HHRF Research Grant Application Cover Page

I Title/Cover page **Submission Date:** 01/30/2023

Title of Project: A proof of concept study of functional near infrared spectroscopy to elucidate neurophysiological mechanism of action of equine-assisted services

Primary focus area of the investigation: Neurophysiological mechanisms underlying benefits of equine-assisted services

Principal Investigator Name and Title: Beth Lanning, PhD. Associate Chair, Professor of Public Health

Dr. Beth Lanning is a Professor of Public Health at Baylor University. Her research areas are Human-Animal interactions and interpersonal violence and trauma. Dr. Lanning has experience leading research exploring the benefits of EAS for veteran, military service members, and children with Autism. She has managed over $1 million in externally funded projects. Dr. Lanning will function as the Principal Investigator for the project and will assist with all areas of the project including data collection, data analysis, manuscript development, IRB application, and budget management.

Contact Name and Title: Beth Lanning, PhD. Associate Chair, Professor of Public Health
Institute: Baylor University, Department of Public Health, One Bear Place, 97343, Waco, Texas 76798
Address: Same

Phone Number: 254-710-4027 **Email Address**: Beth_Lanning@baylor.edu

Co-Investigator(s):
Cory Smith, PhD. Baylor University
Cory_M_Smith@Baylor.edu

William (Bill) Marchand, MD, LFAPA, University of Utah and VA Salt Lake City Health Care System
William.Marchand@va.gov

Elena Nazarenko, MS, VA Salt Lake City Health Care System
Elena.Nazarenko@va.gov

II. Introduction: Equine-assisted services (EAS) are used for civilian[1] and military [2-6] trauma survivors. However, the field is in the early stages of scientific development and rigorous research is lacking.[7] Further, the neurophysiological mechanisms underlying EAS benefits are unknown. [8] An understanding of neural adaptations responsible for positive outcomes will allow for further development of optimal treatment methodologies. Thus, studies are needed to disambiguate mechanisms of action. This innovative pilot study will move the field forward by enhancing our ability to measure neurophysiological mechanisms of action. In this study, functional near-infrared spectroscopy (fNIRS) will be used to evaluate changes in brain activation. Heart rate variability (HRV) and psychological data will be collected, and analyses will explore associations between psychological and physiological results. This study will lay the groundwork for future rigorous studies aimed at disambiguating neurophysiological changes associated with EAS participation. Specifically, this pilot project will determine: 1) whether fNIRS methodology can be used to explore neural responses to EAS participation and 2) if so, will fNIRS results correlate with HRV and psychological instrument outcomes, thus demonstrating utility as a tool to assess neural mechanisms underlying the benefits of EAS.

a. Background: PTSD rates approach 30%[9, 10] among Veterans and currently treatments have high non-response and attrition rates [11-13, 14, 15] Military sexual trauma (MST) may impact up to 15% of female veterans[16] and conventional interventions may not address complex effects of MST. [17] Thus, there is a need to develop interventions for trauma survivors that may enhance treatment engagement and outcomes.[18, 19]

While focusing on Veterans, this project will evaluate methodologies that can be used to explore neurophysiological mechanisms of action across the field of EAS by concentrating on four commonly used EAS activities:1) equine grooming 2) equine leading 3) group processing and 4) meditation. The research intervention for this study is modeled after Whispers with Horses (WwH), which is an EAS intervention with preliminary evidence of effectiveness, [20] and includes the four commonly used EAS activities. WwH integrates mindfulness and self-compassion training in the context of a horse-human relationship for Veterans with PTSD. WwH is based upon evidence that mindfulness [21] and self-

compassion[22] are beneficial for recovery from trauma as well as preliminary work of one of the investigators.[7, 20, 23-25]

To conduct a preliminary assessment of the fNIRS methodology it is necessary to evaluate whether changes in brain activation are correlated with either physiological measures (HRV) and/or psychological outcomes. This investigation will focus on four psychological outcomes associated with EAS: 1) increased positive affect; [20, 23, 24] 2) decreased negative affect; [20, 23, 24] 3) decreased anxiety;[23,26] and 4) increased psychological flexibility (PF). [20, 24, 25] PF facilitates pursuing goals despite feeling distress,[27] is closely related to mindfulness, and [28, 29] evidence[6, 30-33] suggests that enhanced PF is a mechanism of action for EAS associated with reduction of depression,[34] and anxiety,[1, 35] as well as enhanced quality of life[2, 36-40] and interpersonal interactions.[39] Thus, we hypothesize enhanced PF underlies benefits of EAS and that changes in PF will correlate with changes in affect, anxiety, brain activation,[41, 42] and HRV.[43]

