UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ELECTROCORTICAL ACTIVITY DIFFERENCES BETWEEN SINGLE AND DOUBLE LEG GRADED EXERCISE CYCLING TEST: AN EEG STUDY

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

By

CAMERON DALE OWENS
Norman, Oklahoma
2020

ELECTROCORTICAL ACTIVITY DIFFERENCES BETWEEN SINGLE AND DOUBLE LEG GRADED EXERCSIE CYCLING TEST: AN EEG STUDY

A THESIS APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY THE COMMITTEE CONSISTING OF

Dr. Rebecca D. Larson, Chair

Dr. Daniel J. Larson

Dr. Jeremy M. Kellawan

TABLE OF CONTENTS

Acknowledgements…… **vii**

Abstract…… **viii**

Chapter I: Introduction…….... **1**

Significance…… 2

Purpose/Research Question/Hypotheses…… 3

Delimitations/Limitations…… …… 4

Assumptions/Operational Definitions…… 5

Chapter II: Literature Review…… **6**

2.1: Motor Cortex Anatomy and Function…… 6

2.2: EEG Use and Accessibility During Whole-Body Movements…… 8

2.3: EEG Recording During Double Leg Cycling…… 9

2.3a: Constant Load…… 10

2.3b: Graded Exercise…… 13

2.4: Single Leg Cycling……..…… 15

2.5: Bilateral Asymmetry in Multiple Sclerosis…… 16

Gaps in Literature…… . 18

Summary…… . 19

Chapter III: Methodology…… **21**

Participants/Sample…… 23

Research Design…… 22

Measurement Tools/Procedures/Procedure Development…… . 23

Reliability and Validity…… 24

Internal and External Validity Threats…… …… …… 25

Minimization of Threats/Data Collection Procedures/Procedures for Data Management…. 26

Data Analyses/Data Analysis Software/Previous Literature Data Collection…… 27

Chapter IV: Results…… **29**

a) Subject Characteristics…… 29

Table 1: Subject Characteristics Pre-Post…… 30

b) EEG Power in θ, α, β, γ…… 30

i. Rest 1 vs. Rest 2…… 30

Table 2: Whole Brain EEG Power…… 31

Table 3: C4 EEG Power…… 32

Table 4: Whole Brain and C4 Power Difference Scores…… 32

Figure 1: Whole Brain and Right Motor Cortex Power Difference Scores…. 33

i. Rest 1 vs. Stage 1…… 35

Table 5: Subject characteristics Pre-Stage 1…… 36

Table 6: Whole Brain EEG Power…… 36

Table 7: C4 EEG Power…… 37

Table 8: Whole Brain and C4 Power Difference Scores…… 37

Figure 2: Whole Brain and Right Motor Cortex Power Difference Scores …… 38

c) Case Studies…… 40

i. Case Study A…… 40

Figure 3: Subject A Whole brain and C4 theta power values before, during, and after GXTs in SL and DL…… 41

Figure 4: Subject A Whole brain and C4 alpha power values before, during,

and after GXTs in SL and DL…… 42

Figure 5: Subject A Whole brain and C4 beta power values before, during, and after GXTs in SL and DL…… 43

Figure 6: Subject A Whole brain and C4 gamma power values before, during, and after GXTs in SL and DL…….. 44

ii. Case Study B…….. 45

Figure 7: Subject B Whole brain and C4 theta power values before, during, and after GXTs in SL and DL…… 45

Figure 8: Subject B Whole brain and C4 alpha power values before, during, and after GXTs in SL and DL…… 46

Figure 9: Subject B Whole brain and C4 beta power values before, during, and after GXTs in SL and DL…… 47

Figure 10: Subject B Whole brain and C4 gamma power values before, during, and after GXTs in SL and DL…… 48

d) Summary……. 48

Chapter V: Discussion…… 50

a) Whole Brain: Case Studies, R1-R2 and R1-S1…… 50

b) Motor Cortex: Case Studies, R1-R2, and R1-S1……. 51

c) Prefrontal Cortex: R1-R2, and R1-S1…… 53

d) *Exercise and Long-term Potentiation (LTP)*…… 55

e) Multiple Sclerosis and Exercise …… 56

f) Limitations…… 57

Chapter VI: Conclusion…… 58

References…… 61

Appendix…… 66

ACKNOWLEDGEMENTS

I would like to start by thanking Dr. Rebecca Larson for allowing me to participate in her lab as an undergraduate research assistant. You helped me find my initial interest in exercise physiology and multiple sclerosis research. Because of your guidance through these three years as an undergraduate and graduate student, I have a strong passion to continue doing exercise research in the area of multiple sclerosis and neuroscience. I would also like to thank Dr. David Lantis for allowing me to be so hands on during his dissertation project. This allowed me to see what conducting research was like and was a huge factor in my decision to pursue this degree. I would also like to thank my committee members, Dr. Daniel Larson and Dr. Jeremy Kellawan, for their input on this project

Grant Chesbro and Brian Pribble, you were always there to answer any questions that I had during these two years and I greatly appreciate your leadership in the lab, as well as your immense help with this project. Braylon Warrior and Morgan Delp, these two years in the lab would truly not have been as enjoyable without the two of you and I greatly appreciate both of your support and the great times we've had. I would also like to thank Travis Richardson for piloting this study countless times. Going through this process together made this project much more enjoyable. To my mom, Parthena, dad, Roger, and girlfriend, Jamie, your continued encouragement throughout my academic endeavors has been essential to my success and I would not be here without your support.

Lastly, I would like to thank Dr. Michael Wenger, Lisa De Stefano, and Melody Reese. Thank you all for your immense help during this project. Without you all this would not have been possible.

ABSTRACT

Single leg (SL) cycling has been reported to show increases in cardiac output, neuromuscular activity, power output, and oxidative molecules when compared to double leg (DL) cycling. While these peripheral variables have been investigated, to date there is no published data regarding the response of the central nervous system (brain activity) between DL and SL cycling. Previous research has conducted electroencephalography (EEG) measures during DL cycling in a variety of protocols. More research needs to be conducted to elucidate how the brain responds to a graded exercise test (GXT). With the previous SL vs. DL peripheral data (during cycling), understanding the CNS response during a GXT and at max exercise is a step toward understanding if peripheral adaptations are centrally mediated in SL cycling. In addition, it could be a way to separate out the occurrence of central vs. peripheral fatigue. **Purpose:** The purpose of this study was to examine different conditions (SL cycling and DL cycling) and assess EEG activity in the whole brain and motor cortex before, during and after GXT during SL and DL cycling. **Methods**: 26 subjects were recruited to participate in this study. After inclusion criteria was met, and preprocessing and processing of data was complete, 8 subject's data remained usable for analysis. All subjects completed two GXT's, one SL and one DL. During SL testing, the workload started at 0.5W/kg body weight (BW) and increased 0.25W/kgBW every minute until volitional fatigue. DL testing workload started at 1W/kgBW and increased 0.5W/kgBW every minute until volitional fatigue. **Results:** Pre (R1) to post exercise (R2) in whole brain showed significant increases for DL cycling in theta (θ) ($p = 0.035$), alpha (α) ($p = 0.012$), beta (β) ($p = 0.0006$), and gamma (γ) ($p = 0.013$) and during SL cycling in α ($p = 0.023$), β ($p = 0.049$). Large effect sizes (ES) for whole brain analysis were seen in γ ($d = 0.96$, SL vs. DL = 0.00 ± 0.02 vs. 0.02 ± 0.02, $p= 0.15$), and β ($d=1.28$, SL vs. DL= 0.02 ± 0.01 vs. 0.03 ± 0.02 $p=0.09$). Significant increases for DL and SL cycling from R1 to stage 1 (S1) were seen in DL theta (θ) ($p= 0.025$) and SL theta (θ) ($p= 0.047$). DL cycling elicited significant increases in right motor cortex (C4) activity from R1-R2 in β ($p= 0.021$), γ ($p= 0.019$). Notable ES were seen between SL and DL from R1-R2 in C4: θ ($d= 0.7$, SL vs. DL= 0.01 ± 0.06 vs. 0.06 ± 0.07, $p= 0.24$) and β ($d=0.66$, SL vs. DL= 0.01 ± 0.01 vs. 0.02 ± 0.01, $p=0.16$). Significant increases for DL cycling from R1-S1 in C4 were seen in γ ($p= 0.03$). A medium ES in C4 was found between SL and DL from R1-S1 in θ ($d=0.61$, SL vs. DL= 0.10 ± 0.14 vs. 0.17 ± 0.10, $p=0.27$). **Conclusion**: Our results are consistent with previous literature indicating elevated power values at rest. Gamma and beta increases in DL compared to SL whole brain analysis indicated by a large ES is expected due to beta and gammas proposed role of increased cortical activity. Theta activity was the only significant increase in both groups in whole brain from R1-S1. These results are consistent with previous work and allows us to infer theta's possible role in initiating motor movement. We did see greater neural activity in C4 during DL rather than SL cycling. This allows us to infer that non-CNS mechanisms that have been previously reported are possibly activating the muscles during SL cycling to a greater extent, regardless of decreased C4 activity. Our results indicate that DL possibly has greater activation in the right motor cortex compared to SL cycling. Future research should assess DL vs SL with a larger sample size during recumbent maximal cycling to explore the activation of the peripheral and central nervous system. This could allow for clarity of the central and peripheral fatigue phenomena.

CHAPTER I: INTRODUCTION

Electroencephalography (EEG) has recently become a common method for analyzing cortical activity during exercise tests (Perrey & Besson, 2018). Single leg cycling, which has shown numerous beneficial neuromuscular adaptations compared to double leg cycling (Abbiss et al., 2011) (MacInnis et al., 2017), has never been done while measuring electrocortical activity. Understanding whether double leg or single leg cycling taxes the central nervous system (CNS) to a greater extent during exercise is pertinent to understanding whether previously reported neuromuscular single leg adaptations (neuromuscular activity and power output) are centrally or peripherally mediated. The importance of this falls on the CNS diseased patients who lose and have limited function in their periphery. Multiple sclerosis (MS), an immune-mediated disease which degenerates myelin in the CNS, has shown asymmetry in strength, O_2 uptake, and workload between limbs while cycling (Larson et al., 2013). If single leg cycling neuromuscular adaptations are not centrally mediated, these patients could be able to benefit from the protocol to limit their asymmetries and regain increased function. However, exercise has been shown to induce long-term potentiation (LTP)(enhancement of synaptic connection and increased excitatory postsynaptic potentiation) in human and animal models and regenerate myelin in mice (Singh et al., 2014) (Jensen et al., 2018). If single leg cycling is centrally mediated, MS patients could benefit from these previous CNS findings, as well as the neuromusculature

Previous double leg cycling tests measuring electrocortical activity have mixed results and protocols, with some studies using graded exercise tests (GXT) (increasing workload while maintaining the same pace/revolutions per minute (rpm), and others using constant load cycling (Enders et al., 2016)(Hilty et al., 2011)(Ludyga et al., 2015)(Brümmer et al., 2011)(Bailey et al.,

2008). Literature that has reported decreased cortical activity have attributed this to central fatigue (indicator of central inhibition for continued movement during exercise) (Ludyga et al., 2015)(Hilty et al.,2011). However, other research has shown increases until the end of exercise in cortical activity (Bailey et al., 2008)(Enders et al., 2016)(Brümmer et al., 2011). This could possibly be due to exercise protocol with decreases in activity being in longer duration cycling studies (Ludyga et al., 2015)(Hilty et al.,2011) and increases in activity being in shorter duration, higher intensity cycling (Bailey et al., 2008)(Enders et al., 2016)(Brümmer et al., 2011). With the limited research in the area of EEG and cycling, we sought to contribute to the field in the current study by examining the CNS through EEG to assess whether there is increased or decreased activation in the CNS at the end of exercise when the periphery has ceased to allow for muscular contraction.

Significance

Peripheral physiological factors have been examined during single and double leg cycling in diseased and healthy population but central variables, such as electrocortical activity, has not been examined during single leg cycling tests (Abbiss et al., 2011) (MacInnis et al., 2017) (Larson, McCully, Larson, Pryor, & J.White, 2013). Examination of electrocortical activity via EEG comparing single vs double leg cycling will be important to determine the central nervous systems (CNS) response as well as provide a greater understanding of the neuromuscular peripheral adaptations previously reported.

The understanding of brain-body interaction can provide information as to whether the CNS and PNS have different activation times, as well as how the activity changes prior to volitional fatigue. In future studies, neurologically diseased populations, such as multiple sclerosis (MS), could be tested to determine whether there are hemispheric activity differences

that relates to subjects more affected limbs. If single leg tests are seen to increase CNS neuronal activity compared to double leg, this could be a steppingstone in showing that single leg cycling taxes areas of the CNS to a greater extent. This could be applicable for rehabilitation/disease maintenance in CNS diseased populations due to increasing CNS activation, and possible long-term excitatory signal transmission through synaptic connections (Purves, Augustine, & Fitzpatrick, 2001)(Singh et al., 2014).

Purpose

The purposes of this study were to investigate whether healthy individuals exhibited: (1) differences in electrocortical activity during single leg or double leg cycling; (2) increased electrocortical activity during single leg cycling in motor areas of the brain with increasing workloads at similar rates as seen during double leg cycling.

Research Questions

1. Are there differences in whole brain electrocortical activity during single leg and double leg cycling with increasing workloads?
2. Is there increased motor cortex electrocortical activity with increasing workloads in single or double leg cycling?
3. Are there differences in whole brain activity between single leg and double leg pre to post exercise?
4. Are there differences in motor cortex activity between single leg and double leg pre- to post-exercise?

Hypotheses

1. Double leg cycling will elicit increased whole brain EEG activity compared to single leg cycling.

2. There will be increased contralateral motor cortex EEG activity during single leg cycling compared to during double leg cycling.
3. Double leg cycling will elicit increased whole brain EEG activity post exercise compared to single leg cycling.
4. There will be increased contralateral motor cortex EEG activity post-exercise in single leg cycling compared to double leg cycling.

Delimitations

The delimitations for the following study were:

1. The findings of this study are applicable to healthy individuals between the ages of 18-35.
2. The findings of this study are applicable to whole body double leg and single leg cycling.
3. Individuals without asymmetric orthopedic limitations.
4. Individuals without multiple risk factors for cardiovascular diseases.
5. Individuals who are not current recreationally or competitively trained cyclist (cycling < twice per week)
6. Individuals without respiratory, cardiovascular, neuromuscular, neurological or psychiatric disorders.

Limitations

The limitations for the following study were:

1. The participants will be volunteers from the Norman and Oklahoma City areas and will not represent a true random sample
2. Testing will occur on multiple testing visits and daily fatigue and mental states will be variable between and within individuals.

Assumptions

The assumptions of the following study included:

1. Participants will give maximal effort for all single and double leg maximal GXTs.
2. Participants will provide accurate medical information and health history.
3. Participants will comply with the directions and guidelines provided prior to testing.

Operational Definitions

1. Electrocortical activity: Electrical activity in the cerebral cortex
2. Electroencephalography (EEG): Functional measurement of electrocortical activity (Perrey & Besson, 2018).
3. Electromyography (EMG): Functional measurement of neuromuscular activity (MacInnis et al., 2017).
4. Multiple Sclerosis (MS): Chronic and progressive autoimmune disease of the central nervous system (Larson, McCully, Larson, Pryor, & J.White, 2013).
5. Single leg cycling: Cycling with one leg while the contralateral leg is kept stable on an apparatus outside of the cycle ergometer.
6. Theta (θ) (4.00-7.99 Hz): increased activity with task complexity
7. Alpha (α) (8.00-12.99 Hz): increased activity= decreased cortical activity
8. Beta (β) (13.00-29.99 Hz): increased beta activity represents increased cortical activity
9. Gamma (γ) (30.00-80.00 Hz): increased gamma activity represents increased cortical activity

CHAPTER II: LITURATURE REVIEW

Introduction to Literature Review

Using EEG (electroencephalography) as a method of recording cortical changes during full body exercise, such as cycling, has been of increasing, but still limited use in research. There has been no published research measuring the difference in cortical activation via EEG between double leg and single leg cycling. The following literature review will cover areas of previous literature pertinent to this area of study of which there is no known research being conducted. The organization of this review will list subheadings (2.1-2.6) with the relevant topic indicating the information covered in the subsection. At the end of the literature review, gaps in research findings will be reviewed, as well as a summary of the information covered.

2.1: Motor Cortex Anatomy and Function

Voluntary stimuli must come from the cerebral cortex which can evaluate, plan, coordinate, and initiate motor movements. From this voluntary stimuli, alpha motor neurons innervate muscles, and spinal circuits integrate neuronal signals as well as facilitate reflex actions (Knierim, 2019).

The motor cortex is anterior to the central sulcus which includes the primary motor cortex and the precentral gyrus (Purves, Augustine, & Fitzpatrick, 2001). Anterior to the primary motor cortex is the supplemental motor area and the premotor cortex (Knierim, 2019). Activity in the motor cortex is involved in activations of muscles on the contralateral side of the body. Different areas of the motor cortex allow for innervation of particular spinal neurons which innervate muscles and facilitate movements of the body. The control of legs, feet and toes are represented as the most medial area of the motor cortex and do not occupy a large cross-sectional area (Purves, Augustine, & Fitzpatrick, 2001). Movements of the face and hand take up much more

space in the motor cortex due to the increased neuronal connection needed to control the fine/precise movements of these muscles (Knierim, 2019). The ability of the motor cortex to influence motor control on specific areas of the body is represented by a 'map'. This map shows anatomical locations through sulci and gyri on the motor cortex that control movement of specific body parts, as well as facilitating organized movements of different areas through neural circuitry of nearby motor regions (Purves, Augustine, & Fitzpatrick, 2001). A previous theory for cortical activity was neurons that control one muscle were all grouped together in the cortex and functioned to activate that muscle (through spinal innervation). Now it is largely accepted that stimulation of specific areas of the primary motor cortex doesn't simply activate single muscles, but rather activates coordinated movements of body parts (Knierim, 2019).

Neurons of the primary motor cortex fire about 5-100 milliseconds (ms) prior to initiating movement. This is due to the time it takes for signals of the cortex to reach alpha motor neurons, resulting in appropriate innervation and subsequent contraction of muscles. As previously discussed, the primary motor cortex encodes for proper organization of movement and not for individual muscle contraction. Keeping this in mind, it makes sense that the primary motor cortex also encodes for the overall force of organized movement but not the individual muscle force. Direction, extent and speed of movement is also coordinated by the primary motor cortex. These functions of the primary motor cortex are all regulated through mechanisms that influence inhibition and excitation of particular neurons/neuronal networks (Knierim, 2019). While the cortex controls voluntary motor action, the periphery does influence contraction through involuntary muscular, vascular and metabolic mechanisms ("How does the nervous system work?," 2016).

Other areas of the cerebral cortex are also important in motor control and movement through actions that do not directly control the execution of movement. The premotor cortex is involved in planning for movement and the supplementary motor area activates to a greater extent during movements that seem to be remembered. The supplementary motor area has also been seen to be activated while mentally visualizing movements without actually performing the movements. The prefrontal cortex is one of the main areas of the cerebral cortex that is involved in executive processing and is involved in motor control and movement through ensuring that movements are appropriate for desired behavior or action. The somatosensory cortex plays a role in proprioception, which makes the brain aware of the bodies state, influencing future motor efferent pathways (Knierim, et al., 2019).

2.2: EEG Use and Accessibility During Whole-Body Movements

EEG has become the most commonly used method during whole body exercise tests to locate real time cortical changes of neuronal activity (Perrey & Besson, 2018). When looking for specific activity within the brain, PET, fMRI, or EEG would be the best methods to use due to their ability to locate neuronal activity levels. As expected, there are drawbacks to each instrument. PET and fMRI have better spatial resolution when compared to EEG but have much worse temporal resolution (Reisberg, 2016). PET and fMRI also have an infeasibility factor as well as non-portable access that cannot be used with subjects performing complex whole-body movements (Perrey & Besson, 2018). EEG, through electrodes placed on a patient/subject's head, has the ability to record electrocortical changes during whole body exercise (Reisberg, 2016) (Perrey & Besson, 2018). Electrocortical readings are meant to be precise recordings of temporal changes of neuronal activity. The recordings from the EEG are represented by frequency waves that represent the cognitive state of individuals, such as: theta, alpha, beta, and

gamma (Reisberg, 2016) (Perrey & Besson, 2018). Theta activity is increased when tasks are more complex (Grunwald et al., 2001). Increased alpha activity represents decreased cortical activity and inhibition of cortical functions (Grimshaw et al., 2014) (Hilty et al., 2011). Beta and gamma activity increases are thought to indicate increased cortical activity (Moraes et al., 2007) (Kandel et al., 2013) (Abhang et al., 2016).

