role of AE to prime the brain (i.e., to create an environment conducive to plasticity) prior to engaging in activities such as motor skill learning or, specific to rehabilitation after stroke, task-specific therapy.

Numerous studies have investigated the ability of an acute bout of AE to modulate brain plasticity, focusing primarily on motor regions. Typically, transcranial magnetic stimulation (TMS), a form of non-invasive brain stimulation, is employed to examine AE-related effects on the brain. Briefly, single-pulse TMS can be used to generate a stimulus response (S-R) curve to assess the excitability of the corticospinal tract, including neurons in M1. Paired-pulse paradigms can probe changes in intra-cortical inhibitory and facilitatory networks (Chen 2000, 2004; Ilic et al. 2002; Rossini et al. 2015). Indeed, using TMS, numerous studies have demonstrated altered levels of corticospinal excitability and/or changes in the degree of intra-cortical inhibition or facilitation following moderate-intensity AE (Lulic et al. 2017; Singh et al. 2014b; Smith et al.

2014; Yamaguchi et al. 2012). These studies have established the acute effects that AE can have on motor regions in the brain. However, a better understanding of how various exercise-related factors modulate this effect could optimize AE prescription and therapeutic use. For instance, the majority of studies have focused on moderate-intensity AE, and as such little is known about the overall effect of varying AE intensities on driving brain plasticity in motor regions for the purpose of priming the brain. Specifically we were interested in knowing if there was a lower limit at which AE would cease to drive changes in corticospinal excitability. In the context of using AE as a means to prime the brain prior to a bout of rehabilitation therapy, it is critical that patients not be fatigued following the AE, as they need to engage in the task specific therapy component of the intervention immediately thereafter. The exercise intensities and durations used in previous work (e.g., 20 min of AE at 70% of age-predicted HR_{max} or $\sim 50\%$ HRR, and 30 min of AE at 40% HRR), while considered low-moderate for healthy and 'fit' individuals, may not be ideal for this priming application in clinical practice owing to relative deconditioning and propensity for fatigue in the post-stroke population (Cumming et al. 2016; Duncan et al. 2012; Elf et al. 2016; MacKay-Lyons and Howlett 2015).

A second factor that may be important to consider relates to the characteristics of the individual performing AE. For instance, Lulic and colleagues reported that moderate-intensity AE altered intra-cortical networks in participants from groups with both high and low physical activity levels but increased corticospinal excitability was observed in the high activity group only (Lulic et al. 2017). Similarly, Cirillo and colleagues showed increased excitability in highly active compared to sedentary participants following the application of paired associative stimulation, a technique used to induce neuroplasticity (Cirillo et al. 2009). While this evidence indicates that there is an overall plasticity effect of AE on motor regions of the brain,

considerable variability was observed amongst study participants. This variability may well result from issues related to measuring physical activity, including insensitivity to the type of activity the participants were engaging in (e.g., low vs. high intensity; aerobic vs. anaerobic), as well as reporting biases inherent to the assessment tool (Dyrstad et al. 2014; Fogelholm et al. 2006). One individual level characteristic that may better predict the cortical response to AE is aerobic fitness (Newson and Kemps 2006), which represents the functional capacity of the cardiovascular system. Aerobic fitness is often quantified in terms of the maximum oxygen uptake ($\text{VO}_{2\text{max}}$), which represents the limit of the body to use oxygen as an energy source, and is often used to measure changes after aerobic training. There is little information about how aerobic fitness, as opposed to level of physical activity, modulates the cortical response to a single bout of AE.

Here we performed two experiments; the first sought to determine if a bout of low intensity AE can drive changes in corticospinal excitability, and the second examined the relationship between aerobic fitness and corticospinal excitability. In both studies we used single-pulse TMS to probe changes in corticospinal excitability from pre- to post-exercise. In experiment 1, participants performed AE at 30, 40 and 50% of their HRR, with corticospinal excitability assessed pre- and post-exercise. We hypothesized that corticospinal excitability would be increased following exercise at each of the three intensities, replicating prior work and demonstrating that lower intensities of AE are effective at modulating corticospinal excitability. In experiment 2, participant's aerobic fitness was determined via their $\text{VO}_{2\text{max}}$, and corticospinal excitability assessed before and after a bout of AE at 50% of their HRR. We hypothesized that there would be a positive relationship between aerobic fitness and corticospinal excitability, whereby, relative to individuals with lower aerobic fitness, individuals with higher aerobic

fitness would: 1) have increased corticospinal excitability (i.e., pre-exercise); and 2) have a greater change in corticospinal excitability in response to AE (i.e. pre- to post-exercise). By investigating factors that influence the AE-based priming effect, we aimed to generate a better understanding of the effect of AE on corticospinal excitability, and in-turn, help direct the use of AE in applications promoting brain plasticity for motor learning and rehabilitation.

METHODS

Participants

We recruited 15 (8 females, 26.9 ± 3.8 years) and 29 (14 females, 25.9 ± 2.8 years) young, healthy individuals for experiments 1 and 2, respectively. Participants only performed the study they were recruited for (i.e., no participants performed both experiments). Informed consent was obtained from all participants prior to undergoing their respective experimental protocol. All participants were screened for their suitability to perform exercise using the Physical Activity Readiness-Questionnaire (PAR-Q) (Thomas et al. 1992), and each were free of contraindications for TMS (Rossi et al. 2009). Participant's physical activity was determined using the short version of the International Physical Activity Questionnaire (IPAQ; available at <https://sites.google.com/site/theipaq/>) (Craig et al. 2003; Fogelholm et al. 2006). Both experiments received approval from the Research Ethics Board at Dalhousie University.

Experimental design

Experiment 1. Participants attended four experimental sessions in a seven-day period. At the first, participants underwent a maximal graded cycle exercise test (GXT) to determine their HR_{max} . Sessions two through four involved assessing corticospinal excitability using TMS before

and after completion of a 20 min bout of AE at one of three intensities: 30, 40, or 50% HRR, that were randomly assigned. Figure 1A depicts the timeline for the experiment.

Experiment 2. Participants attended two experimental sessions in a one-month period. At the first, participants underwent a maximal GXT to determine their HR_{max} and aerobic fitness (as measured by their VO_{2max} , described below). The second session involved assessing corticospinal excitability using TMS before and after completion of a 20 min bout of AE at 50% HRR. Figure 1B depicts the timeline for the experiment. A 20 min bout of AE was utilized in both experiments in order to closely approximate methods from two related studies (Lulic et al. 2017; Singh et al. 2014b).

In both experiments, instructions were given before exercise testing based on the American College of Sports Medicine (ACSM) recommendations (Pescatello 2014). Participants were asked to wear comfortable clothing and refrain from ingesting food, alcohol and caffeine within 3 hours of testing. Participants were also asked to avoid significant exertion or exercise on the day of testing and to get adequate sleep the night before. In experiment 2 participants were asked if there were any significant changes in their training regimens between the first and second sessions.

Figure 1 here.