b. Physiological measures: fNIRS neuroimaging will quantify the levels of brain activation in the prefrontal and motor cortices throughout the intervention. fNIRS methodology is ideal for EAS outcome studies because it is not impacted by movements and can be used in real-world EAS environments as demonstrated by several studies.[44-46] We are aware of only one neuroimaging study[47] of EAS outcomes. That investigation of Veterans with PTSD demonstrated that symptom reduction was correlated with changes in functional connectivity as demonstrated by functional magnetic resonance imaging (fMRI). fNIRS is comparable to fMRI in terms of demonstrating brain activation but is much more suited to EAS research given the portability and the ability to capture participants in motion.

EAS participation is thought to reduce stress and improve well-being. [26, 48] The stress response is regulated by the hypothalamus-pituitary adrenal axis and the autonomic nervous system (ANS), made up of both the sympathetic and parasympathetic nervous system. HRV is a key marker for the ANS[49] sympathetic stress response. HRV allows researchers to understand how the body responds to stress [50] and has been used in studies of PTSD.[51] This study will use Polar systems equipment, which have been found to be an acceptable to collect HRV data.[52, 53]

b. ***Specific objectives:*** The overarching aim of this innovative study is to conduct preliminary work necessary to prepare for future rigorous studies. Specifically, this project will evaluate fNIRS methodologies that can be used to explore neurophysiological mechanisms of action across the field of EAS. Further, this study will help move the EAS research field forward by supporting a new collaboration of investigators from Baylor University and the Salt Lake City VA/University of Utah.

Primary objective 1: Determine if fNIRS can be utilized to elucidate changes in human brain activation, connectivity and/or neuroplasticity associated with EAS participation.

Hypothesis and significance (1): fNIRS can be used to evaluate cortical activation and functional connectivity that occurs in association with EAS interventions using a targeted hemodynamic assessments approach with high temporal and spatial resolution.[54, 55] If so, this portable and movement-compatible methodology will be a powerful tool that can be used across the field of EAS in rigorous studies to disambiguate neurophysiological mechanisms of action associated with EAS.

Primary objective 2: Evaluate whether the changes in human brain activation demonstrated by fNIRS are associated with changes in HRV, anxiety, affect, and psychological flexibility.

Hypothesis and significance (2): Brain activation demonstrated with fNIRS methodology will correlate with changes in HRV, anxiety, affect and psychological flexibility. Thus, fNIRS methodology will not only demonstrate neural changes associated with EAS but will also allow future investigations in larger studies to parse the cortical, autonomic and psychological mechanisms underlying the benefits of EAS.

Secondary objective 1: Assess whether psychological flexibility increases pre- to post-session and if so whether this change predicts improvements in HRV, anxiety and affect.

Hypothesis and significance (3): Enhancement of PF is an underlying mechanism of action of some EAS interventions. Enhanced PF will be associated with positive changes in HRV, improved affect and decreased anxiety. If so, future more rigorous studies can explore what therapeutic components contribute to improved PF to facilitate the development of more effective interventions.

III Materials and Methods:

a. ***Experimental Design:***

Location: Recruitment, enrollment, and collection of Health Electronic Record (HER) data will occur at the VA Salt Lake City Health Care System (VASLCHCS). The intervention and collection of data will occur at a Salt Lake City, Utah equine facility. Data analyses and writeup will occur at the VASLCHCS and Baylor University located in Waco, Texas.

Sample size: Fifteen subjects will provide acceptable results. Twenty subjects will be enrolled to allow for attrition. An a priori power analysis from the fNIRS neuroimaging techniques indicated an n = 14 is required to reach a power of 0.80 based on the fNIRS amplitude of the deoxygenated hemoglobin changes during cognitive motor skills in a healthy, male population (effect size: 0.76, alpha: 0.05, correlation among measures of 0.50).[56, 57]

Subjects: Male subjects will be studied in this pilot study to eliminate any gender-related confounding factors.[58-60] *Inclusion criteria*: Male, enrolled for services at VASLCHCS, diagnosis of PTSD, and age range of 21 – 55. *Exclusion criteria*: Diagnosis of cognitive impairment or psychotic spectrum illness or active substance use disorder. Use of beta-blockers or other heart rate limiting medications. Any traumatic brain injury within the past 6 months.

