The Effect of Acute Aerobic Exercise on the Consolidation of Motor Memories

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Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Abstract

Acute aerobic exercise performed prior to training may assist with motor skill acquisition through enhancement of motor cortical plasticity. In addition, high intensity exercise performed after training improves retention, although the mechanisms of this are unclear. We hypothesized that acute continuous moderate intensity exercise performed post-motor training would also assist with motor skill retention and that this behavioural change would be positively correlated with neural markers of training-related cortical adaptation. Participants (n=33; assigned to an exercise (EXE) or control (CON) group) completed a single visuomotor training session using bilateral wrist movements while movement-related cortical potentials (MRCPs) were collected. After motor training, the EXE group exercised for 20 minutes (70% of heart rate reserve (HRR)) and the CON group read for the same amount of time. Both groups completed two post-training tests after exercise/rest: 10 minutes and ~ 30 minutes once heart rate returned to resting level in EXE. Retention and transfer tests were both completed 1 and 7 days later. MRCPs measured training-related neural adaptations during the first visit and motor performance was assessed as time and trajectory to the target. The EXE group had better performance than CON at retention (significant 7 days post-training). MRCP amplitudes increased from early to late motor training and this amplitude change was correlated with motor performance at retention. Results suggest that moderate intensity exercise post-motor training helps motor skill retention and that there may be a relationship with motor training-related cortical adaptations that is enhanced with post-motor training exercise.

1.0 Introduction

There is growing interest in the potential use of aerobic exercise to enhance cortical excitability associated with the acquisition and consolidation of motor skill learning in healthy adults. An acute bout of moderate-intensity aerobic exercise performed prior to a single session of motor training enhanced the excitability of the trained effectors within the primary motor cortex (M1) but did not improve behavior (Singh et al. 2016). Interestingly, an acute session of high intensity interval exercise performed immediately after motor training has been shown to enhance the consolidation of motor memories in a visuomotor tracing task (Roig et al. 2012). The association between cortical adaptations during skill acquisition and the consolidation of the motor memories of these skills is important to fully understand in healthy adults as it informs motor learning paradigms and has potential applications in sport, rehabilitation and occupational skills training.

Synaptic plasticity is thought to be an underlying mechanism of motor learning. Early long-term potentiation (E-LTP) causes the addition of more AMPA receptors into the postsynaptic membrane, allowing more glutamate to bind to these newly available receptors, resulting in a larger postsynaptic response the next time glutamate is released into the synapse. With enough stimulation, late LTP (L-LTP) results in the addition of a new dendritic spine which requires genetic modifications and protein synthesis signalled through second-messenger systems. Acute exercise is associated with increases in LTP-related biomarkers, such as BDNF, catecholamines, and lactate, some of which are positively correlated with acquisition and retention rates (Winter et al. 2007; Skriver et al. 2014). This suggests that exercise could improve motor memory consolidation through the elevation of LTP-related compounds to prime the cortex for synaptic plasticity.

Consolidation is the process through which the encoded motor memory is stabilized in the sensorimotor network. Memories are thought to be consolidated at two levels: synaptic consolidation, associated with L-LTP, and systems consolidation, a longer-term change in the location of the storage of a memory (Dudai 2012). It is likely that consolidation involves not just M1, but the whole sensorimotor network, including motor planning regions such as the dorsal premotor cortex (Meehan et al., 2013), and sometimes cognitive areas including the dorsolateral-prefrontal cortex (Kantak et al., 2010, 2011).

Motor learning relies on the interaction among many cortical and subcortical structures. The underlying mechanisms of these changes in brain activity likely involve LTP processes occurring in various brain regions dependent on the task and phase of learning across the sensorimotor networks. During a single motor training session, there is increased functional connectivity between many sensorimotor and cognitive areas including: M1-premotor cortex (PMC)-supplementary motor area (SMA), as well as prefrontal cortex (PFC)-PMC (Sun et al. 2007). This increase in functional connectivity between these regions allows communication from different areas to prepare, plan, and execute a new movement. Within one session of practicing a novel visuomotor task, fMRI shows learning-related increases in the PMC, SMA, parietal areas, basal ganglia, and cerebellum, and decreases in the PFC, M1, and preSMA (Dayan and Cohen 2011). EEG studies have found increases in brain activity, represented as an enhanced early component of the MRCP in motor preparatory areas, during a single session of bimanual motor training (Smith and Staines 2006, 2010, 2012). This increased excitability is likely a combination of a reduction in GABA-mediated intracortical inhibition and increase in excitatory postsynaptic potentials (EPSPs) and makes the induction of LTP more likely to occur. Research has suggested that beta-band event-related desynchronization (ERD) may be a neural marker for the consolidation of motor memories (Pollok et al. 2014). Dal Maso et al. (2018) recently measured the effects of post-motor training exercise on the consolidation of motor memories and found that a decrease in beta-band ERD correlated with enhanced retention. These findings suggest that changes in sensorimotor areas indicative of consolidation processes are related to behavioural performance.

Using transcranial magnetic stimulation (TMS), researchers have discovered that acute exercise can enhance the intracortical excitability of M1 and as a result, prime M1 for LTP-like plasticity (Singh et al. 2014a,b; Smith et al. 2014). Similarly, exercise is also thought to enhance cortical activity in the motor planning regions of the sensorimotor network, specifically the SMA (Thacker et al. 2014) and cerebellum (Mang et al. 2016b), as well as increase the functional connectivity of resting-state sensorimotor networks (Rajab et al. 2014). Behavioural studies have shown that moderate intensity exercise performed prior to motor training can enhance the acquisition but not the retention of a motor skill (Snow et al. 2016; Statton et al. 2015), and both the acquisition and retention of learned motor adaptations (Neva et al., 2019). Thus, pre-motor training exercise may prime the neural mechanisms responsible for encoding the motor memory. When exercise is performed after motor training, improvements in the retention of motor skills are observed which suggests that post-motor training exercise primes consolidation mechanisms (Roig et al. 2012; Thomas et al. 2016a,b,c). While the type of exercise does not have an effect on this relationship (Lundbye-Jensen et al. 2017; Thomas et al. 2016a), the timing of exercise relative to motor training seems to play a role as studies have found that the long-term retention of the motor skill is improved when exercise is performed immediately after motor training (Roig et al. 2016, 2012; Thomas et al. 2016b). The intensity of exercise is also related to the long-term retention of the motor skill as it has been shown that high intensity interval exercise leads to superior retention scores compared to low intensity interval exercise (Thomas et al. 2016c). The effects of a continuous moderate intensity exercise have not yet been examined. Pre-motor training high intensity exercise can also assist with consolidation in addition to enhancing acquisition (Mang et al. 2014, 2016a; Stavrinos and Coxon 2017; Winter et al. 2007). In this case it affects retention as well as acquisition likely because high intensity exercise has longer-lasting effects that temporally overlap with consolidation mechanisms. Roig et al. (2012) compared whether acute high intensity exercise influenced motor acquisition and retention, and also whether the timing of exercise relative to motor training changed this relationship. Exercise performed before or after motor training both improved performance when tested 1 day or 1 week following the training, but importantly, exercise performed after motor training, compared to before, enhanced skill performance at the 7 day retention test to a greater degree. These results suggest that aerobic exercise performed after motor training may enhance motor memory consolidation (Wanner et al., 2020).