EEG during whole-body movements is most appropriate to use when measuring the sensorimotor system which involves the body/brain interaction of sensory stimuli which can be relayed into a motor action. Placement of EEG electrodes must correspond with accepted anatomical landmarks to allow for correct electrocortical reading when looking at what area of the cortex the reading is coming from. Cycling on a cycle ergometer is a good exercise to analyze cortical activity via EEG because the head can stay relatively steady which can lessen the effects of movement artifacts during the EEG recording. (Perrey & Besson, 2018).

2.3: EEG Recording During Double Leg Cycling

The use of EEG during double leg cycling has been of limited use in examining the cortical response in relation to a specific cycling protocol. The addition of more research examining cortical changes in response to exercise prescription, specifically the primary motor cortex and the structures that influence it, is needed. This could allow for greater understanding of cortical activation and its relation to exercise intensity during whole body movements (Perrey & Besson, 2018). Results of brain activation and exercise could be useful for athletic and neurologically impaired populations by showing exercise intensities/protocols which have the highest CNS activation. Increases in CNS activation from exercise has been linked to increasing long term strengthening of signal transmission between neurons and decreasing intracortical inhibition (Singh et al., 2014). Of the few studies which have examined this area of research,

some studies have used a recumbent cycle ergometer to limit movement artifacts of the EEG and others have used upright cycling for their subject positioning (Enders et al., 2016) (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011) (Ludyga, Gronwald, & Hottenrott, 2015) (Bailey, Hall, Folger, & Miller, 2008) (Brümmer, Schneider, Strüder, & Askew, 2011). Also, the use of graded exercise, or constant load has differed in recent literature (Enders et al., 2016) (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011) (Ludyga, Gronwald, & Hottenrott, 2015) (Bailey, Hall, Folger, & Miller, 2008) (Brümmer, Schneider, Strüder, & Askew, 2011). This review of EEG during double leg cycling is split with literature recording cortical changes during constant load cycling (2.3a) and literature using GXT (2.3b).

2.3a: Constant Load

Enders et al 2016 measured cortical activity/changes via EEG during a high intensity cycling exercise at a constant load of 85% of subject's maximum power output. Electrocortical clusters were analyzed through independent component analysis (IC) allowing for better spatial resolution of the EEG recording. It was hypothesized that EEG power (amplitude of EEG signal) would be increased as fatigue developed during the constant load exercise. Cessation criteria of the test was either dropping below 70 rpm or going 15 rpm under the subject's initial revolution speed. EEG was measured before and during exercise in 10 experienced male cyclists. Results showed significant increase in EEG power ($p<0.05$) when fatigued in the areas of the superior frontal gyrus and precentral gyrus, frontal cortex, and superior and inferior parietal lobe. After analyzing changes from before exercise to during exercise at fatigued state, significant increase in alpha, beta, and gamma frequencies ($p<0.05$) were seen in the left frontal cortex. Increases in the supplementary motor area and left parietal cortex showed significant increases in alpha and beta frequencies while the right parietal cortex only showed increases in

alpha frequency ($p<0.05$). Increases in the alpha, beta and gamma frequencies during EEG power analysis showed that there was a significant effect of high intensity cycling on frontal and parietal areas of the brain associated with motor planning, execution and sensorimotor processing. The increase in EEG power in these brain areas as duration and fatigue increased suggests that with increased demand, the brain is sending more signals, and receiving increased proprioceptive inputs during a fatigued state. These results indicate that the CNS is responding to the higher demand of the test and when the body is peripherally fatigued (Enders et al., 2016).

Analyzing the increases in EEG power during high intensity exercise is essential to understanding the effect of intensity on motor cortical activation. Analysis of communication through areas which process sensory information, which is thought to project this information to the motor cortex, is also valuable in understanding the complex integration of signals in the brain (Enders et al., 2016) (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011). Research which looked at the intracortical communication between sensory and motor areas of the brain in response to constant load cycling wanted to see whether there was increased communication between the motor cortex and the mid/anterior insular. The criteria the researchers used to indicate increased communication between the structures was increased lagged phase synchronization. Researchers hypothesized that the lagged phase synchronization was increased compared to the beginning of exercise and results showed an enhanced lagged phase synchronization represented an increase communication of the motor cortex and mid/anterior insular. 16 healthy males averaging 26 years old who trained 3 hours per week aerobically performed a GXT to obtain each subjects VO_2 peak. Four days later subjects performed a constant load test at 60% of their VO_2 peak at 70rpms until volitional exhaustion. Mean exercise time was 35.5 minutes. The electrodes placed for regions of interest were determined by

Montreal Neurological Institute coordinates, specifically marking motor areas associated with the legs, and an area in the insular cortex associated in limiting physical performance during muscle fatigue. Findings from EEG showed that lagged phase synchronization increased during the end of exercise compared to the beginning of exercise ($p<0.001$ & $p<0.004$ respectively). These findings of synchronicity do not relate to EEG power but the time for communication. There was no definitive way to know if the communication was in the direction of sensory to motor or vice versa, but from anatomical examination in the mid/anterior insular, efferent pathways to the motor cortex have been seen. Therefore, the increased communication associated with fatigue was thought to occur from mid/anterior insular to the motor cortex (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011).

In studies conducted by Hilty & Enders, subjects exercised until exhaustion (either by protocol or voluntary fatigue) (Enders et al., 2016) (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011). EEG power increased in areas of interest and whole brain (Enders et al., 2016). In a study to examine the differences in cortical activity in men and women during a constant load cycle test, certain areas of the cortex, such as frontal, central, and parietal, were measured. Subjects in this study included 13 males and 13 females averaging 28 years old. These subjects had a weekly aerobic training of 7 hours per week. Male and female subjects exercised at their anaerobic threshold (determined by a lactate power curve during a GXT) for 30 minutes. Subjects anaerobic threshold was determined by a GXT. During the 30-minute exercise test, continual EEG analysis and 5 blood lactate measurements were recorded. Researchers believed they would see a decrease in alpha and beta power due to the probability that central fatigue would be induced during the submaximal test. The researchers also hypothesized that there would be a greater decrease in male EEG alpha and beta power because they expected more

fatigue in males. Results of the study showed that there were no significant differences between men and women cortical activity during the cycling exercise ($p>0.05$). Diminished levels of alpha and beta frequencies were seen in all regions of the brain toward the middle/end of exercise ($p<0.014$). This decrease in alpha and beta during prolonged duration exercise at submaximal intensity was, as these researchers defined, an indication of central fatigue. Central fatigue is an indicator of central inhibition for continued movement during exercise, and this study's results indicated that their subjects showed decreased CNS activation with increased duration (Ludyga et al., 2015).

2.3b: Graded Exercise

During a GXT, the intensity increases, which allows for EEG recordings to measure the change in cortical activity in real time. Research has used changes in cortical activity to indicate increased activity of alpha, beta, and theta frequencies. Also, specific regions of interest (4 electrodes in frontal, 2 in central, and 2 in parietal) were looked at to examine whether certain areas of interest have increased activation over other areas (Bailey, Hall, Folger, & Miller, 2008). In a study conducted by Bailey et al., 2008, 20 male subjects with a mean age of 24 years old with an average VO_2 (oxygen consumption) of 40 mL/kg*min participated in the study. The protocol for the GXT started initially at 50 W, increasing 50 W every 2 minutes until volitional fatigue. EEG measurements were taken before and within the final minute of each stage, as well as immediately post exercise and 10 minutes post exercise. This study used a recumbent cycle ergometer to limit the noise of the EEG found during previous recordings of whole-body exercise on the treadmill. Results of this study showed increases in alpha, beta and theta activity in all leads during exercise/fatigue ($p<0.05$). Significant increases occurred around 150-200 W which were typically the final two stages of the exercise prescription. There were no

hemispheric differences in localization of electrocortical activity on either side ($p>0.05$). There were no regional differences in increased EEG power of the different frequencies ($p>0.05$). The alpha/beta ratio was increased during exercise in the frontal electrodes (no significant change), but not parietal and central. The alpha/beta ratio was increased in all leads immediately post exercise ($p<0.05$). Findings of this study show that during high intensity exercise that all theta, beta, and alpha frequency activities are increased at all electrode sites, but the alpha/beta ratios differed during exercise (Bailey et al., 2008).

Investigation of changes in cortical activity during a high intensity cycling exercise protocol had regions of interest examined to see if the incremental cycling test produced any changes in motor cortex, pre-frontal cortex and somatosensory cortex. The changes in regions of interest were relative to the changes in the respective lobe. The researchers hypothesized that there would be increases in the primary motor cortex and decreases in the pre-frontal cortex when close to exhaustion. The pre-frontal cortex is associated with motivation, intuition, decision making and exercise preference, which is why the researchers believed this area would be less active close to exhaustion. In a study conducted by Brümmer et al., 2011, 14 males and 4 females ~ 26 years old participated in a GXT test which started with an EEG recording at rest on the cycle ergometer (in upright position). After the initial baseline reading, the test started at 50W and increased every 5 minutes by 50 W until volitional fatigue. Lactate and heart rate were measure during the test as well as pre and post. All EEG readings were recorded while eyes were closed. Results showed that the activity in the primary motor cortex increased with increasing exercise intensity ($p<0.01$) and sensory cortex and pre-frontal cortex were not altered with exercise. Lactate was significantly increased during each stage ($p<0.001$). These findings show the probability that the motor cortex is required to increase activity during movement

execution and force production and the other regions of interest measured are not pertinent to this exercise execution. Sensory demand was expected to increase from pre-exercise to stage one, but this did not increase. This could possibly be due to a pre-activation at rest on the cycle ergometer. The pre-frontal cortex was also expected to decrease activity during exhaustion, but no changes were recorded. This could be due to measurements of EEG activity in the 4^{th} minute of each stage because most subjects went 1-2 minutes after there last EEG recording. This indicates that true exhaustion was not measured for some subjects (Brümmer, Schneider, Strüder, & Askew, 2011).

2.4: Single Leg Cycling

Examination of single leg cycling, and the effects of this intervention on cortical activity has yet to be examined. However, physiological measures such as: oxygen uptake (VO_2), carbon dioxide production (VCO_2), expired ventilation (VE), electromyographic activity (EMG), metabolic molecules, and power output have been compared between single and double leg cycling (Abbiss et al., 2011) (MacInnis et al., 2017).

In a study that examined the metabolic and power parameters of single leg versus double leg cycling, nine experienced male cyclists preformed a cycle ergometer training protocol. A wash out period was included (42 days) to limit the learning effect on the cycle ergometer. Results showed that when looking at power output for single and double leg, the single leg mean power output was more than half of the total mean power output for double leg ($p<0.05$). Metabolic molecules, such as oxidative enzymes, also increased in subjects who performed single leg cycling when compared to double leg cycling subjects ($p<0.05$). VO_2max values were similar across the two training groups ($p>0.05$). These results showed greater increases in power output as well as metabolic molecules while reaching the same VO_2max (Abbiss et al., 2011).

During an analysis of single leg cycling versus double leg cycling, incremental, continuous and interval cycling were used to compare VO_2, VCO_2, VE, EMG signals, and power output. Results showed in 12 healthy males that when normalizing power outputs, during incremental and continuous cycling, power outputs were greater for single leg versus double leg ($p<0.001$). EMG responses were similar for single and double leg in all muscles except the semitendinosus which had significantly more activity in single leg cycling compared to double leg ($p<0.005$). VO_2, VCO_2, VE, heart rate, and rating of perceived exertion were all lower in single leg versus double leg (MacInnis et al., 2017).

2.5: Bilateral Asymmetry in Multiple Sclerosis

As previously mentioned, single leg cycling, and the comparison of cortical activity during single leg vs. double leg cycling has yet to be evaluated. In certain neurological diseases, such as MS, bilateral asymmetry of the lower limbs has been evaluated through results such as oxygen uptake, workload and strength (Larson, McCully, Larson, Pryor, & J.White, 2013).

MS is a CNS disease which results in demyelination and inflammatory responses to CNS axons. Through many postmortem examinations, it is widely accepted that characteristic signs of MS are multiple lesions in varying sites showing demyelination of axons. Motor weakness of MS is said to be due to cortical/spinal lesions, and inflammatory cells or antibodies that have increased circulation in the cerebral spinal fluid allowing for increased access to axons. Demyelination has been said to decrease action potential propagation down the axon, causing a lessening of signals to be transmitted to postsynaptic neurons. Also, CNS axons are thought to be destroyed in MS through inflammatory action, as well as the loss of supporting factors which contribute to long term pre and postsynaptic interaction (Purves, Augustine, & Fitzpatrick, 2001). Due to doctors and researchers not understanding the exact etiology and mechanisms of

MS, research in the area of alternative treatments, such as exercise, are important to improve patient's quality of life. Exercise has been documented to increase long term synaptic strength in the motor cortex in healthy participants (Singh et al., 2014). Future research to investigate possible changes in cortical synaptic mechanisms due to exercise prescription in CNS diseased individuals is an interesting area needed for increased investigation.

During a study of oxygen uptake, workload and strength, eight MS and 7 non-MS participated in a cycling (GXT) and strength protocol (MVIC) to examine these parameters. Bilateral assessment of the leg in MS subjects showed significant asymmetry of muscle strength, oxygen uptake and workload ($p<0.05$). No significant differences were seen in Non-MS subjects during examination of bilateral asymmetry ($p>0.05$). After between group analysis of Non-MS and MS subjects, MS subjects showed significantly greater asymmetry for strength, O2 uptake, and workload compared to Non-MS subjects ($p<0.05$) (Larson, McCully, Larson, Pryor, & J.White, 2013).

Single leg cycling has been documented as less tasking to the respiratory system, but has shown increased activation in the respective limb, relative to double leg cycling (Abbiss et al., 2011) (MacInnis et al., 2017). By using single leg cycling with EEG monitoring in an MS population, the motor weakness, due to lesions of the corticospinal tracts, might be visually explained (Purves, Augustine, & Fitzpatrick, 2001). Because of exercise's role of increasing synaptic strength in the brain, single leg cycling could be a possible rehabilitation area of interest for neurodegenerative patients needing to regain motor strength and movement (Sleiman et al., 2016). By testing neurodegenerative diseased subjects until volitional fatigue, researchers and doctors can observe if there is a disconnect with central nervous system activation during exercise and peripheral performance.

Gaps In literature

While there has been research conducted with EEG during double leg cycling, there has been no current literature to my knowledge studying the effects of cortical activity induced through single leg cycling. Single leg cycling has been shown to increase neuromuscular variables compared to double leg cycling but CNS activation has never been compared between the two tests (Abbiss et al., 2011) (MacInnis et al., 2017). Understanding of how the CNS responds to single leg cycling could be important for athletic and neurologically impaired individuals due to possible increases in synaptic connections (Singh et al., 2014). All studies which have been published have only had subjects perform one bout on the cycle ergometer, either recumbent or upright, and either at a constant submaximal load or GXT to volitional fatigue. To study whether there are significantly different effects on motor cortical activity during single leg cycling when compared to double leg cycling, subjects will need to perform a minimum of two bouts: one double leg, and one single leg. With this protocol, subjects can act as their own control for cortical activity during double leg cycling, which allows for the analysis of differences in single leg vs. double leg cortical activity. Also, since there has been no literature with multiple cycling tests during EEG analysis, washout periods have not been examined for between exercise bouts. Washout period examination is critical to ensure that there is no neuronal adaptation to the cycling test through peripheral or central mechanisms.

Asymmetry in lower limbs of MS population is of recent findings in the literature. Cortical activity has not been examined through EEG during cycling protocol in MS population due to this being a newly studied area. 'Normal' changes in cortical activity through cycling intervention need to be concluded before this is looked at in diseased population. Due to lower limb bilateral asymmetry being a new discovery in MS population, cortical activity examination

through single leg cycling could be of use to discover methods to increase MS quality of life, and decrease functional differences compared to Non-MS population.

Summary

Cortical activity examination via EEG during a cycling test is a relatively new area of examination in the field of exercise physiology. Examination of EEG power and motor areas of interest in the cerebral cortex have been shown to increase during constant load cycling exercise to volitional fatigue (Enders et al., 2016) (Hilty, Langer, Pascual-Marqui, Boutellier, & Lutz, 2011). In a constant submaximal load study on the cycle ergometer which was timed and did not end in volitional fatigue, showed decreased cortical activity (Ludyga, Gronwald, & Hottenrott, 2015). This could possibly be due to not letting subjects reach a volitional fatigue state, allowing movements to become more 'routine' causing a deactivation of cerebral structures due to motor areas of the cortex being involved in evaluating, planning, coordinating, and initiating motor movements (Knierim, 2019). This however is only speculation and is an area that is needed for further research. During GXT to volitional fatigue, increases in cortical activity with increasing intensity were seen (Brümmer, Schneider, Strüder, & Askew, 2011) (Bailey, Hall, Folger, & Miller, 2008). When looking at certain cerebral areas, primary motor cortex activity was seen to increase with increasing activity when compared to the activity of the entire frontal lobe (Brümmer et al., 2011). Although there has been no research in the area of examining cortical activity during single leg cycling, certain physiological measures have indicated increases in power output, EMG activity, VO_2, VCO_2, VE, and oxidative enzymes (Abbiss et al., 2011; MacInnis et al., 2017). These changes in physiological parameters indicate that there are differences between double and single leg cycling, which indicate the need for investigation for possible differences in cortical changes. There has been previous literature examining cortical

changes during double leg cycling in healthy population, but no cycling tests while measuring EEG recordings have been investigated in MS population. Bilateral asymmetries have been seen in MS patients compared to non-MS patients, furthering the need for investigation of the effects of cortical activity induced through single and double leg cycling (Larson et al., 2013). Cortical changes during single vs. double leg cycling need to be examined further in healthy population before examining individuals in a diseased state.

CHAPTER III: METHODOLOGY

Participants

Subjects between the ages of 18-35 were recruited through word of mouth, flyers and snowballing. Delimitations of the study included individuals without previous or current competitive or recreational cycling experience (< cycling twice per week), asymmetric orthopedic limitations, multiple risk factors for cardiovascular disease, respiratory, psychiatric or neuromuscular/neurological disorders which impair motor movement. Inclusion criteria for this study included: (1) subjects completing the physical activity readiness questionnaire (PAR-Q) with all answers being no or having a doctor's note indicating readiness for activity; (2) subjects completing an extended health history questionnaire indicating no current limitations, conditions, or diseases. Subjects who did not meet these criterions were excluded from participating/being included in the study/results.

Sample

26 subjects were recruited to participate in this study. Following the inclusion criteria, 25 subjects were included in data preprocessing. Data preprocessing through manual rejection of artifact and rejection based on independent components through ICLabel (>15 components removed = subject rejected) rejected 15 participants (Appendix 2. ICLabel; 1. Cleaned EEG Data). 10 subjects' data was used during data processing which indicated the number of epochs for each stage during exercise. 2 subjects were rejected due to insufficient epochss (<20) at all stages/rest (Gudmundsson et al., 2007). Following the inclusion criteria, data preprocessing, and data processing, 8 participants (4 male, 4 female) were included in data analysis.

Research Design

This study was a quasi-experimental design which implemented a GXT on an upright cycle ergometer with EEG recordings before, during, and after exercise. This study included three visits which included a familiarization day (visit 1) lasting 30 minutes where subjects were fitted on the cycle ergometer and completed consent forms: PARQ, POMS-B (profile of moods state), and extended health history questionnaire. Subjects also performed one stage of double leg and single leg cycling. This allowed them to understand the movements required during future visits and become comfortable cycling in both protocols. Visits two and three were randomized between single leg and double leg cycling and lasted between 60-90 minutes. During visits two and three, subjects completed a POMS-B form then had the EEG cap fitted and placed on their heads prior to sitting on the cycle ergometer. Once seated on the cycle ergometer (Appendix 3. Subject Positioning), subjects had a 2-minute baseline recording with their eyes open and focused on the RPM (revolution per minute) device, which was approximately 1 foot in front of them. To limit eye movement during the test, subjects were instructed to fixate their eyes on the RPM device for the duration of the test (before, during and post exercise). Also, subjects were instructed to limit any facial movement to the best of their ability before, during and post exercise to limit electromyographic activity recorded by electrodes that reside on the face/forehead.

Double Leg Cycling (Randomized Visit 2 or 3)

Immediately after the 2-minute seated recording, subjects began the GXT, starting at their body weight (BW) in kilograms (kg), with an increase of 0.5W/kgBW every 1 minute. EEG recordings were continuous from pre cycling through post cycling.

Single Leg Cycling (Randomized Visit 2 or 3)

Immediately after the 2-minute seated recording, subjects began the GXT starting at half their BW in kg, with an increase of 0.25W/kgBW every 1 minute. EEG recordings were continuous from pre cycling through post cycling.

