ACUTE EXERCISE CAN AID OFFLINE LEARNING

A Dissertation

by

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ABSTRACT

Evidence revealing the importance of chronic exercise for improving general physical health and maintaining long-term successful cognitive function is widely available but less information addressing the potential efficacy of an acute bout of exercise for learning perceptual-motor skills is available. The present study examined the efficacy of a short bout of moderately intensive exercise to protect knowledge of a newly acquired motor sequence. Previous work revealed that sleep-dependent offline gains in motor sequence performance could be decreased by practicing an alternative motor sequence in close temporal proximity to the original practice with the target motor sequence. Three experiments were conducted in an attempt to investigate the effect of acute exercise bout on offline learning and accompanying neurophysiological changes. In Experiment 1, a brief bout of exercise was inserted at two different temporal locations between practice of a to-be-learned motor sequence and the interfering practice that occurred 2-hr later. Exercise inserted close to alternative sequence practice minimized the negative quality of interference and facilitated the advent of offline gain. When exercise occurred immediately after acquisition of the target motor sequence, practice with the subsequent motor sequence led to interference which in turn reduced offline gain. In Experiment 2, an examination of the role of Brain Derived Neurotrophic Factor (BDNF) for the offline gain observed in Experiment 1 was conducted. Blood samples were collected before and after an acute exercise bout.

The relationship between blood lactate and plasma BDNF levels and offline learning was addressed. Although an acute exercise bout caused changes in blood lactate and plasma BDNF levels, those changes were not correlated with offline learning. The purpose of Experiment 3 was to examine the effect of an acute exercise bout performed immediately before motor sequence learning to consider the role of exercise for both fast and slow motor learning stages. An acute exercise bout was inserted prior to initial target sequence practice and the result revealed that prior exercise influenced the initial acquisition of motor sequence but did not impact the extent of offline enhancement. Thus, while exercise can contribute to post-practice consolidation, there appears to be a fragile interplay between spontaneous memory consolidation occurring after task practice and the consolidation processes induced via exercise. Moreover, the effect of an acute exercise bout inserted before practice appears to be quite transient and not important for offline learning.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Our ability to acquire, maintain, and generalize new motor skills is essential for meeting the demands of even the most mundane tasks we execute on a regular basis. Many of the motor skills central to everyday behavior consist of a series of simple movements that must be executed in a specific order with precise timing. Our capacity to display these abilities is taxed regularly when we for example play tennis, attempt to master a complex musical piece on the piano or reach for our cell phone when it rings. Not surprisingly then considerable experimental effort has been devoted to understanding the evolution of sequential motor behaviors as a result of practice and related experiences and a good deal of our insight into this process has emerged from work that addresses motor sequence learning (Doyon, Korman, Morin, & Dostie, 2009).

Motor sequence learning involves both *fast* and *slow* stages (Karni et al., 1998). The fast learning is characterized by rapid improvements in performance within a practice session whereas slow learning is reflected in delayed, incremental gains associated with continued practice across additional training that may occur over days, months, or even years. This slow stage learning involves memory consolidation that stabilizes and enhances memories. Stabilization refers to the process by which acquired knowledge becomes stable and resistant to interference after initial learning. This process is thought to be time dependent, as there is a 2 to 6 hour critical period for stabilization of acquired knowledge to occur (McGaugh, 2000; Walker, 2005). In addition to changes in motor sequence performance that are directly a consequence of practice, there is evidence indicating that knowledge used to govern the performance of a motor sequence can be further refined or enhanced if the learner is provided an opportunity to sleep between training and test. This sleep-dependent enhancement via further consolidation appears to be especially true for motor sequence learning that occurs explicitly (Diekelmann & Born, 2007; Press, Casement, Pascual-Leone, & Robertson, 2005; Robertson, Pascual-leone, & Miall, 2004; Wright, Rhee, & Vaculin, 2010).

Consolidation of Sequence Knowledge

Walker and colleagues offered initial evidence for sleep-dependent enhancement for motor sequence knowledge (Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). They had participants execute a short motor sequence as rapidly and accurately as possible for twelve 30s trials each separated by 30s of rest. A test was administered after either a 12-hr wake or sleep period. The critical finding was an approximate 20% increase in motor speed with no loss of accuracy following a sleep but not awake-filled period (Diekelmann & Born, 2007; Kuriyama, Stickgold, & Walker, 2004; Walker, Brakefield, Hobson, & Stickgold, 2003). It is important to recognize that Walker, et al. (2003) identified a number of factors that mediated the robustness of sleep related offline enhancement for motor sequence performance. For example, practice of a target motor sequence immediately followed by training with novel motor sequence resulted in retention of the target motor sequence being restricted to just a small improvement in speed only. This was despite the fact the learners were all given a night of sleep.

However, robust offline improvement from sleep returned when practice with the second motor sequence was administered about 6-hr after the completion of the practice with the motor sequence of interest. Based on these data Walker et al. (2003) proposed that during the interval of approximately 10 minutes to 6-hr period following practice of a motor sequence, memory consolidation occurs that is designed to *stabilize* acquired motor sequence knowledge such that it becomes resistant to interference from a competing memory. This initial form of consolidation appears necessary for further consolidation to occur, during sleep, which presumably induces changes in the sequence knowledge such that offline improvements in performance are revealed.

The importance of sufficient time to stabilize motor sequence knowledge as a precursor to the emergence of offline facilitation has been verified (Korman et al., 2007). Korman et al. (2007) administered training with an alternative novel motor sequence training either 2 or 8-hr following practice of the target motor sequence. As anticipated, an 8-hr interval between practice bouts was sufficient to sustain overnight performance improvement for the target motor sequence. Spacing the original and alternative task practice only 2-hr apart led to the sleep-dependent benefits being almost completely eliminated. Of greatest interest for the present work however was Korman et al.'s demonstration that inserting a 90 minutes nap between practice of the target and alternative motor sequence, removed the vulnerability of the target sequence to interference from exposure to practice with the alternative task and led to a reliable overnight gain. This was true even when the interfering practice was only delayed 2-hr after practice of the target sequence. In essence, the nap provided rapid protection for the memory of the target motor sequence allowing the post-practice consolidation processes to be completed and delayed enhancement to be attained.

Exercise and Cognition

A primary goal of the present work was to extend the aforementioned work addressing offline improvements in motor sequence learning by examining the effectiveness of alternative means of protecting motor sequence learning. Specifically this work focuses on the use of an acute bout of aerobic exercise, as opposed to naps, as an alternative strategy to enhance the performance of motor sequences (Korman et al., 2007; Walker et al., 2003). The use of exercise to potentially facilitate memory for a procedural skill is not as speculative as one might first assume. It has long been known that exercise is important for lowering the risk for cardiovascular disease, colon and breast cancer, as well as obesity (Warburton, Nicol, & Bredin, 2006a, 2006b). It is also known that regular aerobic exercise improves neural processing via direct and indirect pathways. Anatomically, exercise recruits specific neural systems such as motor cortex, basal ganglia and cerebellum. Physiologically, exercise causes changes in hormonal, immune, and vascular response which will in turn influence various areas of the brain (Ide, Schmalbruch, Quistorff, Horn, & Secher, 2000; Pedersen & Hoffman-Goetz, 2000).

A more recent development is that exercise is used as a means to combat cognitive impairments caused by a variety of mental disorders including depression and anxiety as well as age-associated neurodegeneration related to Alzheimer's disease and vascular dementia (Hillman, Erickson, & Kramer, 2008; Penedo & Dahn, 2005). Congruent with this clinical use for exercise, during the last decade, the use of cardiovascular activity as a means to improve a wide range of cognitive function including long term and short term memory for spatial and verbal information in both healthy and non-healthy population has also been verified (Cotman & Berchtold, 2002; Voss, Nagamatsu, Liu-Ambrose, & Kramer, 2011). Moreover, facilitation of general cognition performance as a result of physical activity is associated with reliable and extensive adaptation in brain structure and function (Cotman, Berchtold, & Christie, 2007; Cotman & Engesser-Cesar, 2002; Voss et al., 2011). For example, Erickson et al. (2011) reported a 2% increase in hippocampal volume, a critical neural site for establishment of long-term memory, following an exercise training regime compared to the anticipated age associated loss in this brain structure evident for a no-exercise control (Erickson et al., 2011).

To date, the majority of human studies that have examined how participation in cardiovascular exercise exerts a positive influence on cognitive performance have relied heavily on neuropsychological tasks to focus on processes such as attention, decision-making, and processing speed across the lifespan (Roig, Nordbrandt, Geertsen, & Nielsen, 2013). This is not altogether surprising given that a meta-analytic study using data from healthy but sedentary older adults indicated that fitness training was most beneficial for executive processes such as planning, inhibition, and task or event scheduling (Colcombe & Kramer, 2003). Such benefits are most frequently observed following relatively long-term exposure to aerobic training with the largest benefits being observed for an aged population (i.e., >65 years). Also, potential cognitive benefits across all facets of the lifespan, including a protection from age related cognitive dysfunction, are being addressed (Colcombe & Kramer, 2003; Erickson et al., 2011; Kramer et al., 2003; Rhyu et al., 2010).

Acute Exercise and Cognitive Performance

Despite the noted benefit of chronic exercise for cognitive performance much less attention has focused on the influence of an acute bout of exercise on memory performance. Acute exercise involves a single bout of exercise used to impact cognitive performance immediately after to less than 24 hours following the exercise session. For example, Tomporowski (2003) reported that an acute bout of exercise can exert a positive influence on memory performance. Moreover, Coles and Tomporowski (2008) proposed that, for learning, acute exercise influences post practice processes including memory consolidation. When considering the impact of acute exercise on learning, the intensity of the given exercise is an important factor. Tomporowski and Ellis (1986) argued that an exercise bout with an intensity that is too light or too heavy has no effect on learning and only intermediate intensity of exercise has a positive learning effect (Draper, McMorris, & Parker, 2010). For the present work, an intermediate intensity was defined as 70~80% of maximum performance capacity of the subject based on heart rate measure. In addition, the intensity of exercise was determined based on the maximum performance capacity of each person (see methods). To accomplish this, in each subject, a preliminary test of maximum aerobic performance for each individual was given in advance and exercise loads experienced while learning were adjusted accordingly.

Effect of acute exercise bout on memory based cognitive function

Mixed evidence exists regarding the effect of acute exercise bout on short term memory task. Improvements in short term memory tasks such as digit span task, paced auditory serial addition, and verbal memory task following an acute exercise bout have been reported (Potter & Keeling, 2005; Salas, Minakata, & Kelemen, 2011; Stones & Dawe, 1993; Tomporowski, Phillip D. & Ganio, 2006). However, less evidence exists for improvement in word recall (Miles & Hardman, 1998) or in paced auditory serial addition task (Dietrich & Sparling, 2004) as a result of being exposed to acute exercise. These mixed results indicate that the influence of an acute exercise bout on short term memory is mediated by the type of memory task, nature of the exercise bout and, the intensity of the exercise. A common manipulation used in studies examining the effect of acute exercise bout on short term memory was the placement of the exercise intervention being prior to the learning episode (Roig et al., 2013).

In contrast to the effect of an acute exercise bout on short term memory, relatively less examination of the effect of acute exercise on long term memory task performance was occurred. Improved long-term recall in lexical memory tests and image recall test were reported (Segal, Cotman, & Cahill, 2012). For example, Stones & Dawe (1993) reported that low intensity aerobic exercise provided before learning influenced the long-term performance on meaningfully cued memory and Segal et al.,(2012) reported that an acute cycling exercise bout after learning induced improvement in long-term image recall.

Effect of acute exercise bout on motor skill learning

While evidence exists supporting the chain that an acute bout of exercise can facilitate both short-term and long-term verbal memory, we know of only one study to date that has evaluated if a single bout of exercise, a moderate to intense bout of cycling, can improve memory for a procedural skill - visuomotor tracking skill (Roig, Skriver, Lundbye-Jensen, Kiens, & Nielsen, 2012). Roig et al. afforded exercise either prior to or just after training with the tracking task. Performance for the visuomotor skill was subsequently assessed 1-hr, 24-hr, and 7 days following the completion of practice. Compared to individuals that merely rested, participants that exercised displayed superior retention of the tracking task after longer retention intervals (i.e., 24-hr and 7 days). This benefit however was more reliable if the bout of cycling occurred after practice with the visuomotor skill and was more pronounced for the most delayed test administered a week later. Giving exercise prior to practice had a reduced impact on the rate of improvement of tracking performance. Roig et al. proposed that exercise influenced long-term memory for tracking behavior by regulating post-training consolidation processes, as opposed to influencing arousal, which might influence initial encoding (Korman et al., 2007; Roig et al., 2013; Walker et al., 2003).

Biological Contributions to Consolidation Following Exercise

One primary approval to explain how exercise aids memory performance have focused on biological contributions. One growth factor in particular has been the subject of extensive examination with respect to its role for memory and related to cognitive function is Brain Derived Neurotropic Factor (BDNF). BDNF is a neurotrophic factor that helps cell growth in various neuronal area especially hippocampus that play a key role in organizing the acquired memory (Bekinschtein, Cammarota, & Medina, 2014). The brain produces BDNF mostly when a human is sleeping but exercise also upregulates production of BDNF (Huber, Tononi, & Cirelli, 2007). Following exercise increased blood BDNF level has been reported to remain elevated for approximately an hour after the exercise session (Seifert et al., 2010). A prolonged increase in BDNF concentration has been argued to be instrumental in stabilizing existing synapses while allowing for the increment of synaptic terminals and induction of additional dendritic outgrowths (Kovalchuk, Hanse, Kafitz, & Konnerth, 2002). According to Levin et al., (1995) a single bout of moderate and high intensity aerobic exercise can increase circulating BDNF levels in humans which in turn may facilitate memory consolidation process during motor sequence learning which then may increase offline benefits. This possibility is the subject of Experiment 2 in the present work.

Experimental Overview and Expectations

In an attempt to further elucidate how an acute bout of exercise influences memory consolidation and offline performance gains during motor learning the present work includes three experiments. Experiment 1 was designed to examine how an acute bout of exercise influences the learning of target sequence if practice of an alternative task was provided at the time point when the initial learning is known to be susceptible to interference (see Walker, Brakefield, Hobson, et al., 2003). An acute exercise bout was inserted at two different temporal locations between target sequence practice and supplemental practice with an alternative motor sequence. One condition involved exercise immediately after the target sequence practice (EX_{IMM}) and a second condition had exercise near to the end of the 2 hours duration (EX_{DELAY}) just prior to practice with the alternative motor sequences. It was hypothesized that the acute bout of exercise will protect memory of the initial target sequence from interference from the alternative practice despite the close temporal proximity of the two training sessions and that susceptibility to interference would be different depending on the location of acute exercise bout. Specifically, an offline improvement between the performance of target sequence practice and delayed test of target sequence is expected to be larger with an acute exercise bout located closer to an alternative sequence practice (EX_{DELAY}) than the condition with an acute exercise bout immediately following a target sequence practice (INT_{DELAY}). This should occur because the acute exercise bout immediately following practice of a motor sequence will likely disrupt spontaneous consolidation. Thus, providing the learner with some time to initially consolidate acquired knowledge prior to administration of acute exercise is assumed to maximize the effectiveness of the exercise and an acquired knowledge that underwent prolonged consolidation process is expected to have more benefit from acute exercise bout (Cohen & Robertson, 2007; Coles & Tomporowski, 2008; Diekelmann & Born, 2007).