Few studies have investigated the relationship between the neural modulations and behavioural changes associated with the combination of exercise and motor learning (Dal Maso et al. 2018; Mang et al. 2014; Singh et al. 2016; Stavrinos and Coxon 2017). The findings of these studies have not shown conclusive correlations, possibly due to small sample sizes that are not able to capture the effects. Despite this, there are some significant findings and correlations that informed the current study. Singh et al. (2016) found that exercise performed before motor training increased cortical excitability more so than training or exercise alone. However, no immediate behavioural improvements in the motor task were observed. Instead, their results demonstrated that exercise and motor training alone both contribute to excitability changes in the cortex. The effects of exercise are more global throughout M1, and the effects of training are specific to the involved muscles. Mang et al. (2014) did not find any correlations between exercise-enhanced responses to paired associative stimulation (PAS) and exercise-related improvements in motor retention. One recent study investigated the cortical modulations underlying improvements in retention associated with post-motor training exercise (Dal Maso et al. 2018). The exercise group had a better retention of the skill 24 hours later and they had increased functional connectivity between sensorimotor areas, some increased beta-band corticomuscular coherence (CMC), and a decrease in beta-band ERD in the contralateral sensorimotor area. Beta-band ERD was the only physiological measure correlated with skill retention. Beta-band ERD is thought to represent neural activity related to planning and execution of movements. Thus, a decrease in beta-band ERD suggests that exercise assists motor learning by making the neural networks more efficient. That is, less neural activity is needed to perform the task. Continuous moderate intensity exercise has previously been shown to induce cortical modulations (Singh et al. 2014a,b, 2016; Thacker et al. 2014). Additionally, no known studies have examined the effects of post-motor training continuous moderate intensity exercise on retention of motor skills. Addressing this gap in the literature is important because high intensity interval exercise is not accessible for all populations.

The objectives of the current study were to first examine whether a session of continuous moderate intensity aerobic exercise performed after motor training would improve motor skill consolidation and second, to investigate the relationship between training-related cortical adaptations during skill acquisition and behavioural measures reflective of skill consolidation. We hypothesized that the exercise group would retain the skill better than the control group over a 1 week period, similar to previous research (Lundbye-Jensen et al. 2017; Roig et al. 2012). We also predicted that there would be a correlation between within-session training adaptations

(MRCP increases) and changes in performance at retention. This hypothesis was based on recent findings that demonstrate a correlation between cortical activity in motor preparatory areas and motor skill retention (Dal Maso et al. 2018).

2.0 Methods

2.1 Participants

Thirty-four young healthy adults (aged 18-35, 19 females/15 males) were recruited from the University of Waterloo community. One participant was excluded because they did not meet the minimum physical activity cut-off. Participants were randomly assigned to an exercise (EXE, n=17) or control (CON, n=16) group. Exclusion criteria assessed by the University of Waterloo Health History Questionnaire consisted of the following: injury that makes exercise uncomfortable, any medications affecting the central nervous system, history of seizure/epilepsy, any neurological injury/disease, and recent history of concussion. Previous research has shown that an individual's physical activity level can affect their cortical response to acute exercise (Lulic et al., 2017). To limit variability between participants, individuals accumulating less than 600 metabolic equivalents (METs) per week as assessed by the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) were excluded from the study. The Get Active Questionnaire (GAQ) (Canadian Society for Exercise Physiology, 2017) was used to determine if the individual could safely participate in exercise. Study procedures were approved by the University of Waterloo Office of Research Ethics and participants provided informed written consent before beginning the study.

2.2 Procedures

Prior to collection, participants completed the Positive and Negative Affect Schedule (PANAS) (Watson et al., 1988), the Stanford Sleepiness Scale (SSS) (Hoddes et al., 1971), and the Edinburgh Handedness Questionnaire (EHQ) (Oldfield, 1971) to assess their current affect, level of sleepiness, and handedness respectively. Affect and sleepiness may contribute to arousal levels which could influence learning and performance of the motor task. Handedness is not likely to affect the task since it is bimanual, however it would provide some explanation should one hand be dominant in the task. Participants were also asked to provide an estimate of their weight and height so body mass index (BMI) could be calculated. Participants were randomly assigned to one of two groups: CON or EXE. Participants were informed of the group they were assigned to, given instructions for the task and were oriented and familiarized with the custom-made bimanual motor training (BMT) device used for the motor training. After this, as illustrated in Figure 1, participants performed the baseline test and then completed the motor training. It is not likely that the knowledge of their group membership impacted performance on the training task as it has been shown that anticipation of exercise does not affect cognitive control (Bergelt et al., 2020). The CON group performed the motor training and then rested for a period of 30 minutes. During the rest period they read while they were in a seated position on the cycle ergometer. After motor training the EXE group performed a session of aerobic exercise for 30 minutes. Both groups then completed a second PANAS scale to assess any mood differences post-exercise or rest. Two post-training tests were completed, one 10 minutes after the rest/exercise session (post-training test 1), and one once HR had returned to baseline in the EXE group (within 5 bpm of resting HR) and 30 minutes after rest in the CON group (post-training test 2). This second post-training test was to account for fatigue and arousal effects that may have affected performance in the first post-training test. During the time between the post-training test 1 and post-training test 2 both groups were provided with reading material. Participants returned to the lab 1 day and 7 days later to complete retention and transfer tests.

Two participants, one of which was in the EXE group, and one of which was in the CON group, returned 2 days and 8 days later due to campus closure. Prior to completing the retention and transfer tests participants completed the PANAS scale and the SSS. On visit 2 they also completed the St. Mary's Hospital Sleep Questionnaire (SMHSQ), which provided an indication of the quality and quantity of sleep the night after the experimental session. All three visits for each participant were completed around the same time of day. Consistent with past studies, participants were required to refrain from physical activity 2 hours before each visit and 4 hours after the first visit (Borota et al. 2014; Thomas et al. 2016a,b). Participants were instructed to maintain their typical caffeine intake and avoid sleeping for 4 hours after the first visit.

---- *Figure 1 near here* ----

2.3 Exercise

Prior to exercise a baseline HR was collected in a seated position using a Polar HR monitor (Léger and Thivierge 1988). Rate of perceived exertion (RPE) was assessed using the Borg scale (Borg 1970). Target RPE was between 12-15 to reflect a moderate intensity. The exercise was a 20 minute continuous moderate intensity session on a stationary recumbent bike. During exercise arms were resting on the handlebars and participants were instructed to keep their arms relaxed to avoid fatiguing the muscles involved in the motor training task. Cycling was chosen based on past literature that has demonstrated that lower body aerobic exercise can modulate excitability in the motor cortical representations for the upper body (Singh et al. 2014a). Participants completed a 5-minute self-paced warm-up to get their HR up to a moderate intensity level, which was calculated as 70% of their HRR based on their age-predicted HR max and resting HR (i.e., $70\%\ HRR = 0.7(HR_{max} - HR_{rest}) + HR_{rest}$). Once they reached this HR at the end of the 5 minute warm-up, they exercised at this intensity for 20 minutes. HR was monitored continuously throughout the session and RPE was reported every 5 minutes. Participants kept a pace of 55-65 revolutions per minute (RPM) and adjusted the bike's resistance level to maintain target HR. After 20 minutes at this intensity participants completed a 5-minute cool-down after which HR and RPE measures were taken again. The intensity and duration of exercise were selected to be less intense than high intensity interval training and more intense than a low intensity interval or continuous exercise bout. This intensity was meant to challenge participants but not exhaust them.

