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Investigation of the effects of transcutaneous electrical stimulation on physiological stress, marksmanship, and cognitive performance

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INVESTIGATION OF THE EFFECTS OF TRANSCUTANEOUS ELECTRICAL STIMULATION ON PHYSIOLOGICAL STRESS, MARKSMANSHIP, AND COGNITIVE PERFORMANCE

by

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ABSTRACT

Military training and operations can place significant demands on cognitive and physical resources of service members, resulting in heightened stress and fatigue, elevated risk of accidents and injuries, and diminished cognitive and occupational performance. Transcutaneous electrical stimulation (TES) is a novel, non-invasive neuromodulatory technique being investigated as a means to improve alertness and preserve performance under stress with few-to-no side effects. Despite the recent increase in research using TES, few studies have explored the effects of stimulation of the trigeminal nerve on cognition and the human stress response. Therefore, the aims of this study were to elucidate the effects of TES on biochemical and physiological responses to stress, cognition, and marksmanship performance under cognitive load.

Participants in this repeated measures, crossover-design study included 23 healthy male (n = 18) and female (n = 5) civilians and members of the military ranging in age from 19 to 37 (mean 24.00 ± 5.65) years. Study procedures occurred in the afternoon on

five consecutive days, including two testing days involving administration of active or sham TES to the right supraorbital region of the face using a commercially-available device (Thync One, Cerevast Therapeutics). To evaluate the effects of TES on the stress response, participants were required to complete a prolonged, cognitively challenging target discrimination task using a simulated firing range, which has been previously demonstrated to induce a reliable stress response in human research volunteers. Computer-assisted cognitive tasks were administered before and after rifle marksmanship in order to provide complementary assessment of functional domains challenged during the marksmanship task. Salivary markers of cortisol and α-amylase were collected at several time points during the testing day, and electrocardiography (ECG) and photoplethysmography (PPG), both markers of heart rate variability and stress responding, were monitored continuously. Linear mixed models with random slopes were used to analyze the effect of stimulation condition (active versus sham TES) on marksmanship and cognitive, physiological, and salivary outcomes across the testing period and at each measurement time point.

No significant effects of stimulation condition or the interactions between stimulation condition and measurement time point were found for salivary stress biomarkers (p_{unadj} range 0.12 – 0.98) or for cognitive (p_{unadj} range 0.25 – 0.88) and physical workload (p_{unadj} range 0.31 – 0.79). There were no significant effects of stimulation condition on time-series indicators of heart rate variability (p_{unadj} range 0.10 – 0.96) except for pNN50 when measured with PPG (β = -4.97, p_{unadj} = 0.04, p_{adj} = n.s., d < 0.01). There were, however, significant stimulation condition by time interaction effects

on mean heart rate, mean R-R interval, SDNN, RMSSD, and pNN50 (p_{unadj} range 0.12 – 0.98, d range < 0.01 – 0.02), indicating that trigeminal TES using the Thync One device increased activity of both the sympathetic and parasympathetic nervous systems during marksmanship and cognitive testing. Similar effects were noted on frequency-series indicators of heart rate variability using both ECG and PPG, in which stimulation condition effects were noted on ECG high frequency absolute ($\beta = 8.50$, $p_{unadj} < 0.01$, $p_{adj} = 0.01$, $d < 0.01$) and relative powers ($\beta = -8.54$, $p_{unadj} < 0.01$, $p_{adj} = 0.01$, $d < 0.01$), as well as PPG very low frequency power ($\beta = -367.98$, $p_{unadj} < 0.01$, p_{adj} = n.s., $d = 0.12$). Effects of the interaction between stimulation condition and measurement time point were noted on very low, low, and high frequency powers (p_{unadj} range < 0.01 – 0.048, d range < 0.01 – 0.21), as well as the ratio of low- to high-frequency powers in ECG (p_{unadj} range < 0.01 – 0.048, $d < 0.01$ for all). These results also suggest that trigeminal TES increased activity of both the sympathetic and parasympathetic nervous systems during marksmanship and cognitive testing.

Furthermore, significant effects of stimulation condition were noted on marksmanship shot accuracy ($\beta = 0.14$, $p_{unadj} = 0.01$, p_{adj} = n.s., $d = 0.60$) and distance of shots from the targets' center of mass ($\beta = -0.08$, $p_{unadj} = 0.02$, p_{adj} = n.s., $d = 0.56$), indicating that trigeminal TES impaired shot accuracy. There were also significant condition-by-time interaction effects on target detection latency ($\beta = 220.46$, $p_{unadj} = 0.04$, p_{adj} = n.s., $d = 0.49$); significant impairments in shot latency observed during the first marksmanship session in the active TES condition only resolved by the second marksmanship session. There were no significant effects of TES on accuracy or response

times for neuropsychological tasks assessing response inhibition, sustained attention, and working memory (p_{unadj} range 0.09 – 0.98). Active trigeminal TES did, however, significantly reduce the standard deviation of response times on a measure of sustained attention and response inhibition (β = -16.29, p_{unadj} = 0.045, p_{adj} = n.s., d = 0.43).

Although the literature suggests that TES may benefit stress and performance, these results do not support that conclusion. Overall, these analyses found that TES using a commercially available device did not influence chemical biomarkers of stress, but did influence markers of physiological stress, as well as cognitive and marksmanship performance under high cognitive load. TES was associated with impairments in marksmanship performance as well as increases in both sympathetic and parasympathetic nervous system activity. Further studies using different stimulation parameters, including multiple sessions of stimulation, will be necessary to more fully characterize possible influences of trigeminal nerve stimulation on stress responding and marksmanship performance or other military relevant tasks. In addition, this project underscores the need for more investigation into the mechanisms of effect of the Thync One device and other devices applying TES of the trigeminal nerve.

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5-HT	Serotonin
ABVN	Auricular branch of the vagus nerve
ACh	Acetylcholine
ADHD	Attention-Deficit/Hyperactivity Disorder
ADIOL	5-androsten-3β,17β-diol
AIC	Akaike Information Criterion
ANAM4	Automated Neuropsychological Assessment Metrics, version 4
ANOVA	Analyses of variance
AR	Autoregressive Transformation
BC	Before Christ
C/cm^2	Coulombs per centimeter squared
Ca2+	Calcium
CI	Confidence interval
Cl-	Chloride
d’	d-prime
DA	Dopamine
DBS	Deep brain stimulation
df	Degrees of freedom
DHEA	Dehydroepiandrosterone
DMNV	Dorsal motor nucleus of the vagus nerve

DRN	Dorsal raphe nucleus
DRS-15	Dispositional Resilience Scale
DV	Dependent variable
ECG	Electrocardiography
ECT	Electroconvulsive therapy
EDA	Electrodermal activity
EPI	Epinephrine
EST	Engagement Skills Trainer
FFT	Fast Fourier Transformation
GABA	γ-aminobutyric acid
gradCPT	Gradual Onset Continuous Performance Test
Grit-S	Grit Scale – short version
HF	High frequency
HPA	Hypothalamic-pituitary-adrenal
HR	Heart rate
HRV	Heart rate variability
HVT	High value target
Hz	Hertz
IL-6	Interleukin-6
iOS	Apple operating system
IUD	Intrauterine device
IV	Independent variable

IZOF	Individual Zones of Optimal Functioning
K+	Potassium
kHz	Kilohertz
LC	Locus coeruleus
LF	Low frequency
LF/HF	Ratio of low- to high-frequency heart rate variability
LOT-R	Life Orientation Test – revised
m	Meters
M4	Carbine, military model 4
mA	Milliamperes
mA/cm^2	Milliamperes per centimeter squared
MCS	Mental component score
Min	Minutes
msec	Milliseconds
mV	Millivolts
N or n	Number of individuals in a group
Na+	Sodium
NAc	Nucleus accumbens
NaN	Not a Number
NASA-TLX	NASA Task Load Index
NE	Norepinephrine
NPY	Neuropeptide Y

n.s.	Not significant
NSI	Neurobehavioral Symptom Inventory
NSSC	Natick Soldier Systems Center
NTS	Nucleus of the tractus solitarius
n.u.	Normal units
p	*p*-value
P3 wave	P300 wave (EEG)
p_{adj}	*p*-value adjusted for multiple comparisons correction
PANAS	Positive and Negative Affect Scale
PCS	Physical component score
PFC	Prefrontal cortex
pNN50	Percentage of successive R-R intervals that differ by more than 50 msec
PNS	Parasympathetic nervous system
PPG	Photoplethysmography
PPN	Pedunculopontine nucleus
PSS-10	Perceived Stress Scale
PTSD	Posttraumatic stress disorder
p_{unadj}	*p*-value unadjusted for multiple comparisons correction
RMSSD	Root mean square of successive differences between heart beats
R-R	Beat-to-beat heart rate intervals