Experiment 2 was designed to evaluate if the exercise benefits revealed in Experiment 1 can be accounted for by a contemporary biological accounts for consolidation specifically in the upregulation of BDNF that has been proposed as a key contributor to improved cognitive function following exposure to exercise (Erickson et al., 2011; Ferris, Williams, & Shen, 2007; Seifert et al., 2010). The experimental procedures regarding the exercise and motor sequence learning during Experiment 2 were identical to those used in Experiment 1. These procedures were supplemental with blood samples being collected immediately before and after an exercise for the with-exercise condition (EX_{DELAY}+BD). For a without-exercise condition (INT_{DELAY}+BD), blood samples were collected at the matching temporal location of the blood draws to EX_{DELAY}+BD condition. Blood lactate level and serum BDNF level were measured from the collected blood samples (Rojas Vega et al., 2006; Skriver et al., 2014). It was hypothesized that the blood lactate level and serum BDNF level will be increased with exercise and the increment in BDNF will be positively correlated with the extent of offline learning (Rojas Vega et al., 2006; Seifert et al., 2010; Skriver et al., 2014).

Experiment 1 and 2 focused on the slow stage of motor learning such as consolidation and enhancement process that takes place immediately after initial practice to possibly hours and days later. The purpose of Experiment 3 was to determine if an acute bout of exercise influences the fast stage learning of a motor sequence learning that is most appropriately captured by performance of the motor skill immediately after an exercise bout. This addressed the possibility that an acute bout of exercise may exert a proactive influence on subsequent motor performance, a result observed in Experiment 2. In addition, recall that Roig, et al. (2012) reported a benefit of an acute bout of exercise provided prior to the practice of a visuomotor tracking task, although this observed benefit was weaker than the benefit observed when exercise followed target sequence practice. Experiment 3 had two experimental conditions and all the conditions began with an acute bout of exercise immediately followed by target sequence practice.

Any condition with practice of an alternative motor sequence was included as the focus of this experiment was on exercise occurring before exposure to practice with the target sequence. It was hypothesized that the performance of initial practice of target sequence after exercise would be superior to the performance of the initial practice of target sequence with no preceding exercise bout (Roig et al., 2013). It was also hypothesized that offline performance improvement would be present for delayed tests but it was less clear if exposure to exercise prior to practice would influence the extent of improvement observed in the no exercise condition.

CHAPTER II

EXPERIMENT 1

Introduction

In an initial study intended to extend the work of Roig et al., (2013) and Korman et al., (2007), we examined if an acute bout of exercise can be used to protect newly acquired motor sequence knowledge from interference experienced from practice of an alternative motor sequence in close temporal proximity to the original training (Walker et al., 2003). Participants were assigned to one of two exercise conditions each of which involved a bout of moderately intensive cycling in the 2 hours interval between the practice bouts of two separate motor sequences. The exercise conditions differed with respect to the exact temporal locus of the exercise within the 2 hours interval between practice bouts. Specifically an individual completed exercise either immediately after the completion of practice with the target sequence (EX_{IMM}) or just prior to the beginning of the second bout of practice which was approximately 2 hours after practice with the first motor sequence (EX_{DELAY}).

Four additional control conditions were also included one of which merely required the participants to practice a motor sequence and complete the test phase 24 hours later (CONTROL) while a second condition involved a group of test of target sequence immediately after completion of the initial practice bout of target sequence ($TEST_{IMM}$). Performance on the additional three trials in this condition provided an opportunity to determine the improvement that was expected of each participant given the additional practice associated with the test trials. Importantly, performance for these CONTROL and $TEST_{IMM}$ conditions provided the basis from which to assess if offline improvements emerged. That is, two interference conditions were included to assess how offline enhancement during training can be compromised by practice of an alternative sequence. The temporal locus of alternative sequence training was varied such that one condition involved the additional practice occurring 2 hours later after initial training (INT_{DELAY}) and another condition involving additional practice occurring immediately after the target sequence training (INT_{IMM}). Participants in both INT_{DELAY} and INT_{IMM} conditions completed the test trials 24 hours after initial practice was completed. The control conditions provided the opportunity to replicate the presence (CONTROL vs $TEST_{IMM}$ conditions) and partial elimination (INT_{DELAY} vs CONTROL conditions) of offline enhancement for the 24 hours delayed test (Korman et al., 2007; Walker et al., 2003). The INT_{IMM} condition provided an opportunity to examine time-dependent manner of motor sequence learning including consolidation and offline enhancement.

The critical question of interest was one or both of the exercise conditions, despite the location of an acute exercise bout, would support the return of offline gain. That is, does the inclusion of exercise lead to motor sequence test performance congruent with that of the CONTROL not the INT_{DELAY} condition. If the critical determinant of the efficacy of exercise for enhancing motor performance was that it occurs after a bout of training, as suggested by Roig et al. (2013), then both the EX_{IMM} and EX_{DELAY} conditions should support recovery of offline gain even in the presence of interfering practice. On the other hand, if a certain amount of time to stabilize an acquired knowledge is the critical determinant, the offline performance gain of EX_{IMM} and EX_{DELAY} condition may exhibit similar pattern of improvement to those of INT_{IMM} and INT_{DELAY} conditions.

Methods

Apparatus

The apparatus for an acute exercise bout consisted of a Bicycle Ergometer (MONARK Ergomedic 828E) that was used for the intensity controlled exercise bout and a heart rate monitor (POLAR E600) that was used to measure participant's heart rate. Motor sequence tasks used in this experiment were programmed using E-Prime 2.0 Professional (Psychology Software Tools, Inc., Sharpsburg, PA) and executed on standard PC system with Microsoft Windows OS using standard PC monitor and keyboard.

Participants

A total of seventy two (72) individuals served as participants in Experiment 1. Participation in this study fulfilled a research requirement for an undergraduate class in the Department of Health and Kinesiology. The participants had no prior experience with the experimental task and were informed of the specific purpose of the study. Informed consent and a physical readiness checklist approved by the Institutional Review Board for the ethical treatment of experimental participants at Texas A&M University were obtained prior to any participation in the experiment.

Tasks

Graded exercise test

On Day 1, a graded exercise test was administered to all individuals assigned to an exercise condition (EX_{IMM} and EX_{DELAY}) in order to measure each individual's base performance of fitness and to calculate designed intensity for each individual during a later acute bout of exercise. The test consisted of a subject sitting on a bicycle ergometer (Ergomedic 828E, Monarch) for 2 minutes of rest during which time heart rate (HR) was assessed using a Heart Rate monitor (Polar E600) (Seifert et al., 2010). Once acclimated, the participant cycled for 3 minutes with an initial resistance of 0W maintaining a cycling frequency of 75 rpm. Every 3 minutes until exhaustion, the resistance was increased by 37.5 W, and each minute Heart Rate was recorded, as well as a rating of perceived exhaustion (RPE)(Borg, 1998). Individuals cycled until they were exhausted which was operationally defined as not able to maintain the required cycling frequency of 75 rpm for 1 minute or when the participant voluntarily terminated the test. At the conclusion of resistance applied cycling the participant continued to cycle against 0 W for an additional 3-min. Heart rate and RPE score were recorded every minute from rest to completion of the cool down period.

Acute exercise bout

On Day 3, individuals assigned to the exercise condition experienced an acute bout of exercise either at the beginning (EX_{IMM}) or close to the end (EX_{DELAY}) of a 2 hours interval between practice of a target and interference motor sequences (described in the next section). The intensity of the bout of exercise experienced by a participant was based on their performance during the Graded Exercise Test (described in the previous section). A linear regression equation[1] describing the relationship between Heart Rate and resistance during the Graded Exercise Test was calculated separately for each individual. The predicted resistance at 80% of maximum heart rate was used as the workload for 18 minutes of the acute exercise bout. Prior to exposure to this workload the participant was afforded 2 minutes of rest while seated on the cycle ergometer. Participants started cycling at 0W and the resistance increased gradually once a cycling frequency reached at 75 rpm. After 18 minutes with cycling at a resistance associated with average ~80% max HR, the workload was increased to the resistance associated with max HR for an additional 3 minutes. During the acute bout of exercise the participant was required to maintain a cycling frequency of 75 rpm. Each participant continued to cycle against 0W for an additional 3 minutes after completion of the acute exercise bout. Throughout rest, exercise, and cool-down periods, HR was recorded. The duration of the resistance associated cycling was 21 minutes and total duration of an acute bout of exercise was 24 minutes.

Motor sequence tasks

All participants performed a target sequence, 4-1-3-2-4, on a standard PC keyboard using the V, B, N, M keys where "1" was the leftmost key (V key) and "4"

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[1] During a subject performing a graded exercise test, heart rate was recorded every minute while the resistance was being increased every 3 minutes. After completion of graded exercise test, a simple linear regression analysis was performed to obtain a slope and intercept for a simple linear regression equation for each individual (i.e., RESISTANCE = SLOPE * HEART RATE + INTERCEPT). Then 80% of maximum heart rate was calculated and entered into the equation to calculate 80% of maximum resistance for each individual.

was the rightmost key (i.e., M key) with their non-dominant hand. The task involved type the required sequence repeatedly as quickly and accurately as possible for 30 s. In addition, all individuals performed the target sequence during the delayed test administered 24 hours after the completion of training with the target sequence except the $TEST_{IMM}$ condition which conducted the test session as an extended training immediately after completion of target sequence training. Some individuals (INT_{DELAY}, INT_{IMM}, EX_{IMM}, EX_{DELAY}) were administered an extra bout of practice with an alternative sequence, 2-3-1-4-2, 2 hours after completion of target sequence training, again performed with the non-dominant hand to potentially induce interference. (see Walker, Brakefield, Hobson, et al., 2003).

On execution of motor sequence learning, a required sequence to be entered was displayed across the top third of the computer display and four boxes were displayed across the bottom third of the computer display that were spatially compatible with the fingers placed on the V, B, N, and M keys on a standard PC keyboard. Each key press resulted in the appearance of a black dot in the box above the key that should be pressed. All features of this experiment were programmed using E-Prime® 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA). Prior to practice individuals were informed of the nature of the sequence of key presses that constituted the required task.

Procedures

Prior to participation in the Experiment 1 all participants completed an informed consent. The general sequence of events followed by each experimental condition is depicted in Figure 1. Specifically, 48 hours prior to any practice with the target motor sequence individuals assigned exercise condition (EX_{IMM}, EX_{DELAY}) performed a graded exercise test. These individuals returned to the laboratory at least 48 hours later to perform the initial training of target sequence whereas individuals in no exercise conditions (CONTROL, $TEST_{IMM}$, INT_{DELAY}, and INT_{IMM}) started their target sequence training on the first visit to the laboratory. The initial training of target sequence

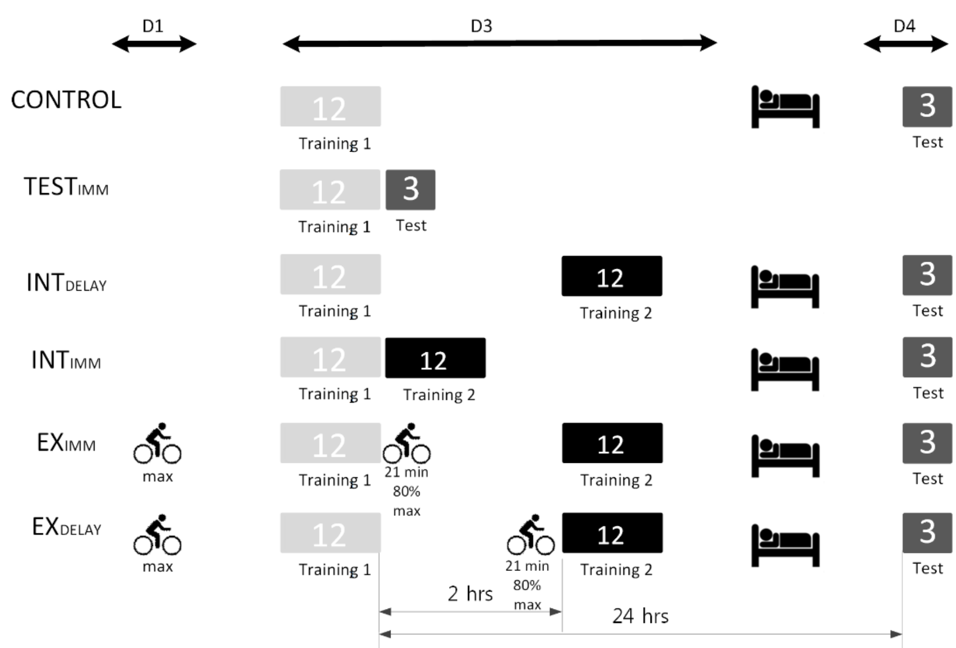

Figure 1. Illustration of the six experimental conditions in Experiment 1. Top two conditions (CONTROL, $TEST_{IMM}$) consist of target sequence training and 3 trials of test only. The middle two conditions (INT_{DELAY}, INT_{IMM}) have additional training of alternative sequence as interference at immediately or 2 hours after completion of target sequence training. Bottom two conditions (EX_{IMM}, EX_{DELAY}) conditions have an acute exercise bout in between target sequence training and alternative sequence training.

consisted of repeatedly executing the target sequence for 30 seconds followed by 30 seconds rest. Twelve 30 seconds practice trials of the target sequence were completed by each participant. Two hours later, additional practice of a novel motor sequence, using the same 30 seconds of practice followed by 30 seconds of rest was administered to all participants except CONTROL and $TEST_{IMM}$ conditions (see Figure 1).

Individuals assigned to the exercise conditions (EX_{IMM}, EX_{DELAY}) experienced a single bout of exercise described in a previous section. The locus of the exercise bout during the 2 hours break between initial training and alternative training depended on the assigned condition. Individuals in the EX_{IMM} condition started an exercise bout immediately after the initial target sequence training and an interfering target sequence training followed approximately 90 minutes after an exercise bout. On the other hand, individuals in the EX_{DELAY} condition started an exercise bout after 90minutes of target sequence training and an interfering target sequence training followed immediately after completion of an exercise bout.