2.4 Motor Task

The motor training task was a modified version of the BMT task that has been validated by past motor learning studies from our lab that have examined behavioural and cortical modulations (Neva et al. 2012; Singh et al. 2016; Smith and Staines 2006, 2010, 2012) (Figure 2a). The task required participants to hold two handles, one in each hand, that were connected to potentiometers to measure their movement with a customized LabVIEW program that allowed for the movement of the handles to control movement of the cursor on a screen. Flexion/extension of the right wrist moved the cursor vertically and of the left wrist moved the cursor horizontally. During a single trial, participants first saw the cursor (black circle) and moved it to the start position (x) in the bottom right corner (Figure 2b). When this occurred, the cursor disappeared and 1 of 3 possible targets (30°, 45°, and 60° from the y-axis) appeared in the top left corner. After a 2 s delay the cursor reappeared which was the cue for participants to move it to the specified target location. This ensured the measurement of the early cued MRCP component that starts approximately 2 s before movement. The participant then had 2 s to move the cursor to the target. After this, feedback was given in the form of a response time (RT), calculated as the time between when the cursor reappeared and when the cursor reached the target. Participants started the next trial when they were ready. The movement from the start position to the 3 targets required participants to perform simultaneous (in-phase) wrist flexion movements with slightly different endpoint positions (Singh et al. 2016). This motor training task was specifically selected because of past research that has demonstrated that inphase BMT enhances the amplitude of the early MRCP component (Smith and Staines 2010) and can also increase cortical excitability of M1 (Singh et al. 2016) suggesting the beginning of early plasticity processes. Participants were told the objective of the task was to flex both wrists simultaneously to move the cursor onto the target and to perform the task as quickly and as accurately as possible within the 2 s timeframe.

The motor training was a random variable practice structure consistent with previous studies (Kantak et al. 2010, 2011). Participants completed 180 trials of training of which 50% were the test target (45°), 25% were a target located 30° from the y-axis, and the remaining 25% were a target located 60° from the y-axis. This equated to 90 trials of the test target, and 45 trials each of the other two targets. The 180 trials were completed in a randomized order. Before training participants completed 5 familiarization trials and 5 baseline trials to the test target. Post-training test 1 and retention test 1 and 2 were identical to the baseline test. Post-training test 2 consisted of a larger number of trials (50 trials) to the test target to allow the generation of the MRCP trace. Transfer test 1 and 2 were 5 trials of a new target (approximately 37.5° and 52.5° from the y-axis). The transfer test was included as an alternative measure of learning. During the baseline, post-training, retention, and transfer tests no feedback of RT was given.

---- Figure 2 near here ----

2.5 Data acquisition

Behavioural data was collected in the form of RT and the trajectory of the cursor. Event codes from the customized LabVIEW program indicated the start of the trial, when the target appeared, cue to move, start of cursor movement, and the end of the trial. Voltage data from the potentiometers and cursor location were recorded in the customized LabVIEW program. This data was collected at a sample rate of 40 Hz.

EEG was recorded during the training and post-training test 2 at visit 1 using a 32 channel cap (Quik-Cap, Neuroscan, Compumedics, NC, USA) according to the International 10-20 system and referenced to the linked mastoids. Specifically, 11 electrodes recorded electrical activity over frontal and sensorimotor areas (FP1, FZ, F3, F4, FC3, FCZ, FC4, C3, CZ, C4, PZ) guided by previous studies using a similar bimanual training task (Smith and Staines, 2010, 2012). Impedances were maintained below 5 kΩ and continuous EEG data was collected, filtered (DC-200 Hz, 6dB octave roll-off) and digitized (1000 Hz, SynAmps2, Scan 4.5, Compumedics Neuroscan, Charlotte, NC) before being stored on computer for off-line analysis.

2.6 Data analysis.

To assess accuracy, movement trajectory of the cursor to the target was measured by the deviation from a straight (ideal) path to the target, represented by the root mean square (RMS) of this difference. Minimal deviations from a straight line were considered markers of good motor performance. Response time (RT) was calculated as the time between the cue to move and when the cursor reached the target. If the participant did not reach the target their RT was recorded as 2000 ms which was the maximum amount of time participants had to reach the target.

For the behavioural data (RMS and RT), at each timepoint (baseline, early training, late training, post-training 1, post-training 2, retention 1, retention 2) 5 trials to the test target were averaged to represent performance. The baseline, post-training 1 and retention tests all only consisted of 5 trials to the test target. The first 5 trials and the last 5 trials to the test target in the motor training session were averaged to represent early training and late training performance respectively. For post-training 2, the first 5 trials were averaged to represent performance at this time point. The last 5 trials of post-training 2 were averaged and used to normalize the retention and transfer scores to their performance at the end of visit 1. While post-training 2 was not intended to be a second motor training session, it may have served as extra practice for participants since it consisted of 50 trials. Therefore, the last 5 trials of post-training 2 were the most accurate representation of performance at the end of the first data collection session. For the transfer tests, the 5 trials to the new targets were averaged.

The EEG data was analyzed in Neuroscan (Compumedics Neuroscan, NC, USA) software. MRCPs were extracted from the digitally filtered (100 Hz low pass) EEG data by averaging artifact-free baseline-corrected (to the initial 200 ms: -2 to -1.8 s) individual epochs that were time-locked to the onset of cursor movement and extended from 2 s before to 500 ms after the onset of cursor movement. Prior to averaging epochs containing artifacts (i.e. from eye blinks, muscle contraction) were removed by first identifying deflections greater than 80 μV and then screening all remaining epochs by visual inspection. Averaged MRCP traces representing cortical activity at early training, late training, and post-training 2 were created for each participant. The first 60 trials of training were used to represent early training, and the last 60 trials of training were used to represent late training. All 50 of the post-training 2 trials were used to represent post-training 2. An average of 6 epochs were removed from each average trace due to artifacts. From each averaged MRCP trace an area report was used to sum all data points of the trace together from the time window of -1250 to 0. This time was selected to include cortical activity during planning and execution of the skill. This sum provided a measure of the amplitude of the early MRCP. MRCP amplitudes were maximal over the midline electrodes, specifically FCZ and CZ, so were measured here for statistical analysis.

2.7 Statistical analysis

Our main objective was to examine whether a session of continuous moderate intensity aerobic exercise performed after motor training would affect the consolidation of the motor skill as measured by performance at retention measures. To do this a mixed-model analysis of variance (ANOVA) for each behavioural measure (normalized RMS, RT) was used with time (retention 1, retention 2) as the within-subjects factor and group (EXE/CON) as the between-subjects factor. Pre-planned contrasts were used to test the hypothesis that exercise would enhance the retention of performance relative to rest at the retention time points. Specifically, it was hypothesized that retention would be enhanced in the exercise group both at 1 day and at 1 week but to a greater degree at 1 week following the training session (Lundbye-Jensen et al. 2017; Roig et al. 2012). Performance measures (RMS and RT) for retention were normalized in each participant to the scores of the last 5 trials of post-training 2. To test our assumption that there would be significant improvements in RMS and RT at late training and post-training measures and no differences between the groups at these time points, a mixed-model ANOVA for each behavioural measure was used with time (baseline, early training, late training, post-training 1, post-training 2) as the within-subjects factor and group (EXE/CON) as the between-subjects factor. Our second objective was to investigate the relationship between cortical adaptations during skill acquisition and behavioural measures reflective of skill consolidation. To test our prediction that both the EXE and CON groups would have a larger amplitude of the early MRCP component at the late training measure compared to the early training measure we used a mixed-model ANOVA. The within-subjects factor was time (early training, late training, and post-training 2) and the between-subjects factor was group (EXE/CON). Pre-planned contrasts were used to compare early and late training time points. To investigate whether there was a relationship between behavioural measures and MRCP changes we examined the correlation between change in the amplitude of the early component from early to late training and change in RMS and RT from the last 5 trials of post-training 2 to retention. Correlational analysis was conducted using the Pearson correlation. A t value was calculated from the coefficient and a one-tailed t test, with Bonferroni corrections applied for each dependent measure, was run to test for significance. To test that the two groups did not have significantly different physical activity or fitness levels a two-tailed t test was run on the average MET-minutes per week and on the average BMI for each group. To confirm that the time period after exercise prior to starting post-training 2 was not significantly different between groups, a two-tailed t test was run on the group differences in time from exercise/rest to post-training 2. Affect scores from the PANAS scale were compared using a mixed-model ANOVA with time (pre-intervention, post-intervention, day 2, day 3) and group (EXE/CON) as the factors. For all ANOVA tests and assumptions of normality, homogeneity of variance, and sphericity, SAS University Edition was used. Significance was taken as $p < 0.05$.