Intervention: The research intervention will consist of four activities common across EAS programs. These are: A) equine grooming; B) equine leading; C) meditation; and D) processing/discussion. Activities A and B will include the horse, an equine specialist (ES) and participant, while activities C and D will include the horse, participant, ES, and a mental health professional. For activities C and D, the equine will be in the area close to the participant but will not be directly involved in the activity. Equine grooming will consist of instruction by the ES on how to properly groom a horse and subsequent practice. The leading activity will include instruction on how to lead a horse safely through an obstacle course. The meditation will be a guided mindfulness medication led by the mental health professional. It will focus on awareness of breath, body and sounds and keeping attention in the present moment without judgement. The processing/discussion activity will be led by the mental health professional and include discussion of

previous or planned interactions with the equines. Each intervention session will last 40 minutes with subjects participating in each of the four activities for ten minutes. Each activity will be conducted in a standardized manner and the order of activities will be randomized. Each subject will complete the intervention twice on two separate days. Fidelity of the protocol will be monitored by the PI using a protocol fidelity tool.

Data collection: The following data will be collected: 1) Subject demographic and diagnostic information; 2) data from self-report psychological measures, pre- and post- each intervention session. 3) HRV data will be collected throughout each session. 4) fNIRS data will be collected throughout each session.

Outcome measures: (See Appendix 2 for more details).

Functional near infrared spectroscopy (fNIRS): The fNIRS apparatus will be placed on the head of each participant at the beginning of each session. Continuous wavelet fNIRS will be used to quantify components of brain activity during the four sessions. All fNIRS measurements will be performed using an OctaMon+ (Artinis Medical Systems, Lieden, Netherlands).

Heart rate variability: Heart rate variability will be measured throughout each session using a Polar heart rate chest strap system.

Psychological measures:

1) The **Acceptance and Action Questionnaire II** (AAQ-II) [61] will be used to measure psychological flexibility.

2) The **State-Trait Anxiety Inventory** (STAI: Y – 6 item) [62] will be used to measures\ state anxiety.

3) The **Positive and Negative Affect Scale** (PANAS) will be used to measure both positive and negative affect.

Data analyses:

Primary Objective 1: Determine the mean $fNIRS_{amp}$ and $fNIRS_{fc}$ changes over time for each activity type by examining results for the appropriate 10 minute epoch lengths.

Primary Objective 2: Two generalized linear mixed effects regression models will be utilized with fixed effects for time, psychological, HRV (RMSSD, pNN50, and TINN), and baseline $fNIRS_{amp}$, $fNIRS_{fc}$ (pre-intervention for day one) factors and random effects intercepts for participants to examine $fNIRS_{amp}$, $fNIRS_{fc}$ factors.

Secondary Objective 1: The analysis is similar to that in primary objective 1. However, we will have three generalized mixed effects model for HRV, anxiety and affect based on psychological flexibility and time predictors.

Study team:

1. Dr. Beth Lanning, Principal Investigator. See Cover Page for details.

2. Dr. Cory Smith is an applied physiologist specializing in aerospace and environmental physiology, physiological-based multimodal sensor data fusion, and neurophysiological research. Dr. Smith has experience in emergency medicine, neurophysiological clinical assessment techniques, myoelectric prosthetic algorithms, warfighter performance monitoring, and extreme environmental physiology.

3. William (Bill) Marchand, MD is a Clinical Professor of Psychiatry at the University of Utah and Director of Research and Equine-Assisted Services for the Whole Health Service at the VHASKCHCS. He is certified by Eagala to provide psychotherapy incorporating horses and by PATH, Intl. as an ESMHL. He is an experienced horseman, mindfulness teacher, and senior VA investigator with nearly 70 peer-reviewed publications, four book chapters and two books. His research experience is primarily in the areas of human functional neuroimaging, mindfulness, Veteran mental health, and equine-assisted services. He will contribute to all aspects of the project and lead the data collection portions of the study in Salt Lake City, UT.

4. Elena Nazarenko, MS. is a Sr. Research Analyst and Study Coordinator for the Whole Health Service at the VHASLCHCS. Ms. Nazarenko will work with Drs. Lanning, Marchand and Smith to analyze the data collected as well as provide local study coordination and method support in Salt Lake City throughout the life of the project.