Maximal graded exercise test (GXT)

Experiment 1. Participants performed the GXT on a stationary electronically braked cycle ergometer (Corival 2003, Lode B.V., AN) using a ramped protocol that was controlled externally via Lode Ergometry Manager software (v 10.4.4, Lode B.V., AN). Prior to the start of the GXT,

resting HR (RHR) was obtained following 5 min of rest (sitting on the bike with no distractions). Participants then performed a 5 min warm-up at a workload of 50 Watts. Following this, the workload increased by 20 Watts/min until cessation of the test. Throughout the test, HR was measured via a wrist-mounted monitor (Mio global, 2014, Physical Enterprises Inc., USA) that is a valid measure of HR (Stahl et al. 2016). Every 2 min participants were asked to rate their perceived exertion (RPE) using the modified Borg scale [anchored by the values 0 (rest) to 10 (maximal exertion)]. Participants were asked to provide a cue to indicate when they believed they had approximately 1 min remaining in the test, at which time the final measurements of HR and RPE were made. To be considered a true maximal test, participants were required to have a final RPE on the Borg scale ≥ 7 and a $HR_{\max}$ equal to or within 12 beats of the age-predicted (estimated) $HR_{\max}$ determined using the equation: $Estimated\ HR_{\max} = [206.9 - (0.67 \times Age)]$ (Tanaka et al. 2001).

Experiment 2. Participants performed the GXT on a stationary electronically braked cycle ergometer (Ergoselect 200P, Ergoline, Bitz, DE) using a ramped protocol that was controlled externally via JLab (LABManager v 5.3.04, Jaeger®, DE). Resting HR was obtained as in experiment 1. For the GXT, power output increased every minute. Workload was determined by the participant's fitness level, based on the International Physical Activity Questionnaire (IPAQ) score (Wasserman 2012). The goal was for participants to reach exhaustion within 8-12 min. Participants with low physical activity levels began with a 3 min warm up at 25 W, followed by a 15 W increase every minute. Participants with high physical activity levels performed a 3 min warm-up at 50 W followed by a 20 W increase every minute. An additional protocol consisting of a 3 min warm up at 75 W and a GXT beginning at 100 W and increasing by 25 W every

minute was used for participants with high self-reported levels of vigorous physical activity.

The volume and content of expired oxygen and carbon dioxide were analyzed using the Oxycon Mobile® (VIASYS Healthcare, GmBH, DE) portable metabolic unit. Data were collected on a breath-by-breath basis during the GXT and averaged in 20 s for further analysis. In addition to VO_2 and VCO_2 , the Oxycon Mobile® measured HR, respiratory rate, and O_2 saturation, as well as calculated the respiratory exchange ratio (RER). A secondary measure of HR was obtained using the wrist-mounted monitor as described above. During the GXT, participants were asked every 2 min for their RPE using the original Borg scale [anchored by the values 6 (rest) to 20 (maximal exertion; (Borg 1982))]. The $\text{VO}_{2\text{max}}$ value was identified as the highest 20 s average when a plateau in VO_2 was observed, where a plateau in VO_2 was defined as a difference of $< 2.1 \text{ ml/kg/min}$ in the last minute of the GXT. As many individuals do not attain a VO_2 plateau, other criteria were used to support the judgement of achieving a $\text{VO}_{2\text{max}}$ (Heyward, 2010). If a plateau in VO_2 was not observed, 2 of the following 3 criteria must have been achieved (McArdle et al. 2010): 1) reaching a peak HR $\geq 95\%$ of age predicted maximum (Tanaka et al. 2001); 2) a final RPE on the Borg scale ≥ 17 ; or 3) a resting exchange ratio (VCO_2/VO_2) ≥ 1.15 .

TMS protocol

In both experiments 1 and 2, single-pulse TMS was applied to the cortical representation of the right extensor carpi radialis (ECR) muscle, consistent with prior work examining the effect of AE on cortical excitability of a non-exercised upper limb muscle (Singh et al. 2014b). Stimulation was delivered through a 70mm figure of eight coil connected to a MagStim BiStim system (The Magstim Company, Whitland, UK). BrainSight neuronavigation (Rogue Research

Inc., Montreal, CA) was used to guide the positioning and orientation of the coil over the target motor region. For the neuronavigated TMS, prior to each of the experimental sessions, three anatomical landmarks (nasion, right and left pre-auricular points) were digitized for each participant and co-registered with a template MRI (MNI152_T1_1mm). Motor evoked potentials (MEPs) were obtained from the ECR using surface electrodes (1×3 cm; Q-Trace Gold; Kendall-LTP, USA) in a bi-polar configuration (1 cm interelectrode distance) placed overlying the muscle belly 5cm distal to the radiohumeral joint with the ground electrode placed on the olecranon process. For localization of the motor hotspot electromyography (EMG) was recorded using vendor-supplied hardware (Brainsight EMG Isolation Unit and Amplifier Pod). All subsequent EMG was sampled using Signal software (Signal 6.03c, Cambridge Electronic Design Ltd., UK) at 1000 Hz with a bandpass of 10-1000 Hz (1902 and Power 1401; Cambridge Electronic Design, UK) and stored for offline analysis. For all TMS procedures, the TMS coil was held in close proximity to the skull with the handle pointing posteriorly and laterally at an angle of approximately 45° to the mid-sagittal line.

To localize the motor hotspot of the right ECR, a 5×5 cm grid (7.5 mm spacing) was placed overlying the cortical surface of the template brain with the mid-point (2, 2) centered on the estimated location of the forearm muscle representation of the left M1. Starting at point 2, 2, points on the grid were stimulated to determine the location(s) that produced the highest amplitude MEPs in the resting muscle for 5 out of 10 stimulations at the lowest stimulator output as assessed by MEP peak-peak amplitude. Once the hotspot was located, resting motor threshold (RMT) was determined as the lowest stimulation intensity required to elicit an MEP with a minimum peak-peak amplitude of $50\mu\text{V}$ for 5 out of 10 consecutive stimuli (Kleim et al. 2007; Rossi et al. 2009). With the exception of single-pulse TMS for hotspot localization and

determination of RMT, during which the stimulator was under manual control, delivery of stimuli (i.e., control of the stimulator) was based on a custom script programmed using Signal software.

After the RMT was determined, a S-R curve was generated. Ten single pulses were delivered over the motor hotspot with a fixed inter-stimulus interval of 3 s at stimulus intensities of 100, 110, 120, 130, and 140% of RMT, with the order of delivery of the single pulses randomized. Throughout the TMS procedures, participants were seated comfortably in a chair with their arms at rest (positioned on pillows in their lap).

Aerobic exercise

Participants performed a brief warm-up at 50 Watts following by 20 min of continuous stationary cycling at a percentage of their HRR, where $HRR = HR_{max} - RHR$. Participants were asked to maintain their HR within 10 beats/min of the target HR (where target HR = [% exercise intensity $\times$ (HRR)] + RHR) throughout the 20 min of cycling. The experimenter performed workload adjustments (i.e., increased or decreased workload) to facilitate this. Participants were instructed to rest their arms comfortably by their sides and not to grip the handlebars to avoid any muscle activity in the upper limbs as both experiments aimed to test the excitability of a non-exercised upper limb muscle. Heart rate was sampled once per second using the Mio watch and participants were asked for their RPE every 2 min throughout the cycling exercise. TMS measures were collected just prior to exercise (pre), immediately following exercise completion (post 1) and again 30 min following exercise completion (post 2; experiment 2 only). This latter time point (post 2) was included in experiment 2 to mirror previous work that has shown evolution of the effect of AE on plasticity in motor regions (Singh et al. 2014b). To

negate the effect of order, the intensity of AE (30, 40 or 50% HRR) to be performed at each of the three sessions in experiment 1 was randomized. As indicated above, participants in experiment 2 performed AE in a single session only, at 50% HRR. For both experiment 1 and 2, the TMS equipment and cycle ergometer used for the AE sessions were located in close proximity, with less than 1 min required to move between the two.