For all trials with either the target or interfering motor sequence, *speed,* defined as the correct number of sequences executed in 30 seconds and, *error rate,* defined as the percentage of erroneous key presses in 30 seconds, were recorded and subsequently used as the primary dependent variables of interest (Krakauer & Shadmehr, 2006). For the present work, offline learning is defined as a positive performance improvement that is larger than that observed for the mean performance observed for those individuals that experienced trials 13-15 immediately following trials 1-12 (i.e., the $TEST_{IMM}$ condition). The purpose of Experiment 1 was 1) to examine whether the acute exercise bout has effect on motor sequence learning similar to that observed for a 90 minutes nap which accelerated the consolidation process in Korman et al.'s study (Korman et al., 2007), and 2) to assess the time course of the influence of exercise for consolidation of motor sequence knowledge.

Results

Demographics of the EX_{IMM} and EX_{DELAY} exercise condition

Table 1 displays demographics and performance data from the graded exercise test conducted on Day 1 for participants in the EX_{IMM} and EX_{DELAY} exercise conditions. Each variable was submitted to single sample t-tests which revealed no significant differences between exercise conditions for any variables.

Condition	EX_{IMM}	EX_{DELAY}
Age	20.13±0.55	19.67±0.47
Max Heart Rate	189.63±4.25	188.56±3.46
Heart Rate Recovery Ratio in 2 minutes	22.74±2.82	22.77±1.88
VO_2 Max	31.72±1.52	32.52±0.98

Table 1 Demographics of graded exercise test results for EX_{IMM} and EX_{DELAY} condition.

Performance of the target sequence during the initial practice phase

To assess performance during the initial practice phase *speed* and *error rate* were calculated for each individual in each of the six experimental conditions (i.e., two exercise and four control conditions) for each trial of the target sequence. These data were subjected to a 6 (Condition: CONTROL, $TEST_{IMM}$, INT_{DELAY}, INT_{IMM}, EX_{IMM},

EX_{DELAY}) x 12 (Trial: 1-12) analysis of variance (ANOVA) with repeated measures of the last factor. Figure 2 displays mean speed (top panel) and error rate (bottom panel) for the target sequence collapsed across condition for the 12 trials of initial practice.

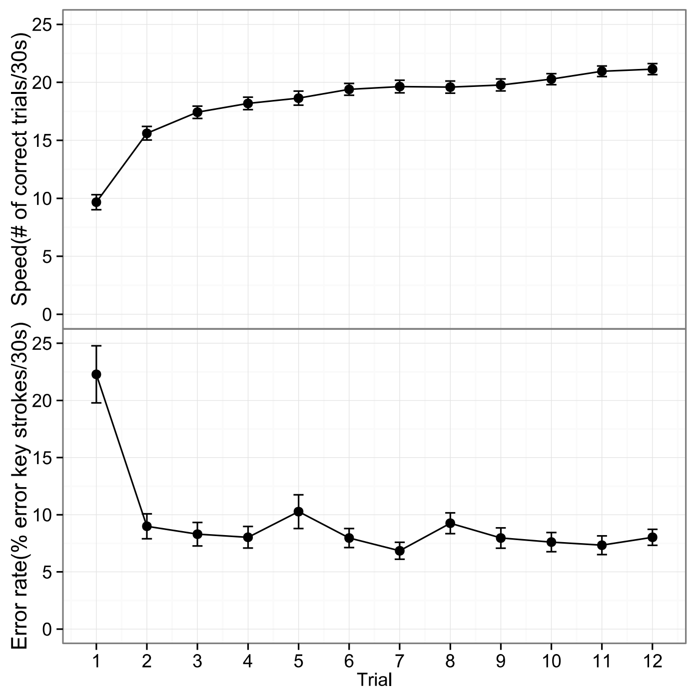

Figure 2 Mean performance speed (top panel) and error rate (bottom panel) of target sequence practice for all individuals in Experiment 1. Each trial was separated by a 30-s rest interval. This data indicated speed and error rate were changed over trials.

Formal analysis of speed revealed a significant main effect of Condition, $F(5,60) = 2.88$, $p<.05$, and Trial, $F(11,659) = 80.06$, $p<.01$ (See Figure 2). For error rate only the main effect of Trial, $F(11,659) = 14.13$, $p<.01$ was significant. Thus, as expected, and congruent with the significant effect of Trial, general performance of the target sequence improved with practice (i.e., ~118% increase in speed or an additional 11.5 sequences per 30 seconds; 64% reduction in error rate with practice). Individuals assigned to the $TEST_{IMM}$ condition (M = 20.3 sequences/30s) exhibited greater mean speed than observed for the subjects in the INT_{IMM} (M = 15.8 sequences/30s) and EX_{IMM} (M = 16.8 sequences/30s) conditions. Mean speed for participants in the $TEST_{IMM}$ condition did not differ reliably from that observed for those in the CONTROL(M = 18.5 sequences/30s) and EX_{DELAY} (M = 19.5 sequences/30s) conditions. Furthermore, mean speed did not differ significantly for the CONTROL, INT_{DELAY}, EX_{IMM}, and EX_{DELAY} conditions. Improvement was similar across Condition for speed and error rate as evidenced by the lack of significant Condition x Block interaction speed, $F(55,659) = 1.12$, $p=.27$; error rate, $F(55,659) = 0.93$, $p=.63$.

Performance of target and interfering task during the initial practice phase

For individuals assigned to the INT_{DELAY}, INT_{IMM}, EX_{IMM}, and EX_{DELAY} conditions, additional practice of an interfering motor sequence was experienced. Speed and error rate was calculated for each individual for each trial of the interfering sequence in a manner previously described for the target sequence. For the purpose of analysis these data were combined with the 12 trials from practice with the target motor sequence and subjected to a 4 (Condition: INT_{DELAY}, INT_{IMM}, EX_{IMM}, EX_{DELAY}) x 2 (Sequence: Target, Alternate) x 12 (Trial: 1-12) ANOVA with repeated measures on the last two factors. For the analysis of speed, there was a marginally significant main effect of Condition for mean speed $F(3,38) = 2.63$, $p=0.06$ and Trial for mean speed $F(11,416) =$

64.68, $p<.01$. For error rate, a significant main effect of Trial, $F(11,416) = 6.74$, $p<0.01$ and Sequence $F(1,38) = 4.12$, $p<.05$ were observed. No other effects were reliable. These data indicate that similar improvements in performance were made across practice of both the target and interfering motor sequences for individuals in the INT_{DELAY}, INT_{IMM}, EX_{IMM}, and EX_{DELAY} conditions.

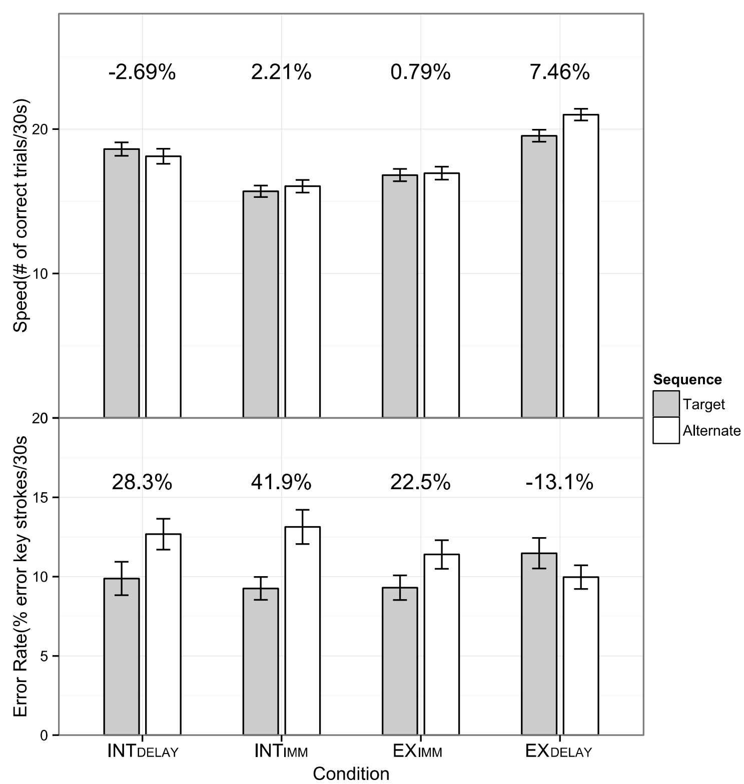

Figure 3 Mean performance of speed (top panel) and error rate (bottom panel) of target sequence training (grey bars) and alternative sequence practice (white bars) for each individuals experienced an alternative sequence practice. These data indicated the possibility of time dependent post practice consolidation process (different amount of changes between INT_{DELAY} and INT_{IMM} condition) and proactive benefits of an acute exercise bout (different amount of changes between EX_{IMM} and EX_{DELAY} condition).

Even though no significant effect between sequences for mean speed were observed, the marginally significant main effect of Condition indicated a difference in the performances of interfering sequences across conditions. This outcome is depicted in Figure 3 revealing better performance of the interference sequence for the EX_{DELAY} condition compare to other conditions. While there was no significant interaction for Condition x Sequence observed, the performance of alternative target sequence practice for the EX_{DELAY} condition which was relatively higher than those of other conditions may indicate the possibility that an acute bout of exercise offered same benefit to the learning of sequence practiced after the exercise session (Roig et al., 2012). This issue will be revisited in Experiment 3.

Assessment of offline learning: End of practice versus test trial comparison

The assessment of offline learning followed procedures previously adopted in studies addressing consolidation of motor sequence knowledge (Walker et al., 2003; Wright et al., 2010). This involved a comparison of performance (mean speed and error rate) at the conclusion of practice and during the test. Recall that for the present work, offline learning was defined as greater performance improvement (i.e., increased speed and/or reduction in error) from the conclusion of training to the delayed test trials compared to that observed for the individuals that experienced trials 13-15 immediately following trials 1-12 (the $TEST_{IMM}$ condition). Mean speed and error rate were separately calculated for each individual for the last three 30 s trials of practice of the target motor sequence (e.g., Trials 10-12) and the three test trials. These data were submitted to a 6 (Condition: CONTROL, $TEST_{IMM}$, INT_{DELAY}, INT_{IMM}, EX_{IMM}, EX$_{DELAY}$) x 2 (Phase: Practice, Test) ANOVA with repeated measure of the last factor. Figure 4 depicts mean speed (top panel) and error rate (bottom panel) for the end of practice and test phases as a function of Condition. Analysis of mean speed revealed a significant Phase main effect, $F(1,62) = 24.24$, $p<.01$ and a significant Condition x Phase interaction, $F(5,62) = 3.73$, $p<.01$. The Phase main effect was a result of greater mean speed during test ($M = 22$ sequences/30 s) compared to that observed during practice ($M = 20.6$ sequences/30 s). Also the significant Condition x Phase interaction suggests that the change in speed from the end of training to test differed across Condition. Simple main effects analysis indicated that the CONTROL, $F(1,62) = 7.98$, $p<.0.01$, INT$_{DELAY}$, $F(1,62) = 4.05$, $p=.05$, and EX$_{DELAY}$, $F(1,62) = 9.2$, $p<.01$, conditions revealed significantly greater mean speed during the test compared to the practice phase. This was not the case for the TEST$_{IMM}$, $F(1,62) = 0.13$, $p>.05$, INT$_{IMM}$, $F(1,62) = 0.45$, $p>.05$, and EX$_{IMM}$, $F(1,62) = 2.25$, $p>0.05$, conditions, for which the improvement in mean speed was not significant.

As was the case for mean speed, the analysis of error rate, revealed a significant Phase main effect, $F(1,62) = 8.53$, $p<.01$ and a significant Condition x Phase interaction, $F(5,62) = 2.42$, $p<.05$. The Phase main effect was a result of a reliably lower error rate during the test ($M = 6.6\%$ of total key-presses) than during practice ($M = 8.1\%$ of total key-presses). The significant Condition x Phase interaction suggests that the reduction in mean error rate from practice to test was different across conditions. Simple main effects analysis indicated that this interaction was a result of reliably lower mean error rate during the test compared to the practice phase for the participants in the EX$_{DELAY}$,

$F(1,62) = 7.01$, $p<.01$ and INT$_{DELAY}$, $F(1,62) = 2.84$, $p=.09$ conditions. In contrast, subjects exposed to the CONTROL, $F(1,62) = 1.1$, $p>.05$, TEST$_{IMM}$, $F(1,62) = 0.13$, $p>.05$, INT$_{IMM}$, $F(1,62) = 0.24$, $p>0.05$ and EX$_{IMM}$ conditions, $F(1,62) = 1.98$, $p>.05$, failed to show a reliable change in mean error rate from practice.

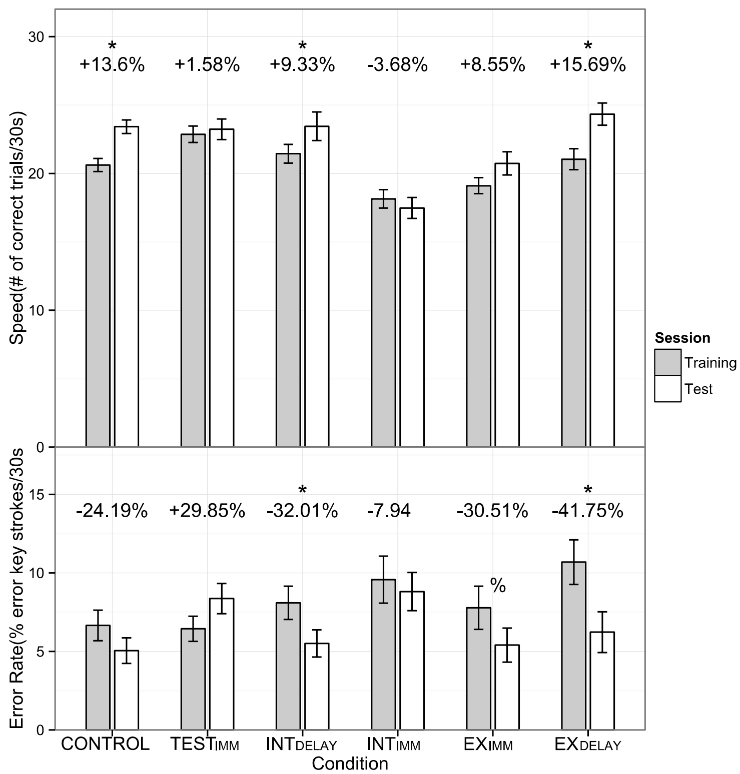

Figure 4 Mean performance of speed (top panel) and error rate (bottom panel) of target sequence training (grey bars) and 24 hours delayed retention test of target sequence (white bars) for all conditions in Experiment 1. These data indicated the consolidation process after initial practice requires time for stabilization. Offline improvement for INT$_{DELAY}$ condition was decreased compared to CONTROL condition but the knowledge of target sequence was still maintained compared to TEST$_{IMM}$ and INT$_{IMM}$ conditions and this time requirement for the initial stabilization seems to be the case for the offline benefits that an acute exercise bout facilitates.