Measurement Tools/Procedures

All measurements were analyzed from EEG recordings. A cycle ergometer (Lode Excalibur Sport) was used during the GXT, and subjects cycled until volitional fatigue. EEG electrodes were placed on the subjects' head in accordance with the International 10-20 system (Appendix 4. 10/20). All leads recorded impedances below 50Kohms. Continuous measurement of EEG via 32 electrode EEG cap (HydroCel Geodesic Sensor Net) started 2 minutes prior to cycling while seated on the cycle ergometer, during the GXT, and for 2 minutes following the end of exercise. A grounding strap (Nasafes Grounding Cord, Cable, Strap) was attached to participant's wrists to lower impedance levels during the test, due to the electrical current flowing from the cycle ergometer.

Double leg cycling was performed using typical bilateral cycling technique. Single leg cycling was performed using a counterweight system. The counterweight system allowed for less stress on the exercising leg on the upward motion of cycling which would not normally be felt during double leg cycling. Because of the counterweight system, it elicits the same mechanics for the exercising leg as it would during double leg cycling (Abbiss et al., 2011).

Procedure Development

Currently there is very limited literature regarding a gold standard or specific protocol for assessing electrocortical activity during a GXT. In Brummer et al., 2011 study, workload durations were 5 minutes to allow for subject familiarization and recordings were done on the 4th minute. Bailey's study had a duration of 2 minutes, measuring that electrocortical activity in the

last minute of the stage. In the current study, we shortened the stages to 1 minute, allowing for increases in intensity at a faster pace and constant EEG recordings before, during, and after the GXT. Brummer's study failed to get readings of many subjects' last stage due to failure before the 4th minute. Therefore, by shortening the stages and continuous recording, this can allow for recording of increased neuronal activity due to the changing of overall force output and motor pattern movement. Maximal effort criteria for our study was the last stage that the subject fully completed. This criterion was in place for: 1) to ensure enough EEG data for the stage; 2) Many subjects start to us more body movement during the end of exercise especially during a GXT where the work rate is increasing towards maximal effort. This extra movement creates unusable EEG data due to the movement artifact.

Reliability and Validity

EEG during whole-body movements is most appropriate to use when measuring the sensorimotor system. Electrodes are placed on subject's heads and temporally precise recordings of neuronal activity is recorded. Placement of EEG electrodes must correspond with accepted anatomical landmarks to allow for correct electrocortical reading when looking at what area of the cortex the reading is coming from. Cycling is a good exercise to analyze cortical activity via EEG because the head can stay relatively steady, which can lessen the effects of movement artifacts on the EEG. PET and fMRI scans have better spatial resolution of the brain but are much worse temporally and they are infeasible due to their limited portability (Perrey & Besson, 2018).

While EEG as an instrument has been shown to be a valid measurement tool in measuring electrocortical current density of the brain (Perrey & Besson, 2018), exercise protocols for cycle ergometer have yet to be established as reliable in conjunction with EEG.

Different results and protocols have been used in graded exercise tests and continuous exercise tests, furthering the need for testing to establish a reliable protocol (Bailey el al., 2008)(Brummer et al., 2011)(Enders et al., 2016)(Ludyga et al., 2015).

Due to the amount of inevitable movement that occurs during cycling, there is a great deal of movement artifact during EEG pre-processing. Epoched data is said to be reliable with at least 20 seconds of epoched data (Gudmundsson et al., 2007). With the limited duration of this study, 20 one-second end epochs were the minimum required amount of data per stage to be analyzed.

Internal and External Validity Threats

Internal Threats

Due to non-random sampling, selection of subjects for this study was an internal threat to validity. Due to many of the subjects being a part of the Health and Exercise Science Department at the University of Oklahoma, subjects were not representative of the population. Testing was another threat to internal validity because multiple GXTs were conducted. Subjects could learn the procedure throughout multiple tests and neuronal adaptations could occur from repetitive motor movements.

External Threats

Interaction effects of selection bias and experimental treatment could occur during this study due to subjects having been recruited through convenience. This interaction could affect external validity by trying to generalize results when only testing subjects within a certain department/university.

Minimization of Threats

Level of training was controlled for prior to participation, and subjects who were above the maximum training level were excluded from the test. Due to measures of this test solely being electrocortical activity, external and internal validity threats about generalization and representation of the population, respectively, should be limited. Internal threats due to testing was limited by developed washout period. The washout period was 48 hours between GXT's. Also, subjects perform different types of GXT's (left/right and both legs) for each day subjects came to test, which limited testing threats.

Data Collection Procedures

This study was a one-week study minimum with day one consisting of familiarization, informed consent, PAR-Q, POMS-B, and extended disability forms being completed. Day two through three were separated by 48 hours minimum to allow for wash out period. I am the principle investigator and conducted all subject experiments and data collection procedures.

The data that was collected in this study included EEG frequencies (continuous), EEG power (continuous), duration until volitional fatigue (continuous) and workload stage failure (ordinal).

Procedures for Data Management

Once subjects signed up for the study, they were assigned a subject ID number. Their ID number was the only way to identify each subject. All participant forms were kept in a locked room and file cabinet in the University of Oklahoma Visual Neuroscience Laboratory. All EEG and workload computers were kept on password protected computers in the Visual Neuroscience Laboratory.

Data Analyses

By performing a sample size calculation in G*Power the effect size was moderately large (d=0.7) (Cohen, 1988), giving a total sample size of 20 subjects. The level of significance was set at $p<0.05$. Therefore, we aimed to recruit and test 25-30 individuals. All effect sizes are consistent with Cohen, 1988.

1. Research question: Are there differences in whole brain electrocortical activity during single leg and double leg cycling with increasing workloads (stages)? Repeated Measures
2. Research question: Is there increased motor cortex electrocortical activity with increasing workloads in single or double leg cycling? Repeated Measures
3. Are there differences in whole brain activity between single leg and double leg pre to post exercise? Paired t-test
4. Are there differences in motor cortex activity between single leg and double leg pre- to post-exercise? Paired t-test

Data Analysis Software

SPSS for Mac version 26 was used for all statistical analyses. Matlab R2018b was used for all EEG data pre processing and processing.

Previous Literature Data Collection

Previous literature showed similar data collection procedures and research design with respect to measuring ROI vs respective lobe activity and electrocortical activity measurements with increasing workloads in double leg cycling tests (Bailey et al., 2008)(Robertson et al., 2015). Other procedures that were conducted in the current study which involve the single leg

variable have yet to be investigated in previous literature so there were no procedures and designs to compare to.

CHAPTER IV: RESULTS

This section will be broken down into four sections: a) subject characteristics, b) frequency results, c) case studies, and d) summary of results. Subject characteristics encompass descriptive data for subjects who were included in pre and post exercise and for subjects included in pre and during exercise. Frequency results show the analysis of EEG activity (power) in pre and post exercise (sub section Rest 1 vs. Rest 2), and pre and during exercise (sub-section Rest 1 vs. Stage 1). All data in the frequency results section were compared using paired sample t-tests. The case studies section consists of two case studies: case study A and case study B. Each case reports individual data from two subjects who completed all stages for double leg (DL) and single leg (SL) GXT. Each cases reports EEG power data for theta (θ), alpha (α), beta (β), and gamma (γ). The summary of results sections will summarize data presented in frequency results.

a) Subject Characteristics

Descriptive data (age, height, weight, sex), analyzed by one-way ANOVA, for pre- and post-exercise data are reported in Table 1 as means $\pm$ standard deviation (SD). There was a significant difference between male and female height (cm) (males: 174.88 ± 4.17, females: 160.50 ± 6.94, $p = 0.018$) for the pre- to post-exercise sample. Descriptive data for pre-exercise to stage one is reported in Table 5. The number of subjects are different in pre-post exercise analysis and pre-stage 1 analysis because usable data differed between subjects in pre-exercise, stage 1, and post-exercise. POMS-B questionnaire results from repeated measures ANOVA showed no significant differences in vigor or fatigue between visits. Repeated measures ANOVA results from sleep diaries showed no significant differences between visits or between visits and their average sleep for testing duration.

Table 1: Subject Characteristics Pre-Post

Variable	Whole Sample (n=7)	Males (n=4)	Females (n=3)
Age (years)	25.14 ± 1.95	24.25 ± 0.96	26.33 ± 2.52
Height (cm)	168.71 ± 9.16	174.88 ± 4.17 *	160.50 ± 6.94*
Weight (kg)	69.46 ± 8.56	74.03 ± 8.70	63.37 ± 3.01

Values are displayed as mean ± SD.
(*) indicates a significant difference ($p < 0.05$) between males and females

b) EEG Power in θ, α, β, γ

i. Rest 1 vs. Rest 2

Results of whole brain and C4 electrode (right motor cortex) within and between leg EEG power changes are shown in Tables 2, 3 and 4. EEG activity for DL and SL in the whole brain showed statistically significant increases in power from rest 1 (R1) to rest 2 (R2): DL: theta(θ) ($p = 0.035$), alpha(α) ($p = 0.012$), beta (β) ($p = 0.0006$), gamma (γ) ($p = 0.013$); SL: α ($p = 0.023$), β ($p = 0.049$). Power difference scores (Δ R2-R1) between legs (DL vs. SL) showed no significant differences ($p>0.05$). While there were no significant differences between DL and SL notable effect sizes (ES) were seen in γ ($d = 0.96$, SL vs. DL= 0.00 ± 0.02 vs. 0.02 ± 0.02, $p = 0.15$), and β ($d =1.28$, SL vs. DL = 0.02 ± 0.01 vs. 0.03 ± 0.02, $p = 0.09$). DL in C4 showed statistically significant power increases from R1 to R2 in: β ($p = 0.021$), γ ($p = 0.019$). No significant differences were seen in SL from R1 to R2 or between SL and DL power difference scores. While there were no significant differences between SL and DL, notable ES were seen in θ ($d = 0.7$, SL vs. DL = 0.01 ± 0.06 vs. 0.06 ± 0.07, $p = 0.24$) and β ($d =0.66$, SL vs. DL= 0.01 ±

0.01 vs. 0.02 ± 0.01, p = 0.16). Power difference scores for the whole brain and C4 in SL and DL groups is shown in Figure 1 as mean ± standard error of the mean (SEM).

Table 2: Whole brain EEG power before and after SL and DL GXT (n=7)

	Single Leg Power (μV^2)		Double Leg Power (μV^2)	
Frequency (Hz)	Rest 1	Rest 2	Rest 1	Rest 2
theta (θ)	0.37 ± 0.12	0.41 ± 0.08	0.36 ± 0.14*	0.46 ± 0.22*
alpha (α)	0.39 ± 0.26*	0.45 ± 0.25*	0.33 ± 0.16*	0.38 ± 0.20*
beta (β)	0.12 ± 0.03*	0.13 ± 0.02*	0.10 ± 0.02#	0.13 ± 0.03#
gamma (γ)	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.02*	0.06 ± 0.03*

Values are displayed as mean ± SD. R1 indicates rest 1 (before exercise). R2 indicates rest 2 (after exercise). Whole brain indicates the average of all 32 electrodes.
(*) indicates a significant difference within leg between R1 and R2 within frequency ($p < 0.05$)
(#) indicates a significant difference within leg between R1 and R2 within frequency ($p < 0.01$)

Table 3: C4 EEG power before and after SL and DL GXT (n=7)

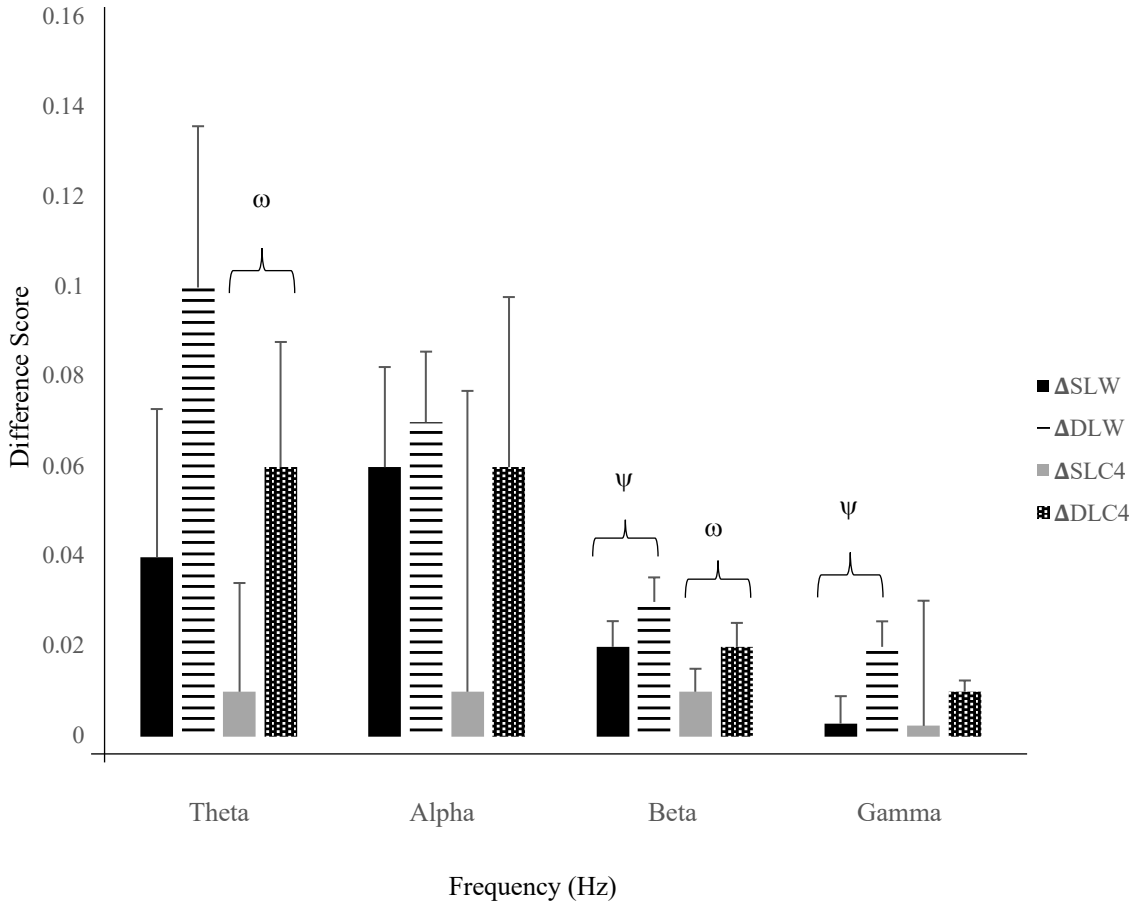
	Single Leg Power (μV^2)		Double Leg Power (μV^2)	
Frequency (Hz)	Rest 1	Rest 2	Rest 1	Rest 2
theta (θ)	0.33 ± 0.13	0.34 ± 0.14	0.33 ± 0.14	0.39 ± 0.16
alpha (α)	0.52 ± 0.39	0.53 ± 0.33	0.42 ± 0.32	0.48 ± 0.39
beta (β)	0.11 ± 0.04	0.12 ± 0.04	0.09 ± 0.04*	0.11 ± 0.03*
gamma (γ)	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.02*	0.03 ± 0.02*

Values are displayed as mean ± SD. R1 indicates rest 1 (before exercise). R2 indicates rest 2 (after exercise). C4 indicates the single C4 electrode representative of the right motor cortex.
(*) indicates a significant difference within leg between R1 and R2 within frequency ($p < 0.05$)

Table 4: Whole brain and C4 power difference scores [Rest 2 minus Rest 1] in SL and DL (n=7)

Frequency (Hz).	ΔSLW	ΔDLW	ΔSLC4	ΔDLC4
theta (θ)	0.04 ± 0.09	0.10 ± 0.09	0.01 ± 0.06	0.06 ± 0.07
alpha (α)	0.06 ± 0.04	0.07 ± 0.06	0.01 ± 0.18	0.06 ± 0.10
beta (β)	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
gamma (γ)	0.00 ± 0.02	0.02 ± 0.02	0.00 ± 0.02	0.01 ± 0.01

Values are displayed as mean ± SD. Δ indicates R2-R1. SL indicates single leg. DL indicates double leg. W indicates the average power of all 32 electrodes. C4 indicates the single C4 electrode representative of the right motor cortex.



No significant differences between SL and DL. W= whole brain. C4= electrode indicating right motor cortex. Δ=R2-R1. SLW= single leg whole brain. DLW= double leg whole brain. SLC4= single leg right motor cortex. DLC4= double leg right motor cortex
(ω) indicates a medium effect size (d=0.5-0.79)
(ψ) indicates a large effect size (d= >0.8)

Figure 1: Whole brain and right motor cortex (C4) power difference scores (Rest2-Rest1) (n=7) (mean ± SEM)

Results comparing EEG power means for averaged C3 and C4 electrodes (left and right motor cortex) between R1 and R2 showed a statistically significant increase from R1 to R2 in β ($p = 0.028$). Power difference scores were also calculated for each electrode (C3 and C4) and compared. There were no significant differences between C3 or C4 power difference scores in DL.

F3 and F4 (left and right dorsolateral prefrontal cortex (DLPFC) electrode power results were averaged and statistically significant increases in power from R1 to R2 were seen in: DL: β ($p = 0.018$), γ ($p = 0.042$); SL: θ ($p = 0.03$), α ($p = 0.03$), β ($p = 0.013$). Averaged DLPFC difference scores for each leg (SL vs. DL) were compared and there were no significant differences between SL and DL. While there were no significant differences between SL and DL, notable ES was seen in α ($d= 0.84$, SL vs. DL= 0.0683 ± 0.06 vs. 0.02 ± 0.05, $p= 0.10$). Within leg power difference scores for individual electrodes (F3 and F4) were compared and there was no significant difference between F3 and F4 power difference scores within leg (SL and DL). While there were no significant differences between electrode, notable ES was seen in DL β ($d = 0.74$, F3DL vs. F4DL = 0.01 ± 0.01 vs. 0.02 ± 0.02, $p = 0.18$). Power difference scores for individual electrodes were also compared between legs (F3 SL vs F3 DL; F4 SL vs F4 DL). There were no significant differences in SL and DL groups within electrode, although there were notable ES for F3 α ($d =0.76$, F3SL vs. F3DL = 0.06 ± 0.06 vs. 0.02 ± 0.07, $p = 0.07$), and F4 α ($d =0.72$, F4SL vs. F4DL = 0.07 ± 0.08 vs. 0.02 ± 0.05, $p = 0.2$).

F7 and F8 (left and right ventrolateral prefrontal cortex (VLPFC) electrode power results were averaged and statistically significant increases in power from R1 to R2 were seen in: DL α ($p = 0.029$), β ($p = 0.009$), γ ($p = 0.008$); SL θ ($p = 0.002$), α ($p = 0.004$). Averaged VLPFC difference scores for each leg (SL vs DL) were compared and showed no significant differences between SL and DL. While there were no significant differences between legs, notable ES were seen in α ($d =0.65$, SL vs. DL = 0.09 ± 0.05 vs. 0.06 ± 0.05, $p = 0.15$). Within leg power difference scores for individual electrodes (F7 and F8) were compared and there were no significant differences between F7 and F8 within leg (SL and DL). Although, notable effect sizes were found for DL β ($d =0.57$, F7DL vs. F8DL = 0.02 ± 0.02 vs. 0.03 ± 0.02, $p =0.06$), and DL θ

(d =0.51, F7DL vs. F8DL = 0.05 ± 0.15 vs. 0.12 ± 0.09, p =0.2). Power difference scores for individual electrodes were also compared (F7 SL vs F7 DL; F8 SL vs F8 DL). There were no significant differences in SL and DL groups within electrode, however notable effect sizes include: F7 α (d =0.67 F7SL vs F7DL = 0.09 ± 0.06 vs. 0.05 ± 0.07, p = 0.09)

ii. Rest 1 vs. Stage 1

Table 5 represents subject descriptive data. One-way ANOVA showed no significant differences in age, height or weight between sexes. Results for whole brain and C4 electrode within leg and between leg EEG power changes are shown in Tables 6, 7 and 8. DL and SL in whole brain showed statistically significant increases in power from R1 to S1 in: DL Theta (θ) ($p = 0.025$); SL Theta(θ) ($p = 0.047$). Difference scores (Δ S1-R1) between legs (DL vs. SL) showed no significant differences. DL in C4 showed a statistically significant increase in power from R1 to S1 in γ ($p = 0.03$). No significant differences were seen in SL from R1 to S1 and no significant differences were seen between SL and DL power difference scores. Notable ES for SL vs DL in C4 include θ (d = 0.61, SL vs. DL= 0.10 ± 0.14 vs. 0.17 ± 0.10, p = 0.27). Power difference scores in whole brain and C4 in SL and DL groups is shown in Figure 2 as mean ± SEM.