Timeline:

Notification of award	IRB and IACUC approvals	Recruitment and preparation	Intervention and data collection	Data analyses	Write up results for publication
	Months 1 and 2	Month 3	Month 4	Month 5 and 6	Month 7 - 9

c. ***Ethical Approvals:*** IRB approval will be obtained through the University of Utah, which serves as the primary IRB for the VASLCHCS. IACUC approval will be obtained by the VASLCHCS Research and Development Committee. The University of Utah will serve as the primary IRB for this study.

IV Impact: This innovative proof-of-concept study will lay the groundwork for future rigorous fNIRS studies aimed at disambiguating neurophysiological mechanisms of action across the field of EAS.

V Literature Cited: See Appendix 1

VI Budget: A budget of **$10,000** is requested for the proposed project.

A. Personnel: As per award guidelines, no money will be allocated for personnel.

B. Other Personnel: None.

C. Equipment/Facility: The equine facility fee is **$2,000** per week. We plan to collect data over one week.

D. Travel: $6,500. The PI (Lanning) and Co-investigator (Smith) and his PhD student will travel to Salt Lake City, Utah to collect the data. Travel expenses will include airfare, rental car, hotel, and food. The travel expense is necessary to support the unique professional collaboration required to achieve the project aims.

E. Participant Support: $1,500. A total of 15 participants will be paid $50/session for 2 sessions, total $100 per participant.

Appendix 1 – literature cited:

1. Earles JL, Vernon LL, Yetz JP. Equine-assisted therapy for anxiety and posttraumatic stress symptoms. *J Trauma Stress.* 2015;28(2): 149-152.

2. Romaniuk M, Evans J, Kidd C. Evaluation of an equine-assisted therapy program for veterans who identify as 'wounded, injured or ill' and their partners. *PLoS One.* 2018;13(9): e0203943.

3. Ferruolo DM. Psychosocial Equine Program for Veterans. *Soc Work.* 2016;61(1): 53-60.

4. Steele E, Wood DS, E JU, Applegarth DM. TRR's Warrior Camp: An Intensive Treatment Program for Combat Trauma in Active Military and Veterans of All Eras. *Mil Med.* 2018;183(suppl_1): 403-407.

5. Lanning, BA, Wilson, AL, Krenek, N. Beaujean, AA. Using Therapeutic Riding as an Intervention for Combat Veterans: An International Classification of Functioning, Disability, and Health (ICF) Approach. *Occupational Therapy in Mental Health.* 2017. http://dx.doi.org/10.1080/0164212X.2017.1283282

6. Burton LE, Qeadan F, Burge MR. Efficacy of equine-assisted psychotherapy in veterans with posttraumatic stress disorder. *J Integr Med.* 2019;17(1): 14-19.

7. Marchand WR, Andersen SJ, Smith JE, Hoopes KH, Carlson JK. Equine-Assisted Activities and Therapies for Veterans With Posttraumatic Stress Disorder: Current State, Challenges and Future Directions. *Chronic Stress (Thousand Oaks).* 2021;5: 2470547021991556.

8. NE F. Horses in the Treatment of Trauma. In: Jenkins PTMA, ed. *Transforming trauma : resilience and healing through our connections with animals.* West Lafayette, Indiana: Purdue University Press; 2019.

9. Hoge CW, Castro CA, Messer SC, McGurk D, Cotting DI, Koffman RL. Combat duty in Iraq and Afghanistan, mental health problems, and barriers to care. *N Engl J Med.* 2004;351(1): 13-22.

10. Suris A, Lind L. Military sexual trauma: a review of prevalence and associated health consequences in veterans. *Trauma Violence Abuse.* 2008;9(4): 250-269.

11. Schottenbauer MA, Glass CR, Arnkoff DB, Tendick V, Gray SH. Nonresponse and dropout rates in outcome studies on PTSD: review and methodological considerations. *Psychiatry.* 2008;71(2): 134-168.

12. Bradley R, Greene J, Russ E, Dutra L, Westen D. A multidimensional meta-analysis of psychotherapy for PTSD. *Am J Psychiatry.* 2005;162(2): 214-227.

13. Hoskins M, Pearce J, Bethell A, et al. Pharmacotherapy for post-traumatic stress disorder: systematic review and meta-analysis. *Br J Psychiatry.* 2015;206(2): 93-100.