In addition to the previous assessment of offline learning which compared the performance improvement between target sequence training and delayed test within group, the performance improvements between groups were compared by calculating the

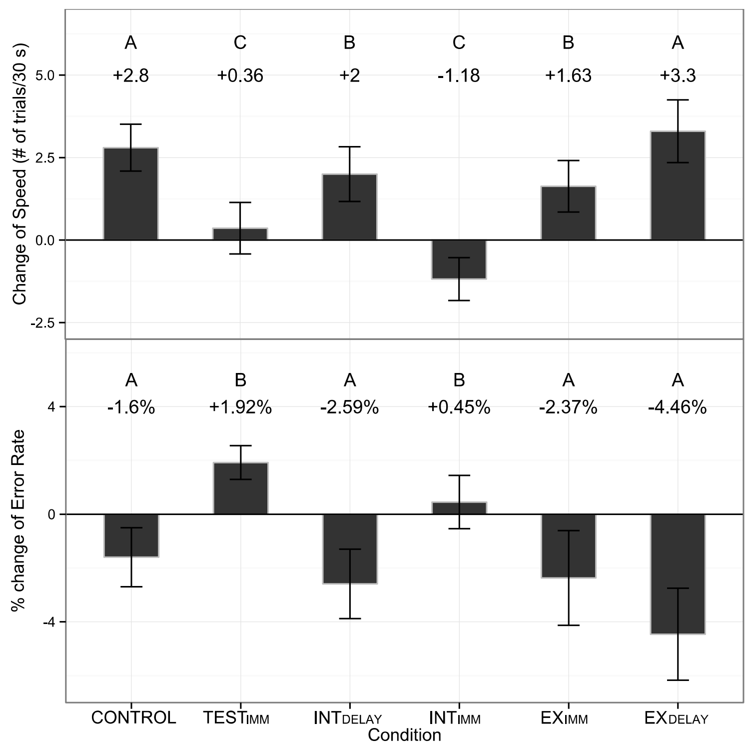

Figure 5 Change score for speed (Top panel) and error rate (Bottom panel) between last three 30-s trials of target sequence training and three trials of test session are displayed. For speed, CONTROL and EX_{DELAY} exhibited significantly larger improvement than the other four groups. On the other hand, INT_{DELAY} and INT_{IMM} exhibited very little or even negative change which indicates that no offline enhancement was acquired. INT_{DELAY} and EX_{IMM} condition shoed moderate improvement for speed. This indicates that a 2-hr duration between target sequence training and alternative sequence training may allow initial target sequence practice to be stabilized. For error rate, $TEST_{IMM}$ and INT_{IMM} conditions exhibited no improvement whereas the other four conditions exhibited improvement.

individual improvement for speed and error rate. Mean speed and error rate change score were separately calculated by subtracting last three 30 s trials of practice of the target motor sequence (e.g., Trials 10-12) from the three test trials. These data were submitted to a 6 (Condition: CONTROL, $TEST_{IMM}$, INT_{DELAY}, INT_{IMM}, EX_{IMM}, EX_{DELAY}) between factor analysis of variance and this analysis revealed a significant main effect on Condition $F(5,60) = 4.22$, $p<0.01$. Figure 5 depicts mean change score of each condition for speed and error rate. For speed, the CONTROL and EX_{DELAY} conditions exhibited biggest improvement which was as expected as a result of offline enhancement. On the other hand, $TEST_{IMM}$ and INT_{IMM} conditions exhibited no improvements indicating motor sequence learning requires a certain amount of time to stabilize and subsequently further consolidate acquired knowledge to reveal offline improvement. In addition, moderate improvement observed from INT_{DELAY} and EX_{IMM} condition indicated that memory stabilization begins almost immediately after completion of initial target sequence practice initiating some resistance to upcoming interference. For error rate, only $TEST_{IMM}$ and INT_{IMM} conditions showed an increased error rate while the other four condition exhibited a decreased error rate.

Discussion

The goal of the Experiment 1 was to evaluate the effectiveness of an acute bout of aerobic exercise as a means of protecting memory for procedural skill that has been shown to be susceptible to interference from competing practice experienced in close temporal proximity (Walker et al., 2003). Previously, by inserting a brief period of sleep (i.e., a nap), Korman et al. (2007) was able to resurrect offline performance gains despite the fact practice with the target sequence was followed by additional practice with an alternative task about 2 hours later. In the present work we substituted the nap with a short, moderately intensive bout of cardiovascular exercise performed on a bicycle ergometer located either (a) immediately after practicing the target task, or (b) right before practice of the interfering activity approximately 2 hours later. To interpret the effectiveness of the exercise for protecting procedural learning it is first helpful to briefly examine the outcomes for the control conditions that were included in the study.

As expected, little change in performance occurred for individuals that merely continued practicing for an additional three trials of practice beyond the twelve afforded all other participants during acquisition (i.e., $TEST_{IMM}$ condition). In contrast, and again as expected, robust offline gains were present, in the form of both a reliable increase in speed accompanied with a reduction in error, for the participants that were privy to a night of sleep (i.e., CONTROL condition). These improvements were significantly greater than observed in the $TEST_{IMM}$ condition. The offline benefit associated with a night of sleep was reduced by completing additional practice of a novel motor sequence. The interfering motor sequence was provided immediately after the completion of target sequence training (i.e., INT_{IMM} condition) or approximately 2 hours after practice of the target task (i.e., INT_{DELAY} condition). The impact of the interfering sequence practice was very similar to that was used in previous studies (Walker et al., 2003). Practice of a novel motor sequence immediately after practice of the target sequence task (i.e., INT_{IMM} condition) completely removed offline enhancement and this indicates that a period of time is needed for the consolidation process to begin for the offline benefits to occur. Taken together, these data are very consistent with the extent literature demonstrating that motor sequence knowledge, if given the opportunity to stabilize, can undergo further consolidation which is manifest as an offline gains in performance. (Korman et al., 2007; Walker et al., 2003; Wright et al., 2010).

With respect to exercise, the present data indicated that an acute bout of moderately intense exercise can reduce the susceptibility of the target motor sequence to the deleterious effect of additional training with the interference motor sequence. However, this protective feature from exercise was dependent on its temporal locus relative to practice with the motor sequence. When exercise occurred toward the end of the 2 hours interval between acquisition of the target motor sequence and the novel sequence training, performance of the target motor sequence not only appeared to be protected from the negative influence of the alternative task practice but exhibited some facilitation manifest as an offline improvement benefit that observed for participants in the CONTROL condition who were exposed to practice with the target sequence only and no alternative sequence practice.

In contrast, when exercise occurred immediately after training with the target motor sequence, performance during the delayed test was very similar, indeed a little worse, to that observed for individuals in the INT_{DELAY} condition. Thus, an acute bout of aerobic exercise immediately following skill training did not afford the same offline return as that observed from the EX_{DELAY} condition. While these data intimate that post-practice consolidation of a motor task can be influenced by exposure to exercise (Coles & Tomporowski, 2008; Roig et al., 2012), they highlight the novel finding that the benefit of acute exercise is dependent on its timing relative to original learning.

It is possible that exercise located closer to the alternative task practice enforced a consolidation of motor memory for target sequence training and operated in a proactive manner on performance of the alternative sequence practice such that it induced "less" interference on memory for the target sequence. Thus, offline enhancement emerged indirectly through diminishing the impact of the interfering alternative task practice. The duration for the acquired memory from target sequence practice to be consolidated appears to be important factor to have an offline enhancement considering no offline improvements were observed from INT_{IMM} and EX_{IMM} condition.

At this point it is difficult to discern exactly why the temporal positioning of the exercise used in the present work was so vital to its effectiveness. However, losing an offline benefits observed from the INT_{IMM} condition suggested that an exercise bout can also work to interfere with the consolidation process of learned target sequence when exercise is provided too close to initial practice. The pattern of losing offline benefits observed for INT_{IMM} and EX_{IMM} condition were different indicating that the effect of interference task and the effect of acute bout of exercise as an interference may work in different manner. For example, practice of interfering sequence at INT_{IMM} condition may work to directly interfere with the acquired memory of the target sequence to prevent consolidation of acquired memory being initiated. In contrast, it is likely that an exercise bout followed immediately after target sequence training, as in the EX_{IMM} condition, worked as indirect interference consuming required cognitive resources for the ongoing consolidation process thus resulting in a relatively suppressed performance improvement similar to that observed from the INT_{DELAY} condition.

At this point it is clear, the specific temporal dynamics of consolidation for stabilization needs to be further clarified. This is clearly one direction in which further work needs to be conducted. Despite this, there was a clear advantage of placing exercise at the end of the 2-hr window prior to experiencing additional training with an alternative motor sequence. It is possible that an acute exercise bout can influence both fast and slow stages of motor sequence learning by (a) accelerating the consolidation of stabilized motor memory to induce further offline enhancement and (b) by helping the acquisition of upcoming alternative sequence learning. However, this advantage on both fast and slow learning only seems possible when certain amount of time for stabilization of initial learning was allowed.

These data then, provide initial support for a protective effect of an acute exercise bout for motor sequence learning was revealed. Experiment 2 examined a possible biological underpinning for the efficacy of exercise for enhancing motor memory.

CHAPTER III

EXPERIMENT 2

Introduction

Current theorizing regarding the locus of exercise-related cognitive benefits, including limited examples of memory for motor skills, has implicated an up-regulation of a number of growth factors important for neurogenesis, in particular, brain-derived neurotrophic factor (BDNF) (Erickson et al., 2011; Voss et al., 2011; Zoladz & Pilc, 2010). Peripheral concentrations of BDNF have been reported to increase anywhere from 12-400% following acute bout of moderate to intense aerobic exercise (Knaepen, Goekint, Heyman, & Meeusen, 2010). However, there is some evidence, albeit limited in humans, that BDNF concentrations generally remain elevated after an acute bout of aerobic exercise for about 15-60 min (Ferris et al., 2007; Gold et al., 2003). Experiment 2 considered the possibility that the retention of the offline benefits reported for the EX_{DELAY} experimental condition in Experiment 1 was associated with BDNF concentration changes in this experimental condition.

Result from the Experiment 1 indicated that the acute exercise bout inserted between the practice trials and the alternative trials reduced the susceptibility of initial learning to alternative practice trials and accelerated a consolidation process of offline performance gains that usually require a night of sleep. The rationale for Experiment 2 was that exercise has been shown to increase the presence of specific neural growth factors including brain-derived neurotrophic factor(BDNF) which had been identified as responsible for improved memory stabilization and offline learning following overnight sleep (Erickson et al., 2011). In this experiment, the change of the peripheral circulating levels of plasma-BDNF as a result of acute bout of exercise was calculated and its' relationship with the result of offline improvement in motor sequence learning was examined.

To examine this relationship, individuals were assigned to one of two experimental conditions. One condition (EX_{DELAY}+BD) was almost identical to the EX_{DELAY} condition of the Experiment 1 with exception of two blood draws prior to and immediately after the acute exercise bout. From the two exercise conditions used in the Experiment 1 (EX_{IMM} and EX_{DELAY}), the condition exhibited largest behavioral improvement (EX_{DELAY} condition) was chosen. The second condition (INT_{DELAY}+BD) was identical to the INT_{DELAY} condition from the Experiment 1 except two blood draws and the time course of blood draws during the delays between target sequence practice and interfering sequence practice was matched to time course of blood draws of EX_{DELAY}+BD condition group. The primary goal of this experiment was to examine the role of BDNF for offline gain. It was assumed that by experiencing an acute bout of exercise would increase circulating levels of BDNF. It was further considered that elevated levels of BDNF would have an influence on consolidation (Bramham & Messaoudi, 2005). These result of enhanced consolidation would be increased offline improvement being related to increased BDNF level.

Methods

Participants

A total of Twenty-four (24) individuals served as participants in this experiment. Participation in this study fulfilled a research requirement for undergraduate class. The participants had no prior experience with the experimental task and were informed of the specific purpose of the study. Informed consent and a Physical readiness checklist approved by the Institutional Review Board for the ethical treatment of experimental participants at Texas A&M University were obtained prior to any participation in the experiment.

Tasks

Graded exercise test

Procedure of the Graded exercise was identical to that described in the Experiment 1.

Acute exercise bout

Procedure of the acute exercise bout was identical to that described in the Experiment 1

Motor sequence tasks

All participants performed a target sequence, 4-1-3-2-4, on a standard PC keyboard using the V, B, N,M keys where "1" was the leftmost key (V key) and "4" was the rightmost key (i.e., M key) with their non-dominant hand. In addition, all individuals (EX_{DELAY}+BD, INT_{DELAY}+BD) were administered an extra bout of practice with an alternative sequence, 2-3-1-4-2, again performed with the non-dominant hand to potentially induce interference (see Walker, Brakefield, Hobson, et al., 2003) as well as delayed test of target sequence administered 24 hours after the completion of target sequence training.

On execution of motor sequence learning, a required sequence to be entered was displayed across the top third of the computer display and four boxes were displayed across the bottom third of the computer display that were spatially compatible with the fingers placed on the V, B, N, and M keys on a standard PC keyboard. Each key press resulted in the appearance of a black dot in the box above the key that should be pressed. All features of this experiment were programmed using E-Prime® 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA). Prior to practice individuals were informed of the nature of the sequence of key presses that constituted the required task.

Procedures for blood draws and post processing of blood samples

Prior to and after an exercise bout, 2 cc of peripheral venous blood was drawn from each individual using aseptic technique and stored to two color-coded tubes. One tube contained powdered sodium fluoride and potassium oxalate to inhibit glycolysis which might cause false-low glucose and compromise measurement of blood lactate level. Blood lactate level was measured to confirm the intensity of the exercise bout for each individual. The second tube contained EDTA[2] as an additives. These additives bind calcium ions to block the coagulation cascade associated with blood clotting.

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[2] Ethylene Diamine Triacetic Acid (EDTA) has a very strong chemical attraction for calcium ions in a medium containing water. When blood is drawn into a tube containing EDTA, calcium ions in the blood are strongly bound to the molecules of EDTA. Calcium ions are a critical component in the biochemical cascade that results in blood clotting.

Using the first tube, a blood lactate assessment was conducted immediately after the second blood draw. The second tubes were blood samples for BDNF assay and were centrifuged to separate serum/plasma from red blood cells at 3500RPM for 20 minutes immediately after the 2nd blood draw after exercise on Day 2. Blood samples collected before exercise (1st blood draw) were stored in the refrigerator during the exercise bout. Separated plasma was extracted and stored at -80F until further analysis. Serum and plasma BDNF levels were measured using commercially available enzyme linked immunosorbent assay kit (Human BDNF ELISA, R&D Systems, WA) (Klein et al., 2011).