Table 5: Subject Characteristics Pre-Stage 1

Variable	Whole Sample (n=4)	Males (n=3)	Females (n=1)
Age (years)	24.25 ± 0.96	24.67 ± 0.58	23.00
Height (cm)	171.75 ± 5.12	173.67 ± 4.16	166.00
Weight (kg)	76.73 ± 11.74	72.50 ± 9.97	89.40

Values are displayed as mean ± SD.

Table 6: Whole brain EEG power before and during exercise in SL and DL GXT (n=4)

	Single Leg (μV^2)		Double Leg (μV^2)	
	Rest 1	Stage 1	Rest 1	Stage 1
Frequency (Hz)				
theta (θ)	0.46 ± 0.14*	0.65 ± 0.23*	0.50 ± 0.15*	0.69 ± 0.23*
alpha (α)	0.28 ± 0.10	0.31 ± 0.11	0.33 ± 0.19	0.34 ± 0.15
beta (β)	0.12 ± 0.05	0.14 ± 0.05	0.12 ± 0.06	0.13 ± 0.04
gamma (γ)	0.04 ± 0.03	0.06 ± 0.03	0.04 ± 0.02	0.06 ± 0.03

Values are displayed as mean ± SD. R1 indicates rest 1 (before exercise). S1 indicates stage 1 (during exercise) Whole brain indicates the average of all 32 electrodes.
(*) indicates a significant difference within leg between R1 and S1 within frequency ($p < 0.05$)

Table 7: C4 EEG power before and during exercise in SL and DL GXT (n=4)

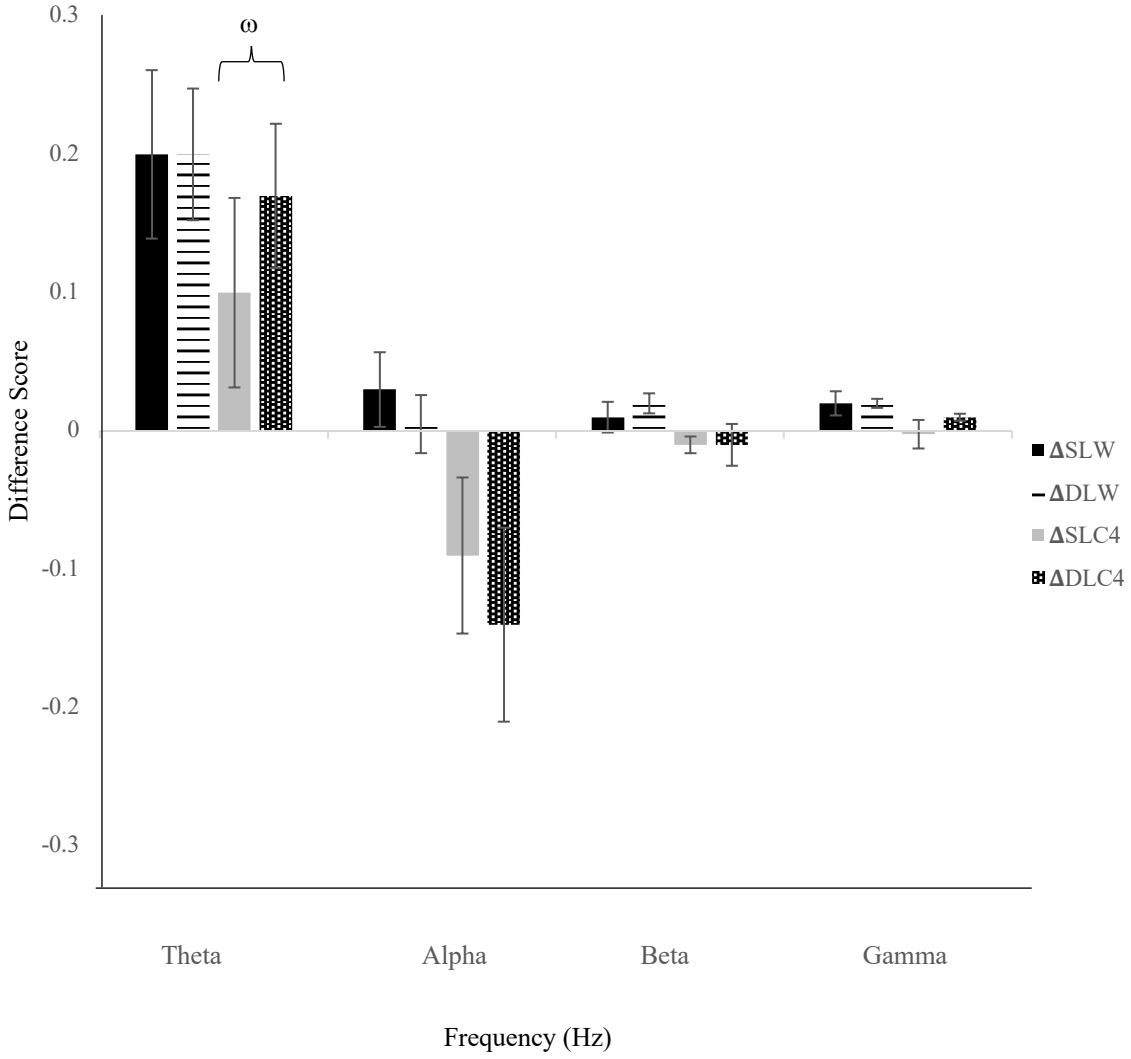
	Single Leg (μV^2)		Double Leg (μV^2)	
	Rest 1	Stage 1	Rest 1	Stage 1
Frequency (Hz)				
theta (θ)	0.40 ± 0.11	0.50 ± 0.13	0.48 ± 0.12	0.65 ± 0.10
alpha (α)	0.34 ± 0.14	0.26 ± 0.07	0.39 ± 0.15	0.25 ± 0.05
beta (β)	0.15 ± 0.09	0.14 ± 0.10	0.12 ± 0.07	0.12 ± 0.05
gamma (γ)	0.06 ± 0.07	0.06 ± 0.06	0.03 ± 0.03*	0.04 ± 0.02*

Values are displayed as mean ± SD. R1 indicates rest 1 (before exercise). S1 indicates stage 1 (during exercise). C4 indicates the single C4 electrode representative of the right motor cortex.
(*) indicates a significant difference within leg between R1 and S1 within frequency ($p < 0.05$)

Table 8: Whole brain and C4 EEG power difference scores [stage 1 of exercise minus pre-exercise] in SL and DL (n=4)

Frequency (Hz)	ΔSLW	ΔDLW	ΔSLC4	ΔDLC4
theta (θ)	0.20 ± 0.12	0.20 ± 0.10	0.10 ± 0.14	0.17 ± 0.10
alpha (α)	0.03 ± 0.05	0.00 ± 0.04	-0.09 ± 0.11	-0.14 ± 0.14
beta (β)	0.01 ± 0.02	0.02 ± 0.01	-0.01 ± 0.01	-0.01 ± 0.03
gamma (γ)	0.02 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01

Values are displayed as mean ± SD. Δ indicates S1-R1. SL indicates single leg. DL indicates double leg. W indicates the average of all 32 electrodes. C4 indicates the single C4 electrode representative of the right motor cortex.



No significant differences between SL and DL. W= whole brain. C4= electrode indicating right motor cortex. Δ=R2-R1. SLW= single leg whole brain. DLW= double leg whole brain. SLC4= single leg right motor cortex. DLC4= double leg right motor cortex
(ω) indicates a medium effect size (d=0.5-0.79)

Figure 2: Whole brain and right motor cortex (C4) power difference scores (Stage1-Rest1) (n=4) (mean ± SEM)

Results comparing EEG power means for averaged C3 and C4 between R1 and S1 showed a statistically significant increase in power in DL θ ($p = 0.017$). Power difference scores (ΔS1-R1) were also calculated for each electrode (C3 and C4) and there were no significant differences between C3 and C4 difference scores in DL.

F3 and F4 electrodes (DLPFC) were averaged and a statistically significant decrease in power from R1 to S1 was seen in DL α ($p = 0.047$). Averaged DLPFC power difference scores for each leg (SL vs. DL) were compared and there were no significant differences between SL and DL, however there were notable effect sizes in γ ($d = 0.90$, SL vs. DL = -0.02 ± 0.03 vs. 0.01 ± 0.01, $p = 0.144$), and α ($d = 1.39$, SL vs DL = -0.02 ± 0.04 vs. -0.08 ± 0.05, $p = 0.074$). Within leg power difference scores for individual electrodes (F3 and F4) were compared via paired t-test. There were no significant differences between F3 and F4 power difference scores within leg (SL and DL), however there were notable ES for SL θ ($d = 0.82$, F3SL vs F4SL = 0.04 ± 0.13 vs. 0.16 ± 0.17, $p = 0.21$). Power difference scores for individual electrodes were also compared between leg (F3 SL vs F3 DL; F4 SL vs F4 DL). There were no significant differences in SL vs DL within electrode, however notable ES include F3 α ($d = 1.2$, F3SL vs F3DL = -0.01 ± 0.04 vs -0.07 ± 0.06, $p = 0.27$), and F4 α ($d = 0.96$, F4SL vs. F4DL = -0.03 ± 0.08 vs. -0.10 ± 0.08, $p = 0.14$).

F7 and F8 were averaged and no significant differences in power were seen from R1 to S1 in SL or DL. Averaged VLPFC power difference scores for each leg (SL vs DL) were compared no significant differences between SL and DL, however there were notable ES in α ($d = 1.06$, SL vs DL = 0.20 ± 0.13 vs. 0.07 ± 0.13, $p = 0.07$) and γ ($d = 0.55$, SL vs. DL = 0.00 ± 0.04 vs. 0.02 ± 0.04 $p = 0.273$). Within leg power difference scores for individual electrodes (F7 and F8) were compared and there were no significant differences between F7 and F8 power difference scores within leg (SL and DL), however there were notable ES in SL γ ($d = 0.72$, F7SL vs F8SL = -0.01 ± 0.03 vs. 0.02 ± 0.05, $p = 0.14$) and SL β ($d = 0.96$, F7SL vs. F8DL = 0.01 ± 0.01 vs. 0.07 ± 0.08, $p = 0.25$). Power difference scores for individual electrodes were also compared between leg via paired t-test (F7 SL vs F7 DL; F8 SL vs F8DL). There were no

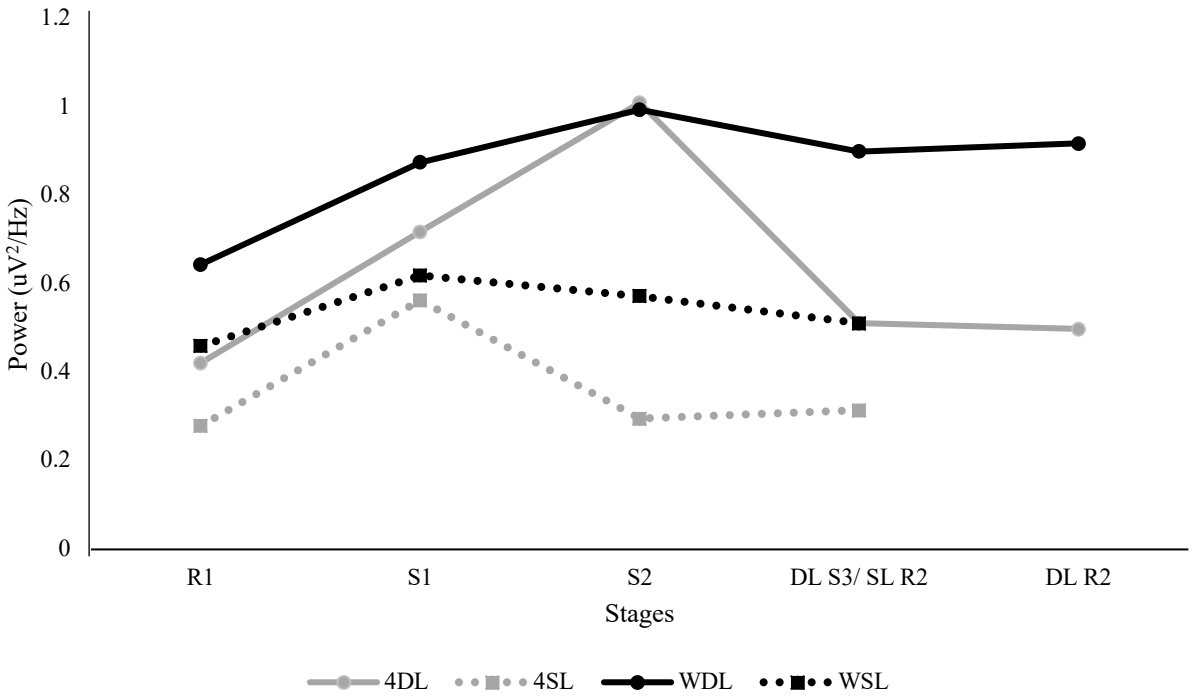
significant differences in SL vs DL within electrode, but there were notable ES for F7 α (d =0.68, F7SL vs F7DL = 0.22 ± 0.25 vs. 0.08 ± 0.17, p = 0.13) and F7 γ (d = 1.13, F7SL vs. F7DL = -0.01 ± 0.03 vs. 0.02 ± 0.03, p = 0.14).

c) Case Studies

Two subjects completed both GXTs (SL and DL) and had sufficient data for before exercise, submaximal and maximal exercise, and post exercise. Each subject has an individual case study (Subject A and Subject B) to show electrocortical changes in power for theta, alpha, beta, and gamma frequencies at rest and during exercise. Figures for each case study have both DL and SL GXT to allow for power visualization between GXTs.

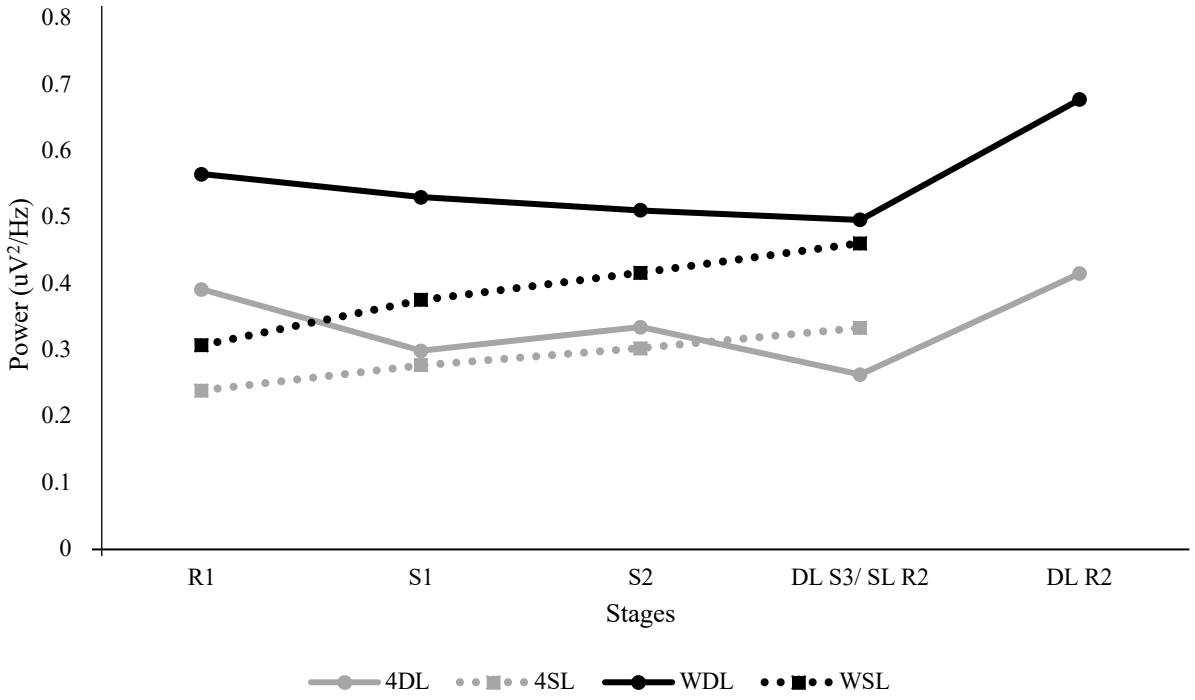
i. Case Study A.

Whole brain and C4 electrode changes in EEG power from SL and DL GXT are shown in Figures 3-6. Data in Figures 3-6 indicates two separate tests (SL GXT and DL GXT). Whole brain data points are mean power values for all 32 channels in θ,α, β, and γ frequencies. C4 values are individual power values for the C4 electrode in θ,α, β, and γ frequencies.



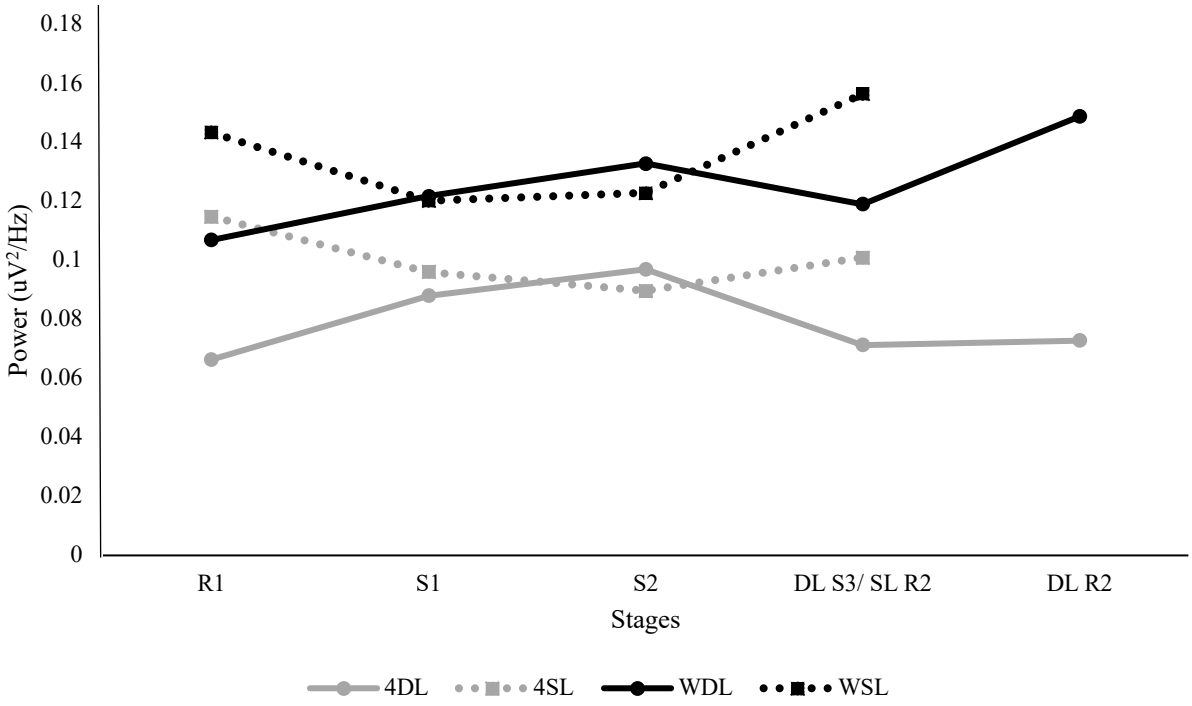
All data is represented as Subject A's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 3: Subject A Whole brain and C4 theta power values before, during, and after GXTs in SL and DL