RSPEC-R	Respiration monitor with electrocardiography amplifier
RV	Research volunteer
SAM	Sympathetic-adrenal-medullary
SDNN	Standard deviation of normal R-R intervals
SE	Standard error
SM	Service member
SN	Substantia nigra
SNS	Sympathetic nervous system
Stim	Stimulation
t	t-statistic
tACS	Transcranial alternating current stimulation
tDCS	Transcranial direct current stimulation
TEQ	Task Engagement Questionnaire
TES	Transcutaneous electrical stimulation
TMS	Transcranial magnetic stimulation
TNF-α	Tumor necrosis factor-α
TSNC	Trigeminal sensory nuclear complex
tVNS	Transcutaneous vagal nerve stimulation
U/mL	Units per milliliter
ug/dl	Micrograms per deciliter
UPPS-P	UPPS-P Impulsive Behavior Scale
µS	Microseconds

USARIEM	United States Army Research Institute of Environmental Medicine
V1	Opthalmic branch of the trigeminal nerve
V2	Maxillary branch of the trigeminal nerve
V3	Mandibular branch of the trigeminal nerve
VLF	Very low frequency
VR-12	Veterans RAND 12-Item Health Survey
VTA	Ventral tegmental area
WA	Washington

CHAPTER ONE: INTRODUCTION

Military training and operations can place significant physical and mental demands on Service Members (SMs). Meeting these demands can tax a SM's available cognitive and physical resources and when resources are overwhelmed, SMs may experience a number of medical illnesses, such as cardiovascular disease, diabetes, and hypertension (Logan & Barksdale, 2008; Mattei, Demissie, Falcon, Ordovas, & Tucker, 2010; McEwen, 1998). SMs may also experience deteriorated mood and increased suicidal behavior (Allan et al., 2017; Bernert, Kim, Iwata, & Perlis, 2015; Wolkow, Ferguson, Aisbett, & Main, 2015) and occupational performance may suffer as a result of insomnia, sleep restriction, and sleep deprivation (Seelig et al., 2016). The effects of stress and allostatic overload on SM health, cognition, and job performance are, therefore, far-reaching and require sophisticated solutions to mitigate.

Among the myriad medicinal and therapeutic interventions for stress is neuromodulation, which is a broad term used to describe a host of techniques that influence neural activity. One such technique is transcutaneous electrical stimulation (TES), in which mild electrical pulses are applied to peripheral nerves through the skin via electrodes placed on the head, neck and/or face. TES is safe, easy to use, and does not require input from medical providers, which is critical for SMs who cannot always access providers easily. However, its effects on cognitive and occupational performance, as well as its mechanisms and effects on the stress response, are currently unknown. Therefore, this study aimed to address this gap by characterizing the impacts of trigeminal TES on

biochemical and physiological markers of stress activity as well as on cognition and marksmanship in order to elucidate the effectiveness and mechanisms of TES.

The Human Stress Response

The demands that SMs face during operations and training may be extreme and include (but are not limited to) sleep loss, alterations in nutrition and hydration status, exposure to chemical hazards and environmental extremes (heat, cold, altitude), deployment, prolonged periods of separation from support systems and combat (Good, Brager, Capaldi, & Mysliwiec, 2020). In addition, interpersonal challenges, such as poor unit cohesion or loss of a battle buddy, and intrapersonal or psychological stress induced by traumatic exposures to combat or other sources can result in or exacerbate existing emotional difficulties such as depression, anxiety, or posttraumatic stress disorder (PTSD).

Exposure of SMs to such extreme situations may induce "allostasis," which Sterling and Ever (1988) defined as the process of "achieving stability through change." This process is not inherently maladaptive but rather returns the body to homeostasis, the physiological state in which the body is functioning within the bounds essential for life, after environmental changes (Cannon, 1932; Romero, Dickens, & Cyr, 2009). Homeostasis itself involves reactive responding to environmental stimuli while staying within the parameters necessary to sustain life (e.g., decreased glucose consumption after an acute stressor; Marik & Bellomo, 2013), while change during allostasis is significantly more variable than homeostatic reactivity because it is not limited to the changes

necessary to sustain life (McEwen & Wingfield, 2010). Indeed, it may allow the individual to adapt to changing circumstances in a more sustained manner (e.g., to maintain high blood pressure in the face of a prolonged stressor; Ramsay & Woods, 2014). These accumulated changes are referred to as "allostatic load", the cumulative somatic "wear and tear" that result from prolonged, repeated, or particularly intense stress-induced elevations of cortisol, norepinephrine (NE), and inflammatory cytokines, for example (Lupien, Juster, Raymond, & Marin, 2018; McEwen & Wingfield, 2010).

When allostatic load is prolonged during chronic stress (referred to as an "allostatic state") (Koob, 2001), an individual may begin to feel overwhelmed, and performance and health may suffer (Romero et al., 2009). This state is referred to as either "homeostatic overload" (Romero et al., 2009) or "allostatic overload" (McEwen & Wingfield, 2010), the term used most frequently by biopsychosocial scientists due to its inclusion of responses to stressful stimuli (Ramsay & Woods, 2014). When allostatic overload is achieved and cognitive demand is high (due to fatigue, time on task, or task complexity), a stress response may occur (Moreira et al., 2018; Solhjoo et al., 2019; Tiferet-Dweck et al., 2016). The human body has two primary pathways by which it responds to stress: the sympathetic-adrenal-medullary (SAM) axis, which forms the basis of the sympathetic nervous system's response to stress, and the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky, Romero, & Munck, 2000). These responses act in coordination to mobilize the body to selectively attend to a stressor and respond accordingly, often referred to as the "fight or flight" response.

The SAM axis typically accounts for the first biological responses to a stressor because it involves direct connections between the brain, adrenal glands, and neural tissues (Sapolsky et al., 2000). It acts by means of the catecholamines, NE and EPI (Sapolsky et al., 2000). In particular, release of catecholamines results in increases in heart rate and an associated decrease in heart rate variability, as well as increases in inflammation, respiration rate, pulmonary bronchodilation, glycogenolysis, and sweat secretion (Carnevali, Koenig, Sgoifo, & Ottaviani, 2018; Mackersie & Calderon-Moultrie, 2016; Piazza, Almeida, Dmitrieva, & Klein, 2010; Sapolsky et al., 2000; Sequeira, Hot, Silvert, & Delplanque, 2009; Yamakawa et al., 2009). Peripheral inflammation, in particular levels of pro-inflammatory cytokines such as interleukin-6 and interleukin-1β, continues to increase for at least two hours following stress induction (Rohleder, 2014; Steptoe, Hamer, & Chida, 2007). Each biological response is intended to increase the body's ability to respond to a stressor by either fighting or escaping (Sapolsky et al., 2000).

A second response to stress, which is slower due to its indirect connections between the brain and adrenal glands, occurs when the HPA axis is activated (Rasmusson & Pineles, 2018; Sapolsky et al., 2000). A cascade of responses allows the release of corticotropin-releasing hormone that stimulates the release of ACTH from the pituitary into the circulation and the subsequent release of cortisol and other neuroactive steroids from the adrenal glands (Sapolsky et al., 2000). Cortisol influences immune system activity and is involved in energy mobilization and regulation of brain (Piazza et al., 2010; Sapolsky et al., 2000). Dehydroepiandrosterone (DHEA) is released alongside

cortisol and has anti-inflammatory, antioxidative, neuroprotective, and antiglucocorticoid effects (Lennartsson, Kushnir, Bergquist, & Jonsdottir, 2012; Piazza et al., 2010; Ventre, Colonna, Smith, Alfano, & Moldow, 2013). The SAM and HPA axis responses interact to appropriately respond to stress, allowing the individual to selectively attend to the stressor and to respond accordingly (Sapolsky et al., 2000).

Activity of the SAM and HPA axes are commonly measured via biomarkers assayed in blood, urine, and cerebrospinal fluid, but can be approximated using non-invasive behavioral and salivary methods as well. For example, SAM axis activity can be measured via salivary α-amylase, which is considered to be an indirect measure of NE activity as levels of α-amylase in saliva increase and decrease alongside NE levels in response to agonist or antagonist administration (Nater & Rohleder, 2009) and is frequently used in studies of stress (Chatterton, Vogelsong, Lu, Ellman, & Hudgens, 1996; Piazza et al., 2010). Sympathetic nervous system activity generally can be measured by monitoring heart rate and heart rate variability, blood pressure, and electrodermal variability (Piazza et al., 2010). Salivary free cortisol and DHEA can be used to estimate HPA axis reactivity (Piazza et al., 2010). Finally, the immune response to stress can be measured via salivary markers of inflammation, including specific inflammatory cytokines (Maldonado et al., 2018). Using these salivary and sympathetic system measures of physiological arousal following an acute stressor, researchers can characterize and quantify physiological response to that stressor (Chatterton et al., 1996; Maldonado et al., 2018; Piazza et al., 2010), if not the individual's perceived emotional stress, which is better assessed by subjective measures.

The Effects of Stress on SM Emotional Health

Military SMs are at a high risk of experiencing stressful and traumatic events by virtue of their participation in the military. Two thirds of Soldiers report experiencing traumatic events, such as seeing dead or seriously injured Soldiers, while 68% report being subject to incoming fire and 53% report experiencing blasts (Mental Health Advisory Team 9, 2013). These experiences lead to chronic allostatic overload and increased activity of the SAM and HPA axes, ultimately leading to a number of medical illnesses, including cardiovascular disease, diabetes, and hypertension (Logan & Barksdale, 2008; Mattei, Demissie, Falcon, Ordovas, & Tucker, 2010; McEwen, 1998). Allostatic overload also can have negative effects on mental health. Sleep deprivation leads to increased cortisol and pro-inflammatory cytokine levels, as well as deteriorated mood and increased suicidal behavior (Allan et al., 2017; Bernert et al., 2015; Wolkow et al., 2015).