Procedures

Prior to participation in the experiment all participants completed an informed consent. The general sequence of events that were followed by each experimental condition is depicted in Figure 6. Specifically, 48 hours prior to any practice with the target motor sequence individuals performed a graded exercise test. At least 48 hours later all participants were required to return to the laboratory to complete practice of the target sequence. A practice trial consisted of repeatedly executing the target sequence for 30 seconds followed by 30 seconds rest. Twelve 30 seconds practice trials of the target sequence were completed by each participant. Two hours later, additional practice of a novel motor sequence, using the same 30 seconds of practice followed by 30 seconds of rest was administered to all participants in the EX_{DELAY}+BD and INT_{DELAY}+BD conditions (see Figure 6).

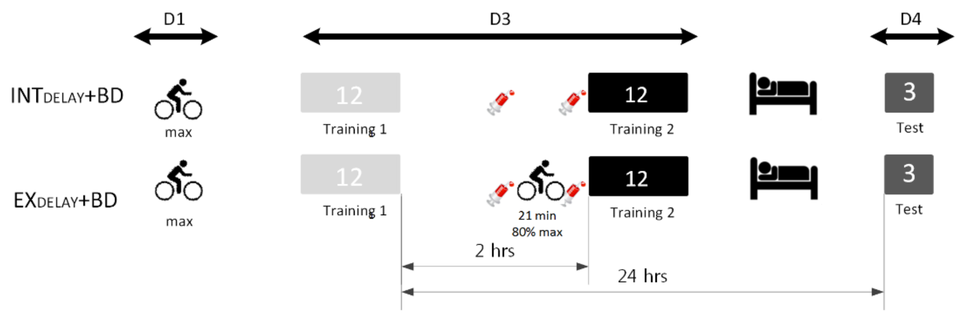

Figure 6 Illustration of the two experimental conditions in Experiment 2. Top condition (INT_{DELAY}+BD) consist of target sequence training, an alternative sequence practice after 2 hours of target sequence training, and 3 trials of test session after 24 hours of target sequence training. The bottom condition (EX_{DELAY}+BD) have an acute exercise bout immediately before an alternative sequence practice. These 2 conditions in Experiment 2 are identical to INT_{DELAY} and EX_{DELAY} conditions in Experiment 1 except 2 blood draws were administered before and after exercise bout (EX_{DELAY}+BD) or at the matching temporal locus to EX_{DELAY}+BD condition (INT_{DELAY}+BD).

Following practice of the target sequence and prior to experience with the alternative motor sequence, individuals assigned to the EX_{DELAY}+BD condition experienced a single bout of exercise similar to that described in Experiment 1. Prior to and immediately following exercise each participant had 2 cc of blood drawn and stored in two septic tubes. At the conclusion of exercise and following the second blood draw participants were administered an additional sequence training trials with the alternative motor sequence. All participants performed a delayed test that involved a set of three 30 seconds trial test of the target sequence 24 hours after the completion of the initial practice of the target sequence.

For all trials with either the target sequence or interfering motor sequence, *speed,* defined as the correct number of sequences executed in 30 seconds and, *error rate,* defined as the percentage of erroneous key presses in 30 seconds, were recorded and subsequently used as the primary dependent variables of interest. Based on findings from Experiment 1, the result of EX_{DELAY}+BD condition was expected to reveal evidence of offline performance enhancement whereas the INT_{DELAY}+BD condition were anticipated to mimic the merely observed in the INT_{DELAY} condition in Experiment 1. It was also expected that the EX_{DELAY}+BD condition would exhibit an increase of peripheral circulating levels of plasma-BDNF from pre to post exercise whereas INT_{DELAY}+BD would not (Knaepen et al., 2010). Finally, it was hypothesized that the change in peripheral plasma-BDNF level would be correlated with performance gains with the extent of protection for offline facilitation from interfering motor sequence practice.

Result

Demographics of the EX_{DELAY}+BD and INT_{DELAY}+BD exercise conditions

Condition	EX_{DELAY}+BD	INT_{DELAY}+BD
Age	21±0.25	20.18±0.18
Max Heart Rate	182.6±2.74	185.91±2.11
Heart Rate Recovery Ratio in 2 minutes	20.98±1.62	24.33±1.36
VO_2 Max	31.51±1.09	32.4±1.11

Table 2 Demographics of graded exercise test result for EX_{DELAY}+BD and INT_{DELAY}+BD condition.

Table 2 displays demographics and performance data from the graded exercise test conducted on Day 1 for participants in the EX_{DELAY}+BD and INT_{DELAY}+BD conditions. Each variable was submitted to single sample t-test that revealed no significant differences between exercise conditions except for age $t(21) = 2.64$, $p<0.05$ which indicated that the participants in the exercise condition were older than their no-exercise condition counterparts.

Performance during the initial practice phase

Speed and error rate for each individual in each of two experimental conditions from Experiment 2 (EX_{DELAY}+BD, INT_{DELAY}+BD) were calculated to assess performance during the initial practice of target sequence along with two control conditions in Experiment 1 (CONTROL, $TEST_{IMM}$). These data were subjected to a 4 (Condition: CONTROL, $TEST_{IMM}$, EX_{DELAY}+BD, INT_{DELAY}+BD) x 12 (Trial: 1-12) analysis of variance (ANOVA) with repeated measures of the last factor. Figure 7 displays mean speed (top panel) and error rate (bottom panel) for the target sequence collapsed across condition for the 12 trials of initial practice.

Formal analysis revealed a significant main effect of Trial for speed, $F(11,473) = 71.80$, $p<.01$ and error rate, $F(11,472) = 18.45$, $p<.01$. Thus, as expected, and congruent with the significant effect of Trial, general performance of the target sequence improved with practice (i.e., ~113% increase in speed or an additional 11.87 sequences per 30 seconds; 75% reduction in error rate with practice). No interaction of Condition x Trial indicates improvement was similar to the EX_{DELAY}+BD and INT_{DELAY}+BD condition when first practice the target sequence.

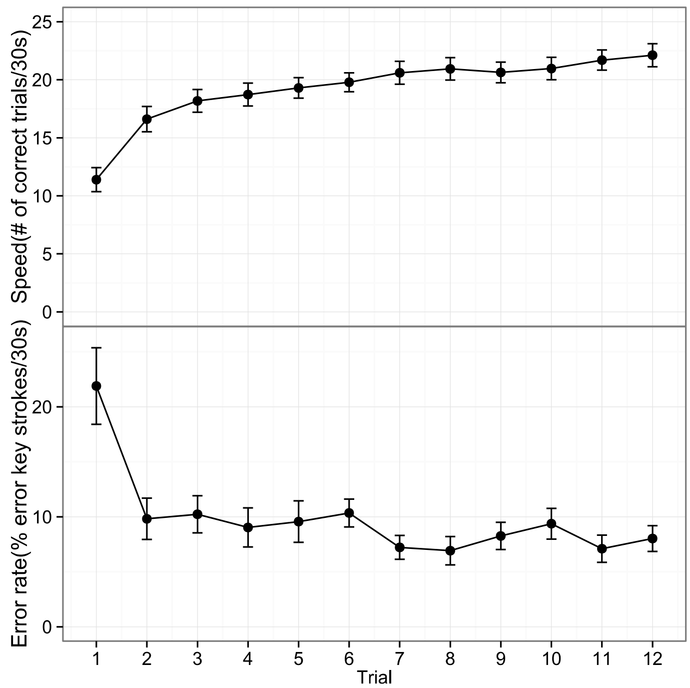

Figure 7 Mean peformance speed (top panel) and error rate (bottom panel) of target sequence training for an individuals in Experiment 2 are displayed. Each trial was separated by 30-s rest interval. This data indicated speed and error rate were changed over trials. The performance of target sequence training had no significant difference between conditions.

Performance of target and interfering task

All individuals in the Experiment 2 experienced additional practice of an interfering motor sequence. Speed and error rate for each individual for each trial of the interfering sequence was calculated with a same manner as the calculation of target sequence. For the purpose of analysis these data were combined with the 12 trials from practice with the target motor sequence and subjected to a 2 (Condition: EX_{DELAY}+BD, INT_{DELAY}+BD) x 2 (Phase: Target, Interference) x 12 (Trial: 1-12) ANOVA with repeated measures on the last two factors. Formal analysis revealed that there was a significant main effect of Trial for the speed, $F(11,231) = 56.34$, $p < .01$ and the error rate, $F(11,231) = 8.42$, $p<.01$ and the Condition x Trial interaction $F(11,231) = 2.72$, $p<.01$. No other significant effects were observed. This performance changes between

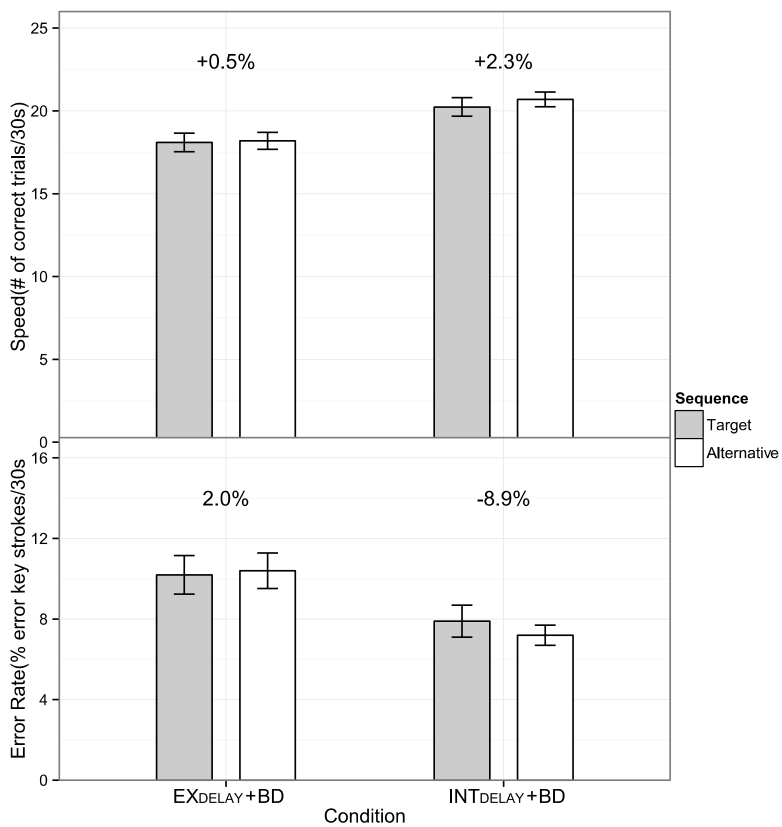

Figure 8 Mean performance of speed (top panel) and error rate (bottom panel) of target sequence training (grey bars) and alternative sequqence practice (white bars) fo each individuals. No siginificant difference between sessions or conditions were observed.

target sequence practice and alternative sequence practice were not different across conditions indicates that, unlike Experiment 1, an acute bout of exercise did not benefit the practice of alternative sequence followed after an exercise bout in Experiment 2 (see Figure 8).

Assessment of offline learning: End of practice vs Test

Offline learning was assessed in same manner as described in the Experiment 1 which involved a comparison of performance (mean speed and error rate) at the conclusion of practice and during the test. Mean speed and error rate were separately calculated for each individual for the last three 30 s trials of practice of the target motor sequence (e.g., Trials 10-12) and the three test trials of test session. In order to assess the effect of practicing an alternative sequence (INT_{DELAY}+BD) and the effect of acute exercise bout to combat susceptibility to interference (EX_{DELAY}+BD), the two control conditions from Experiment 1 (CONTROL and $TEST_{IMM}$) were again included in the analysis. These data were submitted to a 4 (Condition: CONTROL, $TEST_{IMM}$, EX_{DELAY}+BD, INT_{DELAY}+BD) x 2 (Phase: Practice, Test) ANOVA with repeated measure on the last factor. Figure 9 depicts mean speed (top panel) and error rate (bottom panel) for the end of practice and test phases as a function of Condition. Analysis of mean speed revealed a significant Phase main effect, $F(1,43) = 21.88$, $p<.01$. This Phase main effect was a result of greater mean speed during test ($M = 23.6$ sequences/30 seconds) compared to that observed during practice ($M = 21.8$ sequences/30 seconds). For the error rate, a significant Condition x Phase interaction $F(3,43) = 2.80$, $p=0.051$ indicates possible difference in offline improvement between conditions. Simple main effect analysis for error rate exhibited no significantly lower error rate from any of four conditions.

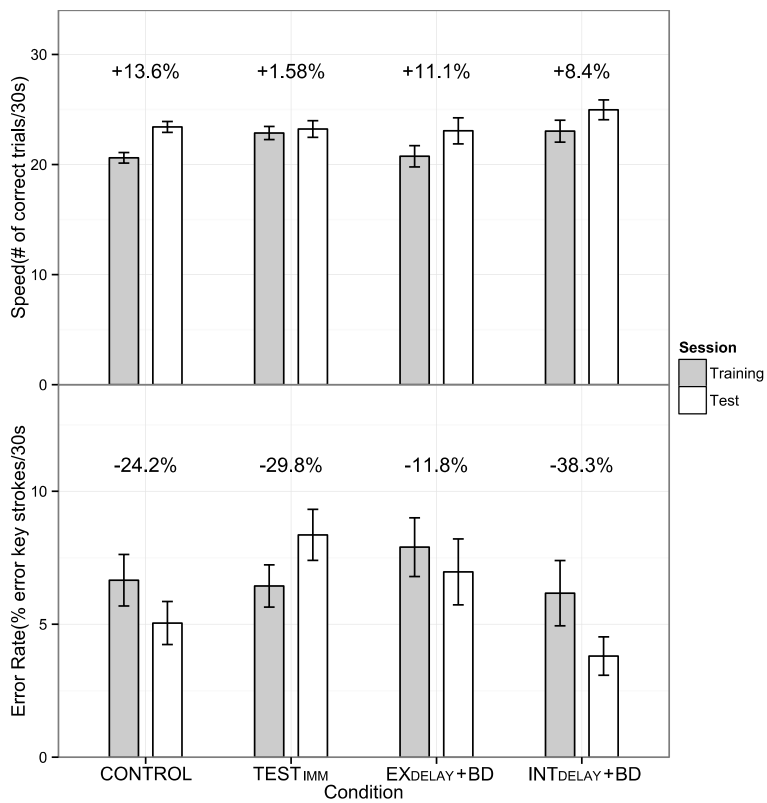

Figure 9 Mean performance of speed (top panel) and error rate (bottom panel) of target sequence training (grey bars) and 24 hr delayed retention test of target sequence (white bars) for all conditions in Experiment 2.