14. Kantor V, Knefel M, Lueger-Schuster B. Perceived barriers and facilitators of mental health service utilization in adult trauma survivors: A systematic review. *Clin Psychol Rev.* 2017;52: 52-68.

15. Kazlauskas E. Challenges for providing health care in traumatized populations: barriers for PTSD treatments and the need for new developments. *Glob Health Action.* 2017;10(1): 1322399.

16. Kimerling R, Street AE, Pavao J, et al. Military-related sexual trauma among Veterans Health Administration patients returning from Afghanistan and Iraq. *Am J Public Health.* 2010;100(8): 1409-1412.

17. Steenkamp MM, Litz BT, Hoge CW, Marmar CR. Psychotherapy for Military-Related PTSD: A Review of Randomized Clinical Trials. *JAMA.* 2015;314(5): 489-500.

18. Imel ZE, Laska K, Jakupcak M, Simpson TL. Meta-analysis of dropout in treatments for posttraumatic stress disorder. *J Consult Clin Psychol.* 2013;81(3): 394-404.

19. Najavits LM. The problem of dropout from "gold standard" PTSD therapies. *F1000Prime Rep.* 2015;7: 43.

20. Marchand WR, Lackner R, Hartquist A, Finnell L, Nazarenko E. Evaluation of a mindfulness and self-compassion-based psychotherapy incorporating horses for Veterans who have experienced trauma. *Complement Ther Med.* 2023;72: 102914.

21. Marchand WR, Sandoval K, Lackner R, et al. Mindfulness-based interventions for military veterans: A systematic review and analysis of the literature. *Complement Ther Clin Pract.* 2021;42: 101274.

22. Meyer EC, Szabo YZ, Frankfurt SB, Kimbrel NA, DeBeer BB, Morissette SB. Predictors of recovery from post-deployment posttraumatic stress disorder symptoms in war veterans: The contributions of psychological flexibility, mindfulness, and self-compassion. *Behav Res Ther.* 2019;114: 7-14.

23. Marchand WR, Joubert K, Smith J, et al. A Pilot Observational Study of Implementing an Equine-A ssisted Services Program Within a VA Medical Center Residential Substance Use Disorder Treatment Program. *Mil Med.* 2022.

24. Marchand WR, Smith J, Hoopes KH, et al. A pilot observational study of horsemanship skills training for Veterans with posttraumatic stress disorder. *Complement Ther Med.* 2022: 102910.

25. Marchand WR, Sullivan-Sakaeda L. A pilot observational study of a psychotherapy incorporating equines resiliency intervention for staff at a large medical center. *Complement Ther Clin Pract.* 2022;49: 101660.

26. Lanning, BA, Wilson, AL, Woelk, R, Beaujean, AA. Therapeutic horseback riding as a complementary intervention for military service members with PTSD. *Human-animal interaction bulletin.* CABI International. 2018;6(2): 58-82. 10.1079/hai.2018.0013.

27. Kashdan TB, Disabato DJ, Goodman FR, Doorley JD, McKnight PE. Understanding psychological flexibility: A multimethod exploration of pursuing valued goals despite the presence of distress. *Psychol Assess.* 2020;32(9): 829-850.

28. Silberstein L, Tirch D, Leahy R. Mindfulness, Psychological Flexibility and Emotional Schemas. *International Journal of Cognitive Therapy.* 2012;5(4): 406-419.

29. Biglan A. Increasing Psychological Flexibility to Influence Cultural Evolution. *Behavior and Social Issues.* 2009;18: 15-24.

30. Marchand WR, Klinger W, Block K, et al. Safety and psychological impact of sailing adventure therapy among Veterans with substance use disorders. *Complement Ther Med.* 2018;40: 42-47.

31. Marchand WR, Klinger W, Block K, et al. Mindfulness-based Therapeutic Sailing for Veterans With Psychiatric and Substance Use Disorders. *Mil Med.* 2021.

32. Meinersmann KM, Bradberry J, Roberts FB. Equine-facilitated psychotherapy with adult female survivors of abuse. *J Psychosoc Nurs Ment Health Serv.* 2008;46(12): 36-42.

33. Kemp K, Signal, T., Botros, H., Taylor, N., & Prentice, K. Equine facilitated therapy with children and adolescents who have been sexually abused: A program evaluation study. *Journal of Child & Family Studies.* 2014;23(3): 558-566.