In military personnel, emotional disorders are between two and four times more common in individuals assessed 120 days after returning from combat compared to their non-combat-exposed colleagues (Bliese, Wright, Adler, Thomas, & Hoge, 2007). Reports of mental health problems increase from 5% to 20% within 10 months after deployment; in fact, the longer someone is deployed, the more likely they are to experience emotional consequences (Mental Health Advisory Team 9, 2013). Unhealthy behaviors, such as alcohol and drug use, are present in 2% to 8% of all Soldiers (Mental Health Advisory Team 5, 2008), while 26% of married Soldiers say they are considering divorce (Mental

Health Advisory Team 9, 2013). The events SMs experience in combat also lead to a high prevalence of PTSD, for which the rate is around 32% of those injured in combat compared to 14% of those never injured (Hoge, Terhakopian, Castro, Messer, & Engel, 2007). Finally, 13% of Soldiers report considering suicide in the last month (Bernecker et al., 2019). Clearly, the impact of stress is far-reaching and life-changing in a way that necessitates preventative intervention.

Meanwhile, a number of factors protect against the negative effects of stress on emotional health; these include exercise, leadership, family and marital support, training, use of medications such as propranolol, personality characteristics, and coping skills (Giustino, Fitzgerald, & Maren, 2016; Harms, Krasikova, Vanhove, Herian, & Lester, 2013; Hourani et al., 2016; Pitts et al., 2019). Indeed, while negative affect and neuroticism are associated with greater stress in life and on the job (Parrish Meadows, Shreffler, & Mullins-Sweatt, 2011), hope, optimism, and grit are each associated with reduced stress and job turnover (Bressler, 2010). Optimism and positive mindset lead to perceiving stressors as a challenge rather than a threat or loss, which is associated with fewer somatic complaints (Schaubroek, Riolli, Peng, & Spain, 2011). In contrast, venting emotions and emotional disengagement predict worse somatic or physical health complaints (Day & Livingstone, 2001). Decreases in PTSD over 12 months (Solomon, Mikulincer, & Avitzur, 1988) and lowered likelihood of suicide completion (Lester, McBride, Bliese, & Adler, 2011) are predicted by the use of problem- and emotion-focused coping skills. Moreover, hardiness, which reflects an ability to respond in an adaptive manner to stress, is associated with more positive health outcomes and

emotional well-being (Bartone, 1999; Bartone & Priest, 2001), as well as job retention and better occupational performance (Maddi, Matthews, Kelly, Villareal, & White, 2012).

The Effects of Stress on SM Cognition

For over a century, psychologists have theorized that stress and physiological arousal associated with the "fight or flight" response have an impact on cognitive performance. One of the most well-known theories is the Yerkes-Dodson law, also called the "Inverted-U Hypothesis," which states that as physiological arousal increases, performance improves until a peak is reached, after which physiological arousal continues to increase but performance decreases to the point of exhaustion, poor health, panic, and anxiety. Though this has become a well-known concept, the Yerkes-Dodson law falls short in reflecting the complexity of the relationship between stress and performance, and neglects major factors that influence performance under stress, including characteristics of the stressor and the ability of the individual to adapt to stress and perform optimally at different levels of stress (Hancock & Ganey, 2003; Hancock & Warm, 1989). Nevertheless, the Yerkes-Dodson law does allow for simplified categorization of seven different "stress states" that contribute to an individual's cognitive performance (Bourne & Yaroush, 2003), as follows (**Figure 1**):

a. **No stress**, in which the individual is completely unstressed and under-stimulated, leading to a low level of performance,
b. **Facilitation**, in which low levels of stress enhance performance,

c. **Optimum**, in which moderate levels of stress lead to the best performance,

d. **Mobilization**, in which higher levels of stress combined with increased motivation leads the individual to perform at a high (but not optimal) level,

e. **Degradation**, in which increasing levels of stress begins to decrease performance gradually,

f. **Choking**, in which stress continues to increase and performance on well-learned tasks becomes poor and slow,

g. **Panic**, in which high levels of stress degrade performance to the extent that thoughts are "primitive" and about survival or dissociative (Bourne & Yaroush, 2003; Bracha, 2004; Dörner & Pfiefer, 1993; Lehner, Seyed-Solorforough, O'Connor, Sak, & Mullin, 1997; Norman & Bobrow, 1975).

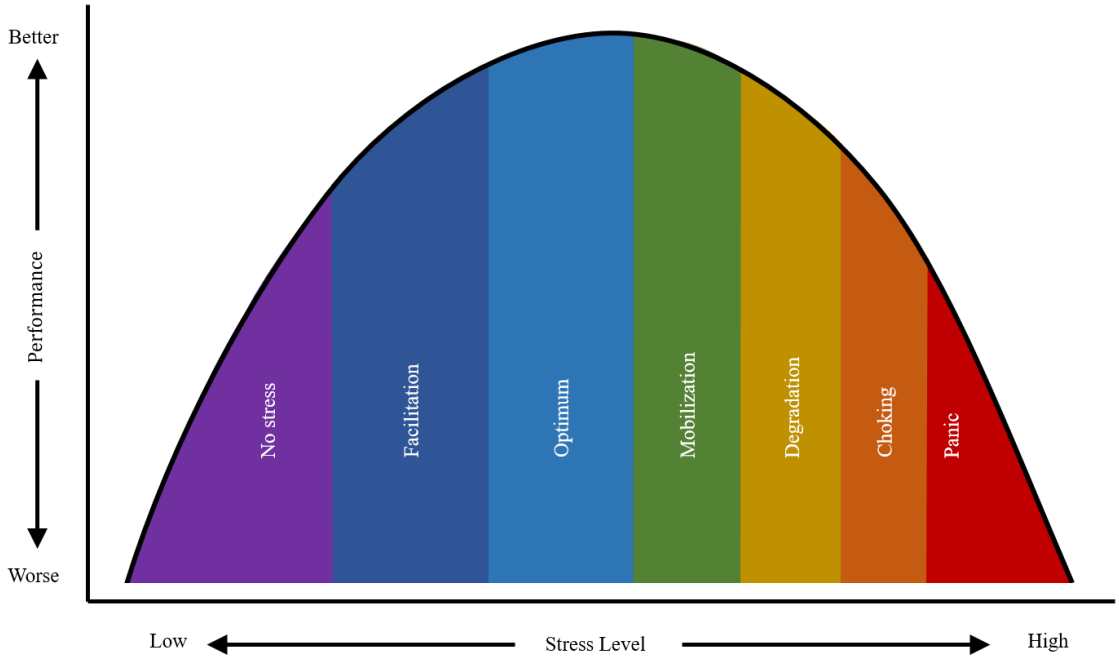


Figure 1. Representation of the various stress states.
Adapted from Bourne and Yaroush (2003).

Building upon the overly simplistic characterization of stress effects presented by Yerkes-Dodson law, several newer theories have emerged that better encompass the complex nature of the association between stress and cognitive performance. Cognitive Appraisal Theory, for example, states that stressful events do not actually create stress unless the individual considers them to be threatening. Thus, the way that one considers an event determines how one responds, behaves, and copes (Bourne & Yaroush, 2003; Lazarus, 1991). Processing Efficiency Theory, Attentional Control Theory, and Cognitive Interference Theory each posit that perceived anxiety about a stressor overloads working memory and attentional capacity, such that it reduces the individual's ability to retain information and remain focused on task-related stimuli (Eysenck & Calvo, 1992; Eysenck, Derakshan, Santos, & Calvo, 2007; Sarason, 1988; Wilson, Smith, & Holmes, 2007). This results in a decrease in performance that can often be reversed with increased effort, motivation, or use of compensatory strategies (Eysenck & Calvo, 1992; Eysenck et al., 2007; Sarason, 1988; Wilson et al., 2007).

Additionally, two notable theories have emerged from the sports psychology literature. The Conscious Processing Hypothesis suggests that high levels of perceived anxiety and self-consciousness about performance result in deliberate, conscious performance of well-learned and automatic behaviors, effectively reversing the benefit of fluency achieved through practice and repetition (Masters, 1992; Wilson et al., 2007). Another theory, known as the "Individual Zones of Optimal Functioning" (IZOF) theory states that each individual has a different level of perceived anxiety that is associated with optimal performance in a motor task (Hanin, 1997; Jokela & Hanin, 1999). For some,

optimal performance occurs at lower levels of anxiety, while for others, moderate or high levels of anxiety lead to optimal performance (**Figure 2**). This theory allows for interaction between the individual's perception of a stressor and the level of his or her cognitive arousal in predicting performance on a task. Subsequent research has also demonstrated that somatic anxious arousal, such as sweating and increased heart rate, is more predictive of performance than perceived anxious arousal (Dennis, Bartsokas, Lewthwaite, & Palin, 1993). The Somatic Marker Hypothesis takes this into account, stating that cognition, particularly decision-making, is directed by and dependent upon the neurobiological processes associated with emotion and their feedback to the brain (Damasio, 1996; Dunn, Dalgleish, & Lawrence, 2006).