The EX_{DELAY}+BD condition exhibited greater improvement in speed than INT_{DELAY}+BD condition and this relationship was congruent with the improvement pattern, greater improvement with an acute bout of exercise immediately before the practice of interfering sequence, observed from INT_{DELAY} and EX_{DELAY} condition in

Experiment 1, however, the amount of improvement EX_{DELAY}+BD and INT_{DELAY}+BD were not significantly different. Change of error rate observed from Experiment 2 was not consistent with the results observed from Experiment 1. The error rate change in both conditions in Experiment 2 were not significant but the amount of error rate change in INT_{DELAY}+BD condition (i.e., -38%) was similar to the amount of error rate change in INT_{DELAY} condition in the Experiment 1(i.e.,-32%). However, for the EX_{DELAY}+BD condition, the amount error rate change (-11.8%) was relatively smaller than that of EX_{DELAY} condition in the Experiment 1(-43%).

Assessment of blood lactate level and plasma BDNF level

Two blood samples were collected from all individuals in the EX_{DELAY}+BD and INT_{DELAY}+BD conditions in Experiment 2. Blood lactate level and plasma BDNF level for each individual for each blood draw was measured. These data were analyzed with 2(Condition: EX_{DELAY}+BD, INT_{DELAY}+BD) x 2 (Phase: before, after) ANOVA with repeated measure on the last factor. Blood lactate level assessment revealed significant main effect of Condition $F(1,19) = 146.82$, $p<.01$, Phase $F(1,19)=142.76$, $p<.01$, and Condition x Phase interaction $F(1,19)=140.21$, $p<.01$. Simple main effect analysis revealed that subjects with EX_{DELAY}+BD condition showed significantly greater increase from before to after exercise for blood lactate level $F(1,19) = 233.03$, $p<.01$. As expected, the blood lactate was increased significantly with an acute bout of exercise as expected (see Figure 10).

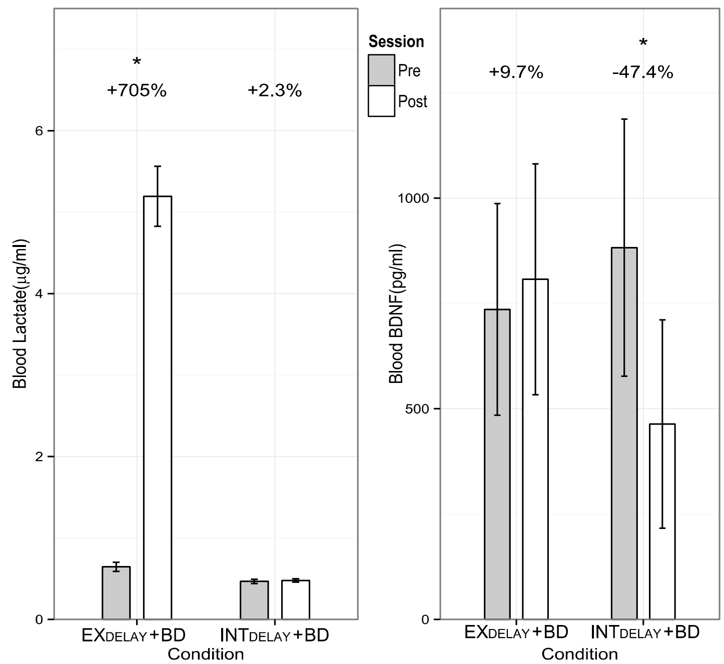

Figure 10 Result of blood draws before (grey bars) and after (white bars) the acute exercise boout for EX_{DELAY}+BD condition or matching temporal locus for INT_{DELAY}+BD condition. Blood lactate level (left panel) increased significantly with an acute exercise bout but did not change in the absence of exercise. Plasma BDNF level (right panel) did not significantly increased with an acute exercise bout but significanlty decreased in the absence condition.

Plasma BDNF level assessment revealed significant main effect of Condition x Phase interaction $F(1,19) = 6.38$, $p<.05$. Simple main effect analysis revealed the EX_{DELAY}+BD exhibited no reliable change from pre-exercise BDNF levels while the INT_{DELAY}+BD exhibited a significant reduction in plasma BDNF level $F(1,30) = 231.11$, $p<.01$. The plasma BDNF level did not exhibit significant change with an exercise bout but decreased significantly without exercise.

Relationship between change in BDNF and change in offline improvement

To assess the relationship between the change in plasma BDNF and lactate level as a result of acute bout of exercise and the effect of acute exercise bout to the resultant offline learning benefits, a Pearson product-moment correlation coefficient between the

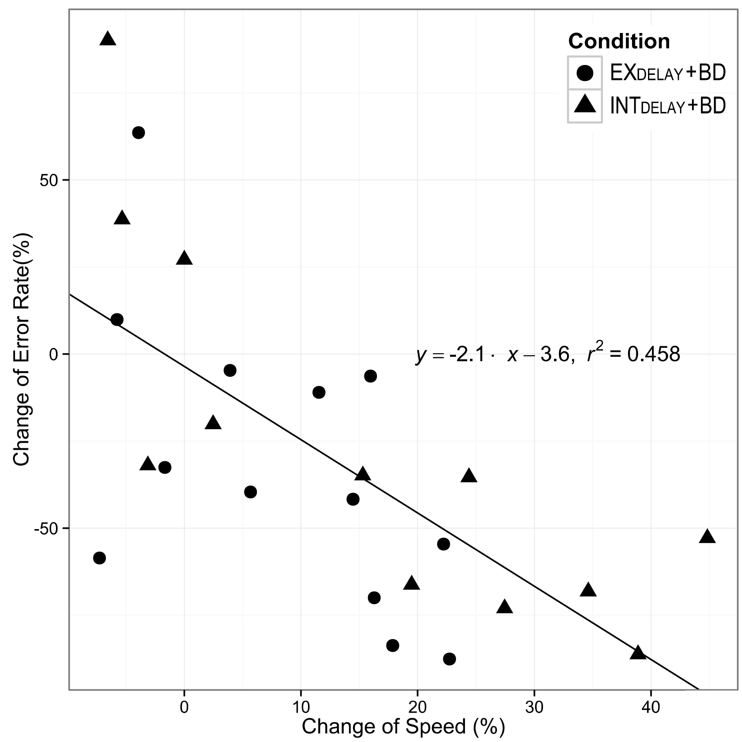

Figure 11 Change of speed and error rate for the two experimental conditions in Experiment 2. Improvements in both speed and error rate during offline delay were observed but there were no group differences for these relationship.

following factors were performed. Percent changes of blood lactate level and plasma BDNF level were calculated across the blood draws before and after exercise bout as well as the percent changes of speed and error rate as an offline improvement between target sequence training and test. These variables were submitted to correlation analysis and Pearson product-moment correlation coefficient for each pair of variables was calculated.

Changes of speed and error rate had moderate negative correlation Pearson $r = -0.4$, $p<.05$. These changes are ratio of difference between the performance of delayed target sequence test and initial target sequence training. The moderate negative correlation between change of speed and error rate depicted in Figure 11 indicates that both speed and accuracy were improved during the offline delay between target sequence training and target sequence test. However, no other strong relationship was found from the calculation and according to the result, BDNF and blood lactate level changes were not correlated with the offline consolidation process of motor sequence learning.

Change in blood lactate level or plasma BDNF level depicted in Figure 12 was not significantly correlated with offline improvement in speed or error rate. In addition, performance improvement pattern observed from the Experiment 2 was not strongly supporting the hypothesis that anticipated greater learning benefits with an exercise involved condition and positive relationship between performance improvement and change of plasma BDNF level.

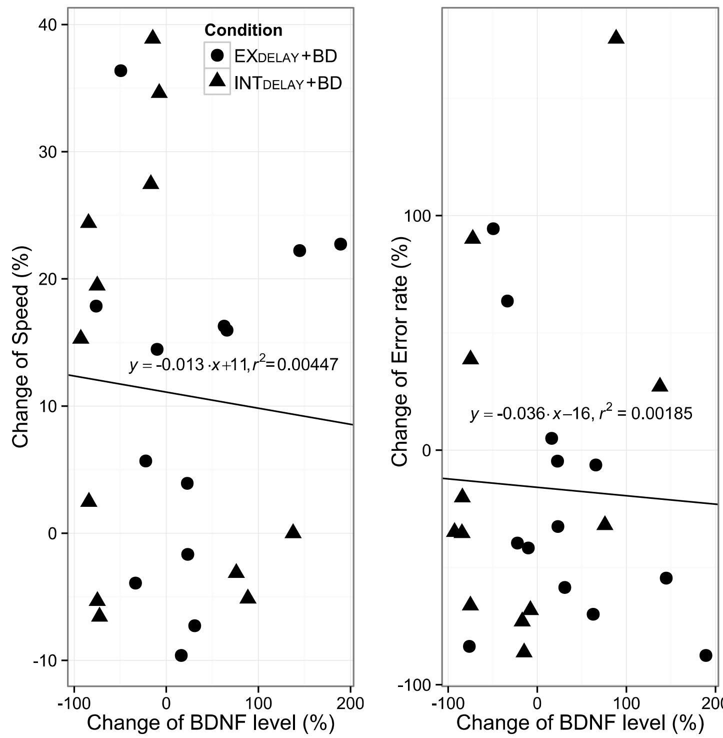

Figure 12 Correlation between Change of BDNF level and Change of Speed (Left Panel) or Change of Error rate (Right Panel) for the two experimental conditions in Experiment 2. This figure indicates that plasma BDNF level change was not correlated with offline improvement of Speed and Accuracy.

Additional correlation analysis

Additional correlation analysis for more variables was required in order to examine whether the distribution of each variable had certain relationship with other variables that can possibly explain lack of support for the proposed hypothesis. In addition to the variables obtained from blood sample analysis, the dependent variables depicting participants' physical fitness level that was measured during an graded exercise test (VO2 Max, Duration of graded exercise test, and Heart rate recovery ratio in 2 minutes after the termination of exercise bout) and categorical independent variables (Condition: EX_{DELAY}+BD and INT_{DELAY}+BD, Year data collected, and Gender) were submitted to the correlation analysis. Duration of graded exercise was the time duration in minutes until subjects was no longer able to maintain required speed as resistance was being increased every 3 minutes. Heart rate recovery ratio was the ratio between maximum heart rate observed during the graded exercise test and the heart rate after 2 minutes of termination of the resistance applied exercise bout. This heart rate recovery ratio is known to one of a key predictor of mortality in a cardiovascular health and also can be an indicator of physical fitness level of a person (Cole, Foody, Blackstone, & Lauer, 2000). The blood lactate level was correlated inversely with Heart Rate recovery ratio Pearson r = -0.67, p<.01, Condition Pearson r = -0.96, p<.01 and Year data collected Pearson r = 0.96, p<.01. Plasma BDNF level showed moderate to strong correlation with Heart Rate recovery ratio Pearson r = -0.4, p<.05, Condition Pearson r = -0.41, p<.05 and Year data collected Pearson r = -0.62, p<.01. Changes in speed had no significant correlation with other variables except error rate Pearson r = -0.4, p<.05. Duration of graded exercise test had strong significant correlation with Gender Pearson r = 0.7, p<.01 and weak marginally significant correlation with Heart rate recovery ratio Pearson r = 0.33, p=.08. Heart rate recovery ratio had weak to moderate correlation with Condition Pearson r = 0.36, p=.05 and Year data collected Pearson r = 0.52, p<.05 (see Table 3).

	% Change Speed	% Change error rate	Recovery Heart Rate in 2 min of exhaustion	Condition	Year
Lactate before Exercise	-0.01 0.98 16	0.19 0.48 16	0.10 0.71 16	-0.47 0.07 16	-0.47 0.07 16
Lactate after Exercise	0.10 0.71 16	-0.03 0.92 16	-0.50 0.05 16	-0.94 <.0001 16	-0.94 <.0001 16
% change of Lactate level	0.15 0.58 16	-0.18 0.50 16	-0.57 0.02 16	-0.77 0.00 16	-0.77 0.00 16
BDNF before Exercise	0.03 0.90 27	-0.10 0.64 27	0.42 0.03 27	0.10 0.62 27	0.50 0.01 27
BDNF after Exercise	-0.08 0.68 27	-0.11 0.58 27	0.34 0.08 27	-0.18 0.38 27	0.28 0.15 27
% change of BDNF	-0.17 0.39 27	0.32 0.11 27	-0.41 0.03 27	-0.42 0.03 27	-0.63 0.00 27
% change of Speed	.	-0.39 0.05 27	0.22 0.27 27	0.12 0.56 27	-0.02 0.92 27
% change of error rate	-0.39 0.05 27	.	-0.33 0.09 27	-0.05 0.82 27	0.03 0.88 27
Recovery Heart rate in 2 minutes of exhaustion	0.22 0.27 27	-0.33 0.09 27	.	0.34 0.08 27	0.51 0.01 27
Condition	0.12 0.56 27	-0.05 0.82 27	0.34 0.08 27	.	0.63 0.00 27
Year Data Collected	-0.02 0.92 27	0.03 0.88 27	0.51 0.01 27	0.63 0.00 27	.

Table 3 Pearson correlation coefficient between factors

Discussion

The goal of the Experiment 2 was to confirm the relationship between the effectiveness of an acute bout of aerobic exercise as a means of protecting memory for procedural skill observed in the Experiment 1 and to consider a potential relationship between physiological changes and offline improvement. In recent years, numerous studies have attempted to examine the effect of exercise and physiological changes such as increased BDNF production in the blood with an endurance training (Seifert et al., 2010; Zoladz & Pilc, 2010). In Experiment 2, blood lactate level and plasma BDNF levels were measured to evaluate the effect of an acute bout of exercise on procedural motor sequence learning. Two experimental conditions, an exercise (EX_{DELAY}+BD) and no exercise (INT_{DELAY}+BD) condition were identical to EX_{DELAY} and INT_{DELAY} condition in the Experiment 1 with the exception that two blood draws immediately before and after an exercise bout for EX_{DELAY}+BD condition and matching temporal locus for INT_{DELAY}+BD condition were included. As expected, the blood lactate level increased significantly and effect not observed across the same time interval for individuals not exposed to exercise.

Interpretation of plasma BDNF level was complicated because the BDNF level did not significantly changed with exercise but was significantly decreased in the INT_{DELAY}+BD condition. This result was not congruent with the previous studies that reported an increased BDNF level with an acute exercise bout (Winter et al., 2007). One possible reason why BDNF level change different from reports in the literature might be related to the time duration that BDNF remains elevated in the peripheral blood after exercise. Although it has been reported that the blood BDNF concentration remains elevated for 30 minutes after a moderate exercise bout (Zoladz & Pilc, 2010), there is considerable debate as to the stability of this finding. According to Vega et al., (2006), blood BDNF level was rapidly decreased and returned back to the level prior to an exercise bout in approximately 10 minutes after the time point when subjects reported being physically exhausted (Rojas Vega et al., 2006).