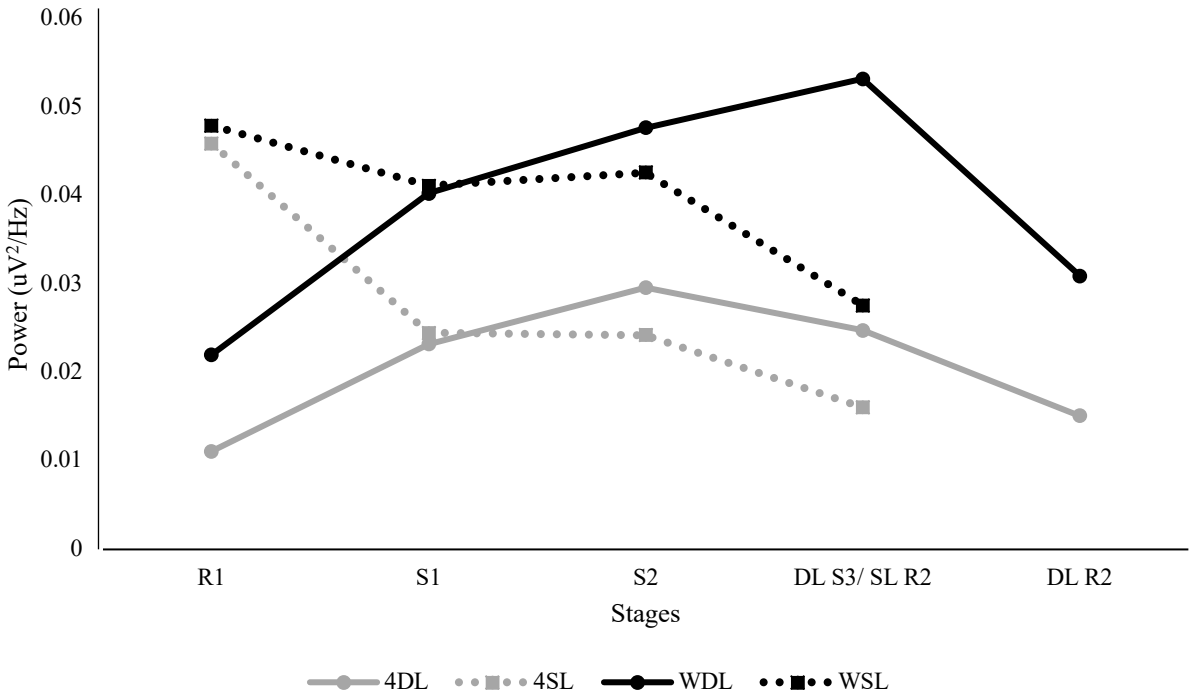
All data is represented as Subject A's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 4: Subject A Whole brain and C4 alpha power values before, during, and after GXTs in SL and DL



All data is represented as Subject A's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 5: Subject A Whole brain and C4 beta power values before, during, and after GXTs in SL and DL

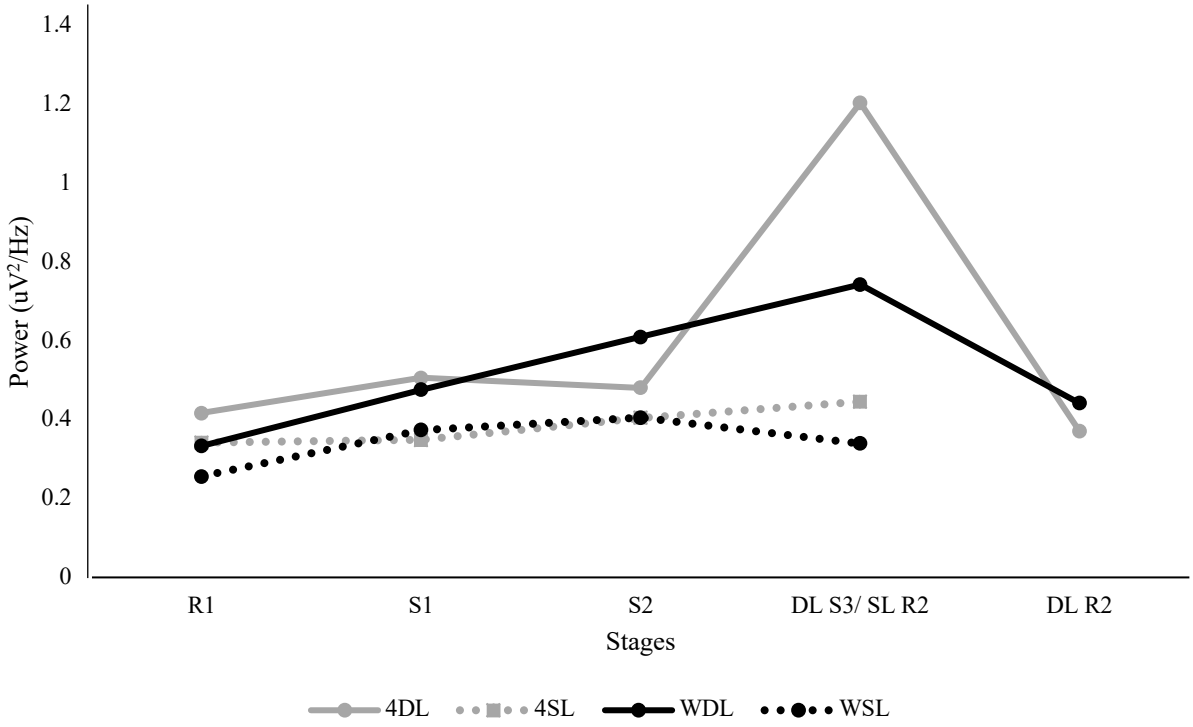


All data is represented as Subject A's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 6: Subject A whole brain and C4 gamma power values before, during, and after GXTs in SL and DL

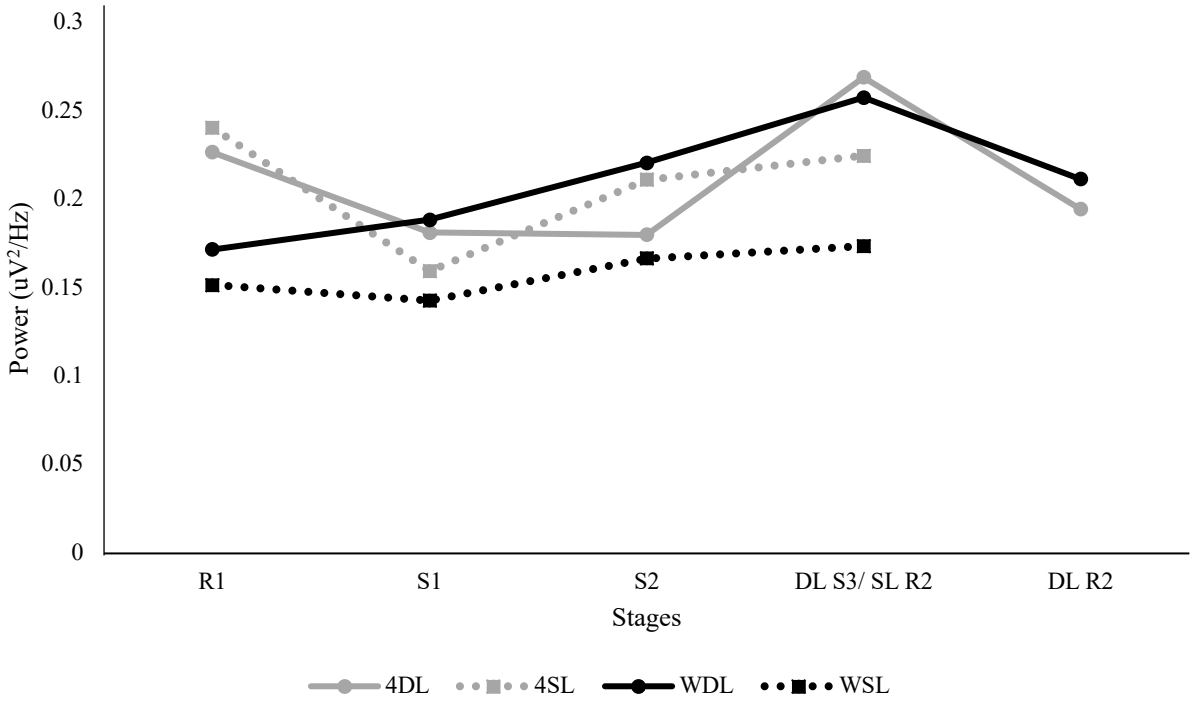
ii. Case Study B.

Whole brain and C4 electrode changes in EEG power from SL and DL GXT are shown in Figures 7-10. Data in Figures 7-10 indicates two separate tests (SL GXT and DL GXT). Whole brain data points are mean power values for all 32 channels in θ,α, β, and γ frequencies. C4 values are individual power values for the C4 electrode in θ,α, β, and γ frequencies.



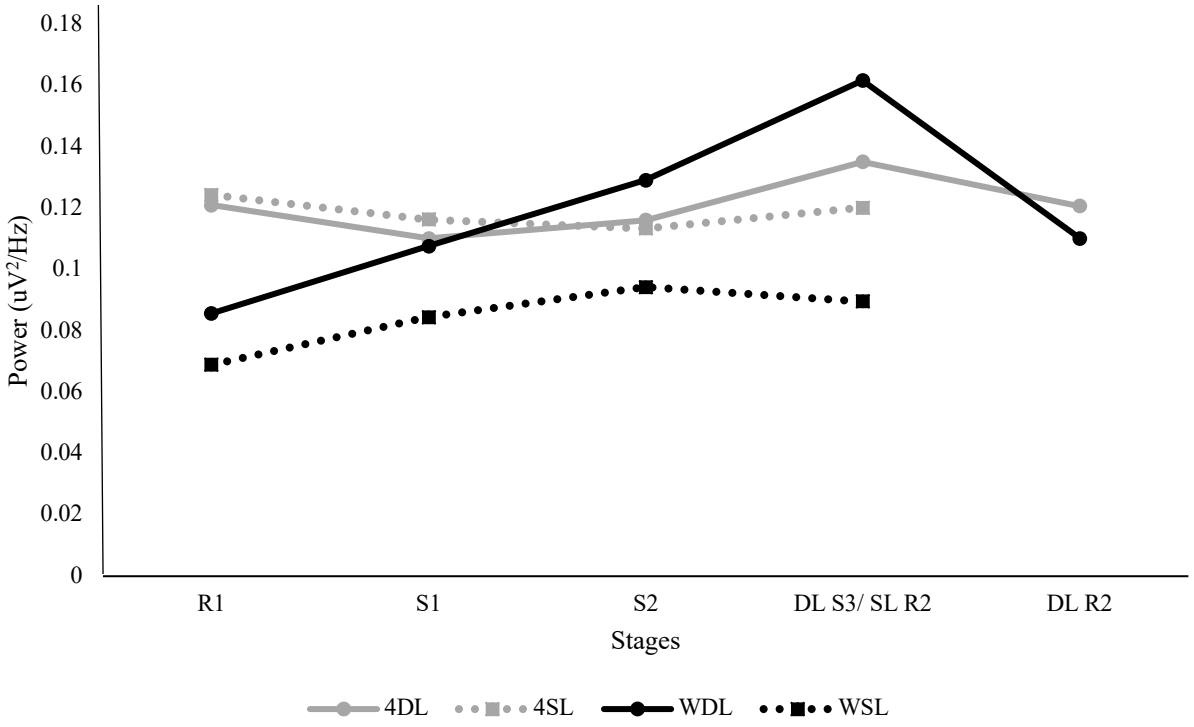
All data is represented as Subject B's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 7: Subject B whole brain and C4 theta power values before, during, and after GXTs in SL and DL



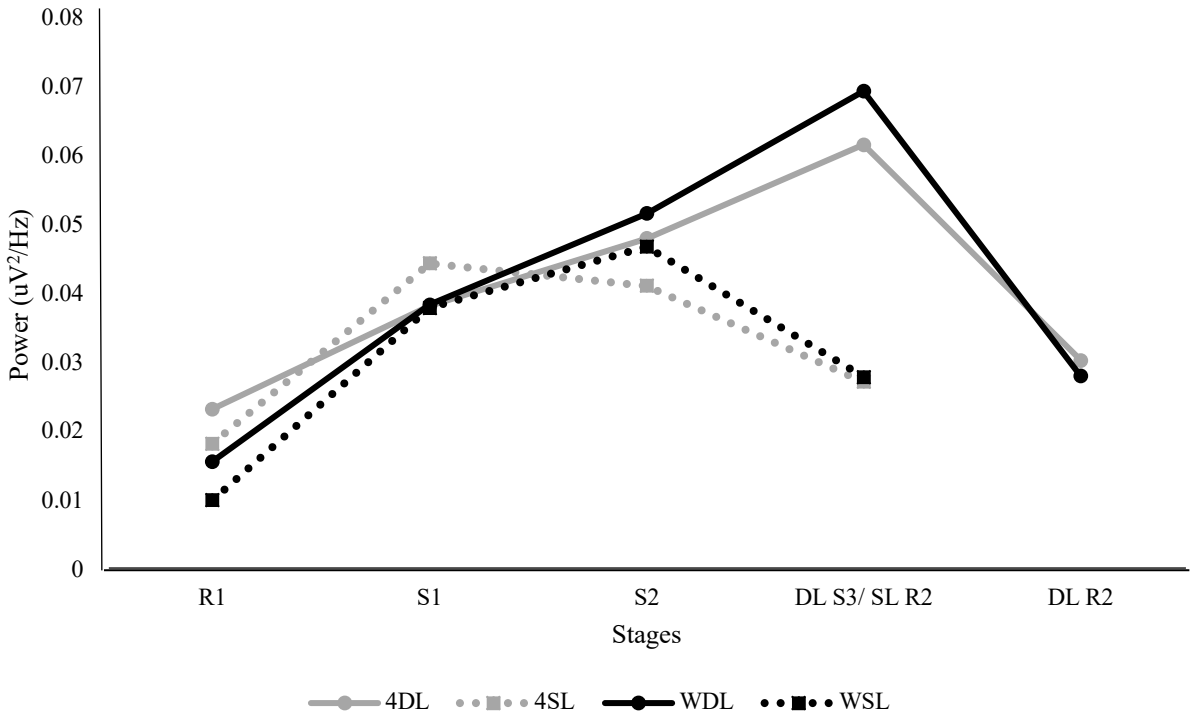
All data is represented as Subject B's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 8: Subject B whole brain and C4 alpha power values before, during, and after GXTs in SL and DL



All data is represented as Subject B's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 9: Subject B whole brain and C4 beta power values before, during, and after GXTs in SL and DL



All data is represented as Subject B's individual data. 4DL= C4 double leg values. 4SL= C4 single leg values. WDL= whole brain double leg values. WSL= whole brain single leg values. R1= resting-pre exercise for DL and SL GXTs. S1 and S2= stages one and two for SL and DL GXTs. DL S3/ SL R2= DL GXT stage 3 and SL resting-post exercise. DL R2= DL resting-post exercise

Figure 10: Subject B whole brain and C4 gamma power values before, during, and after GXTs in SL and DL

d) Summary

i. Rest 1 vs. Rest 2

Whole brain analysis showed increases in activity in R2 compared to R1 in SL and DL. Large effect sizes indicate the possibility of DL exercise producing more activity in whole brain activity compared to SL. The right motor cortex showed increases in activity in in R2 compared to R1 in DL. Moderately large effect sizes indicate the possibility of DL producing more activity in the right motor cortex compared to SL. Overall motor cortex activity increased in DL from R1 to R2. No asymmetries in activity were found between the right and left motor cortex in DL.

ii. Rest 1 vs. Stage 1

Whole brain analysis showed increases in activity in S1 compared to R1 in SL and DL. There were no differences between SL and DL GXTs in whole brain activity. The right motor cortex showed increases in activity in S1 compared to R1. Moderate effect size indicates the possibility of DL producing more activity in the right motor cortex compared to SL. No asymmetries in activity were found between the right and left motor cortex in DL.

CHPATER V: DISCUSSION

The purpose of this study was to utilize EEG to determine if there were significant differences in electrocortical activity between maximal SL and DL GXT in either the whole brain or right motor cortex. It was hypothesized that DL cycling would elicit increased cortical activity compared to SL cycling in the whole brain, and SL cycling would elicit increased cortical activity compared to DL cycling in the right motor cortex. Due to movement artifact and short exercise stages, 8 subjects were included in analysis after data processing. We were able to analyze rest at pre- and post-exercise for 7 subjects and pre-exercise to Stage 1 of exercise in 4 subjects. Two subjects had data for all rest periods and all stages in SL and DL tests. This discussion is outlined as follows: a) whole brain, b) motor cortex, c) prefrontal cortex, d) exercise and LTP, e) multiple sclerosis and exercise, and f) limitations. In subsections a-c, areas discussed include individual case studies (A and B), R1-R2, and R1-S1. Subsections d and e discuss relevant research utilizing a cycling protocol, and our thoughts on future exercise interventions.

a) Whole Brain: Case Studies, R1-R2, and R1-S1

A submaximal constant load (at individuals' anaerobic threshold) cycling study from Ludyga et al., 2015 reported decreases of alpha activity toward the middle and end of exercise in the whole brain. In the current study, Subject A showed an increased gamma power at max exercise in DL and increased alpha, beta, and gamma power at max workload in SL. Subject B showed increases in activity in all frequency bands during SL and DL. While Ludyga's study was submaximal and much longer in duration than the current study, our two case studies did not see similar results. This could be due to the duration of the test itself, with our test being 2-3 minutes of high intensity exercise whereas Ludyga used a submaximal intensity at greater than 30

minutes. When comparing our two cases to Bailey et al., 2008's results, they are much more similar. Bailey's results showed an increase an increase in alpha and activity in all 8 electrodes analyzed. When comparing Bailey and Ludyga's results, the difference in protocol could give rise to the differing neural activity. More research investigating how exercise intensity and duration affects CNS activation needs to be conducted.

From R1 to R2 (resting pre- and post-exercise), Bailey et al, 2008 reported increases at R2 in theta, alpha, and beta activity which is consistent with our results which show increases in theta, alpha, beta and gamma in DL and increases in alpha and beta in SL. Also consistent with Bailey's study, we saw a significant increase from R1 to S1 in theta power, indicating that theta activity possibly plays a role in initiating motor movement. Theta has been found in previous work to be positively associated with executive functions, which could explain the increase in S1 (Trammell et al., 2017). Our findings from comparing SL vs DL is, by indication of a large ES, DL showed increases in beta and gamma activity at R2 compared to SL. Because of beta and gamma's proposed role of increased cortical activation, this was expected due to increased motor movement of both legs during DL cycling (Moraes et al., 2007) (Kandel et al., 2013) (Abhang et al., 2016). This indicates the possibility that the DL cycling elicits increased whole brain activity compared to SL cycling.

b) Motor Cortex: Case Studies, R1-R2, and R1-S1

Afferent signals from the body received by the mid-anterior insular from fatiguing exercise (constant load at 60% peak VO_2 until volitional fatigue) has been shown to increase communication to the motor cortex. This is thought to be a function of the central fatigue response. Therefore, this communication decreases motor cortex efferent signal production to preserve homeostasis (Hilty et al., 2011) (Grimsaw et al., 2014). With this increased

communication, alpha power was seen to decrease in the mid-anterior insular which is believed to be due to alpha's role in cortical inhibition (Hilty et al., 2011) (Uusburg et al., 2013). Enders et al., 2016 looked at non-fatigued (beginning of exercise) and fatigued states (end of exercise) during DL constant load (85% max power) cycling until volitional fatigue. They reported increases in alpha and beta in the supplementary motor area (SMA) in the fatigued condition. Our maximal GXT results from Subject B and Subject A SL GXTs are consistent with alpha and beta increases in C4 (right motor cortex) from stage 1 to max effort. Subject B's DL cycling trial also showed results consistent with Enders, however Subject A showed decreases in alpha and beta at max exercise in DL GXT.

Bailey et al., 2008 saw increases in theta, alpha and beta in the motor cortex pre to immediately post-exercise indicating elevated activity in the motor cortex even after exercise. Our results also saw this increase in DL C4 activity in beta, as well as gamma. We also saw increases at R2 in beta activity in overall motor cortex activity (C3 and C4); both consistent with Bailey's work. Another interesting result from Bailey is in beta 1 (13-17.99 Hz) and alpha frequencies, where there was no difference from immediately post exercise and max intensity in the motor cortex. This indicates the possibility that looking at immediately post exercise values can infer values in the motor cortex at max exercise in these frequencies. Our finding in the current study is that DL showed possible increases, inferred from moderately large ES, in beta and theta post-exercise compared to SL in the contralateral (C4) motor cortex. This result does not explain peripheral results, from a neurological standpoint, which showed SL cycling producing an increased EMG response relative to DL in previous work (MacInnis et al., 2017). However, when compared to DL cycling, increased oxidative molecules in the exercising leg during SL cycling have been reported (Abbiss et al., 2011). Due to a greater increase in

oxidative molecules in SL, as well as increased cardiac output allowing for more O_2 delivery for neuromuscular energy support, differing performance in SL vs DL could be due peripheral adaptations from metabolic, neuronal and cardiovascular components (Abbiss et al., 2011) (Jha and Morrison et al., 2018) (Gordon et al., 2019).