Data analysis

Heart Rate. Data were averaged across the 20 min of AE to allow for comparison with the target HR for the particular session.

S-R Curves. Ten stimulations were performed at each stimulator output intensity (100, 110, 120, 130 and 140% RMT) to generate an S-R curve. The peak-to-peak amplitudes for the resultant MEPs were obtained for each trial using a custom script programmed in Signal. Briefly, the custom script isolated a 50 msec window in which the evoked response occurred, returning the minimum and maximum value (i.e., the peak-peak amplitude) in the specified window. We isolated a window 10 msec following the stimulus (occurring at second 1 in each frame, so 1.01 – 1.06s) as the typical latency of a MEP from the ECR muscle is between 15 and 20 msec. While localization of the windows was performed in an automated manner, data were reviewed manually to ensure the peak-peak amplitude values obtained related to the evoked response (as opposed to artifact for instance). During manual review, each frame was visually inspected to make sure the timing of the stimuli and responses were logical (i.e., the evoked response occurred in the expected latency range following the stimulus).

MEP Inclusion/Exclusion. As volitional activity in the target muscle prior to delivery of the TMS pulse will augment the MEP amplitude, we examined EMG activity in the period immediately preceding the TMS pulse, removing from analysis MEPs where EMG exceeded baseline values.

Specifically, we calculated the average root mean square (RMS) amplitude in a 70 msec window (0.025-0.095) before the TMS pulse, removing any trials in which the average RMS amplitude exceeded the average value at baseline plus one standard deviation. The MEPs obtained for each stimulation intensity (100, 110, 120, 130 or 140% RMT) pre- and post-exercise were averaged within a participant to generate the S-R curve. Participants in whom an *a priori* number of MEPs were not obtained ($< 6/10$) as a response to stimulation at any intensity were excluded from further analysis. For experiment 1, this meant removing the participant from analysis at that given exercise intensity (e.g., if an individual did not have a complete S-R curve at the 40% HRR intensity, but did at 30 and 50% HRR, they would only be excluded from analysis at the 40% HRR intensity), whereas for experiment 2 this meant removing the participant from the analysis entirely. For experiment 2, S-R curves were plotted for each participant at each time-point, and the area under the curve (AUC) determined for each. Briefly, this analysis involves calculating the AUC, where the curve is represented by a series of connected XY points in which the area is computed for a given baseline (in this instance $Y = 0$). Subsequent analysis of S-R curve data utilized the AUC value or the AUC change score, which was calculated by subtracting the AUC value for the pre-exercise condition from the two post-exercise conditions (i.e., post1 and post2).

Statistical Analysis

Experiment 1. A one-sample Kolmogorov-Smirnov test was used to assess normality within each individual combination of conditions (e.g., within each of the 30, 40 and 50% HRR) and Mauchly's Test of Sphericity was performed. To assess changes in corticospinal excitability within the S-R curves, MEP amplitude data were analyzed using three-way repeated-measures ANOVA with time (pre and post1), stimulus intensity (100, 110, 120, 130 and 140% RMT) and exercise intensity (30, 40 and 50% of HRR) as factors. Significant main effects in the ANOVA

were tested with planned follow-up tests using paired t -tests to detect changes from pre- to post-exercise for each dependent measure within each exercise intensity. Finally, we used one-way repeated measures ANOVA with AE intensity as the single factor and Tukey post hoc testing to examine differences in the actual HR values obtained for each of the three AE intensities.

Experiment 2. Normality of the data was examined using the D'Agostino & Pearson omnibus K2 test (D'Agostino 1986). In all instances of a non-Gaussian distribution, outliers were removed to ensure normality of the residuals, as the statistical analyses described below are susceptible to error when the residual values are not normally distributed.

To aid in comparison to prior work looking at AE and corticospinal excitability (Lulic et al. 2017; Singh et al. 2014b; Smith et al. 2014), we examined the general effect of the single exercise session on excitability measures by comparing pre- and post-exercise (post1 and 2) measures using one-way repeated measures ANOVA. To address our specific hypothesis related to the influence of aerobic fitness (i.e., VO_{2max}) on corticospinal excitability we employed linear regression, regressing VO_{2max} values against the pre-exercise AUC values, and the AUC change scores for the post1 and post2 time-points. Where applicable, an *a priori* alpha of $p < 0.05$ denoted statistical significance. For ANOVA, Mauchly's Test of Sphericity was performed to test the assumption of sphericity. Statistical analyses were performed using SPSS v. 23 (IBM Corp., NY, USA and Prism 6 (v. 6.0a, GraphPad Software Inc, CA, USA).

RESULTS

Experiment 1

Physical Activity and Exercise. Physical activity level of the participants was determined by calculating median metabolic equivalent (MET)-min/week, with each participant categorized as

having high (at least 3000 MET-min/week), moderate (at least 600 MET-min/week) or low physical activity according to IPAQ guidelines (2005). Seven participants were considered highly active, with the remainder (five) found to have moderate levels of physical activity. All participants were able to complete the GXT as per the criteria, with HR values within 12 bpm of their respective estimated HR_{max} (Table 1). For the exercise component of the experimental sessions, all participants maintained their HR within range of the target HR for the particular session. Analysis of the actual HR values (Table 1) showed a main effect of exercise intensity ($F_{11, 22} = 66.99, p < .001$), with differences noted between each of the exercise intensities (30, 40 and 50% HRR).

Table 1 here.

TMS. One participant was removed from analysis of two exercise intensities (30 and 50% HRR) because they did not meet the minimum criteria for number of MEPs. Normality tests indicated that all cases were normally distributed. As expected, MEP amplitude increased as stimulation intensity increased, evidenced by a main effect of stimulation intensity ($F_{4, 40} = 61.038, p = .000, \eta^2 = .859$). There was also a main effect of time (pre-post1) relative to exercise ($F_{1, 10} = 11.939, p = .006, \eta^2 = .544$), in that an upward shift in the S-R curve was observed pre-post exercise (Figure 2). No main effect of exercise intensity was observed ($F_{2, 20} = .194, p = .825, \eta^2 = .018$). Planned follow-up tests for the observed main effect of time were performed to assess the difference after each of the three AE intensities. This analysis revealed that MEP amplitude increased significantly after exercising at 40 ($F_{1, 11} = 7.139, p = 0.022, \eta^2 = .394$) and 50% ($F_{1, 11} = 11.939, p = 0.006, \eta^2 = .544$).

$_{10}=21.052$, $p=0.001$, $\eta^2=.678$) of HRR, but not 30% ($F_{1,10}=1.358$, $p=0.271$, $\eta^2=.120$). Table 2 summarizes the pre and post1 MEP amplitude data for each of the exercise intensities.