Alternatively, the elevated BDNF level during exercise was no longer elevated after 15 min following exercise (Skriver et al., 2014). Considering our experiment protocol involved three minutes of cool down after the termination of resistance applied cycling and the existence of at least 5 minutes delay until the second blood draw procedure, it is possible that the elevated blood BDNF level during exercise had already returned close to pre-exercise level at the moment of second blood draw.

Since the change of blood BDNF level and change of performance in sequence learning did not support the proposed hypothesis, correlation analysis that considered potential mediating variables were conducted. These includes physiological measurements (blood lactate level, plasma BDNF level), motor sequence learning (changes in speed and changes in error rate), individual fitness level derived from the performance of graded exercise test (VO_2 Max and Heart rate recovery ratio), and categorical independent variables (Gender, Condition, and Year data collected) was performed in order to verify possible existence of moderating factors. The result of this analysis revealed that an individual's base fitness level was associated with the change in error rate. This result indicated that there was a difference in participants' base fitness level between experimental groups. Moreover, a factor that correlated with the BDNF level change and participants' base fitness level most significantly was the year data collected. Since neuronal uptake level of BDNF is related to level of activity and this high activity in cells can increase both their uptake and transcription of BDNF, the base fitness level difference between groups might be an important contributor as to why the performance of sequence learning task in the Experiment 2 did not match the results of Experiment 1.

There was a significant group difference in the individual's base fitness level between condition and the year data collected, and the base fitness level of subjects was a significant influence on the BDNF level change. In other words, it is possible that the subjects with higher base fitness level tends to be less influenced by the effect of an acute exercise bout provided in the present study and the difference in base fitness level of the subjects between condition and the year the data was collected might be a reason contribute to complicated profile observed in Experiment 2. However, there was no significant relationship between the BDNF level change and offline performance improvement observed. It is interesting to note that Mang et al.,(2014) has also reported that a single bout of exercise, while promoting implicit motor sequence learning, these improvements were not correlated with exercise induced BDNF level change, a result congruent with the relationship between BDNF level change and performance improvement observed in Experiment 2.

In summary, the change of the blood lactate level and plasma BDNF level were changed as a result of acute exercise bout but those changes were not related to the offline improvement. Possible reasons might include but are not limited to (a) higher plasma BDNF level that was observed at first blood draw for no exercise group (INT_{DELAY}+BD) indicating no exercise group have had higher plasma BDNF concentration during the interval after initial target sequence training until first blood draw, a period during which consolidation of target sequence occurred. (b) A higher BDNF level for INT_{DELAY}+BD group likely returned to baseline which would again be higher for the 24 hours after the initial practice that might contribute to offline gain. Despite the speculation as to why the INT_{DELAY}+BD showed evidence of offline improvement, one cannot ignore the lack of relationship between BDNF and offline improvement for the EX_{DELAY}+BD condition. These data suggest BDNF upregulation is not responsible for offline improvement.

CHAPTER IV

EXPERIMENT 3

Introduction

To complete the extension of the present work to that of Roig et al.(2012), Experiment 3 involved changing the temporal location of an acute exercise bout from that used in Experiment 1 and 2 to target the fast learning as opposed to slow stage of learning (Hauptmann & Karni, 2002). Specifically, the acute bout of exercise was completed prior to rather than after exposure to practice of the target motor sequence. Roig et al.,(2012) indicated that exercise prior to learning had positive influence on visuomotor tracking skill improvement, although the improvement was relatively smaller than observed for the group that experienced an exercise bout after practice with a target sequence. Given that emerged from Experiment 1 was consistent with this finding, it is important to revisit this issue. Specifically, in Experiment 1 it was observed that the rate of improvement for the interfering task for the EX_{DELAY} exercise condition exhibited an improvement in performance suggesting that exercise might act in a proactive manner to facilitate performance (i.e., enhance the fast stage of motor learning). In Experiment 1, delayed tests for the interfering task were not included since the focus was on offline benefits for the target task. As such it is not known if the impact of the exercise at this temporal location provides just a temporary arousal change that influences performance during training or a more permanent impact that is manifest as an offline gain. Based on the findings of Roig, et al., (2012) this benefit is assumed to be a temporary effect likely due to increased arousal. It is possible however that the placement of an acute bout of exercise may exert a more permanent effect for the motor sequence task (i.e., on both fast and slow stage of motor learning) used in the present work than observed for a visuomotor tracking task, which is more stimulus driven perceptual motor task (Roig et al., 2012).

To assess this question, individuals were assigned to a condition that was similar to the $TEST_{IMM}$ condition in Experiment 1 with exercise administered prior to initial practice with the target sequence ($_{PE}TEST_{IMM}$: post exercise extended practice) condition which is an extended practice condition that three additional trials followed immediately after the initial training. A second condition referred to as EX+CONTROL condition involved the presentation of an acute bout of exercise prior to practice of the target motor sequence and three trials of retention test after 24-h delay.

Method

Participants

A total of twenty-four (24) individuals served as participants in Experiment 3. Participation in this study fulfilled a research requirement for an undergraduate class. The participants had no prior experience with the experimental task and were informed of the specific purpose of the study on arrival at the laboratory. Informed consent and Physical Readiness Checklist approved by the Institutional Review Board for the ethical treatment of experimental participants at Texas A&M University were obtained prior to any participation in the experiment.

Tasks

Graded exercise test

Procedures for the graded exercise were the same as those used in Experiments 1 & 2.

Acute exercise bout

Procedures for the acute exercise bout were the same as those used in Experiments 1 & 2.

Motor sequence tasks

All participants performed a target sequence, 4-1-3-2-4, on a standard PC keyboard using the V, B, N, M keys where "1" was the leftmost key (i.e., V key) and "4" was the rightmost key (i.e., M key) with their non-dominant hand. In addition, all individuals in Experiment 3 performed additional 3 trials of target sequence practice 24 hours (EX+CONTROL) after or immediately after ($_{PE}TEST_{IMM}$) completion of initial target sequence training.

Procedures

Prior to participation in the experiment all participants completed an informed consent. The general sequence of events that was followed by each experimental condition is depicted in Figure 13. Specifically, 48 hours prior to any practice with the target motor sequence all individuals performed a graded exercise test. At least 48 hours later participants were required to return to the laboratory to complete an acute bout of exercise and target sequence practice. A practice trial consisted of repeatedly executing the target sequence for 30 seconds followed by 30 seconds of rest. Twelve 30 seconds practice trials of the target sequence was completed by each participant. Prior to beginning practice with the target task all participants completed an acute bout of exercise in a manner described previously.

Following completion of the initial training individuals completed three 30 seconds test trials after a pre-determined time delay based on their experimental group. For the participants in the EX+TEST$_{IMM}$ condition an additional three trials of the target task was completed immediately after practice is complete. The participants in the EX+CONTROL condition experience three test 24-h after the completion of the initial practice of the target sequence.

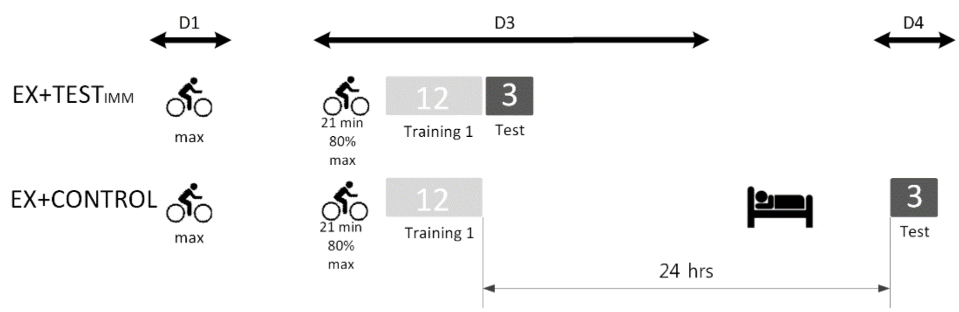

Figure 13 Illustration of the 3 experimental conditions in Experiment 3. All 3 conditions in Experiment 3 consist of an acute exercise bout, a target sequence practice immediately after an exercise bout, and 3 trials of test session that was provided immediately (EX+TEST$_{IMM}$) or 24 hours (EX+CONTROL) after completion of target sequence practice.

For all trials with the target motor sequence, *speed*, defined as the correct number of sequences executed in 30 seconds and, *error rate*, defined as the percentage of erroneous key presses in 30 seconds were recorded. Speed and error rate were subsequently used as the primary dependent variables of interest. For the present work, offline learning was defined as a performance improvement that is larger than that observed for the mean performance observed for individuals that experienced trials 13-15 immediately following trials 1-12 (EX+$TEST_{IMM}$ condition). This expectation is based on using speed as the dependent variable which is expected to increase with practice. Obviously, the reverse effect is anticipated for accuracy which is expected to decrease with practice. If exercise prior to practice of the target sequence has a learning effect we expect the test performance in the EX+CONTROL condition to show significant improvement compare to the performance of training session and the change score of offline improvement would be similar to that observed from the CONTROL condition in Experiment 1. If exercise prior to practice of the target sequence has an arousal effect only we expect the test performance in the EX+CONTROL condition to show significant improvement but its' change score would be similar to that observed from the $TEST_{IMM}$ condition in Experiment 1.

Result

Demographics of the EX+CONTROL and EX+$TEST_{IMM}$ exercise conditions

Table 4 displays demographics and performance data from the graded exercise test conducted on Day 1 for participants in the EX+CONTROL and EX+$TEST_{IMM}$ conditions. Each variable was submitted to single sample t-tests that revealed no significant differences between the conditions for any variables.

Condition	EX+CONTROL	EX+TEST$_{IMM}$
Age	20.3±0.21	22.5±1.58
Max Heart Rate	181±2.99	179.25±4.79
Heart Rate Recovery Ratio in 2 minutes	24.02±1.79	19.58±1.93
VO_2 Max	32.36±1.01	31.13±0.98

Table 4 Demographics of graded exercise test result for EX+CONTROL and EX+TEST$_{IMM}$ condition.

Performance of target sequence training with preceding exercise bout (Experiment 3: EX+TEST$_{IMM}$, EX+CONTROL) vs without preceding exercise bout (Experiment 1: CONTROL, TEST$_{IMM}$)

The performance of initial target sequence training in Experiment 3 (EX+TEST$_{IMM}$, EX+CONTROL) were analyzed along with the two control conditions in Experiment 1 (CONTROL, TEST$_{IMM}$). These data were subjected to a 2 (Exercise: Presence, Absence) x 2 (Test delay: 0, 24 hours) x 12 (Trial: 1-12) analysis of variance (ANOVA) with repeated measures of the last factor. Formal analysis of speed revealed a significant main effect of Trial, $F(11, 440) = 65.1$, $p<.01$ only. This suggested that the speed improved with practice. The analysis of error rate revealed a significant main effect of Trial, $F(11, 440) = 11.38$, $p<.01$ and Exercise x Trial interaction, $F(11, 440) = 4.02$, $p<.01$. In order to verify the significance of changes in dependent variables within each condition, simple main effect analysis on first three trials of target sequence practice for each condition collapsed across Test delay was performed. The simple main effect analysis revealed significant trial main effect for speed in conditions without

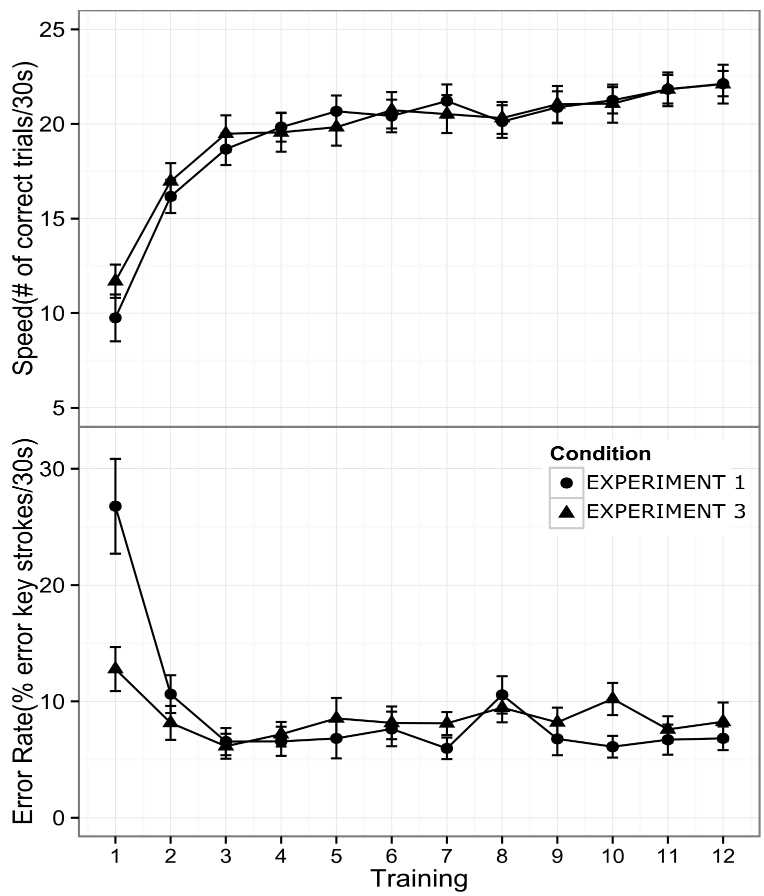

Figure 14 Mean peformance speed (top panel) and error rate (bottom panel) of target sequence training for individuals in Experiment 3 (Triangle) and CONTROL and $TEST_{IMM}$ conditions in Experiment 1 (Circle) are displayed.

preceding exercise bout, $F(2, 84) = 21.22$, $p<0.01$ and with preceding exercise bout, $F(2,84) = 15.66$, $p<0.01$. All four conditions in both Experiment 1 and 2 exhibited significant change in speed. For error rate, main effect in conditions without preceding exercise bout exhibited significant change, $F(2,84) = 23.55$, $p<0.01$ whereas conditions with preceding exercise bout did not exhibit significant difference, $F(2,84) = 2.50$, $p=0.08$. This indicates that the error rate for the conditions in Experiment 3 was significantly faster from first three trials of target sequence training compare to that for the conditions in Experiment 1 which also indicates an acute exercise bout immediately before target sequence training provided benefit on acquisition of the sequence from the beginning (see Figure 14).