When looking at R1-S1 in the current study, we saw a medium ES indicating increased DL theta activity compared to SL at S1. We found significant increases in DL gamma activity from R1 to S1 in C4 and a significant increase in theta activity from DL R1 to S1 in the overall motor cortex with no asymmetry in activity. Previous work which has utilized GXT to volitional fatigue also saw only a significant increase in theta from R1-S1 in the motor cortex (Bailey et al., 2008) indicating theta's possible role in generation of initial movement.

c) Prefrontal Cortex: R1-R2, and R1-S1

While the role of the PFC during exercise is not entirely understood, the PFC's role is believed to coordinate behavioral responses, such as ensuring appropriate movement and attainment of goals. The PFC has many afferent and efferent projections throughout the cerebrum, which allows for the PFC to assume its executive function role through sensory integration to modulate motor cortex activity (Robertson and Marino et al., 2015). The VLPFC is involved in bottom-up processing, including processing sensory and emotional stimuli (Grimshaw et al., 2014). The DLPFC is involved in top-down processing including executive processes such as ensuring a desired behavior or action (Knierim, et al., 2019) (Grimshaw et al., 2014). The DLPFC likely assumes its function through VLPFC input which influences goal directed behavior. Differences in hemispheric activity based on the asymmetric inhibition model have shown increased activity in the left PFC to be associated with inhibition of withdrawal and the right PFC to be associated with inhibition of approach (Grimshaw et al., 2014). Alpha

activity in the PFC is thought to represent cortical inhibition and asymmetries have been reported between hemispheres in alpha power levels (Uusburg et al., 2013). Due to alpha's assumed role in decreased cortical activity (inhibition), left and right activity levels are thought to be decreased with an increase in alpha power (Grimshaw et al., 2014).

In the current study, we measured 4 electrodes which represent the lateral prefrontal cortex (F3, F4, F7 and F8). In our R1 to R2 analysis we did find significant increases in activity in overall (F3 and F4) DLPFC and (F7 and F8) VLPFC in SL and DL, consistent with Bailey et al., 2008, indicating elevated activity in the prefrontal cortex after exercise. We also found that there were greater increases in SL alpha power compared to DL in DLPFC and VLPFC based on large and medium effect sizes, respectively. Our R1 to R2 analysis showed a common trend with SL showing higher levels of activity in alpha in both hemispheres in the DLPFC and the VLPFC showing lower alpha activity in DL. While these results are not significant and are based on moderately large effect sizes, these results show the possibility that DL cycling activates the cortex to a greater extent than SL cycling does.

In our R1 to S1 analysis, we found a significant decrease in DL alpha power in overall DLPFC and no change in any frequency bands in the VLPFC. This result favors alpha cortical inhibition by indicating increased DLPFC activity of goal directed behavior to achieve movement from rest. SL vs DL cycling in the DLPFC showed large effect sizes for increased gamma and decreased alpha in DL compared to SL. Large and medium ES indicate the possibility that alpha power showed a greater increase in SL vs DL and gamma showed greater increase DL vs SL in the VLPFC, respectively.

While looking at asymmetries in activity from R1 to S1, we found no significant differences between hemispheres. Although, in the right VLPFC (F8) we found moderately large

and large ES for increased SL gamma and beta activity in F8 compared to F7, respectively. The right DLPFC (F4) was seen to have a large ES indicating the possibility of F4 having increased theta activity compared to the left DLPFC (F3).

SL vs DL within electrode (ex. F3 vs F3) from R1 to S1, we found moderately large effect sizes for increased alpha activity in F7 SL compared to DL and increased gamma activity in F7 DL compared to SL. Due to alphas inhibitory role (Grimshaw et al., 2014) and gamma indicating increased cortical activity (Abhang et al., 2016), the left VLPFC showed increased neural activity compared to the right. Large ES were seen for F3 and F4 in alpha power, indicating that DL cycling produced increased DLPFC activity in both hemispheres compared to SL.

d) Exercise and Long-term Potentiation (LTP)

LTP has characteristics of functional enhancement of synaptic connections and increased excitatory postsynaptic potentiation. Exercise induced LTP has been extensively studied in animal models with very little work done in humans. LTP in animal models has shown exercise induced increases in N-Methyl- d-aspartate (NMDA) receptor expression in the prefrontal cortex, which is an important area for organizing appropriate actions such as the desired behavior of exercise during a GXT (Loprinzi et al., 2019) (Robertson et al., 2015). Exercise related increases in brain-derived neurotrophic factor (BDNF) has proved a variety of functions, such as upregulating NMDA receptor expression and inducing neurogenesis, both of which may play a role in LTP (Loprinzi et al., 2019). Two human studies utilizing paired associative stimulation (PAS) have been conducted looking at exercise induced LTP. Mang et al., 2014 utilized a GXT on a cycle ergometer and Singh et al., 2015 utilized a 20 min submaximal cycling test at 65-70%

of subjects' age predicted maximal heart rate (HR). Both tests found that exercise increased cortical excitability when paired with PAS (Mang et al., 2014) (Singh et al., 2015).

The current study aimed to see if SL cycling increased motor cortex activity compared to DL cycling. With previous tests showing increases in SL vs DL in relative power outputs, EMG activity, oxidative molecules and cardiac output (Abbiss et al., 2011) (MacInnis et al., 2017)(Gordon et al., 2020), SL vs DL GXT with PAS is an area for future research to assess which group has a greater LTP response. EEG during exercise in the current protocol, followed by PAS is another area for future research to compare power spectral density in different frequencies with PAS induced LTP.

e) *Multiple Sclerosis and Exercise*

Motor weakness in patients with MS is thought to be due to cortical/spinal lesions (demyelination), and inflammatory cells/antibodies that are able to damage axons. Supporting factors associated with LTP are also thought to be destroyed through inflammatory action (Purves, Augustine, & Fitzpatrick, 2001). In certain neurological diseases, such as MS, bilateral asymmetry of the lower limbs has been evaluated through results such as oxygen uptake, workload and strength. MS subjects showed significantly greater asymmetry for strength, O2 uptake, and workload compared to Non-MS subjects (Larson, McCully, Larson, Pryor, & J.White, 2013). Exercise has been seen in animal models as well as limited human studies, to have a role in increasing LTP induction in motor areas of the CNS (Loprinzi et al., 2019). In mice, exercise has also been seen to enhance remyelination of demyelinated axons (Jensen et al., 2018). Due to exercises' proposed role of increasing synaptic strength and enhanced myelination rates, more research documenting these results for potential therapeutic intervention needs to be done. SL cycling has proposed benefits when it comes to peripheral adaptations in neural,

metabolic, and cardiovascular components as well as decreased respiratory exertion. Future research should look at SL vs DL cycling in MS while utilizing EEG and possibly PAS to assess LTP.

f) Limitations

The largest limitation of this study was the time duration of each stage for the GXT. The time duration did not allow for us to get a minimum of 20, 1s EPOCHs for the majority of our subjects which is the minimum number of seconds needed for reliable data (Gudmundsson et al., 2007). Due to our 1-minute exercise protocol, 18 of our 26 participants were rejected due to insufficient amount of data. In future studies, a minimum of 2-minute stages should be done to receive sufficient results.

Movement artifact was our other limitation during this study. This, in conjunction with short duration stages, did not allow for 16 of our subjects to make it past pre-processing. In future studies that want to utilize short duration stages, we suggest utilizing a recumbent cycle ergometer, and possibly utilizing straps over subject's chests to limit movement.

The results of this study can only be generalized to healthy males and females 18-35 years old. The inclusion criteria of this study omitted individuals with existing neurological, cardiovascular, psychological, or orthopedic conditions or disorders.

CHAPTER VI: CONCLUSION

The specific aim of this study was to compare whole brain and C4 EEG response in SL vs DL cycling GXT. Due movement artifact and limited number of EPOCHs (seconds) per stage, analysis was only conducted for R1 to R2 (n=7) and R1 to S1 (n=4). Our research questions included: 1) Are there differences in whole brain electrocortical activity during SL and DL cycling with increasing workloads. We hypothesized that DL cycling would elicit increased activity and we fail to reject the null. 2) Is there increased motor cortex electrocortical activity in SL or DL with increasing workloads? We hypothesized that SL would elicit increased activity and we fail to reject the null. 3) Are there differences in whole brain activity between SL and DL pre- to post-exercise? We hypothesized that DL would elicit increased activity and we fail to reject the null. 4) Are there differences in motor cortex activity between SL and DL pre- to post-exercise? We hypothesized that SL would have increased activity and we fail to reject the null.

The two key findings from whole brain analysis was the increase from R1 to S1 in theta power in DL and SL and increased beta and gamma power, based on moderately large and large ES, in DL compared to SL from R1 to R2, respectively. Previous results from GXT showed increases in theta activity only from R1 to S1 (Bailey et al., 2008) which indicates the possibly of thetas role in movement generation. Beta and gamma increases are consistent with previous literature indicating their role in increased cortical activation (Moraes et al., 2007) (Kandel et al., 2013) (Abhang et al., 2016). This allows us to infer that levels were high at max due to Baiely et al., 2008 showing that EEG activity immediately post exercise remains elevated.

Analysis of C4 showed significant increases in beta from R1 to R2, as well as increases in gamma from R1 to R2 and R1 to S1. While Bailey et al., 2008 did not measure gamma, the beta results are consistent with the previous literature. Novel findings in C4 from the current

study include increased activity in DL compared to SL in R1-R2 and R1 to S1 inferred from moderately large and medium ES, respectively (R1-R2: theta and beta ; R1-S1: theta). This adds interest in theta power due to previous findings of increased theta power with increased stimuli complexity (Grunwald et al., 2001).

Alpha activity has been the most extensively researched frequency due to its role in inhibition of cortical activity (Grimshaw et al., 2014). Novel findings based on moderately large ES indicate the possibility of increased alpha activity from R1 to R2 in F3 and F4, areas associated with attainment of goals (Robertson and Marino et al., 2015), in SL compared to DL. The DLPFCs role in this is not clear as inhibition of both hemispheres seems to indicate inhibition of withdraw and inhibition of approach from the left and right hemispheres, respectively (Grimshaw et al., 2014). While these results are by no means concrete, it is interesting that we also found a significant decrease in DL alpha activity in overall DLPFC and a large ES indicating the possibility that alpha is decreased in F3 and F4 in DL compared to SL from R1 to S1. With these results in mind, they indicate the possibility that alpha activity is decreased in DL cycling compared to SL cycling showing that there is more DLPFC activity in DL cycling compared to SL in both hemispheres.

While the DLPFC is involved in top-down processing, the VLPFC is involved in bottom up processing such as processing sensory and emotional stimuli (Grimshaw et al., 2014). We found significant increases in DL and SL alpha activity at R2 as well as a moderately large ES indicating increased alpha activity in F7 during SL cycling compared to DL in R2 and S1. The inhibited left VLPFC shown by increased alpha in SL shows the likelihood of SL causes greater inhibition of sensory and emotional processing of the left VLPFC compared to DL cycling.

When conducting maximal effort SL vs DL cycling for future research, cycling should be done on a recumbent bike to limit movement artifact. Also, if straps are available, the subject should be strapped into the cycle to further limit movement. Another suggestion when considering this test is to increase the time duration of each stage to at least 2 minutes. 1-minute stages were not enough time to get enough data for the majority of our subjects.

Based on our ES analysis between SL and DL cycling, we found very interesting results indicating the possibility that DL cycling elicited more activity in the contralateral motor cortex compared to SL cycling. Also, prefrontal cortex results indicated the SL cycling condition elicited less activity in DLPFC and VLPFC when compared to DL cycling. With previous research assessing neuromuscular, cardiovascular and metabolic factors during single leg cycling, we can assess the CNS through EEG to shed light on central vs peripheral fatigue. More research needs to be conducted in both areas, EEG and EMG, preferably simultaneously, to assess the complexity of the central and peripheral nervous system during fatiguing exercise.

References

Abbiss, C. R., Karagounis, L. G., Laursen, P. B., Peiffer, J. J., Martin, D. T., Hawley, J. A., … Martin, J. C. (2011). Single-leg cycle training is superior to double-leg cycling in improving the oxidative potential and metabolic profile of trained skeletal muscle. J Appl Physiol, 110, 8.

Abhang, P. A., Bharti, G. W., & Suresh, M. C. (2016). Chapter3-Technical aspects of Brain rhythms and Speech Parameters. In *Introduction to EEG- and Speech- Based Emotion Recognition*. https://www.sciencedirect.com/science/article/pii/B9780128044902000038

Bailey, S. P., Hall, E. E., Folger, S. E., & Miller, P. C. (2008). Changes in EEG during graded exercise on a recumbent cycle ergometer. Journal of Sports Science and Medicine, 7, 505–511.

Brümmer, V., Schneider, S., Strüder, H. K., & Askew, C. D. (2011). Primary motor cortex activity is elevated with incremental exercise intensity. Neuroscience, 181, 150–162. https://doi.org/10.1016/j.neuroscience.2011.02.006

Enders, H., Cortese, F., Maurer, C., Baltich, J., Protzner, A. B., & Nigg, B. M. (2016). Changes in cortical activity measured with EEG during a high-intensity cycling exercise. Journal of Neurophysiology, 115(1), 379–388. https://doi.org/10.1152/jn.00497.2015

Gordon, N., Abbiss, C. R., Maiorana, A. J., Peiffer, J. J. (2019). Single-leg cycling increases limb-specific blood flow without concurrent increases in normalized power output when compared with double-leg cycling in healthy middle-aged adults. *European Journal of Sport Science, 20(2).* https://doi.org/10.1080/17461391.2019.1617789

Grimshaw, G. M., & Carmel, D. (2014). An asymmetric inhibition model of hemispheric differences in emotional processing. *Frontiers in Psychology*, *5*. https://doi.org/10.3389/fpsyg.2014.00489

Grunwald M, Weiss T, Krause W, et al. Theta power in the EEG of humans during ongoing processing in a haptic object recognition task. Brain Res Cogn Brain Res. 2001;11(1):33-37. doi:10.1016/s0926-6410(00)00061-6

Gudmundsson, S., Runarsson T., Sigurdsson S., Eiriksdottir, G., Johnson, K. (2007). Reliability of quantitative EEG features. Clinical Neurophysiology, 118, 2162-217. doi:10.1016/j.clinph.2007.06.018

Hilty, L., Langer, N., Pascual-Marqui, R., Boutellier, U., & Lutz, K. (2011). Fatigue-induced increase in intracortical communication between mid/anterior insular and motor cortex during cycling exercise: Muscle fatigue-induced intracortical communication. European Journal of Neuroscience, 34(12), 2035–2042. (Robertson & Marino, 2015)

Honn, K. A., Hinson, J. M., Whitney, P., & Van Dongen, H. P. A. (2019). Cognitive flexibility: A distinct element of performance impairment due to sleep deprivation. *Accident Analysis & Prevention*, *126*, 191–197. https://doi.org/10.1016/j.aap.2018.02.013

How does the nervous system work? (2016). In *Institute for Quality and Efficiency in Health Care*.

Jensen, S. K., Michaels, N. J., Ilyntskyy, S., Keough, M. B., Kovalchuk, O., & Yong, V. W. (2018). Multimodal Enhancement of Remyelination by Exercise with a Pivotal Role for Oligodendroglial PGC1α. *Cell Reports*, *24*(12), 3167–3179. https://doi.org/10.1016/j.celrep.2018.08.060

Jha, M. K., & Morrison, B. M. (2018). Glia-neuron energy metabolism in health and diseases: New insights into the role of nervous system metabolic transporters. *Experimental Neurology*, *309*, 23–31. https://doi.org/10.1016/j.expneurol.2018.07.009

Knierim, J. (2019). Chapter 3: Motor Cortex. In Neuroscience online. McGovern Medical School at UTHealth Department of Neurobiology and Anatomy.

Larson, R. D., McCully, K. K., Larson, D. J., Pryor, W. M., & J.White, L. (2013). Bilateral differences in lower-limb performance in individuals with multiple sclerosis. The Journal of Rehabilitation Research and Development, 50(2), 215. https://doi.org/10.1682/JRRD.2011.10.0189

Loprinzi, P. D. (2019). The Effects of Exercise on Long-Term Potentiation: A Candidate Mechanism of the Exercise-Memory Relationship. *OBM Neurobiology*, *3*(2), 1–1. https://doi.org/10.21926/obm.neurobiol.1902026

Ludyga, S., Gronwald, T., & Hottenrott, K. (2015). Do Male and Female Cyclists' Cortical Activity Differ before and during Cycling Exercise? Journal of Sport and Exercise Psychology, 37(6), 617–625. https://doi.org/10.1123/jsep.2015-0078

MacInnis, M. J., Morris, N., Sonne, M. W., Zuniga, A. F., Keir, P. J., Potvin, J. R., & Gibala, M. J. (2017). Physiological responses to incremental, interval, and continuous counterweighted single-leg and double-leg cycling at the same relative intensities. European Journal of Applied Physiology, 117(7), 1423–1435. https://doi.org/10.1007/s00421-017-3635-8

Mang, C. S., Snow, N. J., Campbell, K. L., Ross, C. J. D., Boyd, L. A. (2014). A single bout of high-intensity aerobic exercise facilitates response to paired associative stimulation and promotes sequence-specific implicit motor learning. *Journal of Applied Physiology, 117(11),* 1325-1336. https://dx.doi.org/10.1152%2Fjapplphysiol.00498.2014

Moraes, H., Ferreira, C., Deslandes, A., Cagy, M., Pompeu, F., Ribeiro, P., & Piedade, R. (2007). Beta and alpha electroencephalographic activity changes after acute exercise. *Arquivos de Neuro-Psiquiatria*, *65*(3a), 637–641. https://doi.org/10.1590/S0004-282X2007000400018

Perrey, S., & Besson, P. (2018). Studying brain activity in sports performance: Contributions and issues. In Progress in Brain Research (Vol. 240, pp. 247–267). Elsevier. https://doi.org/10.1016/bs.pbr.2018.07.004

Purves, D., Augustine, G., & Fitzpatrick, D. (2001). Box D, Multiple Sclerosis. In *Neuroscience* (2nd ed.). Sunderland (MA): Sinauer Associates.

Purves, D., Augustine, G., & Fitzpatrick, D. (2001). Functional Organization of the Primary Motor Cortex. In Neuroscience (2nd ed.). Sunderland (MA): Sinauer Associates.

Purves, D., Augustine, G., & Fitzpatrick, D. (2001). Increased Conduction Velocity as a Result of Myelination. In Neuroscience (2nd ed.). Sunderland (MA): Sinauer Associates.

Reisberg, D. (2016). Cognition Exploring the Science of the Mind: (6th ed.). W.W. Norton & Company.

Robertson, C. V., & Marino, F. E. (2015). Prefrontal and motor cortex EEG responses and their relationship to ventilatory thresholds during exhaustive incremental exercise. *European Journal of Applied Physiology*, *115*(9), 1939–1948. https://doi.org/10.1007/s00421-015-3177-x

Samuels, C. (2008). Sleep, Recovery, and Performance: The New Frontier in High-Performance Athletics. *Neurologic Clinics*, *26*(1), 169–180. https://doi.org/10.1016/j.ncl.2007.11.012

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

Sleiman, S. F., Henry, J., Al-Haddad, R., Hayek, L. E., Haidar, E. A., Stringer, T., Ulja, D., Karuppagounder, S. S., Holson, E. B., Ratan, R. R., Ninan, I., & Chao, M. V. (2016). Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body b- hydroxybutyrate. *Cell Biology*, 21.