3.0 Results

3.1 Participant Characteristics

A total of 34 participants were recruited for the study (Table 1). The age range of participants was 19-26. All participants were deemed safe to participate in exercise from the

GAQ. There were no significant differences between the groups for MET-minutes per week (t_{31} = 0.89, p = 0.37) or BMI (t_{26} = 1.94, p = 0.06).

---- Table 1 near here ----

3.2 Supplementary Data

Results for affect (PANAS scale) revealed a significant interaction between group and time ($F_{3,93}$ = 11.88, p <0.01). Post-hoc comparisons using the Tukey-Kramer test indicated that there were no differences in the CON group mean scores at any time point (pre-intervention 37.56 ± 3.29; post-intervention 36.81± 3.45; day 2 37.81± 3.37; day 3 38.37± 4.35). The EXE post-intervention group mean score was higher than all of the CON group mean scores and the EXE group mean scores (pre-intervention 39.97 ± 3.34; post-intervention 41.73± 3.50; day 2 38.64± 4.43; day 3 38.20± 3.22). This was significant in every comparison except for the EXE pre-intervention group mean score. The EXE pre-intervention group mean score was significantly higher than CON pre-intervention, CON post-intervention, and CON day 2. There were no significant main effects of time ($F_{3,93}$ = 2.28, p = 0.08) or group ($F_{1,31}$ = 3.06, p = 0.09).

On the SSS all participants rated their degree of sleepiness between a 1-3 except for one participant who during the first visit rated their sleepiness as a 4 (1 = feeling active, vital, alert, or wide awake; 2 = functioning at high levels, but not at peak; able to concentrate; 3 = awake, but relaxed; responsive but not fully alert; 4 = somewhat foggy, let down). According to the SSS a score above 3 is considered 'sleepy' (Berry and Wagner 2014). The mode of all SSS scores was 2. On average participants had 7.25 ± 1.15 hours of sleep the night of visit 1 (from the SMHSQ). The amount of sleep ranged from 4.6-9.5 hours. All participants rated their sleep as ‘fairly well’, ‘well’, or ‘very well’ except for one participant who rated their sleep as ‘fairly badly’.

3.3 Exercise Data

Table 2 summarizes the HR data and time prior to post-training 2, following the motor training and exercise/rest, in visit 1. Two-tailed t tests revealed that there were no between group differences in time from exercise/rest to post-training 2 ($t_{16} = 0.04$, $p = 0.96$). Due to time constraints one participant completed post-training test 2 before their HR was back to resting level.

---- *Table 2 near here* ----

3.4 Behavioural Data

The ANOVA for the RMS retention data revealed a main effect of time ($F_{1,31} = 3.85$, $p = 0.05$) (Figure 3). Post-hoc comparisons using the Tukey-Kramer test revealed no differences between timepoints. There was no main effect of group ($F_{1,31} = 1.56$, $p = 0.22$) or interaction effect between group and time ($F_{1,31} = 0.10$, $p = 0.75$). Planned contrasts revealed there was no difference between the groups at retention 1 ($F_{1,31} = 3.19$, $p = 0.08$). However, the groups were different at retention 2 ($F_{1,31} = 5.01$, $p = 0.03$) and when the retention timepoints were pooled together ($F_{1,31} = 8.10$, $p < 0.01$). There were no main effects or interaction for the RT retention data. The transfer test data was also normalized to the performance at the end of visit 1 (both RMS and RT). There were no significant differences between the groups at transfer 1 and transfer 2 for both the RT and RMS data.

---- *Figure 3 near here* ----

The mixed-model ANOVA on the RMS acquisition and post-training measures (Figure 4 revealed a main effect of time ($F_{4,124} = 98.29$, $p < 0.01$). Post-hoc comparisons using the Tukey-Kramer test revealed that all timepoints were different from one another except for post-training 1 and post-training 2, and late-training and post-training 2. Similarly, there was also a main effect of time ($F_{4,124} = 115.9$, $p < 0.01$) for RT. Post-hoc comparisons revealed that baseline and early training were not different from each other, but they were different from every other timepoint. Late training, post-training 1 and post-training 2 were not different from each other. There were no main effects of group for either RMS ($F_{1,31} = 0.05$, $p = 0.81$) or RT ($F_{1,31} = 0.14$, $p = 0.71$), and no interaction between group and time for either (RMS: $F_{4, 124} = 1.07$, $p = 0.37$; RT: $F_{4,124} = 0.18$, $p = 0.94$).

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3.5 Neurophysiological Data

Mixed-model ANOVAs with time (early training, late training, and post-training 2) and group (EXE/CON) were run on the data from FCZ and CZ electrodes. Results from the FCZ electrode revealed a main effect of time ($F_{2,62} = 10.33$, $p < 0.01$). Post-hoc comparisons using the Tukey-Kramer test revealed that post-training 2 was significantly different than early training and late training. There were no main effects of group ($F_{1,31} = 0.06$, $p = 0.80$) and no interaction between group and time ($F_{2,62} = 2.52$, $p = 0.08$). The planned contrast between early and late training was not significantly different ($F_{1,62} = 3.19$, $p = 0.07$). Results from the CZ electrode

(Figure 5) showed a main effect of time ($F_{2,62} = 3.71$, $p = 0.03$). Grand average traces of EXE and CON are displayed in Figure 5a,b. Post-hoc comparisons using the Tukey-Kramer test revealed that MRCPs in post-training 2 were smaller than late training. There were no main effects of group ($F_{1,31} = 0.23$, $p = 0.63$) and no interaction between group and time ($F_{2,62} = 0.92$, $p = 0.40$). The planned contrast between early and late training showed an MRCP increase (greater negativity) in late- relative to early-training ($F_{1,62} = 4.68$, $p = 0.03$) (Figure 5c).

---- *Figure 5 near here* ----

3.6 Correlational Data

To understand the relationship between training-related neural adaptations during skill acquisition and the consolidation of these skills we correlated the change in MRCP amplitude from early to late training with the change in behaviour from the end of the acquisition visit to the retention time points. This allowed us to examine whether changes in the MRCP amplitudes during motor training were associated with performance at retention scores. In other words, the degree to which the neural representation of skill acquisition predicted participants' ability to consolidate (or retain) the skill. There was a positive linear relationship in the EXE group between the change in MRCP amplitude recorded from early to late training and the change in RT from the last 5 trials of post-training 2 to retention 1 (FCZ / CZ: $r = 0.77$ / 0.60, $p = 0.0001$ / 0.005, $p < 0.05$ Bonferroni corrected) (Figure 6). This correlation was not observed in the CON group (FCZ / CZ: $r = 0.25$ / 0.007, $p = 0.17$ / 0.49). However, when the EXE and CON groups were pooled together, there was a significant correlation at FCZ ($r = 0.58$, $p = 0.007$). There were no significant correlations observed at retention 2 and no significant correlations observed between MRCP change and performance assessed by the RMS scores at retention 1 or 2.