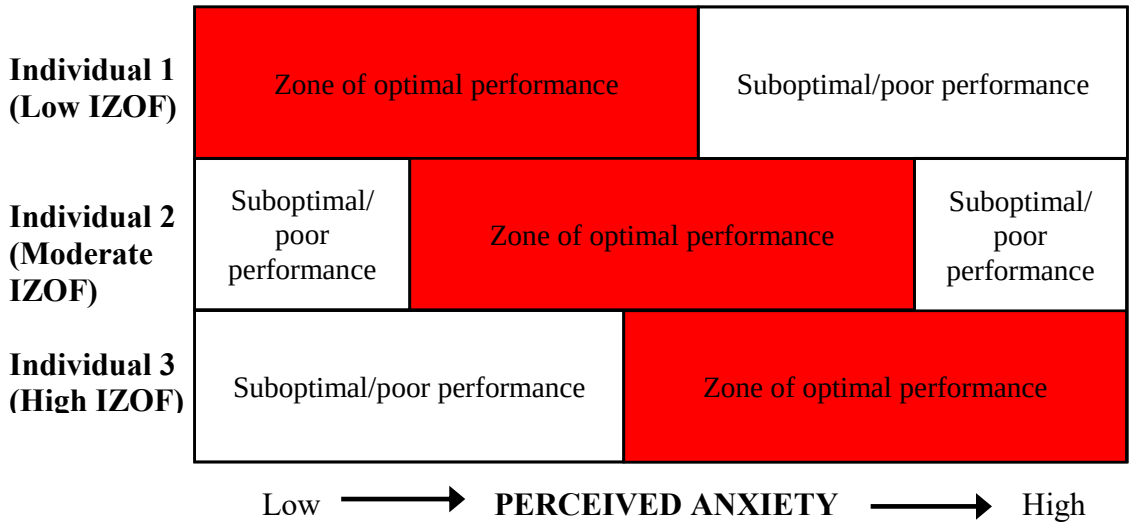


Figure 2. Different individuals' zones of optimal performances under stress. Adapted from Hanin (1997).

Taken together, these theories suggest that performance on cognitive tasks is affected by the anxious arousal (both perceived anxiety and physiological anxiety) created by those tasks, and that each individual performing the tasks may require very

different levels of arousal in order to perform optimally. This accounts for the observation that some people perform better than others at high-stress or difficult tasks (stress under-responders), while others perform more poorly at low-stress or easy tasks (stress over-responders) (Almela et al., 2014; Buchanan & Tranel, 2008; Nater et al., 2007; Raison & Miller, 2003). Moreover, stress reactivity (i.e., the likelihood that an individual responds to stressful events with both immediate and sustained stress responses) and the magnitude of the stress response, predicts risk of depression and sleep problems (Herr et al., 2018), as well as workplace burnout (Wekenborg et al., 2019) and alterations in brain network efficiency (Qi et al., 2021; van Oort et al., 2017; Wheelock et al., 2018; Zhang et al., 2019). Experience—whether one is an expert at a task or a novice—has an added effect, as it modifies the way in which the individual processes the task (Burke et al., 2008). Each of these factors is likely to contribute to cognitive performance during acute stress in a variety of cognitive domains including decision-making, attention, response inhibition, perception, and memory (Bourne & Yaroush, 2003).

Cognitive impairments associated with stress have been demonstrated in executive function (Cox, Ebesutani, & Olatunji, 2015), including working memory (Vrijkotte, Roelands, Meeusen, & Pattyn, 2016), attention and vigilance (Ståhle et al., 2011; Vrijkotte et al., 2016; Zhang & Liu, 2008), response inhibition (Wilke et al., 2019), cognitive flexibility (Wilke et al., 2019), processing speed (Hu, Xiong, Dai, Zhao, & Feng, 2016), and reasoning and decision-making (Ståhle et al., 2011; Vrijkotte et al., 2016), as well as in psychomotor function (Krystow, Beidleman, Fulco, & Muza, 2013;

Proctor, Heaton, Heeren, & White, 2006; Zhang & Liu, 2008), learning (Nation, Bondi, Gayles, & Delis, 2017), and memory (Nation et al., 2017; N. Zhang & Liu, 2008). However, the most common domains in which the effects of stress are noted include decision-making, attention, and working memory.

Decision-Making

Collectively, literature reviews indicate that acute stress has a negative impact on decision-making (Hartley & Phelps, 2012; LeBlanc, 2009; Shields, Lam, Trainor, & Yonelinas, 2016; Staal, 2004; Starcke & Brand, 2012; Starcke & Brand, 2016). Under moderate levels of stress, vigilance, or the maintenance of focused attention over time, increases in order to identify threats and supports decision making whereas under high levels of stress, hypervigilance, or enhanced sensory sensitivity to threat, may promote impulsive and disorganized choices made without enough information or with limited consideration of alternatives and consequences (LeBlanc, 2009; Wemm & Wulfert, 2017). In addition, people under stress tend to make decisions using suboptimal or maladaptive strategies or cognitive heuristics (LeBlanc, 2009). Moreover, stressed individuals may be subject to altered feedback processing, increased reward sensitivity, and decreased punishment sensitivity, leading to disadvantageous decisions centered on reward-seeking and risk-taking (Baradell & Klein, 1993; de Visser, Baars, Lavrijsen, van der Weerd, & van den Bos, 2011; Gray, 1999; LeBlanc, 2009; Miu, Heilman, & Houser, 2008; Starcke & Brand, 2012; Starcke & Brand, 2016; Wemm & Wulfert, 2017). Such effects may lead SMs to make less moral or ethical decisions.

Moral or ethical decision-making is highly associated with environmental and health factors. Exposure to violence and unpredictable, threatening situations change the way that an individual frames actions and consequences in an ethical context and impairs moral judgement and behavior (Merlhiot, Mermillod, Le Pennec, Dutheil, & Mondillon, 2018; Zucchelli & Ugazio, 2019). While acute stress is associated with increased altruistic behavior (Singer et al., 2017), chronic stress is associated with more duty-based ethical choices, which focus on rightness or wrongness of the intention of an action rather than its consequences (utilitarian choices), and taking longer to make choices in emotional dilemmas (Killgore et al., 2007; Timmons & Byrne, 2019; Weippert et al., 2018; Zhang, Kong, Li, Zhao, & Gao, 2018).

Short-term elevations in cortisol are similarly beneficial to moral behavior, but long-term elevations of cortisol and NE are associated with poorer moral judgement and intention (Siedlik et al., 2016). These findings are supported by research indicating that use of propranolol, a β-adrenergic receptor antagonist that blocks the binding of NE and EPI to adrenoceptors, is associated with making more ethical choices (Terbeck et al., 2013). This effect, however, is inconsistent; indeed, other studies have shown that stress results in fewer utilitarian responses to personal moral dilemmas (Youssef et al., 2011). Instead, stress increases egocentric decision-making (Starcke, Polzer, Wolf, & Brand, 2011), suggesting that individuals under stress may act in a manner that benefits themselves above all others. This is illustrated by a startling set of statistics: 33% of Soldiers admit to insulting or cursing at noncombatants, while 6% report acting violent

toward noncombatants when it was not necessary and 4% admit to ignoring Rules of Engagement during missions (Mental Health Advisory Team 5, 2008).

Additionally, marksmanship, which is a skillset required of all SMs, relies on physical and cognitive performance. Physical aspects of marksmanship, such as accuracy and reaction time, are negatively impacted in the short-term as a result of sleep deprivation, heavy load carriage, chemical exposures, and altitude exposure (Bouak, Vartanian, Hofer, & Cheung, 2018; Frykman, Merullo, Banderet, Gregorczyk, & Hasselquist, 2012; Hadid et al., 2017; Muza et al., 2000; Smith et al., 2019; Taylor & Orlansky, 1993). In situations in which working memory demand is high, firing accuracy decreases dramatically (Burke, Oron-Gilad, Conway, & Hancock, 2007). Sleep deprivation, a significant stressor in military training and operational settings, degrades marksmanship accuracy, resulting in larger numbers of errors of commission (firing at targets that should not be fired at) (Head et al., 2017). Seventy-three hours of total sleep deprivation during Navy SEAL Hell Week, which results in periods of physical exhaustion and extended sleep restriction, also degraded marksmanship accuracy by 37.5% and induced a 53% increase in shot latency (Tharion, Shukitt-Hale, & Lieberman, 2003). Shot latency and target discrimination latency also increase, signaling slower response times, and target discrimination accuracy decreases under sleep restriction and high cognitive load (Smith, Cooper, Merullo, Cohen, Heaton, Claro, & Smith, 2019). When under threat of shock, individuals mistake friends as foes (false alarms) more often than under no threat of shock during a friend-foe discrimination marksmanship task. Moreover, this increase in false alarms was associated with increases in high-frequency

heart rate variability indicative of parasympathetic nervous system activity (Gamble et al., 2018). Such deficits in accuracy and response times may be associated with increases in accidents, such as friendly fire, which are more likely to occur during time-pressured operations and as a result of combat stress (Shrader, 1992). Interestingly, there may be gender differences in the effects of sleep deprivation on friendly fire, with men more likely to commit friendly fire than women (Johnson & Merullo, 1999). Women, in turn, may be more likely to commit fail-to-fire errors under sleep deprivation (Johnson & Merullo, 1999).