34. Arnon S, Fisher PW, Pickover A, et al. Equine-Assisted Therapy for Veterans with PTSD: Manual Development and Preliminary Findings. *Mil Med.* 2020;185(5-6): e557-e564.

35. Alfonso SV, Alfonso LA, Llabre MM, Fernandez MI. Project Stride: An Equine-Assisted Intervention to Reduce Symptoms of Social Anxiety in Young Women. *Explore (NY).* 2015;11(6): 461-467.

36. Beinotti F, Christofoletti G, Correia N, Borges G. Effects of horseback riding therapy on quality of life in patients post stroke. *Top Stroke Rehabil.* 2013;20(3): 226-232.

37. Cerulli C, Minganti C, De Santis C, Tranchita E, Quaranta F, Parisi A. Therapeutic horseback riding in breast cancer survivors: a pilot study. *J Altern Complement Med.* 2014;20(8): 623-629.

38. Fields B, Bruemmer J, Gloeckner G, Wood W. Influence of an Equine-Assisted Activities Program on Dementia-Specific Quality of Life. *Am J Alzheimers Dis Other Demen.* 2018;33(5): 309-317.

39. Prieto A, Martins Almeida Ayupe K, Nemetala Gomes L, Saude AC, Gutierres Filho P. Effects of equine-assisted therapy on the functionality of individuals with disabilities: systematic review and meta-analysis. *Physiother Theory Pract.* 2020: 1-16.

40. White-Lewis S, Johnson R, Ye S, Russell C. An equine-assisted therapy intervention to improve pain, range of motion, and quality of life in adults and older adults with arthritis: A randomized controlled trial. *Appl Nurs Res.* 2019;49: 5-12.

41. Smallwood RF, Potter JS, Robin DA. Neurophysiological mechanisms in acceptance and commitment therapy in opioid-addicted patients with chronic pain. *Psychiatry Res Neuroimaging.* 2016;250: 12-14.

42. Aytur SA, Ray KL, Meier SK, et al. Neural Mechanisms of Acceptance and Commitment Therapy for Chronic Pain: A Network-Based fMRI Approach. *Front Hum Neurosci.* 2021;15: 587018.

43. Allen TM, Struemph KL, Toledo-Tamula MA, et al. The Relationship Between Heart Rate Variability, Psychological Flexibility, and Pain in Neurofibromatosis Type 1. *Pain Pract.* 2018;18(8): 969-978.

44. Suzuki M, Miyai I, Ono T, Kubota K. Activities in the frontal cortex and gait performance are modulated by preparation. An fNIRS study. *Neuroimage.* 2008;39(2): 600-607.

45. Holtzer R, Mahoney JR, Izzetoglu M, Izzetoglu K, Onaral B, Verghese J. fNIRS study of walking and walking while talking in young and old individuals. *J Gerontol A Biol Sci Med Sci.* 2011;66(8): 879-887.

46. Yanagisawa H, Dan I, Tsuzuki D, et al. Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test. *Neuroimage.* 2010;50(4): 1702-1710.

47. Zhu X, Suarez-Jimenez B, Zilcha-Mano S, et al. Neural changes following equine-assisted therapy for posttraumatic stress disorder: A longitudinal multimodal imaging study. *Hum Brain Mapp.* 2021;42(6): 1930-1939.

48. B.T. Klontz AB, D. Leinart, T. Klontz, . The effectiveness of equine-assisted experiential therapy: results of an open clinical trial. *Soc Anim.* 2007;15(3).

49. Kim HG, Cheon EJ, Bai DS, Lee YH, Koo BH. Stress and Heart Rate Variability: A Meta-Analysis and Review of the Literature. *Psychiatry Investig.* 2018;15(3): 235-245.

50. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health.* 2017;5: 258.

51. Cheng YC, Su MI, Liu CW, Huang YC, Huang WL. Heart rate variability in patients with anxiety disorders: A systematic review and meta-analysis. *Psychiatry Clin Neurosci.* 2022;76(7): 292-302.

52. K. Georgiou AVL, N.N. Khamis, G.I. Alsuhaibani, Y.A. Alaska, E., Giallafos J. Can wearable devices accurately measure heart rate variability? A

systematic review. *Folia Med.* 2018;60(1): 7-20.

53. Lu G, Yang F, Taylor JA, Stein JF. A comparison of photoplethysmography and ECG recording to analyse heart rate variability in healthy subjects. *J Med Eng Technol.* 2009;33(8): 634-641.