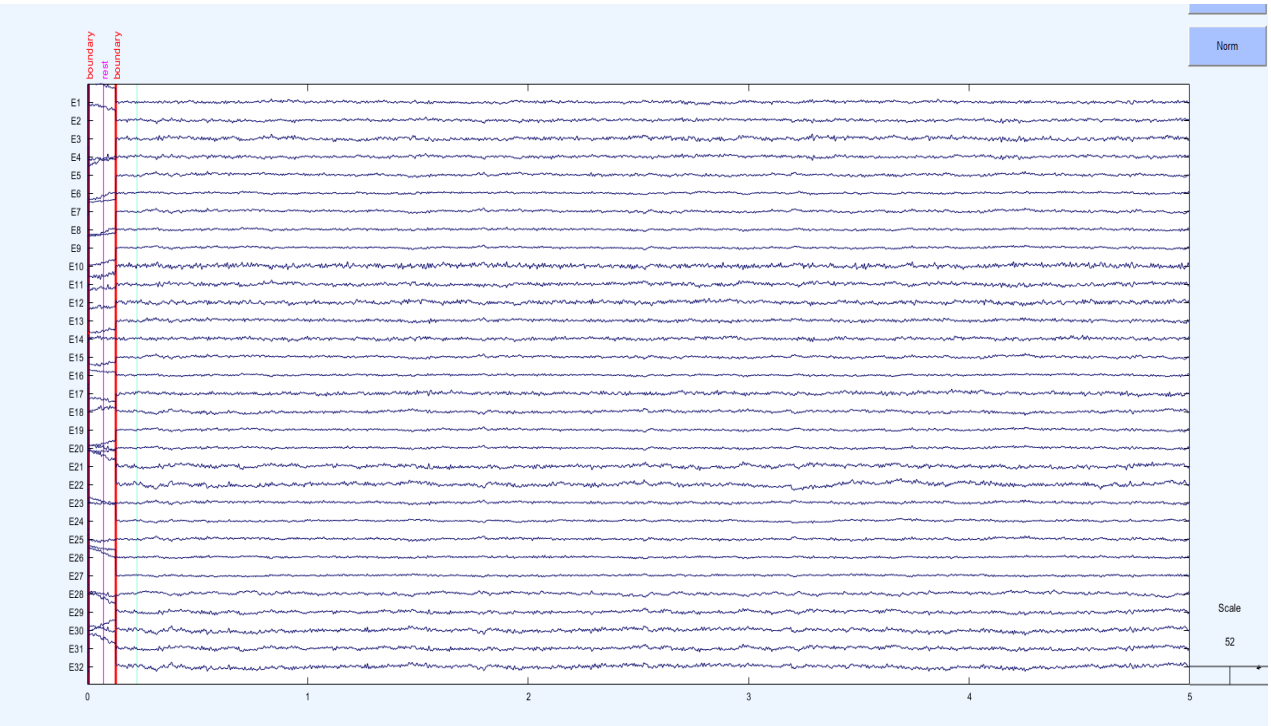
Trammell, J. P., MacRae, P. G., Davis, G., Bergstedt, D., & Anderson, A. E. (2017). The Relationship of Cognitive Performance and the Theta-Alpha Power Ratio Is Age-Dependent: An EEG Study of Short Term Memory and Reasoning during Task and Resting-State in Healthy Young and Old Adults. *Frontiers in Aging Neuroscience*, *9*, 364. https://doi.org/10.3389/fnagi.2017.00364

Uusberg, A., Uibo, H., Kreegipuu, K., & Allik, J. (2013). EEG alpha and cortical inhibition in affective attention. *International Journal of Psychophysiology*, *89*(1), 26–36. https://doi.org/10.1016/j.ijpsycho.2013.04.020

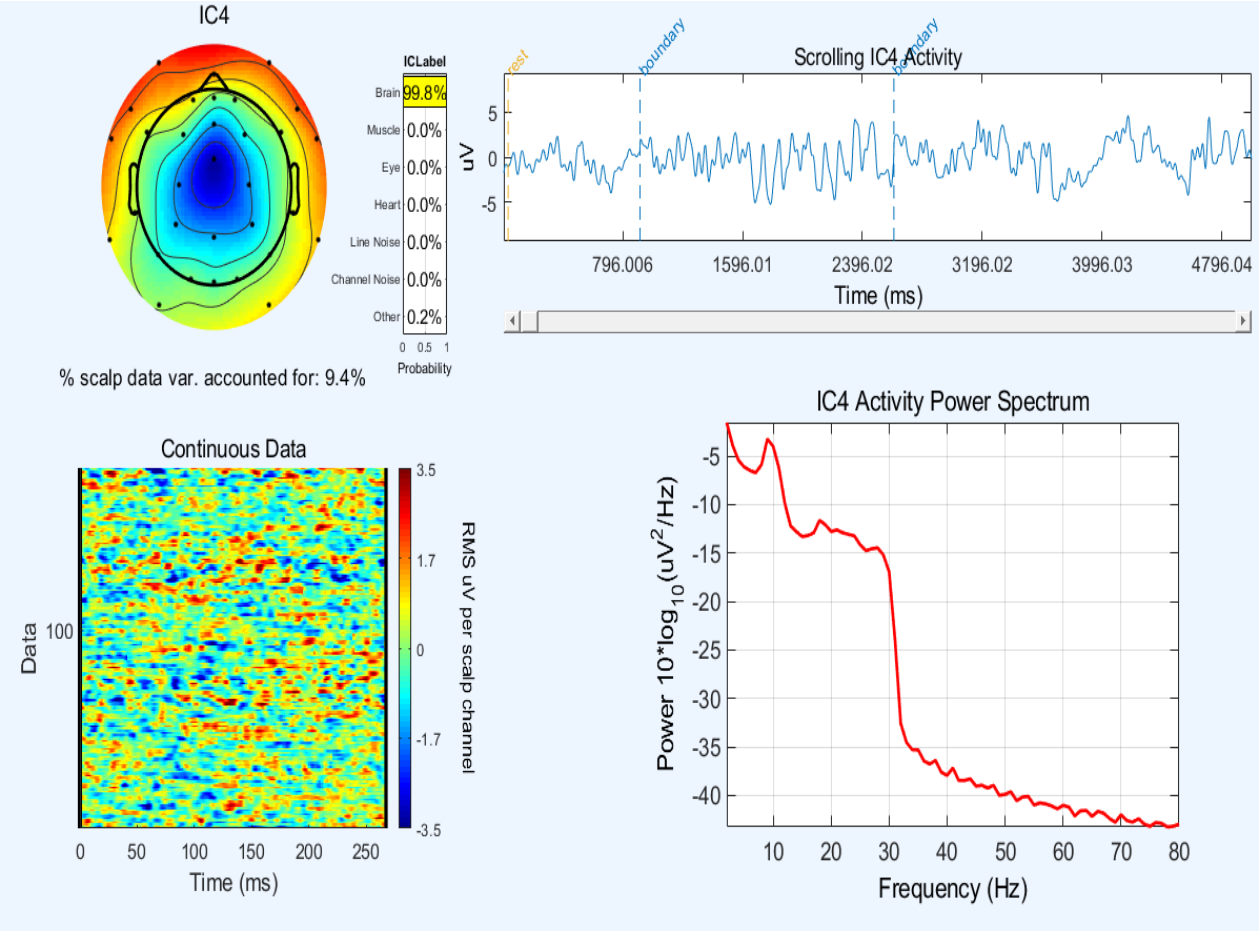
Verweij, I. M., Romeijn, N., Smit, D. J., Piantoni, G., Van Someren, E. J., & van der Werf, Y. D. (2014). Sleep deprivation leads to a loss of functional connectivity in frontal brain regions. *BMC Neuroscience*, *15*(1), 88. https://doi.org/10.1186/1471-2202-15-88

APPENDIX

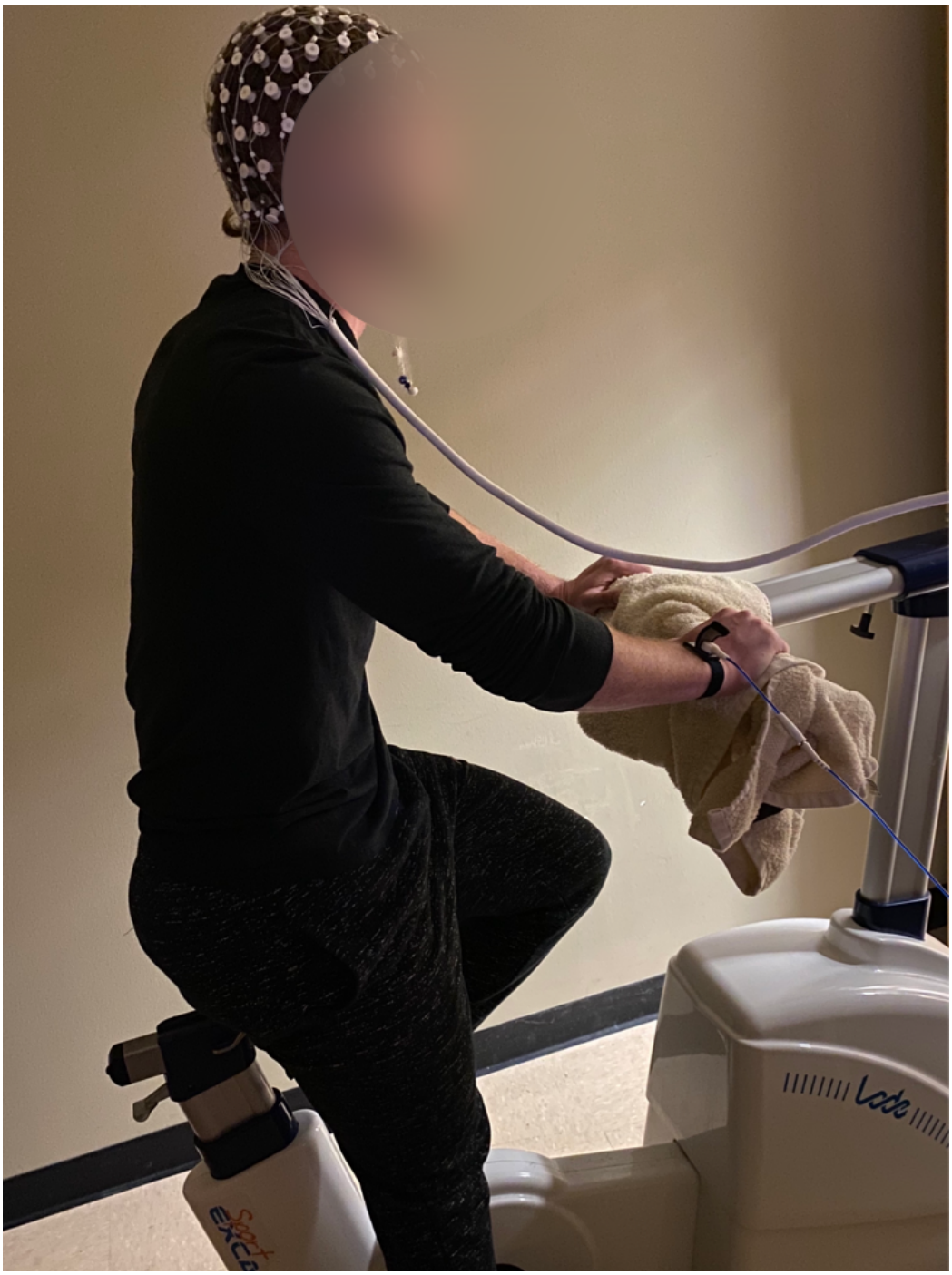
1. Cleaned EEG data



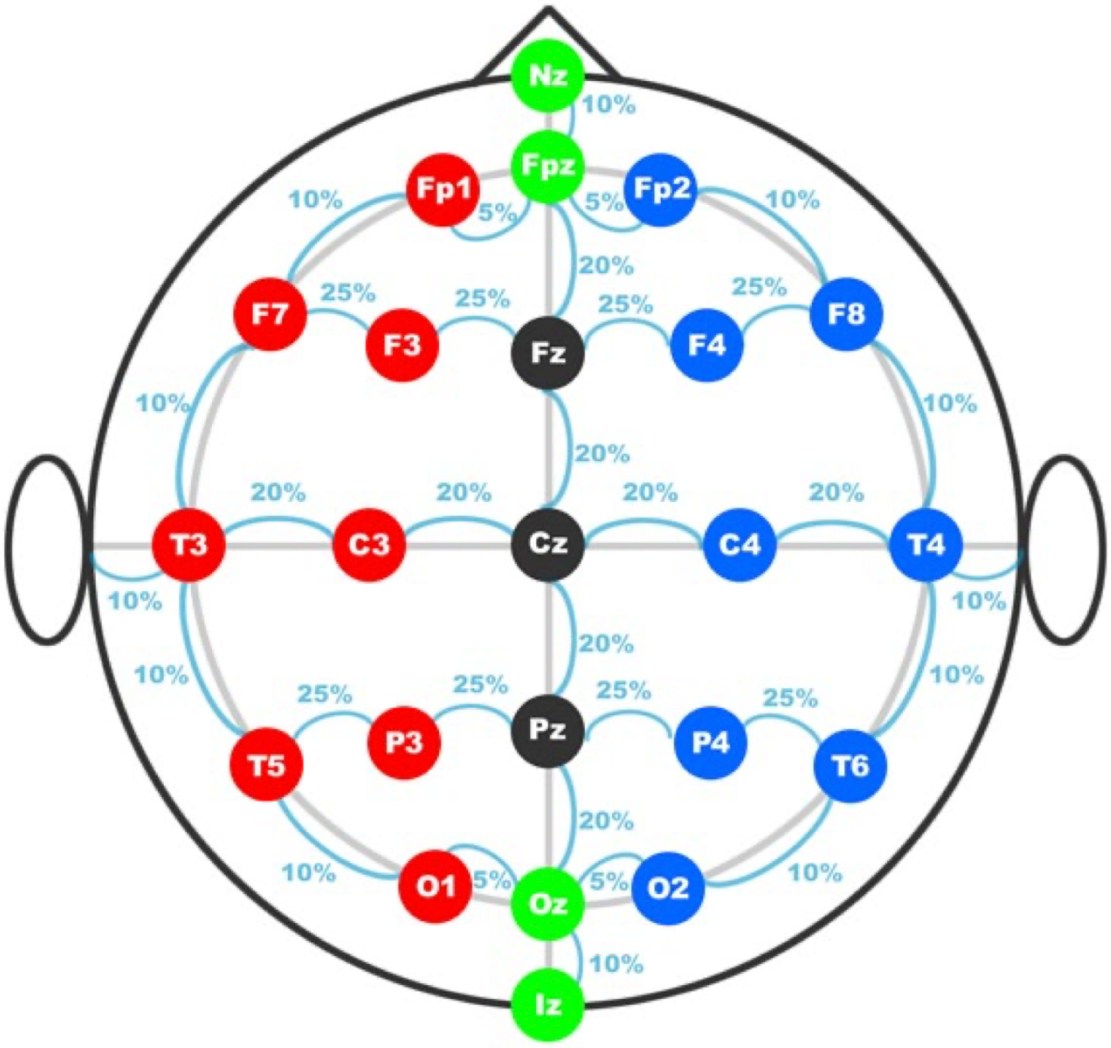
2. ICLabel


IC4
ICLabel
Brain 99.8%
Muscle 0.0%
Eye 0.0%
Heart 0.0%
Line Noise 0.0%
Channel Noise 0.0%
Other 0.2%
0 0.5 1
Probability
% scalp data var. accounted for: 9.4%
Scrolling IC4 Activity
test
boundary
boundary
uV
5
0
-5
796.006
1596.01
2396.02
3196.02
3996.03
4796.04
Time (ms)
Continuous Data
Data
100
3.5
1.7
0
-1.7
-3.5
RMS uV per scalp channel
0
50
100
150
200
250
Time (ms)
IC4 Activity Power Spectrum
Power 10*log10(uV2/Hz)
-5
-10
-15
-20
-25
-30
-35
-40
10
20
30
40
50
60
70
80
Frequency (Hz)

3. Subject Set Up



4. 10/20



http://chgd.umich.edu/wp-content/uploads/2014/06/10-20_system_positioning.pdf

5. IRB Outcome Letter

The UNIVERSITY of OKLAHOMA

Institutional Review Board for the Protection of Human Subjects

Approval of Initial Submission – Expedited Review – AP01

Date: October 23, 2019 **IRB#:** 11317

Principal Investigator: Rebecca Larson, PhD **Approval Date:** 10/22/2019

Status Report Due: 09/30/2020

Study Title: DIFFERENCES IN ELECTROCORTICAL ACTIVITY BETWEEN SINGLE AND DOUBLE LEG GRADED EXERCISE CYCLING: AN EEG STUDY

Expedited Category: 3 & 7

Collection/Use of PHI: Yes

On behalf of the Institutional Review Board (IRB), I have reviewed and granted expedited approval of the above-referenced research study. To view the documents approved for this submission, open this study from the *My Studies* option, go to *Submission History*, go to *Completed Submissions* tab and then click the *Details* icon.

Requirements under the Common Rule have changed. The above-referenced research meets one or more of the circumstances for which continuing review is not required. However, as Principal Investigator of this research, you will be required to submit an annual status report to the IRB.

As principal investigator of this research study, you are responsible to:

- Conduct the research study in a manner consistent with the requirements of the IRB and federal regulations 45 CFR 46.
- Obtain informed consent and research privacy authorization using the currently approved, stamped forms and retain all original, signed forms, if applicable.
- Request approval from the IRB prior to implementing any/all modifications.
- Promptly report to the IRB any harm experienced by a participant that is both unanticipated and related per IRB policy.
- Maintain accurate and complete study records for evaluation by the HRPP Quality Improvement Program and, if applicable, inspection by regulatory agencies and/or the study sponsor.
- **Submit an annual status report to the IRB to provide the study/recruitment status and report all harms and deviations that may have occurred.**
- **Submit a final closure report at the completion of the project.**

If you have questions about this notification or using iRIS, contact the IRB @ 405-325-8110 or irb@ou.edu.

Cordially,

6. Recruitment Flyer

SUBJECTS NEEDED FOR RESEARCH STUDY

Differences in Electrocortical Activity Between Single and Double Leg Graded Exercise Cycling: An EEG Study

We are looking for:

- Males and females, age 18-35
- Subjects who cycle <2 days/week
- Free of injury/disease/conditions that affects neurological function/high intensity exercise

Requires only 3 visits ~ 1 hr each

1st visit → Familiarization

2nd & 3rd visit → Graded Exercise Test

The University of Oklahoma
Department of Health and Exercise Science

The University of Oklahoma is an equal opportunity institution

Cameron Owens 405-974-0618 cdowens@ou.edu

Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu
Cameron Owens 405-974-0618 cdowens@ou.edu

7. Consent Form

Signed Consent to Participate in Research

Would you like to be involved in research at the University of Oklahoma?

I am Cameron Owens, an Exercise Physiology Master's student from the Health and Exercise Science Department and I invite you to participate in my research project entitled Differences in Electrocortical Activity Between Single and Double Leg Graded Exercise Cycling: An EEG Study. This research is being conducted at the University of Oklahoma in the Visual Neuroscience Laboratory. You were selected as a possible participant because you are a healthy male or female who meet the inclusion criteria – meaning you are not a trained cyclist (<2 days/week) and you are free of diagnosed neurological, psychiatric, cardiovascular, orthopedic, and respiratory conditions/diseases. You must be at least 18 years of age to participate in this study.

Please read this document and contact me to ask any questions that you may have BEFORE agreeing to take part in my research.

What is the purpose of this research? The purpose of this research is to assess whether there is increased brain activity in certain regions of interest in the brain during a single leg cycling max effort test compared to a double leg cycling max effort test.

How many participants will be in this research? About 30 people, aged 18-35, who meet the inclusion criteria of being an untrained cyclist and participating in cycling exercise less than twice per week will take part in this research.

What will I be asked to do? If you agree to be in this research, you will be asked to attend 3 visits. The first visit consists of completing paperwork including health, physical activity, and menstrual cycle background questionnaires. You will also be familiarized with the equipment, procedures, and measurements used in this study. You will be given a sleep diary on the familiarization day and will be required to fill out each day of the diary so we can track your sleep, ensuring that you will have a typical night's sleep prior to exercise testing visits. For women participants, the following two visits will correspond with your menstrual cycle based on information you have previously reported during the familiarization visit. During the following two visits you will complete a mood questionnaire and an exercise test on a stationary cycle. The two visits will be randomized between single leg and double leg cycling, meaning that you will perform both tests, but the visit (either 2 or 3) will be random for single or double leg exercise. During these exercise tests, you will be asked to ride against increasing resistance until you cannot meet the desired intensity. Before, during, and after the exercise test, we will have a cap on your head which will measure continuous brain activity. Following the last visit, you will no longer need to track your sleep via the sleep diaries

How long will this take? Your participation will take about 1 hour per visit for 3 visits- equating to around 3 hours.

What are the risks and/or benefits if I participate? Risks involved in this study include moderate soreness. You will be asked to cycle on a stationary bike against increasing resistance until you cannot anymore, which may result in fatigue and soreness the following days. You may find the seat of the bike uncomfortable. This protocol involves increased breathing rate and heart rate, which may result in dizziness

IRB NUMBER: 11317
IRB APPROVAL DATE: 10/22/2019

or faintness. Your safety is the upmost importance, so you will be closely monitored during testing and thoroughly screened beforehand to assure that no complications, injuries or unnecessary discomfort occurs during your participation. There is no medical benefit for participating in this research study.

What do I do if I am injured? If you are injured during your participation, report this to a researcher or the principal investigator, Rebecca Larson, immediately. Dr. Larson can be reached at 352-359-8432 (cell) or 405-325-6325 (work). Emergency medical treatment is available. However, you or your insurance company will be expected to pay the usual charge from this treatment. The University of Oklahoma Norman Campus has set aside no funds to compensate you in the event of injury.

Will I be compensated for participating? You will not be reimbursed for your time and participation in this research.

Who will see my information? In research reports, there will be no information that will make it possible to identify you. Research records with identifiable information will be stored securely in locked file cabinets and research computers, and only approved researchers and the OU Institutional Review Board will have access to the records. You will be assigned a subject identification number, so your identifiable information will be kept confidential.