---- *Figure 6 near here* ----

4.0 Discussion

Our results confirm that a session of continuous moderate intensity aerobic exercise performed after motor training can enhance motor memory consolidation as measured by performance at retention measures. Performing exercise after the motor training led to better retention of motor performance gains one week later. Additionally, we found evidence for a relationship between cortical adaptations during skill acquisition and changes in performance at retention. Specifically, an increase in MRCP amplitude from early to late training was correlated with better performance at retention, indexed by a reduction in response time (RT) to the target, and exercise enhanced this relationship.

4.1 Behavioural Performance

Performance of the task during acquisition and the immediate post-exercise/rest time period in the first session was similar between the groups (Figure 4). However, retention of the skill in the EXE group was significantly better than the CON group. These results suggest that the benefit of exercise post-motor training is that it helps to consolidate a motor memory so that it is resistant to degradation over time. At retention 1 (24 hrs after training) the CON group did not retain the skill as well as the EXE group, however there was no significant difference between the groups at this timepoint. It seems likely that the motor memory formed in the CON group was still strong 1 day after motor training. As a longer period of time went by, the motor memory may have degraded at a faster pace in the CON group than in the EXE group. This is likely why at retention 2, one week post-motor training, the retention score of the CON group was significantly worse than the EXE group. Though the groups were not significantly different from each other at either transfer test, the CON group's transfer test data shows a similar pattern of degrading over time.

Both groups had similar improvements in performance from early to late training (Figure 4), confirming that they acquired the skill similarly. It also suggests that despite some significant differences in PANAS scores between and within the groups at the pre-intervention (before exercise) and post-intervention (after exercise) timepoints, the differences in mood did not affect motor acquisition or consolidation. As predicted, there were no differences between the two post-training tests, and between late training and post-training 2. We did not expect there to be a difference from late training to the post-training tests or between the post-training tests as previous research has demonstrated that skill performance differences between groups do not emerge immediately after the intervention (Lundbye-Jensen et al. 2017; Roig et al. 2012). Consolidation has not yet occurred at the time of the post-training tests as post-training 1 occurs 50 minutes after training, and the post-training 2 occurs roughly an hour and 20 minutes after training. Therefore, if post-motor training exercise enhances the consolidation of the motor memory, a difference in performance between the groups at these timepoints is not to be expected.

4.2 Training-Related Neurophysiological Changes

We predicted from previous research that there would be a training-related enhancement of MRCP amplitude in both groups, which is thought to be a neural marker for skill acquisition (Smith and Staines 2006, 2010). This hypothesis was supported, as shown in Figure 5 the MRCP amplitude was significantly increased as participants acquired the skill in both groups. These results provide evidence that the motor training caused some early training-related neural adaptations. The MRCP represents the electrophysiological correlates of both motor preparation and the execution of the motor command. Specifically, the early portion of the MRCP is associated with motor preparation and the later portion (~ 100 ms before the onset of muscle activity) associated with the execution of movement. The MRCP preceding a self-paced voluntary movement, was been well-described as having an early component beginning up to 2 s prior to EMG onset (the Bereitschaftspotential – BP) representing excitability changes in the supplementary motor area, a late component associated with M1 in addition to the SMA that is representative of a shift from early motor preparation to movement execution, and a sharp negativity beginning ~ 100 ms prior to muscular activity (motor potential – MP) with the main contributor from the contralateral M1 (Shibasaki et al., 2006; Cui et al., 1999; Ikeda et al., 1992). Similarly, preparation of voluntary movements to visual cues generate MRCPs with similar topography but the additional contribution of premotor cortices to the early preparation phase (Smith et al., 2012). In the current study, the bimanual nature of the motor task did not allow the easy separation of the early and late phases so the MRCP reported here, from frontocentral representative sites, has contributors from both motor preparation and execution.

We did not observe an immediate effect of exercise on the MRCP amplitude as this decreased for both groups at the post-training measure (Figure 5). This is inconsistent with Thacker et al. (2014) who found that the BP, a movement-related potential preceding a voluntary self-paced action, was enhanced following an acute bout of exercise. However, differences in the study objectives and tasks likely contributed to the contrasting results. Our study had a motor learning component as our objective was to examine the consolidation of motor memories.

Thacker et al. (2014) examined the effects of exercise on motor cortical areas. Considering these objectives, our task was more complex, requiring participants to complete cued bimanual movements to control a cursor on screen, whereas Thacker et al. (2014) had participants complete self-paced unimanual wrist extension movements. Cued and self-paced MRCPs have slightly different cortical generators (Smith and Staines, 2012) and the complexity of our task likely required additional cortical input from other brain areas. Therefore, it may not have been as easily enhanced through exercise. This was the first study to measure MRCP modulations in an attempt to examine the effects of post-motor training exercise on the consolidation of motor memories, and the relationship between electrophysiological markers of acquisition and performance in retention. Analysis of the spectral components may be better suited to assess markers of consolidation such as the evidence provided by Dal Maso et al. (2018) suggesting that decreases in beta-band ERD post-exercise are associated with skill retention.

There is evidence that the primary motor cortex is involved in motor consolidation similar to what is reported here. Beck et al. (2020) applied low frequency repetitive TMS (rTMS) to the primary motor cortex following motor practice on a visuomotor tracking task to interfere with motor memory consolidation and indeed showed that retention of the skill 24 hours later was worse in those who received active stimulation. However, this reduction in skill retention was counteracted in those who exercised following motor training but prior to the application of rTMS. Similarly, Singh et al. (2016) showed that an acute bout of moderate exercise performed immediately following continuous theta burst stimulation applied to the primary motor cortex counteracted the inhibitory effects of the stimulation.

4.3 Relationship Between Behaviour and Neurophysiological Change

Our correlational analysis revealed a significant correlation between the change in the MRCP amplitude from early to late training during skill acquisition and motor performance during retention, reflected by the response time to move to the target. Specifically, the greater the MRCP negative amplitude increase, the smaller the change in RT score from the last 5 trials of post-training 2 to retention 1. This correlation was only observed in the EXE group. This provides some evidence that there is an underlying relationship between increases in the MRCP amplitude during training and the ability to maintain performance of the skill. This is similar to Smith and Staines (2010) who showed a training-related increase in the MRCP that correlated with a behavioural enhancement, evidenced by a RT reduction in a unimanual task performed immediately following training. Since this relationship was only observed in the EXE group it suggests that exercise may strengthen the early physiological effects of experience-dependent adaptation to consolidate these neural changes which are then translated to performance of the skill. Interestingly, there were no group differences in MRCP amplitude, nor was there maintenance of the enhanced negativity, at the post-training 2 timepoint which represents early retention of the trained skill. To compare our results with that of Dal Maso et al. (2018), the early MRCP change we observed during training represents similar neural activity related to planning and executing movements that the beta-band ERD measures. The correlation of an increased MRCP amplitude during training with performance of the skill at a later time point supports the idea of improved efficiency that is suggested by the findings of Dal Maso et al. (2018). Similarly, Ostadan et al. (2016) showed that practice of a serial reaction time task followed by a bout of exercise enhanced corticospinal excitability, and that the magnitude of increase was correlated with off-line skill gains.

The timing of exercise relative to training may well depend on the type of motor learning that the task involves. Studies that trained participants on a visuomotor adaptation task have shown that exercise performed before training can enhance short-term retention although the timing of these effects are mixed. Using a similar visuomotor rotational adaptation training, Neva et al. (2019) reported retention of the adapted skill 24 hours after training whereas Ferrer-Uris et al. (2017) showed performance improvements at a 1 hour retention time but not at 24 hours.