Figure 2 here; Table 2 here

Experiment 2

Physical Activity and Exercise. The IPAQ was used to determine the physical activity level of the participants as above. Eighteen had a high level of physical activity, ten were classified as moderately active, and the remainder (one) had a low level of physical activity. Although not all participants achieved HR values within 95% of their respective estimated HR_{max} , they all achieved a max RPE ≥ 17 and a RER ≥ 1.15 (Table 3). In the majority of participants ($n=25$) a measurable plateau in VO_2 was observed. VO_{2max} values ranged from 22.1 to 48.2, with an average value of 33.7 ± 7.0 mL/kg/min (Table 3). On average, 19.6 ± 14.5 days separated the first and second experimental sessions. All participants denied significantly changing their training or physical activity routine over the duration of the experiment. Generally, participants were able to maintain their HR within 12 bpm of the target HR during the exercise session. Participants 2 and 29 were <12 bpm under their target HR (15 and 14 beats, respectively), but had high RPE's (11-16 and 13-16, respectively), while participant 19 had an average HR >10 bpm of their target (13 beats) but had a lower RPE (9-12; Table 3).

Table 3 here

TMS. Two participants were removed from analysis because they did not meet the minimum criteria for number of MEPs in the post1 and 2 time points, however their pre-exercise data were retained for analysis related to our first hypothesis for experiment 2. As expected, the average peak-to-peak amplitude for the resultant MEPs increased as a function of stimulation output within a time-point (Table 4).

Table 4 here

Pre- and post-exercise (post1 and post2) values were analyzed independently. Pre-exercise responses were considered baseline values and used to address our first hypothesis (participants with higher aerobic fitness would have greater corticospinal excitability at rest pre-exercise). Post-exercise responses (post1 and post2) were considered the exercise-induced change from baseline and were used to address our second hypothesis (participants with higher aerobic fitness would have a greater increase in corticospinal excitability after exercise). For S-R data, 29 and 27 participants were analyzed for the pre- and post-exercise time-points, respectively. A change score was calculated for post1 and post2 values, with these change scores used in the analysis. Analysis indicated that the residuals for the AUC data were not normally distributed, and thus 3 participants (21, 22 and 28) for the pre-exercise time-point and 5 participants (11, 15, 21, 22 and 28) for the post-exercise time-points were identified as outliers and subsequently removed from the analysis. As a result, data from 26 and 24 participants remained in the analysis of the S-R curve data for the first and second hypotheses, respectively.

While we observed an increase in AUC values in many of the participants at post1 (n=15) and post2 (n=11), we did not observe a main effect of time on AUC values ($F(2,23) = 1.120$, $p =$

0.3268). To address our first hypothesis, linear regression was performed using the $\text{VO}_{2\text{max}}$ and AUC values. The relationship was not significant ($F(1, 24) = 0.6110$, $p = 0.4420$) and the R^2 value was 0.02483. The relationship between $\text{VO}_{2\text{max}}$ and AUC values at pre-exercise showed a negative non-significant relationship (Figure 3A). Figure 3 also demonstrates a similar relationship between $\text{VO}_{2\text{max}}$ and AUC values for the post1 and post2 time-points (Fig 4 B and C, respectively). Results of our planned analysis to address the second hypothesis are shown in Figure 4. Linear regression was performed on the $\text{VO}_{2\text{max}}$ values and AUC change scores for the post1 (A) and post2 (B) time-points. The relationship was non-significant at both time points [post1: ($F(1, 22) = 0.6210$, $p = 0.4391$) and an R^2 value of 0.02745; post2 ($F(1, 22) = 0.01663$, $p = 0.8986$; $R^2 = 0.0007551$)]. Taken together, these results suggest that higher aerobic fitness does not equate to a heightened response to a single session of AE.

Figure 3 and 4 here

DISCUSSION

The overall aim of this study was to explore two factors that have potential to influence the cortical response to AE, namely the intensity of the AE performed and the aerobic fitness of the individual. In experiment 1 we showed that there appears to be a lower bound of AE intensity at which an exercise-induced change in corticospinal excitability is observed. In experiment 2 we showed that prior aerobic fitness, assessed via maximal oxygen uptake or $\text{VO}_{2\text{max}}$, does not influence the cortical response to a single bout of AE.

Generally our work confirms that from previous research, demonstrating a positive effect of AE on motor regions of the brain (Singh et al. 2014a; Singh et al. 2014b; Smith et al. 2014).

Indeed, numerous studies have shown that AE modulates brain plasticity through a number of mechanisms, including increased cerebral blood flow, changes in lactate concentration as well as alterations in the level and activity of neurotransmitters (e.g., gamma aminobutyric acid or GABA and glutamate) and other neuromodulatory agents including brain derived neurotrophic factor (BDNF (Gold et al. 2003; Rojas Vega et al. 2006; Tang et al. 2008); for a comprehensive review of these mechanisms see (Singh and Staines 2015; El-Sayes et al. 2018). Previous work employing TMS to probe AE-related brain plasticity in M1 has shown alterations in intra-cortical inhibitory networks, providing evidence of alterations in activity of GABA_A and GABA_B receptors (short and long interval cortical inhibition respectively; (Lulic et al. 2017; Singh et al. 2014b; Smith et al. 2014; Yamaguchi et al. 2012)). Although conflicting results have been reported, several studies have also shown increased facilitation after AE (i.e., intracortical facilitation), a finding potentially mediated by glutamatergic interneurons, and possibly N-methyl-D-aspartate (NMDA) receptor activity (Liepert et al. 1997; Ziemann et al. 1998). Positive findings related to increased corticospinal excitability (assessed via S-R curves) have been less robust, with several studies showing no significant increase after AE. This lack of change in excitability may be attributed to the large number of central and peripheral mechanisms that modulate the excitability of the corticospinal tract, although it should be noted that other variables (e.g., level of physical activity, type of exercise and intensity) must be considered when interpreting these results, as other studies have shown a change in corticospinal excitability post AE (Lulic et al. 2017).

Experiment 1. The purpose of experiment 1 was to determine if lower intensity AE modulates cortical excitability. If that was the case then AE could be used to prime the brain prior to motor rehabilitative therapy while avoiding fatigue during the subsequent learning task. A recent meta-

analysis reported that estimates of the prevalence of fatigue among survivors of stroke ranged between 25 and 85%, with a pooled prevalence estimate of 50% (Cumming et al. 2016). Prevalence estimates varied little across time-points, suggesting that fatigue is an issue across the time course of rehabilitation. Our results demonstrate that 20 min of AE at 40% and 50% but not 30% HRR has a positive effect on corticospinal excitability. The ACSM classifies intensities of 40% HRR or less as light intensity (Pescatello 2014), therefore our work supports the possibility of using AE as a priming agent for skill acquisition therapy in rehabilitation. The lack of significant findings at the 30% HRR intensity is most likely related to the fact that exercise at this intensity is considered 'very light exercise' (Pescatello 2014). Indeed our participants rated it between 1 and 2 on the RPE scale, representing 'really easy' and 'easy', respectively. The mechanism responsible for increased excitability during low intensity exercise at 40% of the HRR is unclear. Previous work has shown that increased levels of lactate in the brain result from more intense AE, with these increases in lactate corresponding to increased M1 excitability (Coco et al. 2010). Previous research has also shown that higher exercise intensities are associated with a greater release of neuromodulatory substances in the brain (e.g., BDNF (Ferris et al. 2007; Schmolesky et al. 2013)). This suggests that a higher intensity is more likely to produce positive effects on neuroplasticity.