Assessment of offline learning: post exercise target sequence training (Experiment 3: EX+TEST$_{IMM}$, EX+CONTROL) vs target sequence training with no preceding exercise (Experiment 1: CONTROL, TEST$_{IMM}$)

In order to evaluate the offline learning of post-exercise training after 24 hours delay, experimental condition with same 24 hours delay but no preceding exercise (CONTROL in Experiment 1) was compared together along with extended training conditions from both Experiment 1 and 3. These data were submitted to a 2 (Exercise: Presence, Absence) x 2 (Test delay: 0, 24 hours delay) x 2 (Phase: Practice, Test) ANOVA with repeated measure on the last factor. Figure 15 depicts mean speed (top panel) and error rate (bottom panel) for the end of practice and test phases as a function of Test delay. Analysis of mean speed revealed a significant Phase main effect, $F(1,40) = 25.88$, $p<.01$ and Test delay x Phase interaction, $F(1,40) = 6.44$, $p<0.05$. This Phase main effect was a result of greater mean speed during test ($M = 24$ sequences/30 seconds) compared to that observed during practice ($M = 22.2$ sequences/30 seconds). The significant Test delay x Phase interaction indicates that the change of speed between

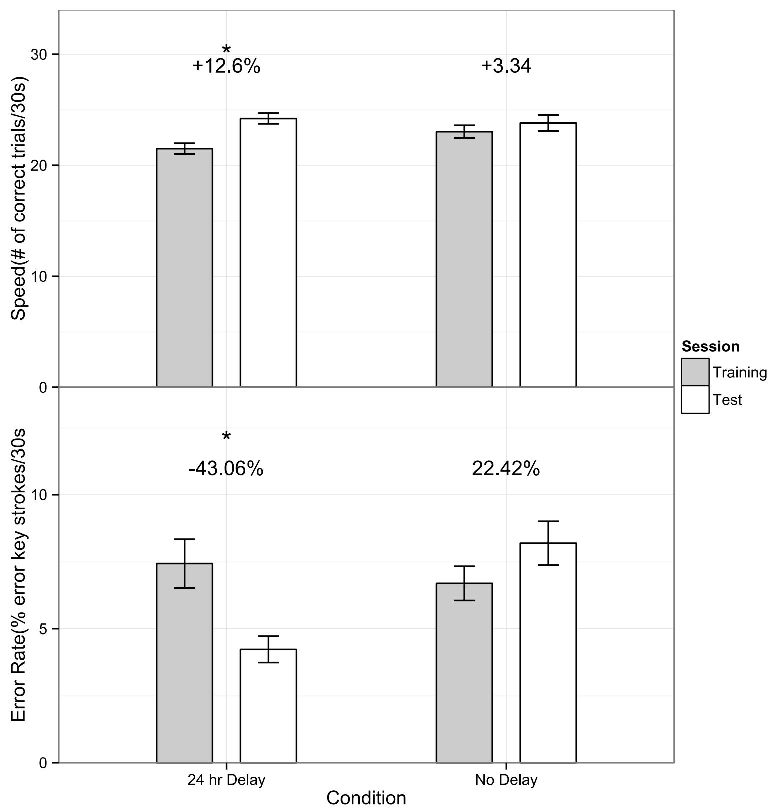

Figure 15 Mean speed (top panel) and error rate (bottom panel) of target sequence training (grey bars) and delayed retention test (white bars) of target sequence of CONTROL and EX+CONTROL conditions collapsed as 24 hours Delay condition and EX+$TEST_{IMM}$ and $TEST_{IMM}$ conditions collapsed as no delay conditions. Significant offline improvement for speed and error rate were observed from the conditions with 24 hours delay presumably involving sleep (CONTROL, EX+CONTROL) and no significant effect of preceding acute exercise bout was observed.

target sequence practice and retention test were different with speed being greater when the test occurred after 24 hours rather than immediately. The analysis of error rate also revealed a significant Test delay x Phase interaction, $F(1,40) = 9.95$, $p<.01$ and this interaction was similar to that observed for speed specifically error rate changes between the conditions with 24 hours delay and extended training groups.

In order to verify the significance of changes in dependent variables within each condition, simple main effect analysis for each level of condition collapsed across exercise was performed. The simple main effect analysis of mean speed revealed significant phase main effect for conditions with 24 hours delay, $F(1,42)=12.62$, $p<.01$ and for error rate, significant main effect for 24 hours delay, $F(1,42) = 10.32$, $p<0.01$ were observed. Both CONTROL and EX+CONTROL groups exhibited significant improvement in speed and error rate as expected whereas TEST_{IMM} and EX+TEST_{IMM} groups exhibited no significant differences. This indicates that the effect of acute bout of exercise immediately before training only influenced motor sequence learning during initial acquisition being faster than no preceding exercise condition.

Discussion

The purpose of the Experiment 3 was twofold. First, to examine the proactive effect of acute bout of exercise on following motor sequence (Roig et al., 2012). Second, to examine if pre-practice exercise influence resultant offline benefit. In both cases, relative performance improvement was observed when an exercise bout was provided prior to initial training. Among four conditions in the Experiment 1 experienced alternative sequence practice**,** EX_{DELAY} condition which temporal locus of an acute exercise bout was immediately prior to interference practice exhibited relatively faster and more accurate performance from the beginning of an alternative sequence practice provided 2 hours after completion of target sequence training. In Experiment 3, two conditions were conducted to identify this improvement observed for interference practice was real learning effect caused by a preceding exercise bout or a temporary influence as a result of an arousal. Both groups experienced an intensity controlled acute exercise bout prior to the target sequence training and the duration of delay between target sequence training and test session was varied to 24 hours or immediately after.

Initial performance in target sequence training in Experiment 3 did not show any significant difference in speed compared to the performance in target sequence training in Experiment 1. However, the accuracy as an error rate of first two trials were significantly lower than that of control groups in Experiment 1. Since, this lower error rate converged to certain level after third trial consistent with the control group, whether this initial benefit was maintained as a learning effect with a resultant offline improvement was not clearly confirmed. Although EX+CONTROL condition exhibited significantly lower error rate compare to the other three groups (CONTROL, $TEST_{IMM}$, and EX+$TEST_{IMM}$), only observed significant interaction between Condition x Phase indicates preceding acute exercise bout has no effect on offline improvement. Therefore, it can be concluded at the moment that proactive benefit of acute exercise bout on following motor sequence learning might due to an arousal that helps initial acquisition of sequence learning but this initial benefit was not maintained to further offline improvement.

Since this initial benefit was only observed from accuracy and not from speed, it also could be argued that proactive benefit of acute exercise bout only applies for certain features of sequence learning. Self-paced finger task with short sequences such as that used in the present study which performance is represented by speed can be considered as skill tasks and it is effector-specific. On the other hand, the task with long embedded sequence which performance is represented by acquisition rate and reaction time against stimulus can be considered as effector-independent (Krakauer & Shadmehr, 2006). The sequence learning task used in the present study is skills task as previously defined and it has effector-specific aspect. However, the accuracy measure represented by error rate can be thought as effector-independent aspect since a subject required to react against perceived error based on acquired knowledge of the task. Hence, proactive benefit of acute exercise bout is limited to effector-independent aspect of sequence learning and moreover, only for the initial acquisition phase.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

In the present study, the effect of an acute bout of exercise on motor sequence learning on both fast (Experiment 3) and slow stage (Experiment 1 and 3) of learning had been investigated (Karni et al., 1998). Slow stage learning is reflected in delayed, incremental gains associated with a practice or an additional trainings over time and an optimization of long-term memory on acquired knowledge during delay which involves memory consolidation process. On the other hand, fast stage learning mostly occurs during practice session and characterized by rapid improvements in performance (McGaugh, 2000; Walker, 2005).

In Experiment 1, we evaluated the effectiveness of an acute exercise bout as a means of protecting memory for a previously learned procedural skill from interference from additional practice of alternative sequence. Offline improvement observed in a CONTROL condition decreased when alternative sequence practice was provided two hours after completion of initial target sequence practice (INT_{DELAY}) and the loss of offline gain was even greater if alternative sequence practice followed immediately after target sequence practice (INT_{IMM}). This indicates that stabilization of memory on acquired information begins immediately after initial training which in turn will become consolidated to have resistance to upcoming interference (Doyon et al., 2009).

Korman et al. (2007) used 90 min nap between initial practice and alternative sequence practice to reduce the effect of interference and we provided an acute exercise bout between the initial training and alternative sequence practice to achieve similar effect to that of nap in Korman's study. During the 2 hour time window of delay, 30 min of acute exercise bout was inserted either immediately after target sequence practice (EX_{IMM}) or close to the end of 2 hours window immediately before alternative sequence practice (EX_{DELAY}). The result indicated that an acute exercise bout provided at the end of the two hour window had the biggest effect on protecting a motor memory from interference from alternative sequence training. An exercise bout provided immediately after initial training exhibited no protective effect against additional target sequence practice.

In order to explain the benefit of an acute exercise bout on learning acquired either before or after exercise, we used the term 'retroactive' or 'proactive' benefit. However, this analogy needs to be redefined since an acute exercise bout followed preceding sequence learning cannot influence the initial acquisition of learning. Following practice, acquired knowledge is stabilized through consolidation. An acute exercise bout following practice presumably accelerates consolidation. This assumption was confirmed Experiment 1 illustrated by the EX_{DELAY} condition exhibiting offline improvement in speed whereas exercise immediately followed target sequence practice did not provide this same offline benefit. Moreover, considering the performance of the EX_{IMM} condition looked similar to that of INT_{IMM} condition, the immediate exercise bout appears to act as a source of interference thus impeding some initial stabilization of acquired memory to occur before the exercise can be useful. An offline improvement pattern of EX_{IMM} condition appears to look similar to that of INT_{DELAY} condition even though the temporal locus of an exercise bout was similar to the temporal locus of alternative sequence practice of INT_{IMM} condition. This indicates that a certain amount of time is required for consolidation of acquired knowledge in order to become resistant against interfering activity (i.e., alternative practice or exercise). In addition, even though an exercise bout in EX_{IMM} condition acted as interference that prevented consolidation of initial target sequence practice, an exercise bout did not completely eliminate the offline improvement that an alternative target sequence practice in INT_{IMM} did. While an alternative target sequence practice in INT_{IMM} interfered directly with the previously learned sequence, an exercise bout might interfere relatively indirectly by demanding a cognitive resource which resulted in decreased improvement.

Experiment 2 was to confirm the relationship between the effect of acute exercise bout on motor sequence learning and accompanying physiological changes such blood lactate level and plasma BDNF level. It was hypothesized that plasma BDNF level, which has been reported to influence memory consolidation (Bramham & Messaoudi, 2005; Skriver et al., 2014), would be increased with an exercise bout. Finding from Experiment 2 have indicated that the plasma BDNF level was not significantly increased with an exercise bout and decreased significantly without exercise. Previous studies have reported that plasma BDNF level can return to pre-exercise level approximately 10 minutes after exhaustion if the intensity of exercise was moderate (Rojas Vega et al., 2006; Skriver et al., 2014). Offline improvement was observed with exercise group (EX_{DELAY}+BD) but this improvement was not related to an increase in BDNF. In addition, the INT_{DELAY}+BD condition revealed an offline improvement. Additional analysis conducted indicated that this result may be due to the participants′ base fitness level which may have mediated the levels of plasma BDNF level for each individual.

According to person product-moment correlation analysis performed between factors of motor sequence learning and other physiological parameters, the participants′ base fitness level and their performance were biased by year data collected. Especially, participants′ base fitness level indexed by recovery heart rate was highly correlated with base plasma BDNF level, suggesting that a participant with healthier cardiovascular condition tends to have overall higher plasma BDNF level. Even though significant different base plasma BDNF level and blood lactate level between the two experimental conditions were observed these results were not associated with significant offline performance gain in Experiment 2. Therefore, the effect of acute bout of exercise for offline improvement was confirmed in Experiment 2 but BDNF was not revealed as a key determinant of the observed offline performance improvement.

Experiment 3 was conducted in order to clarify whether an acute exercise bout can influence a fast stage learning (Karni et al., 1998). This possibility was reported by Roig et al., (2012) and also was observed from the performance of participants in the alternative sequence practice of EX_{DELAY} condition in Experiment 1. Participants in EX_{DELAY} condition revealed relatively superior performance of the alternative sequence and also they exhibited highest offline improvement among other conditions in Experiment 1. Therefore, in Experiment 3, an acute exercise bout was provided immediately before initial target sequence training and retention tests were administered at either 24 hours or immediately after completion of target sequence practice.

Significant offline improvement in speed and error rate when the test occurred after 24 hours delay only. Therefore, preceding acute exercise bout did not benefit the offline improvement of speed nor error rate.

On the other hand, significantly lower error rate was observed from the very first two trials of target sequence training for individuals that experienced an acute exercise bout prior to target sequence practice. This indicates possibility of acute exercise bout influencing initial acquisition in motor sequence learning. However, given no significant offline improvement for error rate was observed, the effect of preceding exercise bout on following motor sequence learning may impart a small influence on the fast stage but no influence on the slow stage of motor learning.

The present series of experiments have some limitations. One was failure to show the relationship between plasma BDNF change and offline performance improvement. This may have been due to huge group difference in participant's baseline BDNF level and fitness level despite random assignment of participants to conditions. Moreover, the present study did not consider the independent effect of acute exercise bout distinct from known effects of sleep. For example, BDNF levels have been reported to change during sleep. Therefore, further investigation of effect of acute exercise bout with accompanying physiological change should be conducted using a paradigm in which any potential role of sleep is removed.

In the present work, we modified a paradigm that included a period of sleep which has been argued to be important for offline gain for explicitly learned motor sequences such as those used in the present study (Walker, Brakefield, Hobson, et al., 2003; Walker, Brakefield, Seidman, et al., 2003). However, in using this approach it is possible that processes induced via exercise may have interacted with those encouraged during sleep and as such the role of exercise per se, for the demonstrated memory improvement, may have been masked. Clearly, assessing the specific role of exercise, independent of sleep, for enhancing procedural skill learning is an important issue to directly addressed in future efforts. Since key interest of the present study was using an acute exercise bout to combat against interference from alternative sequence practice which decreases offline improvement during sleep, sole effect of acute exercise bout separated from the effect of sleep was not investigated. Therefore, additional study would be inserting acute exercise bout in between target sequence training and retention test of target sequence without overnight's sleep in order to examine whether an exercise bout can have offline benefit beyond mere stabilization and consolidation of acquired memory. If BDNF is really a mediating factor as reported by previous studies, an acute exercise bout causing elevated BDNF level would have similar effect of offline improvement to that of overnight's sleep.

In summary, an acute exercise bout can help faster consolidation of acquired motor sequence learning and in turn influence offline improvement for both speed, effector specific aspect of motor learning, and error rate, effector independent aspect of motor learning, if it was provided after certain amount of time affording some initial stabilization to occur. On the other hand, the effect of an acute exercise bout on fast stage learning is due to arousal whose influence is limited to acquisition of effector independent aspect of motor learning. Exercise presented prior to training imparts no positive impact on the resultant offline gains.

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