These effects can also be considered in the context of the SAM- and HPA-axis responses. With respect to the SAM axis, increased EPI, heart rate variability, and electrodermal activity are associated with enhanced decision-making performance in healthy individuals (Czermak et al., 2009; Miu et al., 2008; Ohira, Matsunaga, et al., 2013). Consistently, animal models show that lesions in the NE pathway result in impaired decision-making, especially in target-discrimination performance (Carli, Robbins, Evenden, & Everitt, 1983; Cole & Robbins, 1992; Oke & Adams, 1978; Roberts, Price, & Fibiger, 1976). This association can also be visualized using electroencephalography (Freedman, Keegan, Rodriguez, & Galloway), particularly characterization of the P300 (P3) wave, a positive evoked potential that appears to originate from LC and NE activity at approximately 300 milliseconds after stimulus perception (Nieuwenhuis, Aston-Jones, & Cohen, 2005). P3, especially the P3b wave that is primarily distributed within the central and parietal regions of the brain, has been associated with regulation of neural resources that allow attention to incoming stimuli

and related decision-making (Rufener, Geyer, Janitzky, Heinze, & Zaehle, 2018; Ventura-Bort et al., 2018). Ventura-Bort and colleagues (2018) found that salivary α-amylase levels and P3b amplitude correlate, consistent with a hypothesized link between the P3 wave and NE activity. In contrast, research has demonstrated mixed associations between salivary α-amylase levels and decision-making performance, with some demonstrating correlations between high salivary α-amylase levels and poor decision-making as well as elevated risk-taking behavior, while others showing no association (Labudda, Wolf, Markowitsch, & Brand, 2007; Margittai et al., 2016; Starcke, Wolf, Markowitsch, & Brand, 2008; van den Bos, Taris, Scheppink, de Haan, & Verster, 2013).

With regard to the secondary stress response by the HPA axis, studies have shown that stress-dependent cortisol increases correlate with increased risk-taking behavior and impaired decision-making (Margittai et al., 2016; Pabst, Schoofs, Pawlikowski, Brand, & Wolf, 2013; Putman, Antypa, Crysovergi, & van der Does, 2010; Starcke et al., 2011; Starcke et al., 2008; van den Bos, Golka, Effelsberg, & McClure, 2013). However, other studies, have shown an association between increased stress or higher cortisol levels and improved decision-making (Akinola & Mendes, 2012; Shields, Lam, et al., 2016) consistent with studies showing an association between increased IL-6 levels and poorer decision-making (Frydecka et al., 2015; Marioni, Deary, Murray, Fowkes, & Price, 2010; Ohira, Osumi, Matsunaga, & Yamakawa, 2013; Rubin et al., 2018). If increased cortisol is associated with better decision-making performance as well as decreased systemic inflammation, then it is to be expected that lower levels of inflammatory cytokines will be associated with better decision-making performance (Hannibal & Bishop, 2014).

DHEA is also associated with decision-making performance, such that larger post-stress increases in circulating DHEA predict better decision-making (Hirshman et al., 2003; Ohana et al., 2016; Shields, Lam, et al., 2016). Of note, a metabolite of DHEA, 5-androsten-3β,17β-diol (ADIOL), is neuroprotective and reduces inflammation (Salama et al., 2018; Szalay et al., 2006).

Attention

Another cognitive domain affected by stress is attention. There are many types of attention but three are particularly relevant to stress: selective attention, in which the individual focuses on relevant rather than irrelevant stimuli; sustained attention, in which the individual continues to pay attention to a stimulus for a period of time rather than becoming distracted or losing focus; and divided attention, in which the individual integrates information from multiple incoming sources (LeBlanc, 2009). Under acute stress, attention is focused upon an important task or stimulus and selective attention increases (LeBlanc, 2009; Sato, Takenaka, & Kawahara, 2012; Staal, 2004; Tiferet-Dweck et al., 2016). Stress can also damage attention, as in circumstances in which stimuli are erroneously considered “irrelevant” to the task being performed (Olver, Pinney, Maruff, & Norman, 2015; Staal, 2004). Sustained and divided attention are also typically impaired after exposure to stress, likely because the individual becomes overwhelmed and his or her attentional resources become depleted, leading to distraction or an inability to integrate all of the necessary sources of information (LeBlanc, 2009; Sänger, Bechtold, Schoofs, Blaszkewicz, & Wascher, 2014; Vine, Moore, & Wilson,

2016). However, some studies have demonstrated improvements in sustained attention under threat of shock (Balderston et al., 2017; Grillon, Robinson, Mathur, & Ernst, 2015).

SMs encounter situations involving various attentional processes regularly. Monotonous tasks that require sustained vigilance for long periods of time, such as night watch, become more difficult as concentration and attention are diminished under stress (Gaillard, 2008). The SM's perception of a lack of control over the situation may lead to lower task engagement and, in turn, poor performance as attentional resources deteriorate (Warm, Matthews, & Finomore, 2008). However, when under fire or in an engaging, stressful situation, the SM's physical and mental energy are mobilized so that directed attention and concentration increase in order to focus and act in the stressful situation (Gaillard, 2008). For attention and concentration, the ways in which the brain handles monotony (itself a form of stress) and extreme stress is based on the balance between positive factors (such as motivation, rewards, feedback) and negative ones (distractions, fatigue, time pressure, conflict stress) (Gaillard, 2008). In contrast, stress influences response speed differently depending on the amount or severity of stress as well as the complexity of the task being completed (Harris, Ross, & Hancock, 2008). While stress due to monotony or fatigue does adversely affect speed and accuracy on easy tasks, such as simple reaction time tasks, it does not influence speed or accuracy on complex tasks (Harris et al., 2008). Moderate stress, meanwhile, negatively affects complex tasks as well as easy ones but performance is dependent on the amount of time for which the task is administered as well as the participant's level of control (Harris et al., 2008). Finally,

high stress decreases performance in both easy and complex tasks, regardless of timing of testing and participant's level of control (Harris et al., 2008).

These effects also can be placed in the context of the SAM- and HPA-axis responses elicited by stress. With regard to the SAM axis, increases in EPI levels and heart rate variability are positively correlated with performance on tasks of selective and sustained attention, providing support for the finding that attention is more focused during stress (Folli et al., 1992; Gazzellini et al., 2016; Giuliano et al., 2018; Hansen, Johnsen, & Thayer, 2003; Hansen, Johnsen, & Thayer, 2009; Middleton, Sharma, Agouzoul, Sahakian, & Robbins, 1999; Park, Vasey, Van Bavel, & Thayer, 2013). Furthermore, the NE system has long been associated with attention because of the LC's projections to the prefrontal cortex, a brain region known to be a primary locus for executive function including attention (Clark & Noudoost, 2014). Using non-human primate models, researchers have concluded that the LC and NE are vital to the ability to filter critical information and respond accordingly during new or unexpected situations (Aston-Jones, Rajkowski, & Cohen, 1999). Activity of the LC can be either tonic, (meaning LC neurons fire consistently), or phasic (meaning LC neurons fire transiently or intermittently) (Howells, Stein, & Russell, 2012). Unsworth and Robison (2017) suggest that when LC function is disrupted, optimal tonic firing is not maintained and NE levels are disturbed, leaving the prefrontal cortex and each of its connective brain regions functionally impaired (Unsworth & Robison, 2017). Therefore, when LC firing and NE are too low during an under-aroused period or too high during a stressful period, the prefrontal cortex does not function optimally and attention dysfunction occurs (Aston-

Jones et al., 1999; Unsworth & Robison, 2017). As before, this may be visualized using electroencephalography and the P3 wave, as selective and sustained attentions are among the significant tasks associated with the P3 wave (Kapanci, Merks, Rammsayer, & Troche, 2019; Portin et al., 2000; Sänger et al., 2014).

With regard to the HPA axis, cortisol levels and reactivity are negatively associated with performance and selective, sustained, and divided attention (Bohnen, Houx, Nicolson, & Jolles, 1990; Henckens, van Wingen, Joëls, & Fernández, 2012; Robinson, Ode, & Hilmert, 2014; Roelofs, Bakvis, Hermans, van Pelt, & van Honk, 2007; Sänger et al., 2014; Skosnik, Chatterton, Swisher, & Park, 2000; Vedhara, Hyde, Gilchrist, Tytherleigh, & Plummer, 2000). The association between HPA axis activity and attention is likely because increased cortisol circulation increases emotional interference by altering connectivity between emotion and attentional processing regions of the brain such that attention cannot be focused appropriately (Henckens et al., 2012). However, at least one other study of healthy individuals has found that there is no association between accuracy on a sustained attention task and change in cortisol from before to after an acute stressor (Banks, Tartar, & Welhaf, 2014). Studies have also shown that those with higher levels of inflammation display deficits in selective and sustained attention, and that DHEA levels are positively associated with performance on sustained attention tasks (Marsland et al., 2006; Ritsner & Strous, 2010; Rubin et al., 2018; Ye et al., 2018).