54. Arenth PM, Ricker JH, Schultheis MT. Applications of functional near-infrared spectroscopy (fNIRS) to Neurorehabilitation of cognitive disabilities. *Clin Neuropsychol.* 2007;21(1): 38-57.

55. Choi J, Choi, M., & Bae, H. M. . An efficient data extraction method for high-temporal-and-spatial-resolution near infrared spectroscopy (NIRS) systems. *In 2012 IEEE International Symposium on Circuits and Systems (ISCAS).* 2012.

56. Smith CM, Salmon, O.F., Segovia, M.S. . Impact of Reduced Weight on Motor and Cognitive Function in Astronaut Analogs: a simulated lunar gravity workload study. *Acta Astronautica,.* 2023;in press.

57. Smith CM. Post-Exertion Rate of Reperfusion vs Point-by-Point analysis of skeletal tissue near-infrared spectroscopy during repeated fatigue recovery under normoxia and hypoxemia. *Respir Physiol Neurobiol.* 2023;308: 103985.

58. Cazzell M, Li L, Lin ZJ, Patel SJ, Liu H. Comparison of neural correlates of risk decision making between genders: an exploratory fNIRS study of the Balloon Analogue Risk Task (BART). *Neuroimage.* 2012;62(3): 1896-1911.

59. Saleem S, Hussain MM, Majeed SM, Khan MA. Gender differences of heart rate variability in healthy volunteers. *J Pak Med Assoc.* 2012;62(5): 422-425.

60. Verkuil B, Brosschot JF, Marques AH, Kampschroer K, Sternberg EM, Thayer JF. Gender differences in the impact of daily sadness on 24-h heart rate variability. *Psychophysiology.* 2015;52(12): 1682-1688.

61. Bond FW, Hayes SC, Baer RA, et al. Preliminary psychometric properties of the Acceptance and Action Questionnaire-II: a revised measure of psychological inflexibility and experiential avoidance. *Behav Ther.* 2011;42(4): 676-688.

62. Marteau TM, Bekker H. The development of a six-item short-form of the state scale of the Spielberger State-Trait Anxiety Inventory (STAI). *Br J Clin Psychol.* 1992;31(3): 301-306.

Appendix 2. Outcome Measures

1) *Functional near infrared spectroscopy (fNIRS):* The fNIRS will be placed on the head of each participant at the beginning of each intervention session with sensors located over the prefrontal and motor cortex of the brain to quantify brain activity. The non-invasive, continuous wavelet fNIRS will be used to quantify the amplitude ($fNIRS_{amp}$) and frequency ($fNIRS_{fc}$) components of brain activity during each of the four sessions: equine grooming, equine leading, meditation, and processing/discussion. All fNIRS measurements will be performed using an OctaMon+ (Artinis Medical Systems, Lieden, Netherlands).

2) *Heart rate variability:* Heart rate variability will be measured using a Polar heart rate chest strap system placed at the 5^{th} intercostal space. Heart rate data will be measured throughout the visits and will be analyzed during 5-min periods of non-activity before and after the intervention. The Root mean square of successive RR interval differences (RMSSD), Percentage of successive RR intervals that differ by more than 50 ms (pNN50), and Baseline width of the RR interval histogram (TINN) will be quantified.

3) The *Acceptance and Action Questionnaire II (AAQ-II)*[61] measures psychological flexibility. This is a 10 item, 7-point Likert scale and lower scores indicate greater psychological flexibility and range from 10 to 70. The 3- and 12-month test–retest reliability is .81 and .79, respectively.[61]

4) The *State-Trait Anxiety Inventory (STAI: Y – 6 item*)[62] measures state anxiety. The STAI: Y - 6 item is a 6-item instrument that measures state anxiety. Respondents answer questions, for example, "I feel calm" with options of "not at all," "somewhat," "moderately" and "very much." Some items are reverse scored such that higher scores equals higher levels of state anxiety.

5) The *Positive and Negative Affect Scale (PANAS)* is a 20-item instrument that measures both positive and negative affect. Respondents answer the question, "Indicate the extent that you have felt this way" for items such as "interested," and "excited" on five-point scale ranging from "very slightly or not at all"

to “extremely.” Ten of the items measure positive and the other ten evaluate negative affect and scoring results in both positive and negative affect scores. For positive affect, higher scores equal increased positive affect. For negative affect, lower scores indicate decreased negative affect.