Experiment 2. Single bouts of AE facilitate brain plasticity in M1 via changes in neuromodulatory agents (Singh and Staines 2015). Individual level factors however, such as physical activity and aerobic fitness, are less understood in relation to the response to a single bout of AE. It is generally accepted that routine physical activity leads to physiological changes in numerous body systems, with changes related to the central nervous system manifesting as an increased response to AE. Indeed, previous work provides evidence to support this notion

(Cirillo et al. 2009; Lulic et al. 2017). At first glance, the current findings appear to oppose those of prior work, as we did not observe a relationship between aerobic fitness and corticospinal excitability, either at baseline or post-exercise, refuting our hypotheses. A likely explanation for the incongruity in findings relate to the measure applied, namely aerobic fitness vs. physical activity.

As indicated previously, the criterion measure for aerobic fitness is VO_{2max} . The Fick equation describes VO_{2max} as a product of both central and peripheral factors, specifically, cardiac output and tissue oxygen uptake. Although aerobic fitness is an objective measure of aerobic training, it does not measure or necessarily reflect regular aerobic activity due to a multitude of factors that influence it (e.g., sex, age, genetics and aerobic training; (Heyward and Gibson 2014)). Previous studies have shown there to be a weak positive correlation between aerobic fitness and IPAQ score (Fogelholm et al. 2006; Hagstromer et al. 2006). Our results mirror this prior work; although our independent variable of interest was aerobic fitness level, we collected both VO_{2max} through the GXT as well as MET-min/week of physical activity through the IPAQ. The relationship between participants' VO_{2max} and IPAQ score are shown Figure 5; a low correlation between VO_{2max} and IPAQ score is present, and it is clear that some individuals with low VO_{2max} values were quite active as assessed via the IPAQ. Interestingly, the finding of increased corticospinal excitability in both experiment 1 and 2 (albeit non-significant in experiment 2; Tables 2 and 4; Figure 1) aligns with that of previous work (Lulic et al. 2017), which observed an increase in corticospinal excitability in participants with MET-min/week values exceeding 3000 (i.e., a high degree of physical activity) calculated via IPAQ scores. Here, 7/15 and 18/29 (experiment 1 and 2, respectively) would be considered as having a high level of physical activity, with only one participant overall (i.e., in both experiments 1 and 2) considered

as having a low level of physical activity. Other work examining moderate-intensity AE has not reported an increase in corticospinal excitability post-exercise (e.g., (Singh et al. 2014b), a finding which may be attributed to the fact that their participants were reported as having a ‘moderate level of physical activity’, although no formal assessment of physical activity level was performed (Singh et al. 2014b).

Figure 5 here

There are multiple intrinsic (e.g., genetics and sex) and extrinsic (e.g., training intensity, duration, frequency, etc.) factors associated with both inter-individual differences in aerobic fitness (VO_{2max}) and individualized responses to AE training. Many of the long-term physiological adaptations to AE training, including elevated blood volume, cardiac stroke volume, peripheral oxygen extraction and decreased motor unit activation thresholds are directly related to aerobic fitness (Hellsten and Nyberg 2015; Lundby et al. 2017; MacDonell and Gardiner 2018). However, some individuals have an enhanced genetic predisposition to higher aerobic fitness, regardless of training status, and men tend to have greater VO_{2max} values than women with similar physical and sedentary behavior patterns (Loe et al. 2013; Wang et al. 2010). As such, extrinsic factors related to the mode, intensity, duration and frequency of exercise/physical activity may provide greater insight, than aerobic fitness *per se*, on the corresponding changes in brain plasticity in response to acute bouts of AE. More objective measures of physical (and sedentary) activity patterns are required to help understand the relative impact of these factors on corticospinal excitability and AE-induced brain plasticity.

Limitations. In experiment 1 we only tested three intensities of AE. Given this, we cannot indicate that 20 min of AE at 40% HRR is the ‘lowest common denominator’ for the modulation of plasticity, as we did not test other intensities between 30 and 40% HRR. Thus, further research could be performed to increase the resolution of the data related to the threshold of AE that could still modulate experience-dependent plasticity in M1. While we did not test any intensities lower than 30% HRR, it is not likely they would result in any measurable effect on corticospinal excitability given there were null findings for 30% HRR. Intensities lower than 30% HRR were not examined given they are ‘very easy’ for the population studied, and in many instances would correspond to HR values just above resting levels. Regarding experiment 2, we acknowledge that the analysis was underpowered, largely owing to the magnitude of the variability observed in the AUC values. Given the slope values obtained for each of the regressions (which were low), coupled with the variance of the data points, it is not likely an effect exists. While an effect may be seen with a greatly increased sample size, the real world meaningfulness of such an effect in the general population would be negligible.

We did not control for sex differences or time of day, both of which can impact the findings. Overall our participants included a larger number of females than males. Research has suggested that the phase of the menstrual cycle in females may influence cortical excitability, as excitability can be increased in response to high circulating estradiol levels (Smith et al. 1999). In addition, Kuo and colleagues, in their work using transcranial direct current stimulation, reported that sex affects modulation of cortical plasticity (Kuo et al. 2006). On the other hand, Smith and colleagues suggested that sex has no influence on the amount of short interval intracortical inhibition (Smith et al. 2014). Given conflicting results, the effect of sex on TMS measures is not entirely clear and warrants further study. Regarding time of day, it is known that

cortisol levels fluctuate throughout the day, potentially affecting cortical excitability (Kanaley et al. 2001). Although we did attempt to control for this, due to logistics not all experimental sessions were completed at the same time of day.

CONCLUSION

The findings of this study suggest that AE parameters lower than those previously investigated, namely 40% HRR, are effective in facilitating experience-dependent plasticity in M1. Results further suggest that there appears to be a lower bound AE intensity at which this plasticity is facilitated, as we did not observe a significant change at 30% HRR. Regarding individual characteristics, it appears that aerobic fitness, assessed via $\text{VO}_{2\text{max}}$ is not related to corticospinal excitability either at rest or after moderate intensity AE. Coupled with previous work, these findings suggest that it is the level of physical activity, not aerobic fitness, which may best predict the brain's response to a bout of AE. Collectively these findings will aid in prescription and use of AE in applications promoting brain plasticity for learning and rehabilitation.

*The authors declare no conflicts of interest.

References

2005. Guidelines for data processing and analysis of the International Physical Activity Questionnaire (IPAQ). Available from <https://sites.google.com/site/theipaq/home> [accessed August 11 2015].
- Borg, G.A.V. 1982. Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise* **14**(5): 377-381. Available from https://journals.lww.com/acsm-msse/Fulltext/1982/05000/Psychophysical_bases_of_perceived_exertion.12.aspx [accessed.].
- Chen, R. 2000. Studies of human motor physiology with transcranial magnetic stimulation. *Muscle Nerve Suppl* **9**: S26-32. Available from 11135281 [accessed.].
- Chen, R. 2004. Interactions between inhibitory and excitatory circuits in the human motor cortex. *Exp Brain Res* **154**(1): 1-10. doi:10.1007/s00221-003-1684-1.
- Cirillo, J., Lavender, A.P., Ridding, M.C., and Semmler, J.G. 2009. Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol* **587**(Pt 24): 5831-5842. doi:10.1113/jphysiol.2009.181834.
- Coco, M., Alagona, G., Rapisarda, G., Costanzo, E., Calogero, R.A., Perciavalle, V., et al. 2010. Elevated blood lactate is associated with increased motor cortex excitability. *Somatosens Mot Res* **27**(1): 1-8. doi:10.3109/08990220903471765.
- Cotman, C.W., Berchtold, N.C., and Christie, L.A. 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. *Trends Neurosci* **30**(9): 464-472. doi:10.1016/j.tins.2007.06.011.