Working Memory

A third domain in which cognition is affected by stress is working memory or short-term memory, which includes processes involved in maintaining information while mental operations are being performed (LeBlanc, 2009). Many studies have explored the association between stress and working memory and have produced conflicting results; while some have demonstrated impairment after stress, others have reported that working memory is either unaffected or enhanced by acute stress (Arnsten, 2009; Duncko, Johnson, Merikangas, & Grillon, 2009; Giles, Mahoney, Brunyé, Taylor, & Kanarek, 2014; Luethi, Meier, & Sandi, 2008; Schoofs, Pabst, Brand, & Wolf, 2013; Schoofs, Preuss, & Wolf, 2008; Schoofs, Wolf, & Smeets, 2009; Shansky & Lipps, 2013; Yuen et al., 2009). A recent meta-analysis, however, found that there was a significant, negative effect of stress on working memory (Shields, Sazma, & Yonelinas, 2016). The effects of stress may be long-lasting, with sustained deficits beyond 30 minutes post-stress, and may be sex-specific, with women performing better after stress and men performing worse (Olver et al., 2015; Zandara et al., 2016).

With respect to the SAM axis, higher levels of EPI, greater heart rate variability, and increased electrodermal variability are each associated with improved performance on working memory tasks (Cosand et al., 2008; El-Hage et al., 2013; Hansen et al., 2003, 2009; Laborde, Furley, & Schempp, 2015; Mackenzie et al., 2016; Mosley, Laborde, & Kavanagh, 2018; Smithson & Nicoladis, 2016). Blood pressure is also associated with working memory in an inverted U-shape, such that performance is lowest in both hypo- and hypertensive individuals (Cansino et al., 2021; Duschek, Matthias, & Schandry,

2005). Furthermore, evidence from previous research studies indicates that NE is also vital to the performance of working memory processes under stress because of its role in the functioning of the medial prefrontal cortex and basolateral amygdala (Roozendaal & McGaugh, 2011). While decreases in the levels of NE result in impaired working memory, too much NE can also impair working memory - a potential effect of acute stress that causes increased release of NE in the medial prefrontal cortex (Arnsten, 2009; Roozendaal & McGaugh, 2011). This inverted U-shaped relationship is likely due to NE engagement of different pre-synaptic and post-synaptic receptors during stressful versus non-stressful conditions. As summarized by Arnsten (2009), during non-stressful conditions, NE engages α_{2A}-autoreceptors in the LC to modulate release of NE in the PFC where moderate levels of NE engage high affinity post-synaptic α_{2A} receptors on PFC neurons to enhance working memory; during stressful conditions, high levels of NE released by the LC engage low affinity post-synaptic α_1- and β-receptors on PFC neurons, which impair working memory (Arnsten, 2009). This shifts allows flexible attention during the stressful situation (Berridge & Spencer, 2016). Consistent with the theory put forth by Unsworth and Robison stating that LC neuronal firing and release of NE must neither be too high nor too low in order to promote optimal attentional function, it could be said that when LC neuron firing rates are either too low or too high, optimal levels of NE are not released in the PFC to modulate working memory effectively (Unsworth & Robison, 2017). With regard to the HPA axis, studies on the effects of stress-induced changes in cortisol on working memory show that in healthy individuals, lower cortisol levels are associated with improvements in working memory (Newcomer et al., 1999;

Oei, Everaerd, Elzinga, van Well, & Bermond, 2006; Vedhara et al., 2000). Increases in cortisol after stress, particularly long-lasting increases, are associated with impaired performance on working memory tasks in groups of active-duty military members (Shia et al., 2015; Taverniers, Van Ruysseveldt, Smeets, & von Grumbkow, 2010). Despite these findings, a meta-analysis of the effects of stress-induced cortisol increases on working memory found that the increased cortisol did not moderate the association between stress and working memory, indicating that other factors are more predictive of stress effects (Shields, Sazma, et al., 2016). This is further supported by studies that suggest that higher levels of inflammatory markers, specifically IL-6 and tumor necrosis factor-α (TNF-α), associated with poor working memory performance in clinical populations (Bulzacka et al., 2016; Frydecka et al., 2015; Hildreth et al., 2013; Marsland et al., 2006; Rubin et al., 2018; Shucard, Gaines, Ambrus, & Shucard, 2007).

Moreover, a large number of studies suggest that DHEA may be intrinsically associated with working memory function. One theory in particular suggests that this may be because DHEA affects cortical and hippocampal plasticity during childhood and adolescence and that, because cortico-hippocampal connections are among the pathways vital to working memory, maintenance of those pathways by DHEA is invaluable to working memory performance (Nguyen et al., 2017). Additionally, the amplitude of the P3 wave decreases as load increases during both encoding and retrieval of memory (Morgan, Klein, Boehm, Shapiro, & Linden, 2008). In healthy samples, DHEA levels are correlated with the amplitude of the P3 wave, while decreasing or having no effect on its latency (Braverman et al., 2009; Wolf, Naumann, Hellhammer, & Kirschbaum, 1998).

Despite the effects that DHEA appears to have on the P3 wave, the effect of DHEA on working memory has been inconsistent, with several studies reporting that it has no effect and others showing that higher levels of DHEA are associated with enhanced performance on working memory tasks, particularly as load increases (Braverman et al., 2009; Davis et al., 2008; do Vale et al., 2014; Hildreth et al., 2013; Hirshman et al., 2004; Wolf, Kudielka, Hellhammer, Hellhammer, & Kirschbaum, 1998).

Based on each of these theories and research studies, it can be concluded that decision-making, attention, and working memory are each affected by stress and allostatic overload. Stress neurobiology, particularly activity of the HPA and SAM axes, may act as a mediator of this association. An individual's somatic or physiological arousal, in combination with his or her perceived anxiety, has a direct effect on his or her cognitive performance whether he or she is considered an over- or under-responder to stress. As a result, methods of reducing selected physiological markers of stress may be used in order to preserve performance under stress.

Neuromodulation

Among the many medicinal and therapeutic interventions for stress is neuromodulation, which is a broad term used to describe a host of techniques that influence neural activity. As early as 2,500 BC, Egyptian society applied neuromodulatory techniques to relieve pain (Johnson, 2007). Early in the 20th century, electroconvulsive therapy (ECT), or "shock therapy," in which electricity is used to

induce seizure activity, became popular for the treatment of severe or treatment-resistant depression, suicidality, psychosis, catatonia, and mania (Salik & Marwaha, 2022; Suleman, 2020). More recently, demand for non-invasive methods of neuromodulation has produced techniques such as transcranial magnetic stimulation (TMS), transcranial direct and alternating current stimulations (tDCS and tACS) (Thair, Holloway, Newport, & Smith, 2017), and transcutaneous electrical stimulation (TES). tDCS and tACS may excite or inhibit entire networks of neurons depending on a number of factors such as electrode size and electrical intensity (Davis & Smith, 2019; Ho et al., 2016). A large body of literature has noted that tDCS may enhance cognition in healthy individuals when applied over the dorsolateral PFC, particularly in cognitive domains such as learning (Buch et al., 2017; Choe, Coffman, Bergstedt, Ziegler, & Phillips, 2016; Shin, Foerster, & Nitsche, 2015), executive function (Sellaro, Nitsche, & Colzato, 2016; Shin et al., 2015), attention (Cheng et al., 2021; McIntire, McKinley, Nelson, & Goodyear, 2017), and memory (Matzen, Trumbo, Leach, & Leshikar, 2015). Notably, the beneficial effects of tDCS and tACS on cognition may be due not only to changes in neural activity but also changes in synaptic strength and neurotransmitter activity (Antonenko et al., 2017; Brunoni et al., 2012; Fonteneau et al., 2018). Additionally, research suggests that some of the effects of tDCS and tACS may actually be due to transcutaneous stimulation of peripheral nerves (Asamoah, Khatoun, & McLaughlin, 2019).

Transcutaneous Electrical Stimulation (TES)

When considering the applicability of neuromodulation to SMs, researchers and clinicians must consider the invasiveness, requirements, and portability of a device so that SMs may use the device in limited resource settings. The least invasive neuromodulatory method developed to date is TES, which originated as a pain relief technique for muscle injury and, more recently, has been explored for its potential to improve cognition and decrease depression and anxiety (Johnson, 2007). In TES, mild electrical pulses are applied to peripheral nerves through the skin via electrodes placed on the head, neck and/or face. In contrast to the top-down effects of other cranial stimulation techniques that alter primary cortical activity, it is hypothesized that TES modulates brain activity through bottom-up stimulation of cranial nerves, which then connect to the cortex to influence brain activity and support brain plasticity (Shiozawa, et al., 2014).