You have the right to access the research data that has been collected about you as a part of this research. However, you may not have access to this information until the entire research has completely finished and you consent to this temporary restriction.

Do I have to participate? No. If you do not participate, you will not be penalized or lose benefits or services unrelated to the research. If you decide to participate, you don't have to answer any question and can stop participating at any time.

What will happen to my data in the future? After removing all identifiers, we might share your data with other researchers or use it in future research without obtaining additional consent from you.

Will I be contacted again? The researcher might like to contact you to gather additional data or recruit you into new research.

I give my permission for the researcher to contact me in the future. ___Yes ___ No

Who do I contact with questions, concerns or complaints? If you have questions, concerns or complaints about the research or have experienced a research-related injury, contact me at cdowens@ou.edu or on my cell at 405-974-0618, or you may contact the principal investigator Rebecca Larson at rdlarson@ou.edu, or on her cell at 325-359-8432 or in her office at 405-325-6325.

You can also contact the University of Oklahoma – Norman Campus Institutional Review Board (OU-NC IRB) at 405-325-8110 or irb@ou.edu if you have questions about your rights as a research participant, concerns, or complaints about the research and wish to talk to someone other than the researcher(s) or if you cannot reach the researcher(s).

You will be given a copy of this document for your records. By providing information to

IRB NUMBER: 11317
IRB APPROVAL DATE: 10/22/2019

the researcher(s), I am agreeing to participate in this research.

Participant Signature	Print Name	Date
Signature of Researcher Obtaining Consent	Print Name	Date
Signature of Witness (if applicable)	Print Name	Date

IRB NUMBER: 11317
IRB APPROVAL DATE: 10/22/2019

8. HIPAA Form

AUTHORIZATION TO USE or SHARE
HEALTH INFORMATION[1] THAT IDENTIFIES YOU FOR RESEARCH
An Informed Consent Document for Research Participation may also be required.

Title of Research Project: **Differences in Electrocortical Activity Between Single and Double Leg Graded Exercise Cycling: An EEG Study**

IRB Number: 11317

Leader of Research Team: **Rebecca Larson**

Address: **Department of Health and Exercise Science, 1401 Asp Avenue SJSC 117, Norman, OK 73019**

Phone Number: **405-325-6325**

If you decide to sign this document, University of Oklahoma (OU) researchers may use or share information that identifies you (protected health information) for their research. Protected health information will be called PHI in this document.

PHI To Be Used or Shared. Federal law requires that researchers get your permission (authorization) to use or share your PHI. If you give permission, the researchers may use or share with the people identified in this Authorization any PHI related to this research from your medical records and from any test results. Information used or shared may include all information relating to any tests, procedures, surveys, or interviews as outlined in the consent form; medical records and charts; name, address, telephone number, date of birth, race, government-issued identification numbers, and can include physical findings from questionnaires, electrocortical brain activity, anthropometric measures, and graded-exercise tests.

Purposes for Using or Sharing PHI. If you give permission, the researchers may use your PHI to examine how electrocortical activity differs between single and double leg graded exercise cycling tests via electroencephalography (EEG) recordings.

Other Use and Sharing of PHI. If you give permission, the researchers may also use your PHI to develop new procedures or commercial products. They may share your PHI with other researchers, the research sponsor and its agents, the OU Institutional Review Board, auditors and inspectors who check the research, and government agencies such as the Department of Health and Human Services

[1] **Protected Health Information includes all identifiable information relating to any aspect of an individual's health whether past, present or future, created or maintained by a Covered Entity.**

(HHS), and when required by law. The researchers may also share your PHI with no one outside of the main research team.

Confidentiality. Although the researchers may report their findings in scientific journals or meetings, they will not identify you in their reports. The researchers will try to keep your information confidential, but confidentiality is not guaranteed. The law does not require everyone receiving the information covered by this document to keep it confidential, so they could release it to others, and federal law may no longer protect it.

YOU UNDERSTAND THAT YOUR PROTECTED HEALTH INFORMATION MAY INCLUDE INFORMATION REGARDING A COMMUNICABLE OR NONCOMMUNICABLE DISEASE.

Voluntary Choice. The choice to give OU researchers permission to use or share your PHI for their research is voluntary. It is completely up to you. No one can force you to give permission. However, you must give permission for OU researchers to use or share your PHI if you want to participate in the research and, if you cancel your authorization, you can no longer participate in this study.

Refusing to give permission will not affect your ability to get routine treatment or health care unrelated to this study from OU.

Canceling Permission. If you give the OU researchers permission to use or share your PHI, you have a right to cancel your permission whenever you want. However, canceling your permission will not apply to information that the researchers have already used, relied on, or shared or to information necessary to maintain the reliability or integrity of this research.

End of Permission. Unless you cancel it, permission for OU researchers to use or share your PHI for their research will never end.

Contacting OU: You may find out if your PHI has been shared, get a copy of your PHI, or cancel your permission at any time by writing to:

Privacy Official
University of Oklahoma
PO Box 26901
Oklahoma City, OK 73190

or Privacy Board
University of Oklahoma
201 Stephenson Pkwy, Suite 4300A
Norman, OK 73019

If you have questions, call: (405) 271-2511 or (405) 325-8110

Access to Information. You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study is completely finished. You consent to this temporary restriction.

Giving Permission. By signing this form, you give OU and OU's researchers led by the Research Team Leader permission to share your PHI for the research project listed at the top of this form.

IRB NUMBER: 11317
IRB APPROVAL DATE: 01/21/2020

Participant Name (Print): _____

_____ _____
Signature of Participant Date
or Parent if Participant is a minor

Or

_____ _____
Signature of Legal Representative** Date

**If signed by a Legal Representative of the Participant, provide a description of the relationship to the Participant and the authority to act as Legal Representative:

_____
OU may ask you to produce evidence of your relationship.

A signed copy of this form must be given to the Participant or the Legal Representative at the time this signed form is provided to the researcher or his representative.

9. Medical History Questionnaire

Medical History Questionnaire: Date:

Name: Date of Birth:
Address:
Phone Number: Email:
Age:
Dominant Leg: Blood Pressure:
Height: Weight:
Sex:
Emergency Contact Name and Phone Number:

Please answer the following questions

1. Have you ever/are you diagnosed with an orthopedic limitation, condition, or disease?
   Y N
2. Have you ever/do you have any cardiovascular limitations, conditions, or diseases?
   Y N
3. Have you ever/are you diagnosed with any neurological disorders, such as ones affecting the brain, spine, or other nerves in the body (ex: Multiple sclerosis, brain tumors, epilepsy, Parkinson's disease, Neuropathy, ALS, etc.)
   Y N
4. Have you ever/are you diagnosed with any respiratory limitations, conditions, or diseases (ex: Asthma, COPD, Cystic Fibrosis, etc.)
   Y N
5. Have you ever/are you diagnosed with any psychiatric conditions, disorders, or diseases (ex: Mood disorders, Personality disorders, Schizophrenia, etc.)
   Y N

Please note any additional information related to orthopedic, neurological, cardiovascular, respiratory, or psychiatric conditions, limitations and disorders that you feel the researcher should be aware of:

I certify that these answers are accurate and complete

_____

Your Signature Date

Witness: Date:

IRB NUMBER: 11317
IRB APPROVAL DATE: 10/22/2019

10. Menstrual Cycle Questionnaire

Department of Health and Exercise Science
University of Oklahoma

MENSTRUAL HISTORY QUESTIONNAIRE

Participant ID:_____Date:_____

We are asking you to give us as complete a menstrual history as possible. All information is strictly confidential.

Are you pregnant (circle your response)

YES- Do not complete the rest of this form

NO- Continue to section A.

SECTION A: CURRENT MENSTRUAL STATUS

1. Approximately how many menstrual periods have you had during the past 12 months?
   (please circle what months you have had a period. This means from this time last year to the present month)

   Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

2. What is the usual length of your menstrual cycle (first day of your period to the next onset of your period)?

   _____days. Today is day _____ of your present menstrual cycle.

3. When was the date of the onset of your last period?

4. When do you expect your next period?

5. What is the average length (number of days) of your menstrual flow? _____ days

   How many of these days do you consider "heavy"?_____days

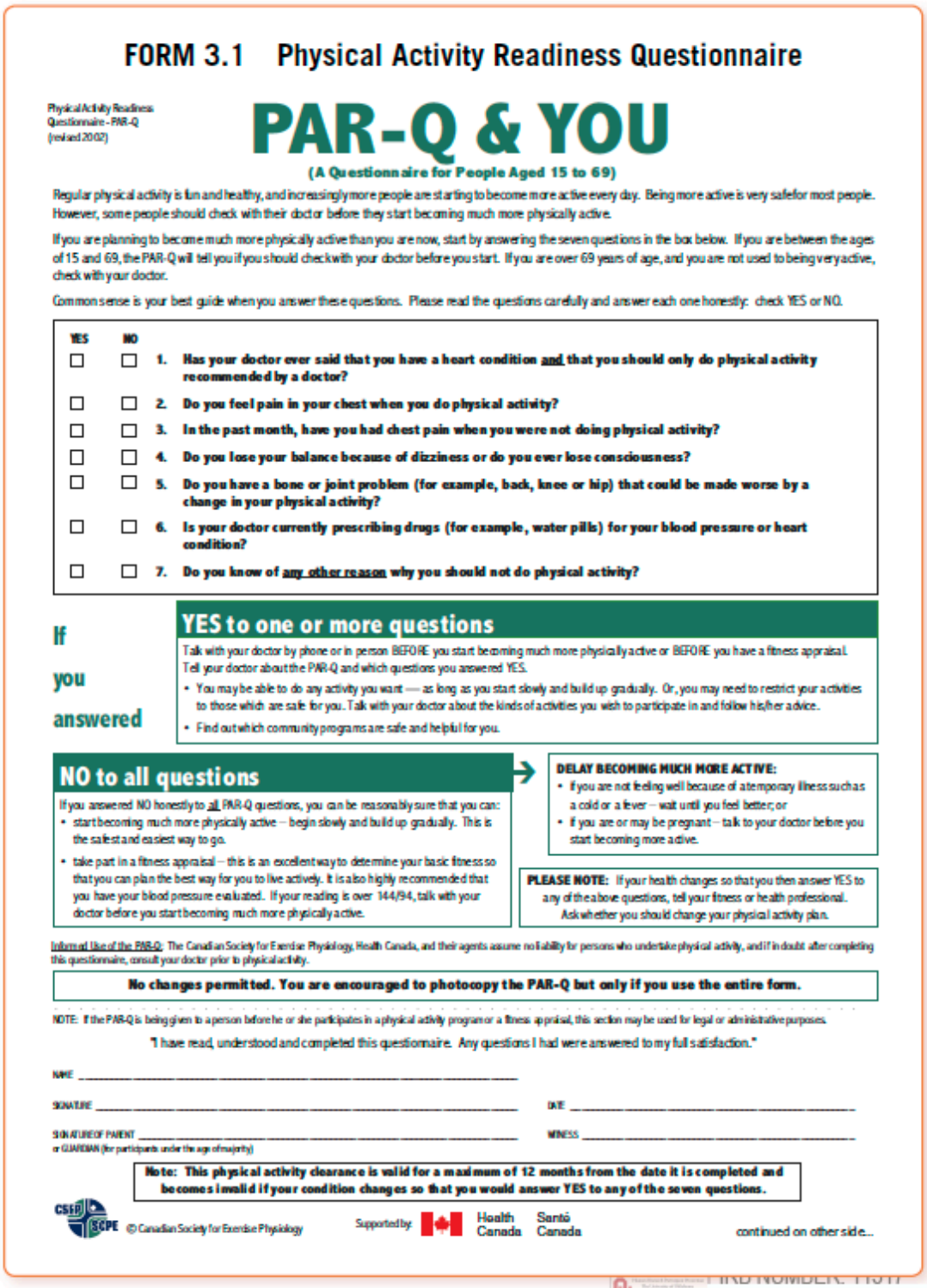
6. Do you take oral contraceptives or any other medication that includes estrogen and/or progesterone?

   If yes, how long have you been taking this medication?_____

   What is the brand name and dosage of this medication?_____

Has this medication affected your menstrual cycle (regularity, length and amount of flow)? If yes, indicate changes.

11. Physical Activity Readiness Questionnaire



FORM 3.1 Physical Activity Readiness Questionnaire

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered

YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- If you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by: Health Canada / Santé Canada

continued on other side...

12. POMS-B Mood Questionnaire

I.D. Number: _____ **Session Number:** _____ **Date:** _____

POMS-B QUESTIONNAIRE

INSTRUCTIONS: Below is a list of words that describe feelings that people have. Please read each word carefully. Then circle the number that best describes:

☐ How you have been feeling during the PAST WEEK, INCLUDING TODAY.

☐ How you feel RIGHT NOW.

	Not At All	A Little	Moderately	Quite a Bit	Extremely
1. Tense	0	1	2	3	4
2. Angry	0	1	2	3	4
3. Worn out	0	1	2	3	4
4. Lively	0	1	2	3	4
5. Confused	0	1	2	3	4
6. Shaky	0	1	2	3	4
7. Sad	0	1	2	3	4
8. Active	0	1	2	3	4
9. Grouchy	0	1	2	3	4
10. Energetic	0	1	2	3	4
11. Unworthy	0	1	2	3	4
12. Uneasy	0	1	2	3	4
13. Fatigued	0	1	2	3	4
14. Annoyed	0	1	2	3	4
15. Discouraged	0	1	2	3	4

PLEASE ANSWER QUESTIONS ON OTHER SIDE

I.D. Number: _____ **Session Number:** _____ **Date:** _____

POMS-B QUESTIONNAIRE

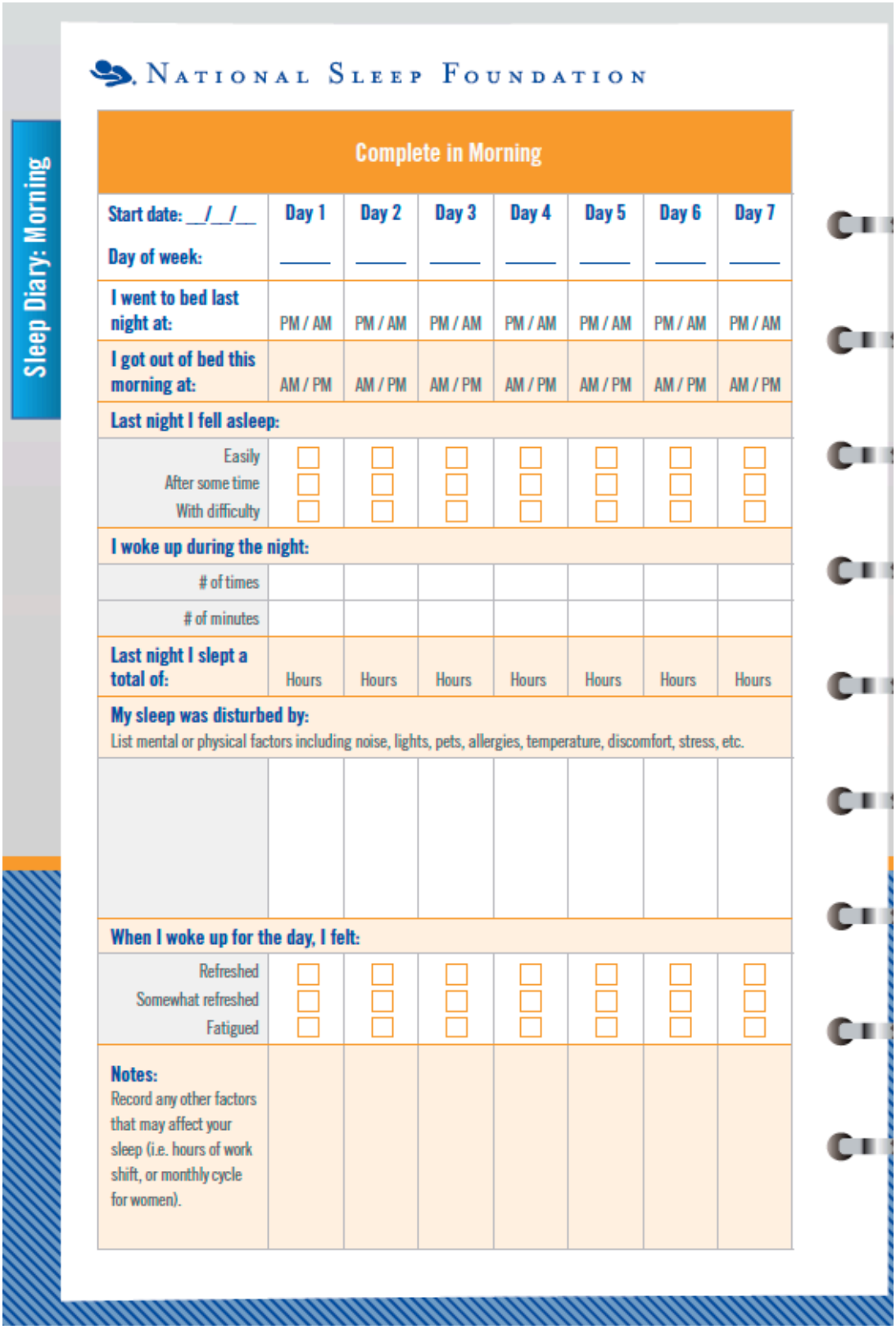
INSTRUCTIONS: Below is a list of words that describe feelings that people have. Please read each word carefully. Then circle the number that best describes:

☐ How you have been feeling during the PAST WEEK, INCLUDING TODAY.

☐ How you have been feeling during the PAST 24 HOURS.

	Not At All	A Little	Moderately	Quite a Bit	Extremely
16. Nervous	0	1	2	3	4
17. Lonely	0	1	2	3	4
18. Muddled	0	1	2	3	4
19. Exhausted	0	1	2	3	4
20. Anxious	0	1	2	3	4
21. Gloomy	0	1	2	3	4
22. Sluggish	0	1	2	3	4
23. Weary	0	1	2	3	4
24. Bewildered	0	1	2	3	4
25. Furious	0	1	2	3	4
26. Efficient	0	1	2	3	4
27. Full of Pep	0	1	2	3	4
28. Bad-tempered	0	1	2	3	4
29. Forgetful	0	1	2	3	4
30. Vigorous	0	1	2	3	4

13. Sleep Diary



NATIONAL SLEEP FOUNDATION

Sleep Diary: Morning

Complete in Morning							
Start date: __/__/__ **Day of week:**	**Day 1** _____	**Day 2** _____	**Day 3** _____	**Day 4** _____	**Day 5** _____	**Day 6** _____	**Day 7** _____
I went to bed last night at:	PM / AM	PM / AM	PM / AM	PM / AM	PM / AM	PM / AM	PM / AM
I got out of bed this morning at:	AM / PM	AM / PM	AM / PM	AM / PM	AM / PM	AM / PM	AM / PM
Last night I fell asleep:							
Easily After some time With difficulty	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
I woke up during the night:							
# of times							
# of minutes							
Last night I slept a total of:	Hours	Hours	Hours	Hours	Hours	Hours	Hours
My sleep was disturbed by: List mental or physical factors including noise, lights, pets, allergies, temperature, discomfort, stress, etc.							
When I woke up for the day, I felt:							
Refreshed Somewhat refreshed Fatigued	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐
Notes: Record any other factors that may affect your sleep (i.e. hours of work shift, or monthly cycle for women).							

Complete at the End of Day							
Day of week:	**Day 1** _____	**Day 2** _____	**Day 3** _____	**Day 4** _____	**Day 5** _____	**Day 6** _____	**Day 7** _____
I consumed caffeinated drinks in the: (M)orning, (A)fternoon, (E)vening, (N/A)							
M / A / E / NA How many?	_____	_____	_____	_____	_____	_____	_____
I exercised at least 20 minutes in the: (M)orning, (A)fternoon, (E)vening, (N/A)							
Medications I took today:							
Took a nap? (circle one)	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No	Yes No
If Yes, for how long?							
During the day, how likely was I to doze off while performing daily activities: No chance, Slight chance, Moderate chance, High chance							
Throughout the day, my mood was… Very pleasant, Pleasant, Unpleasant, Very unpleasant							
Approximately 2-3 hours before going to bed, I consumed:							
Alcohol	☐	☐	☐	☐	☐	☐	☐
A heavy meal	☐	☐	☐	☐	☐	☐	☐
Caffeine	☐	☐	☐	☐	☐	☐	☐
Not applicable	☐	☐	☐	☐	☐	☐	☐
In the hour before going to sleep, my bedtime routine included: List activities including reading a book, using electronics, taking a bath, doing relaxation exercises, etc.							