- Craig, C.L., Marshall, A.L., Sjostrom, M., Bauman, A.E., Booth, M.L., Ainsworth, B.E., et al. 2003. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* **35**(8): 1381-1395. doi:10.1249/01.MSS.0000078924.61453.FB.
- Cumming, T.B., Packer, M., Kramer, S.F., and English, C. 2016. The prevalence of fatigue after stroke: A systematic review and meta-analysis. *International journal of stroke : official journal of the International Stroke Society* **11**(9): 968-977. doi:10.1177/1747493016669861.
- D'Agostino, R.B. 1986. Tests for Normal Distribution. *In* Goodness-of-Fit-Techniques. *Edited by* R.B. D'Agostino, and M.A. Stephens. Marcel Dekker, New York.
- Duncan, F., Kutlubaev, M.A., Dennis, M.S., Greig, C., and Mead, G.E. 2012. Fatigue after stroke: a systematic review of associations with impaired physical fitness. *International journal of stroke : official journal of the International Stroke Society* **7**(2): 157-162. doi:10.1111/j.1747-4949.2011.00741.x.
- Dyrstad, S.M., Hansen, B.H., Holme, I.M., and Anderssen, S.A. 2014. Comparison of self-reported versus accelerometer-measured physical activity. *Med Sci Sports Exerc* **46**(1): 99-106. doi:10.1249/MSS.0b013e3182a0595f.
- El-Sayes, J., Harasym, D., Turco, C.V., Locke, M.B., and Nelson, A.J. 2018. Exercise-Induced Neuroplasticity: A Mechanistic Model and Prospects for Promoting Plasticity. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*: 1073858418771538. doi:10.1177/1073858418771538.

Elf, M., Eriksson, G., Johansson, S., von Koch, L., and Ytterberg, C. 2016. Self-Reported Fatigue and Associated Factors Six Years after Stroke. *PLoS One* **11**(8): e0161942.

doi:10.1371/journal.pone.0161942.

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., et al. 2011.

Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci U S*

A **108**(7): 3017-3022. doi:10.1073/pnas.1015950108.

Fernandes, J., Arida, R.M., and Gomez-Pinilla, F. 2017. Physical exercise as an epigenetic modulator of brain plasticity and cognition. *Neurosci Biobehav Rev* **80**: 443-456.

doi:10.1016/j.neubiorev.2017.06.012.

Ferris, L.T., Williams, J.S., and Shen, C.L. 2007. The effect of acute exercise on serum brain-derived neurotrophic factor levels and cognitive function. *Med Sci Sports Exerc* **39**(4): 728-734.

doi:10.1249/mss.0b013e31802f04c7.

Fogelholm, M., Malmberg, J., Suni, J., Santtila, M., Kyrolainen, H., Mantysaari, M., et al. 2006.

International Physical Activity Questionnaire: Validity against fitness. *Med Sci Sports Exerc*

38(4): 753-760. doi:10.1249/01.mss.0000194075.16960.20.

Gold, S.M., Schulz, K.-H., Hartmann, S., Mladek, M., Lang, U.E., Hellweg, R., et al. 2003.

Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *Journal of Neuroimmunology*

138(1-2): 99-105. doi:10.1016/s0165-5728(03)00121-8.

Gomez-Pinilla, F., and Hillman, C. 2013. The influence of exercise on cognitive abilities.

Comprehensive Physiology **3**(1): 403-428. doi:10.1002/cphy.c110063.

- Hagstromer, M., Oja, P., and Sjostrom, M. 2006. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public health nutrition* **9**(6): 755-762.
- Hellsten, Y., and Nyberg, M. 2015. Cardiovascular Adaptations to Exercise Training. *Comprehensive Physiology* **6**(1): 1-32. doi:10.1002/cphy.c140080.
- Heyward, V.H., and Gibson, A.L. 2014. Advanced Fitness Assessment and Exercise Prescription. Human Kinetics, Champaign, IL.
- Ilic, T.V., Korchounov, A., and Ziemann, U. 2002. Complex modulation of human motor cortex excitability by the specific serotonin re-uptake inhibitor sertraline. *Neuroscience Letters* **319**(2): 116-120. doi:[https://doi.org/10.1016/S0304-3940\(01\)02563-0](https://doi.org/10.1016/S0304-3940(01)02563-0).
- Kanaley, J.A., Weltman, J.Y., Pieper, K.S., Weltman, A., and Hartman, M.L. 2001. Cortisol and growth hormone responses to exercise at different times of day. *The Journal of clinical endocrinology and metabolism* **86**(6): 2881-2889. doi:10.1210/jcem.86.6.7566.
- Kleim, J.A., Kleim, E.D., and Cramer, S.C. 2007. Systematic assessment of training-induced changes in corticospinal output to hand using frameless stereotaxic transcranial magnetic stimulation. *Nat Protoc* **2**(7): 1675-1684. doi:10.1038/nprot.2007.206.
- Kuo, M.F., Paulus, W., and Nitsche, M.A. 2006. Sex differences in cortical neuroplasticity in humans. *Neuroreport* **17**(16): 1703-1707. doi:10.1097/01.wnr.0000239955.68319.c2.

Liepert, J., Schwenkreis, P., Tegenthoff, M., and Malin, J.P. 1997. The glutamate antagonist Riluzole suppresses intracortical facilitation. *Journal of Neural Transmission* **104**(11): 1207-1214. doi:10.1007/BF01294721.

Loe, H., Rognmo, O., Saltin, B., and Wisloff, U. 2013. Aerobic capacity reference data in 3816 healthy men and women 20-90 years. *PLoS One* **8**(5): e64319. doi:10.1371/journal.pone.0064319.

Lulic, T., El-Sayes, J., Fasset, H.J., and Nelson, A.J. 2017. Physical activity levels determine exercise-induced changes in brain excitability. *PLoS One* **12**(3): e0173672. doi:10.1371/journal.pone.0173672.

Lundby, C., Montero, D., and Joyner, M. 2017. Biology of VO₂ max: looking under the physiology lamp. *Acta Physiol (Oxf)* **220**(2): 218-228. doi:10.1111/apha.12827.

MacDonell, C., and Gardiner, P. 2018. Mechanisms and functional implications of motoneuron adaptations to increased physical activity. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et metabolisme*. doi:10.1139/apnm-2018-0185.

MacKay-Lyons, M.J., and Howlett, J. 2015. Exercise Capacity and Cardiovascular Adaptations to Aerobic Training Early After Stroke. *Topics in Stroke Rehabilitation* **12**(1): 31-44. doi:10.1310/rdqm-jtgl-whaa-xybw.

Mang, C.S., Campbell, K.L., Ross, C.J.D., and Boyd, L.A. 2013. Promoting Neuroplasticity for Motor Rehabilitation After Stroke: Considering the Effects of Aerobic Exercise and Genetic Variation on Brain-Derived Neurotrophic Factor. *Physical Therapy* **93**(12): 1707-1716. doi:10.2522/ptj.20130053.