Currently, stimulation of the vagus (tVNS) and trigeminal nerves have received the most attention in the literature. The vagus and trigeminal nerves both have extensive connections to various areas of the brain, via the locus coeruleus (LC) and dorsal raphe nucleus (DRN). The LC and DRN are largely responsible for brain production of the neurotransmitters NE and 5-HT, which have widespread impact on other brain regions, such as the PFC, thalamus, amygdala, and hippocampus. These areas of the brain modulate attention, arousal, decision-making, vigilance, and memory. Therefore, stimulating the trigeminal and vagus nerves may impact many areas of the brain related to cognition and mood. While the mechanisms underlying the effects of transcutaneous vagal nerve stimulation (tVNS) and trigeminal nerve stimulation (tTNS) are still being

investigated, a growing body of literature demonstrates that both tVNS and tTNS result in therapeutic benefits without adverse effects, and applications towards cognitive performance optimization may also prove fruitful (Mercante, et al., 2017).

Mechanisms of TES

TES is applied using a device that contains two electrodes, an "anode" and a "cathode." Electrical current passes through the anode or cathode into the skin over the stimulation target(s) (e.g., cranial nerves). During anodal stimulation, positive electric current is discharged through the anode to the target, then flows back from the target region through the negative cathode; during cathodal stimulation, negative current is discharged from the cathode to the target, and then flows back from the target through the positive anode (i.e., the direction of current runs from negative to positive). The effect of anodal or cathodal stimulation is determined by the concentration of positively- and negatively-charged ions on either side of the neuron's plasma membrane. The neurological impact of the use of anodal or cathodal current distribution also depends on the orientation of the neurons being stimulated. Neurons in the cerebral cortex tend to be oriented with dendrites closest to the skull and skin; as a result, anodal stimulation causes electrical current to flow from the neuron's dendrites to the soma and down the axon, thus depolarizing the neuron. Meanwhile, in cells in which neurons are oriented with their soma nearest to the skull and skin, cathodal stimulation produces the same effect (Reinhart, Cosman, Fukuda, & Woodman, 2016). In a cortical neuron, anodal stimulation

results in an accumulation of anions on the outer surface of the cell, thereby causing the membrane of the cell to become more positive than the surrounding cytosol as K+ ions rush out of the cell, resulting in a depolarization of the cellular membrane (Barnett & Larkman, 2007). In contrast, cathodal stimulation produces cations that push the cell to become more negative than the cytoplasm, resulting in hyperpolarization (Barnett & Larkman, 2007). Meanwhile, in a cerebral cortex neuron, anodal stimulation depolarizes the neuron while cathodal stimulation hyperpolarizes the neuron (Barnett & Larkman, 2007; Bikson et al., 2019). Therefore, for TES of the head, the anode should be placed over the target region of the skin while the cathode should be placed distal to the target. Although the impact of only one "primary" electrode (anode or cathode) is necessary to depolarize or hyperpolarize a neuron, both electrodes are critical for the function of electrical stimulation as electrical current must exit through the electrode opposite the "primary" electrode to minimize safety risks (e.g., skin burning) (Xu, Zhang, Yan, Wang, & Guo, 2021).

TES and Cranial Nerves

The effects of TES on performance depend not only on neuronal activity but also on the functions of the nerves being activated. Although its effects on cranial nerves remain largely hypothetical at the time of this writing, the impact of TES may be demonstrated using functional neuroimaging and by tracking neurotransmitter activity.

Cranial Nerve V (Trigeminal)

Trigeminal nerve stimulation is a form of TES that applies electrical stimulation to the fifth cranial nerve, also called the trigeminal nerve. The trigeminal nerve is the largest cranial nerve and has both sensory (afferent) and motor (efferent) functions. It contains three distinct branches called the ophthalmic (V1), maxillary (V2), and mandibular (V3) branches (Bathla & Hegde, 2013; Jacobson, Marcus, & Sabin, 2014). Each branch is made up of fibers from between five and ten smaller, extracranial nerves that innervate the various regions of the face, head, and neck (Bathla & Hegde, 2013; Marcus et al., 2014). V1 and V2 are sensory nerves that innervate the skin of the superior two-thirds of the face as well as the eyeballs, upper teeth, and oral and nasal mucosa. V3 is a mixed, sensorimotor nerve that innervates the muscles of mastication, the skin of the chin and jaw, the lower teeth, the oral mucosa, and part of the ear (Marcus et al., 2014). Sensory information is transmitted to the central nervous system by four spinal nuclei in the brainstem, namely the spinal trigeminal, trigeminal motor, and chief sensory nuclei in the pons and the mesencephalic nucleus in the midbrain (Marcus et al., 2014). Nerve fibers from V1 and V2 transmit information to each nucleus via nerve fibers that enter the pons and combine with fibers from V3 in a cavity in the middle cranial fossa called Meckel's cave (Bathla & Hegde, 2013; Marcus et al., 2014). Information regarding touch and proprioception is transmitted to the chief sensory and mesencephalic nuclei and up to the ventral posteromedial nucleus of the thalamus via the medial lemniscus and adjacent nerve fibers in a pathway called the trigeminothalamic tract or the trigeminal lemniscus (Marcus et al., 2014). Additionally, pain and temperature information is transmitted to the

spinal trigeminal nucleus and descends to the cervical spinal cord via the descending tract of the trigeminal nerve (Marcus et al., 2014). Meanwhile, the sole purpose of the trigeminal motor nucleus is to innervate the muscles of mastication and it therefore only projects forward to those muscles (Marcus et al., 2014).

Trigeminal TES is typically applied to the supraorbital or ophthalmic branches of trigeminal nerve, which project to areas around the eye, cheek and mouth (**Figure 3**). Some studies apply stimulation to the ophthalmic branch, rather than the mandibular branch, in order to restrict stimulation to the sensory, rather than motor, functions of the trigeminal nerve (Wilson-Pauwels, Akesson, & Steward, 1988). Stimulation is applied using electrodes attached to the right side of the face above the eyes and/or along the jawline for approximately 20 minutes. Although there is considerable cross-study variability, protocols typically recommend rectangular, biphasic pulses, with pulse widths of 250 μS, frequencies of 60-350 Hz, and maximum intensities of 3.5-16 mA for up to 30 minutes (Riederer, Penning, & Schoenen, 2015).

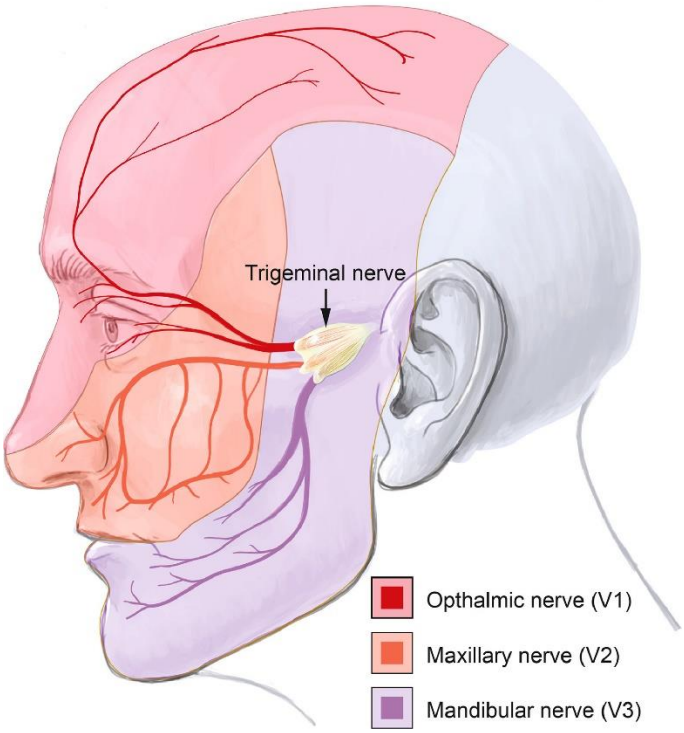


Figure 3. Branches of the trigeminal nerve.
Obtained from Ferneini (2021).

When electrical stimulation is applied to the sensory branches (V1, V2) of the trigeminal nerve, it alters neuronal activity in key brainstem nuclei (**Figure 4**) (Mercante et al., 2017; Vecchio, Gentile, Franco, Ricci, & de Tommaso, 2018), principally the mesencephalic, chief sensory, and spinal trigeminal nuclei, which collectively make up the TSNC (Bradnam & Barry, 2013). The TSNC primarily projects to the thalamus and the cervical spinal cord but has projections to other brainstem nuclei and cortical and subcortical regions as well – in particular, the NTS (Contreras, Beckstead, & Norgren, 1982), an intermediary nucleus that connects to the LC and PPN. While the PPN projects to the thalamus and is involved in arousal (Martinez-Gonzalez, Bolam, & Mena-Segovia, 2011), the LC has many relevant projections to the brainstem (substantia nigra [SN],

ventral tegmental area [VTA], and DRN), basal forebrain (NAc), cortex (including the PFC), and subcortex (thalamus, hippocampus, amygdala, and hypothalamus; **Figure 5**) (Bari, Chokshi, & Schmidt, 2020; Feinstein, Kalinin, & Braun, 2016; Ferrucci, Giorgi, Bartalucci, Busceti, & Fornai, 2013; Hagena, Hansen, & Manahan-Vaughan, 2016; Herman, Cruz, Sahibzada, Verbalis, & Gillis, 2009; Lin & Vartanian, 2018; Nestler, Hyman, Holtzman, & Malenka, 2015). Changes in neuronal activity in these secondary nuclei lead to greater release of the neurotransmitters 5-HT, DA, and NE, which are also involved in arousal as well as attention, motivation, and mood. In TES, stimulation is targeted at the V1 and V2 branches of the trigeminal nerve due to their purely sensory functions, whereas stimulation of the V3 branch, which has additional motor functions, is avoided to prevent significant side effects after stimulation.