These results lead to the question of whether exercise performed prior to and after training would be of benefit for the retention of the motor skill. There is some evidence that exercise performed prior to motor training improves the acquisition of the skill (Snow et al. 2016; Statton et al. 2015). Our data provides some evidence that individuals with larger neural modulations as a result of training are better able to retain the skill. Therefore, if pre-motor training exercise enhanced these neural modulations during motor training then it could be that exercising before and after motor training would further improve the retention of the skill.

4.4 Potential Mechanisms

Our behavioural results suggest that post-motor training moderate intensity exercise assists with retaining the accuracy of a bimanual motor skill. Despite using an inphase bimanual motor task and a moderate intensity of exercise, our results were still consistent with previous studies that examined the effect of high intensity exercise on retention of a unimanual visuomotor accuracy-tracking task (Roig et al. 2012; Thomas et al. 2016a,b,c) and a unimanual handgrip task (Dal Maso et al. 2018). This data suggests that the timing of this exercise enhances consolidation mechanisms. Our neurophysiological data during acquisition showed an increase in amplitude from early to late training, which is reflective of increased excitability. Though we cannot conclude that LTP was occurring during motor training, it is possible that motor training started the induction of LTP in both the EXE and CON groups, and this LTP may have been enhanced and prolonged by exercise in the EXE group. Previous studies suggest that exercise increases the availability of LTP-related compounds (Skriver et al. 2014) and assists with the induction of LTP by reducing cortical inhibition and increasing excitation (Singh et al. 2014b). Studies with similar motor training structures to ours have suggested that BMT can induce early LTP-like plasticity in M1 (Neva et al. 2012). Post-motor training exercise may then enhance these BMT-induced plasticity mechanisms by increasing the availability of LTP-related compounds throughout consolidation. Examples of these LTP-related compounds include BDNF, catecholamine neurotransmitters, and lactate. Increases in levels of these compounds post-exercise may assist with tagging synapses to undergo the transition from E-LTP to L-LTP (Frey and Morris 1998; Redondo and Morris 2011). It could be through these mechanisms that exercise enhances motor memory consolidation resulting in a robust motor memory that is resistant to degradation over time.

4.5 Conclusions and Future Directions

Our main finding from this study is that moderate intensity exercise performed post-motor training assists with the retention of the motor skill in healthy adults. This is an important finding for sports, rehabilitation, and occupational skills training applications as it suggests that exercise could be used as an adjunct to motor practice. Specific recommendations for coaches and clinicians may include structuring practice sessions to first involve practicing a specific technical skill and completing aerobic exercise after this practice. Our results suggest that this may promote consolidation mechanisms to prevent the motor memory from degrading over practice sessions that occur days apart. One recent study had stroke patients complete high intensity interval training post-motor training and found a benefit to motor skill retention (Nepveu et al. 2017). While some neurorehabilitation patients may be able to participate in high intensity exercise, not all may be deemed safe to do so, or be willing to do so. Previous research has suggested that low intensity exercise (45% maximal power output) provides a benefit, but high intensity exercise (90% maximal power output) provides a greater benefit (Thomas et al. 2016c). If there is this proposed dose-response relationship between exercise intensity level and retention benefit, moderate intensity exercise, like the intensity used in the current study (70% of HRR) should provide benefits that are greater than low intensity but less than high intensity. Neurorehabilitation patients who are not able or willing to safely participate in high intensity exercise may start with low intensity exercise, and eventually use moderate intensity exercise to progress to be able to participate in high intensity exercise to get the maximal benefit. To further investigate the relationship between exercise intensity and motor memory consolidation, different intensities should be tested and directly compared. Future research should look to include individuals in special populations including older adults and neurorehabilitation patients. Currently there is a limited amount of research examining the relationship between exercise and motor learning in older adults, and most of this research examines the effect of chronic exercise (Hübner and Voelcker-Rehage 2017). Additionally, in stroke patients, bimanual movements can increase activity between hemispheres (Staines et al. 2001). Considering our results, it is possible that exercising after BMT may enhance this effect. This is a future avenue that should be explored.

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Figure Captions:

Figure 1 – Summary of General Procedures

Figure 2 – *A.* Experimental set-up: Top-down view of a participant performing the behavioural task, grasping the two handles and viewing both the target and cursor movement on the computer screen. *B.* Displays movements made during the bimanual movement training task. Participants began in the bottom right corner and made varying degrees of wrist flexion movements to move the cursor to the remembered visual targets.

Figure 3 – Change in RMS from the last 5 trials of post-training 2 to retention and transfer. Data was normalized to show each group's performance as the difference from the 5 last trials of post-training 2. A lower score is representative of a better performance ('0' representing no difference from post-training 2). Error bars represent standard error of the mean. * indicates significant difference $p < 0.05$.

Figure 4 – RMS scores for baseline, training, and post-training tests (n=5 trials). A lower score is representative of a better performance. Error bars represent standard error of the mean. All timepoints were different from one another except for post-training 1 and post-training 2, and late-training and post-training 2.

Figure 5 – Grand-average MRCP trace of EXE (*A*) and CON (*B*) recorded from electrode CZ. Blue represents early training, green represents late training, and red represents post-

training test 2. Data is time-locked to cursor movement; 0 represents the start of cursor movement. (*C*) Sum of all individual data points from MRCP trace recorded at CZ. Error bars represent standard error of the mean. * indicates significant difference $p < 0.05$.

Figure 6 – Change in RT at retention vs. change in MRCP amplitude. On the x axis is the change in RT from post-training 2 (last 5 trials) to retention 1. A negative score means they performed better than they did at the last 5 trials of post-training 2. On the y axis is the change in the sum of the MRCP amplitude at FCZ (left) and CZ (right) from early to late training. A negative score means their amplitude increased in the negative direction from early to late training. The ⌧ indicates the participant who completed post-training test 2 before their HR was back to resting level.