McArdle, W.D., Katch, F.L., and Katch, V.I. 2010. Exercise Physiology: Nutrition, Energy and Human Performance. Lippincott, Williams & Wilkins, Baltimore, MD.

McDonnell, M.N., Buckley, J.D., Opie, G.M., Ridding, M.C., and Semmler, J.G. 2013. A single bout of aerobic exercise promotes motor cortical neuroplasticity. *Journal of applied physiology* (Bethesda, Md. : 1985) **114**(9): 1174-1182. doi:10.1152/jappphysiol.01378.2012.

Murdoch, K., Buckley, J.D., and McDonnell, M.N. 2016. The Effect of Aerobic Exercise on Neuroplasticity within the Motor Cortex following Stroke. *PLOS ONE* **11**(3): e0152377. doi:10.1371/journal.pone.0152377.

Newson, R.S., and Kemps, E.B. 2006. Cardiorespiratory fitness as a predictor of successful cognitive ageing. *J Clin Exp Neuropsychol* **28**(6): 949-967. doi:10.1080/13803390591004356.

Pescatello, L.S. 2014. American College of Sports Medicine: Guidelines for exercise testing and prescription. Lippincott, Williams & Wilkins, Baltimore, MD.

Ploughman, M., and Kelly, L.P. 2016. Four birds with one stone? Reparative, neuroplastic, cardiorespiratory, and metabolic benefits of aerobic exercise poststroke. *Curr Opin Neurol* **29**(6): 684-692. doi:10.1097/WCO.0000000000000383.

Quaney, B.M., Boyd, L.A., McDowd, J.M., Zahner, L.H., He, J., Mayo, M.S., et al. 2009. Aerobic exercise improves cognition and motor function poststroke. *Neurorehabil Neural Repair* **23**(9): 879-885. doi:10.1177/1545968309338193.

Rojas Vega, S., Struder, H.K., Vera Wahrman, B., Schmidt, A., Bloch, W., and Hollmann, W. 2006. Acute BDNF and cortisol response to low intensity exercise and following ramp

incremental exercise to exhaustion in humans. *Brain Res* **1121**(1): 59-65.

doi:10.1016/j.brainres.2006.08.105.

Rossi, S., Hallett, M., Rossini, P.M., Pascual-Leone, A., and Safety of, T.M.S.C.G. 2009. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* **120**(12): 2008-2039. doi:S1388-

2457(09)00519-7 [pii]

10.1016/j.clinph.2009.08.016 [doi].

Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., et al. 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol* **126**(6): 1071-1107.

doi:10.1016/j.clinph.2015.02.001.

Schmolesky, M.T., Webb, D.L., and Hansen, R.A. 2013. The Effects of Aerobic Exercise Intensity and Duration on Levels of Brain-Derived Neurotrophic Factor in Healthy Men. *Journal of Sports Science & Medicine* **12**(3): 502-511. Available from

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3772595/> [accessed.

Singh, A.M., and Staines, W.R. 2015. The effects of acute aerobic exercise on the primary motor cortex. *J Mot Behav* **47**(4): 328-339. doi:10.1080/00222895.2014.983450.

Singh, A.M., Neva, J.L., and Staines, W.R. 2014a. Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex. *Exp Brain Res* **232**(11): 3675-3685. doi:10.1007/s00221-014-4049-z.

Singh, A.M., Duncan, R.E., Neva, J.L., and Staines, W.R. 2014b. Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BMC sports science, medicine & rehabilitation* **6**: 23. doi:10.1186/2052-1847-6-23.

Smith, A.E., Goldsworthy, M.R., Garside, T., Wood, F.M., and Ridding, M.C. 2014. The influence of a single bout of aerobic exercise on short-interval intracortical excitability. *Exp Brain Res* **232**(6): 1875-1882. doi:10.1007/s00221-014-3879-z.

Smith, M.J., Keel, J.C., Greenberg, B.D., Adams, L.F., Schmidt, P.J., Rubinow, D.A., et al. 1999. Menstrual cycle effects on cortical excitability. *Neurology* **53**(9): 2069. Available from <http://n.neurology.org/content/53/9/2069.abstract> [accessed.

Stahl, S.E., An, H.-S., Dinkel, D.M., Noble, J.M., and Lee, J.-M. 2016. How accurate are the wrist-based heart rate monitors during walking and running activities? Are they accurate enough? *BMJ Open Sport — Exercise Medicine* **2**(1): e000106. doi:10.1136/bmjsem-2015-000106.

Tanaka, H., Monahan, K.D., and Seals, D.R. 2001. Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology* **37**(1): 153-156. doi:10.1016/S0735-1097(00)01054-8.

Tang, S.W., Chu, E., Hui, T., Helmeste, D., and Law, C. 2008. Influence of exercise on serum brain-derived neurotrophic factor concentrations in healthy human subjects. *Neurosci Lett* **431**(1): 62-65. doi:10.1016/j.neulet.2007.11.019.

Thomas, A.G., Dennis, A., Bandettini, P.A., and Johansen-Berg, H. 2012. The effects of aerobic activity on brain structure. *Front Psychol* **3**: 86. doi:10.3389/fpsyg.2012.00086.

Thomas, S., Reading, J., and Shephard, R.J. 1992. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* **17**(4): 338-345.

Wang, C.Y., Haskell, W.L., Farrell, S.W., Lamonte, M.J., Blair, S.N., Curtin, L.R., et al. 2010. Cardiorespiratory fitness levels among US adults 20-49 years of age: findings from the 1999-2004 National Health and Nutrition Examination Survey. *Am J Epidemiol* **171**(4): 426-435.
doi:10.1093/aje/kwp412.

Wasserman, K. 2012. Principles of exercise testing and interpretation including pathophysiology and clinical applications. Lippincott, Williams & Wilkins, Philadelphia, PA.

Yamaguchi, T., Fujiwara, T., Liu, W., and Liu, M. 2012. Effects of pedaling exercise on the intracortical inhibition of cortical leg area. *Experimental Brain Research* **218**(3): 401-406.
doi:10.1007/s00221-012-3026-7.

Ziemann, U., Chen, R., Cohen, L.G., and Hallett, M. 1998. Dextromethorphan decreases the excitability of the human motor cortex. *Neurology* **51**(5): 1320-1324.

TABLES

Table 1. Graded exercise test and participant's target and actual heart rate (HR) for aerobic exercise at 30, 40 and 50% of heart rate reserve (HRR). Parentheses denote standard deviation.

Participant	GXT			30%		40%		50%	
	*RPE	Heart Rate (bpm)							
		Estimated	Actual	Target	Actual	Target	Actual	Target	Actual
1	8	186.8	187	113	115 (2.6)	124	124 (2.5)	135	134 (3.0)
2	10	190.2	185	102	103 (3.0)	113	113 (2.7)	124	124 (2.6)
3	7	185.5	178	113	111 (5.3)	121	122 (1.8)	131	131 (2.7)
4	8	190.2	182	99	98 (5.2)	111	109 (5.6)	122	122 (1.8)
5	7	190.2	186	102	103 (5.3)	113	110 (6.6)	124	120 (4.0)
6	8	188.1	179	102	103 (2.0)	112	111 (3.8)	123	123 (1.8)
7	9	192.8	190	97	98 (1.9)	106	105 (2.0)	116	115 (1.6)
8	7	190.2	183	116	115 (1.4)	125	125 (1.4)	133	133 (1.5)
9	7	188.1	177	108	108 (3.4)	118	117 (2.0)	127	126 (1.8)
10	8	190.2	181	109	108 (2.5)	120	120 (1.9)	131	131 (1.7)
11	8	190.8	187	119	119 (1.7)	127	127 (1.5)	136	136 (1.7)
12	8	183.5	172	100	99 (2.6)	110	113 (2.7)	120	121 (4.9)

*RPE – rating of perceived exertion as per modified Borg Scale.

Table 2. Average MEP peak-peak amplitude values (μV) for single-pulse (S-R curve) TMS measures. Parentheses denote standard deviation.

% Resting Motor Threshold (RMT)										
	100		110		120		130		140	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
30%	275 (224)	275 (127)	393 (265)	410 (157)	426 (290)	492 (184)	541 (232)	541 (232)	571 (311)	638 (213)
40%	258 (254)	309 (303)	336 (327)	384 (327)	430 (355)	482 (358)	504 (364)	558 (370)	547 (371)	650 (350)
50%	249 (193)	313 (231)	356 (235)	382 (251)	393 (216)	453 (276)	442 (237)	487 (258)	491 (238)	578 (255)

Table 3. Graded exercise test (GXT) and participant's target and actual heart rate (HR) for aerobic exercise at 50% of heart rate reserve (HRR). Parentheses denote standard deviation.

Participant	GXT				50% HRR		RPE*
	VO _{2max}	Estimated HR _{max}	% HR _{max} achieved	Max RER	Heart Rate (bpm)		
					Target	Actual	
1	22.1	162	84.9	1.31	116	117 ± 4.0	12-16
2	24.0	174	91.5	1.21	126	111 ± 17.9	11-16
3	25.4	203	106.0	1.31	147	150 ± 4.1	9-13
4	25.7	187	98.7	1.31	127	128 ± 6.7	11-14
5	26.4	174	91.2	1.24	129	133 ± 3.1	10-15
6	26.5	178	94.6	1.22	120	124 ± 4.6	8-13
7	26.9	186	97.5	1.28	134	137 ± 5.7	10
8	27.2	174	92.2	1.38	120	126 ± 5.0	10-12
9	27.6	188	99.2	1.32	138	138 ± 4.6	11-16
10	28.5	180	94.7	1.34	130	134 ± 4.7	9-15
11	31.0	174	92.8	1.36	122	127 ± 2.7	15-18
12	31.3	193	100.8	1.37	133	133 ± 6.3	12-13
13	31.7	191	100.8	1.34	125	126 ± 7.5	11-14
14	33.3	187	102.3	1.42	133	137 ± 5.0	11-13
15	34.1	194	102.0	1.39	134	136 ± 5.9	12-13
16	35.3	163	85.7	1.24	114	120 ± 3.3	12-13
17	35.4	195	102.9	1.24	131	138 ± 5.4	8-13
18	35.5	167	87.2	1.2	118	114 ± 5.7	11-17
19	35.9	177	92.4	1.43	119	132 ± 5.0	9-12
20	37.1	175	92.7	1.36	125	127 ± 3.8	12-15
21	37.2	188	99.9	1.28	122	125 ± 3.7	12-13
22	37.7	181	95.2	1.25	124	123 ± 7.1	11-16
23	40.5	169	88.9	1.31	112	117 ± 6.5	11-15
24	40.9	156	83.2	1.27	107	109 ± 3.8	10-16
25	42.1	194	101.7	1.36	133	139 ± 3.3	11-16
26	42.3	166	88.2	1.37	114	115 ± 4.6	11-13
27	42.8	192	101.0	1.29	133	141 ± 3.4	12-13
28	46.1	190	98.9	1.46	123	126 ± 8.6	9-12
29	48.2	203	108.3	1.23	139	125 ± 6.4	13-16

*RPE – rating of perceived exertion as per the (original) Borg Scale.

Table 4. Average MEP peak-peak amplitude values for single-pulse (S-R curve) TMS measures for pre- and post-exercise. Parentheses denote standard deviation.

% Resting Motor Threshold (RMT)	MEP peak-peak amplitude		
	Pre	Post 1	Post 2
100	261 (129)	325 (154)	275 (132)
110	471 (203)	536 (208)	478 (234)
120	589 (189)	779 (197)	683 (255)
130	730 (235)	862 (182)	735 (191)
140	760 (232)	874 (196)	742 (202)

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FIGURE LEGENDS

Figure 1. Experiment 1 (A). Participants underwent a graded maximal exercise test followed by 3 sessions in which TMS was applied before and immediately after a bout of AE. AE intensity in sessions 2, 3 and 4 was randomized. Experiment 2 (B). Participants underwent a graded maximal exercise test during which aerobic fitness was assessed (via $\text{VO}_{2\text{max}}$), followed by a single session of AE during which TMS measures were assessed before, immediately after and 20 min after AE.

Figure 2. Stimulus-response (S-R) curves pre- and post-exercise at 30 (A), 40 (B) and 50% HRR (C). S-R curves differed between pre- and post-exercise at 40 and 50% HRR, but not 30% HRR.

Figure 3. Area under the curve (AUC) values for each participant, plotted as a function of aerobic fitness (measured by maximal oxygen consumption or $\text{VO}_{2\text{max}}$) prior to ('pre'; A); immediately after ('post1'; B); and 30 min after exercise ('post2'; C).

Figure 4. Area under the curve change scores (ΔAUC) for each participant, plotted as a function of aerobic fitness (measured by maximal oxygen consumption or $\text{VO}_{2\text{max}}$) immediately after exercise ('post1'; A); and 30 min after exercise ('post2'; B).

Figure 5. Relationship between IPAQ score (MET-min/week) and aerobic fitness ($\text{VO}_{2\text{max}}$).

Figure 1. Experiment 1 (A). Participants underwent a graded maximal exercise test followed by 3 sessions in which TMS was applied before and immediately after a bout of AE. AE intensity in sessions 2, 3 and 4 was randomized. Experiment 2 (B). Participants underwent a graded maximal exercise test during which aerobic fitness was assessed (via VO₂max), followed by a single session of AE during which TMS measures were assessed before, immediately after and 20 min after AE.

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Figure 2. Stimulus-response (S-R) curves pre- and post-exercise at 30 (A), 40 (B) and 50% HRR (C). S-R curves differed between pre- and post-exercise at 40 and 50% HRR, but not 30% HRR.

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Figure 3. Area under the curve (AUC) values for each participant, plotted as a function of aerobic fitness (measured by maximal oxygen consumption or VO₂max) prior to ('pre'; A); immediately after ('post1'; B); and 30 min after exercise ('post2'; C).

86x156mm (300 x 300 DPI)

Figure 4. Area under the curve change scores (ΔAUC) for each participant, plotted as a function of aerobic fitness (measured by maximal oxygen consumption or VO_{2max}) immediately after exercise ('post1'; A); and 30 min after exercise ('post2'; B).

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Figure 5. Relationship between IPAQ score (MET-min/week) and aerobic fitness (VO₂max).

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