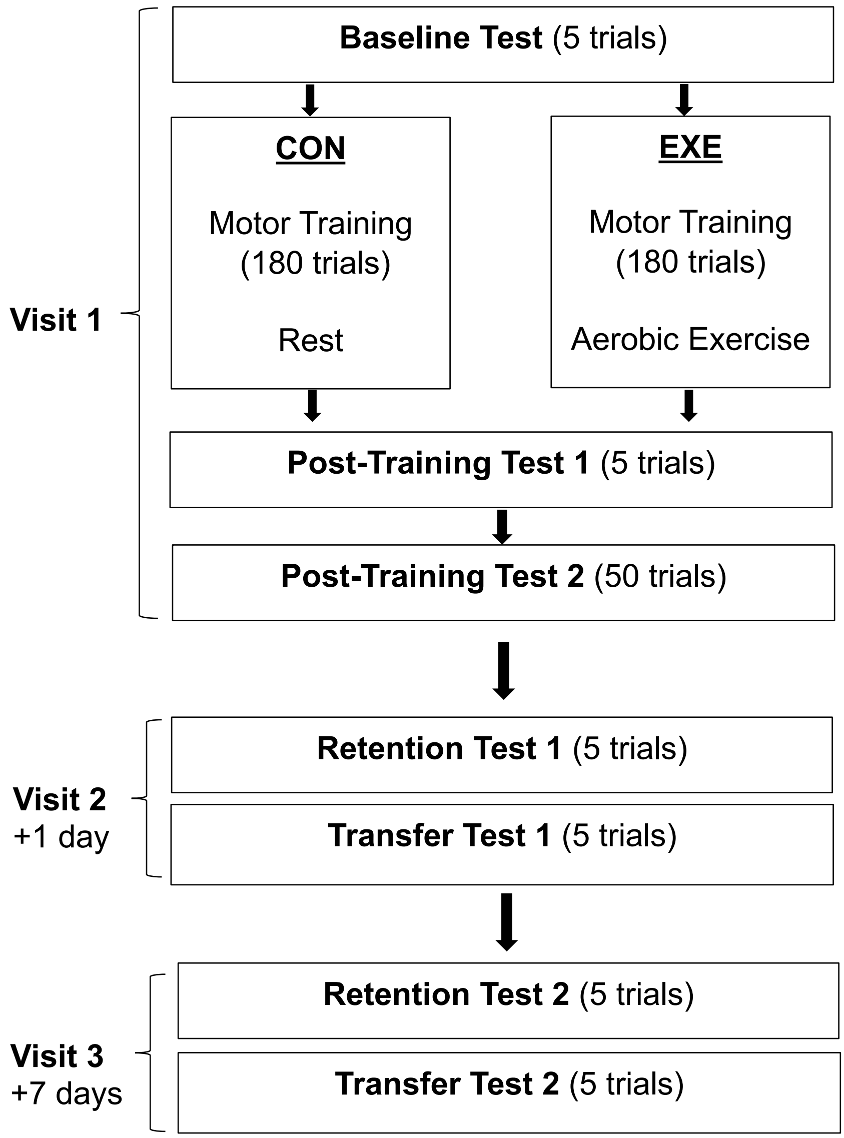
Tables

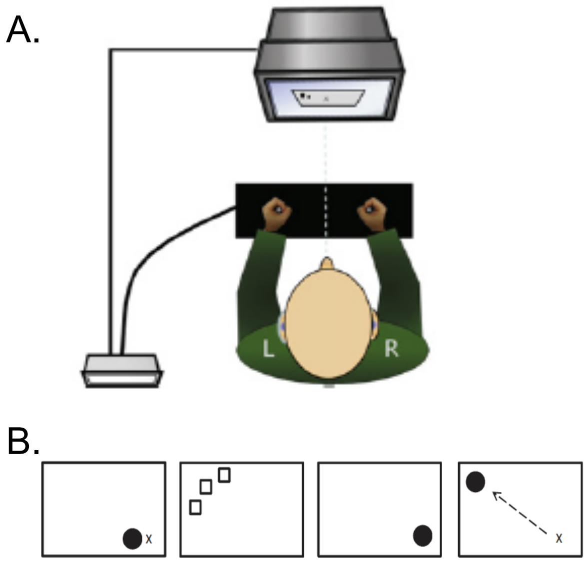
Table 1 – Participant characteristics.

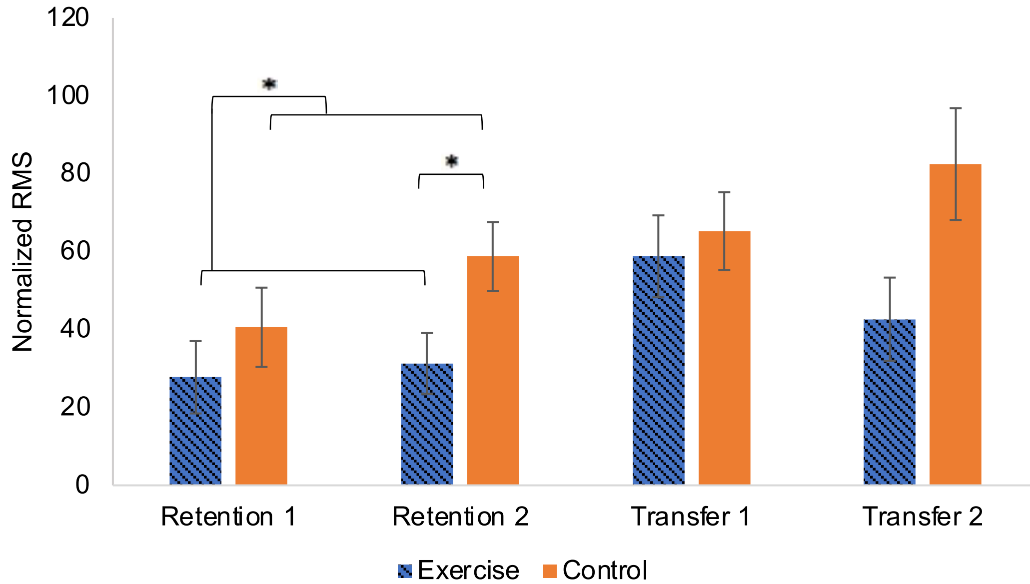
	EXE	CON
Participants	17	16
Sex	10F 7M	9F 7M
Age	21.60 ± 2.26	22.31 ± 2
IPAQ Category	Highly active = 13 Moderately active = 4	Highly active = 10 Moderately active = 6
MET-minutes/week	3263.05 ± 1817.26	2755.81 ± 1408.73
Handedness	14R 2L 1R/L	15R 1L
BMI	24.64 ± 3.40	22.76 ± 2.01

Table 2 – Heart rate (HR) data.

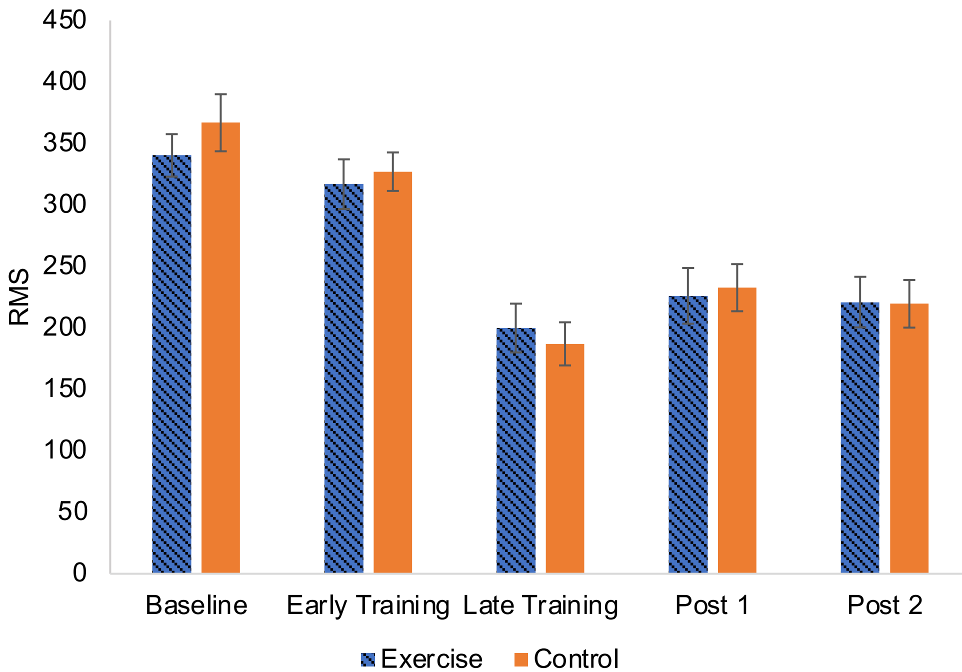
	EXE	CON
Rest HR	66.29 ± 6.19	63.75 ± 9.78
Target HR	158 ± 2.37	-
Exercise/Rest Session AVG HR	156.01 ± 3.82	65 ± 9.73
AVG % of HRR during Exercise	67.91 ± 3.82	-
AVG RPE	14.13 ± 1.05	-
AVG RPM	61.01 ± 2.45	-
HR Post 1	91.82 ± 7.10	63.88 ± 8.80
HR Post 2	71.47 ± 7.68	65.29 ± 10.31
Time from Exercise/Rest to Post 2 (min)	30.35 ± 10.71	30.25 ± 1

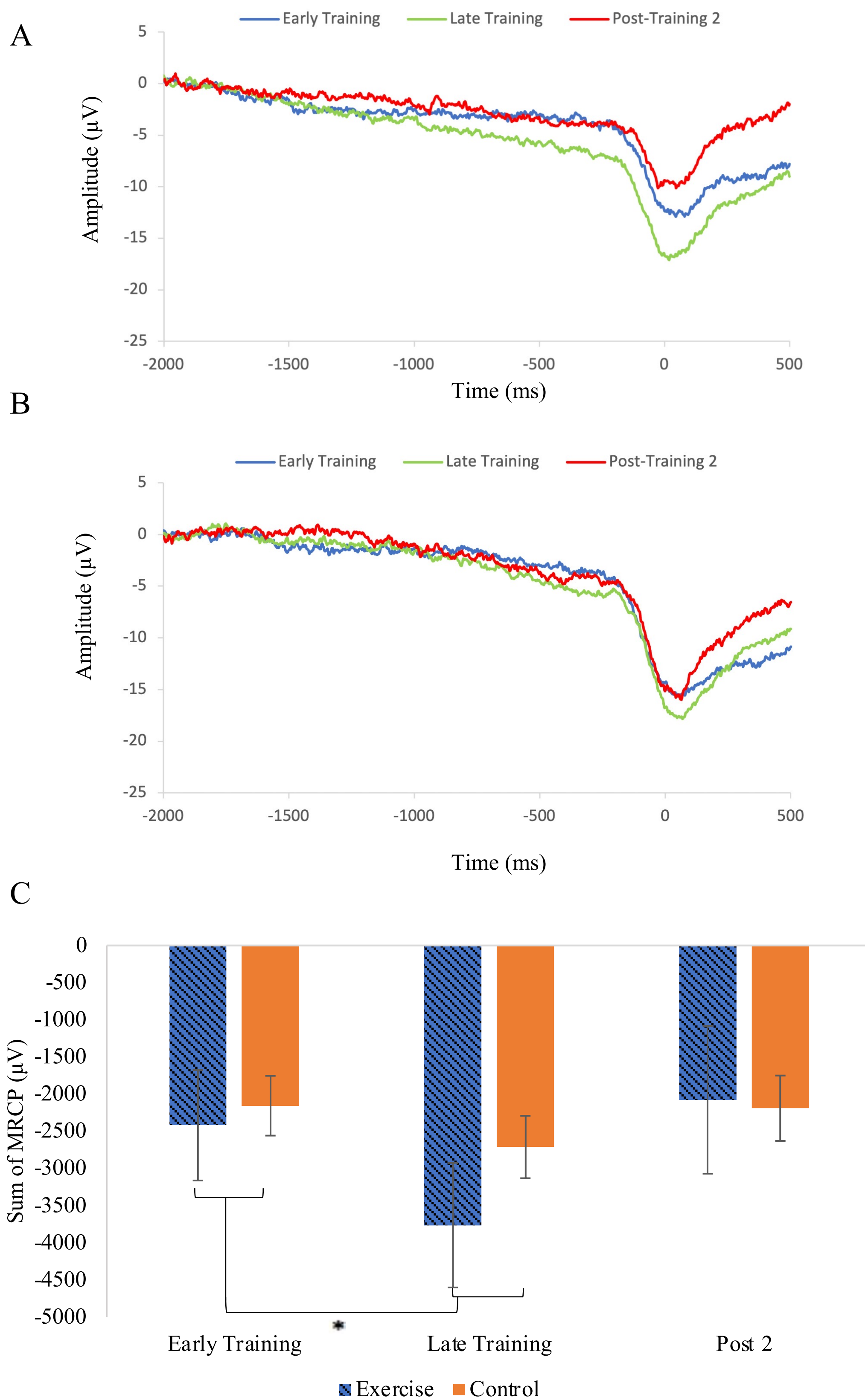

Baseline Test (5 trials)
CON
Motor Training
(180 trials)
Rest
EXE
Motor Training
(180 trials)
Aerobic Exercise
Post-Training Test 1 (5 trials)
Post-Training Test 2 (50 trials)
Visit 1
Retention Test 1 (5 trials)
Transfer Test 1 (5 trials)
Visit 2
+1 day
Retention Test 2 (5 trials)
Transfer Test 2 (5 trials)
Visit 3
+7 days

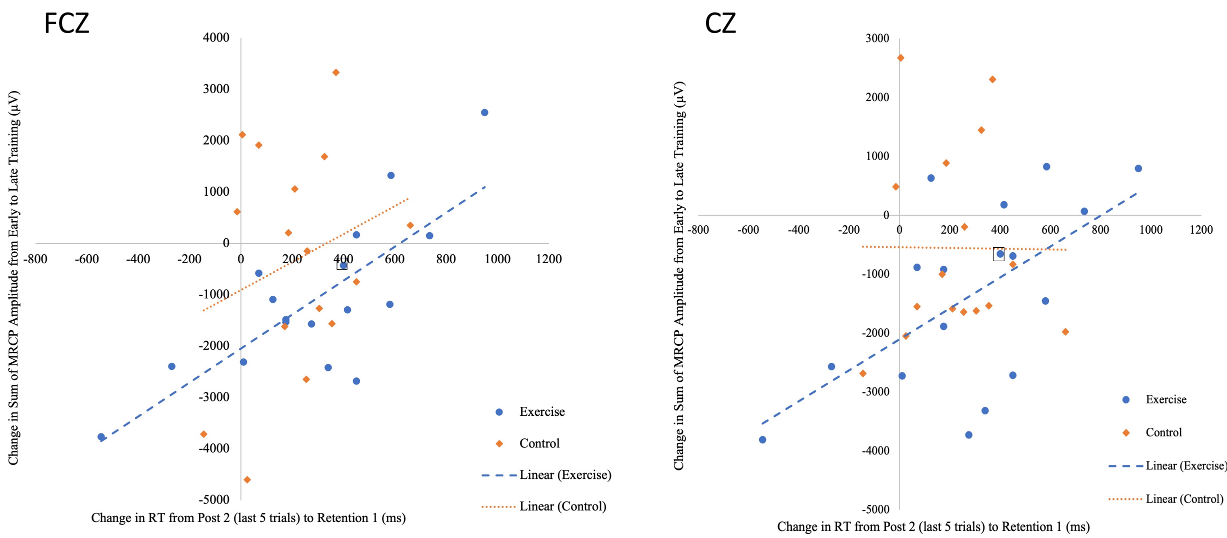

A.
L
R
B.
x
x



120
100
80
60
40
20
0
Normalized RMS
*
*
Retention 1
Retention 2
Transfer 1
Transfer 2
Exercise
Control


450
400
350
300
250
200
150
100
50
0
RMS
Baseline
Early Training
Late Training
Post 1
Post 2
Exercise
Control


A
Early Training
Late Training
Post-Training 2
5
0
-5
-10
-15
-20
-25
Amplitude (µV)
-2000
-1500
-1000
-500
0
500
Time (ms)
B
Early Training
Late Training
Post-Training 2
5
0
-5
-10
-15
-20
-25
Amplitude (µV)
-2000
-1500
-1000
-500
0
500
Time (ms)
C
0
-500
-1000
-1500
-2000
-2500
-3000
-3500
-4000
-4500
-5000
Sum of MRCP (µV)
*
Early Training
Late Training
Post 2
Exercise
Control


FCZ
Change in Sum of MRCP Amplitude from Early to Late Training (μV)
Change in RT from Post 2 (last 5 trials) to Retention 1 (ms)
Exercise
Control
Linear (Exercise)
Linear (Control)
CZ
Change in Sum of MRCP Amplitude from Early to Late Training (μV)
Change in RT from Post 2 (last 5 trials) to Retention 1 (ms)
Exercise
Control
Linear (Exercise)
Linear (Control)