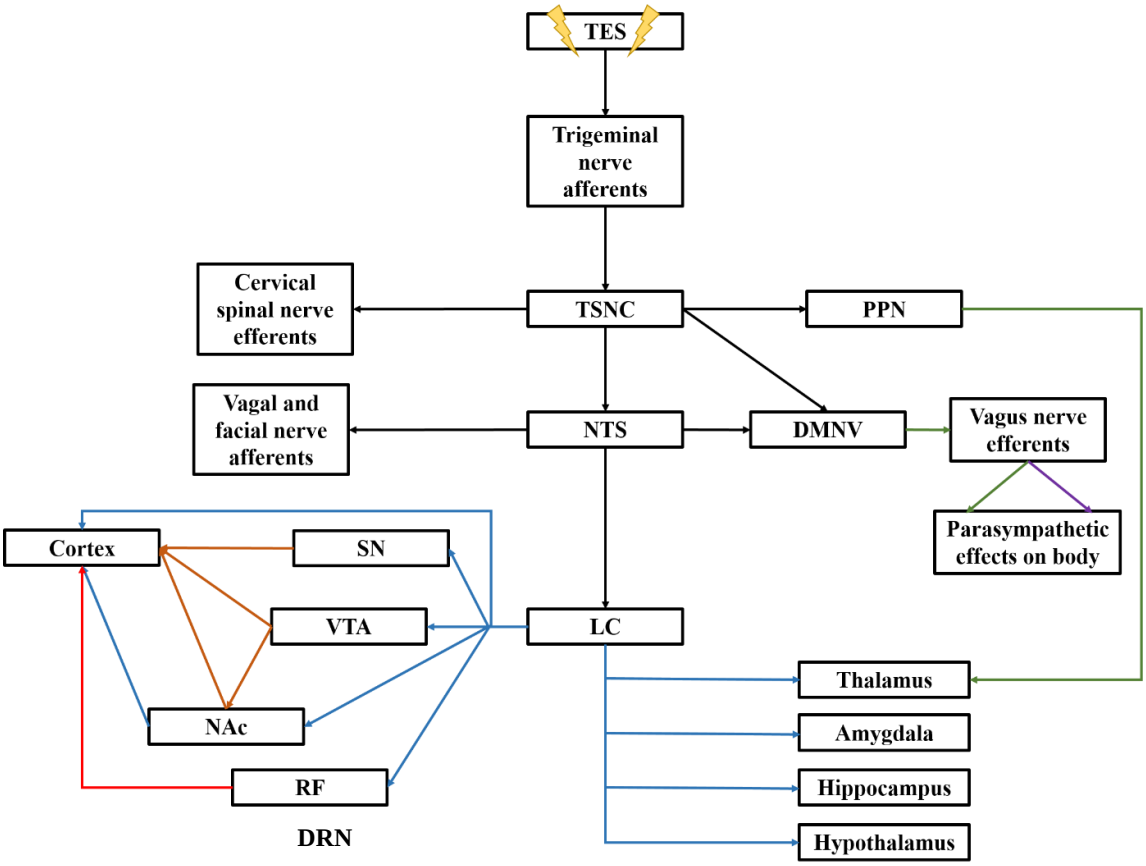


Figure 4. Hypothesized mechanism for effects of TES.
Arrows: red, serotonin (5-HT); orange, dopamine (DA); green, acetylcholine (ACh); blue, norepinephrine (NE); purple, γ-aminobutyric acid (GABA). *Abbreviations:* DMNV, dorsal motor nucleus of the vagus; LC, locus coeruleus; NAc, nucleus accumbens; NTS, nucleus of the tractus solitarius; PPN, pedunculopontine nucleus; DRN, dorsal raphe nucleus; SN, substantia nigra; TES, transcutaneous electrical stimulation; TSNC, trigeminal sensory nuclear complex; VTA, ventral tegmental area.

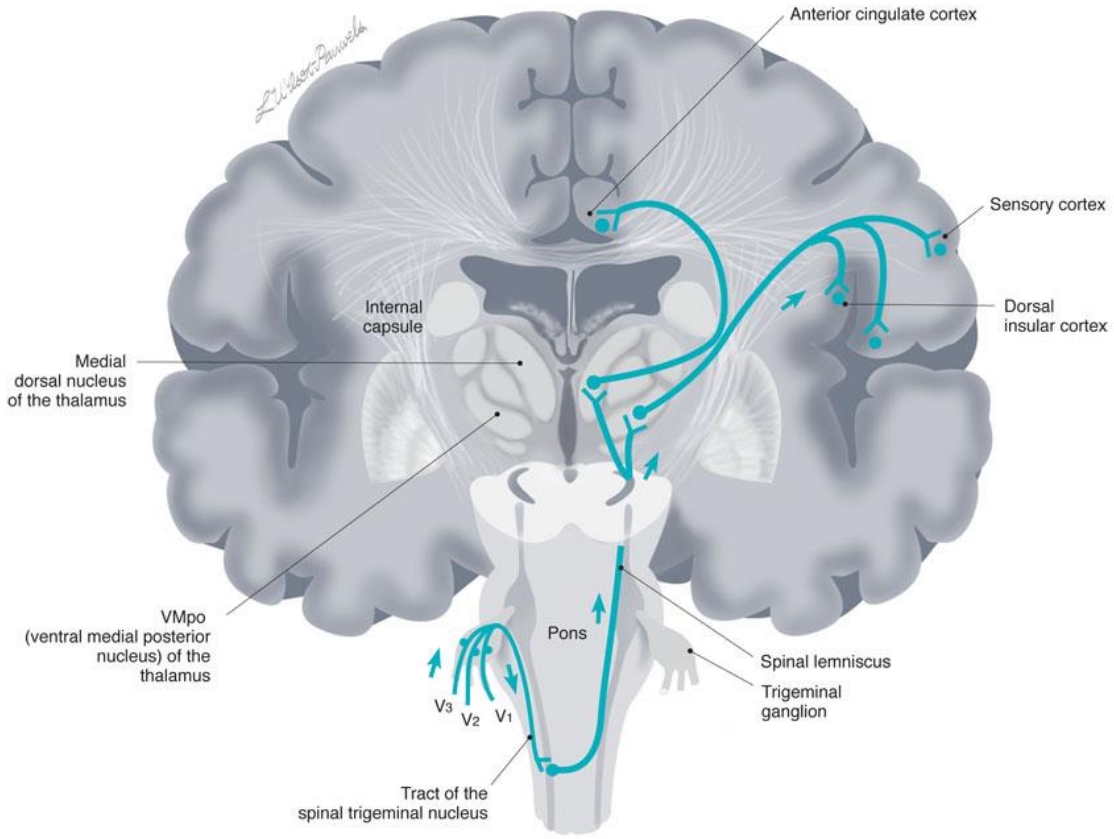


Figure 5. Trigeminal nerve pathway.
Adapted from Wilson-Pauwels (2013).

While TES targets neurons along the trigeminal nerve, it also engages neurons that are a part of other cranial nerve pathways. TES stimulation of the NTS activates projections to the face via the facial nerve (Marcus et al., 2014). The facial nerve is made up of two nerves, a large motor nerve and a small intermediate nerve, which project from the brainstem and specifically from the NTS, the facial motor nucleus, and the salivary nucleus, to the muscles of the face to control facial movement (Marcus et al., 2014). The facial nerve also carries sensory taste information to the thalamus and gustatory impulses

to the taste buds; in addition, it provides parasympathetic input to the salivatory system (Marcus et al., 2014). The NTS also projects forward to the dorsal motor nucleus of the vagus nerve (DMNV) and the NAc (Rogers, Kita, Butcher, & Novin, 1980), which send projections down the spinal cord to have parasympathetic and anti-inflammatory effects on the visceral organs, including the heart, lungs, stomach, intestines, trachea, and esophagus, likely mediated by γ-aminobutyric acid (GABA) and acetylcholine (ACh) (Butt, Albusoda, Farmer, & Aziz, 2019; Capone et al., 2015; Keute, Ruhnau, Heinze, & Zaehle, 2018; Marcus et al., 2014; Van Leusden, Sellaro, & Colzato, 2015). In addition, the vagus nerve projects afferently to the brainstem, cortex, and subcortex (Breit, Kupferberg, Rogler, & Hasler, 2018). The trigeminal and afferent vagal pathways overlap in a number of key regions, including the NTS, but also the LC, amygdala, thalamus, and hypothalamus (Breit et al., 2018). The hypothalamus in turn releases corticotropin-releasing hormone, which triggers a release of adrenocorticotropic hormone from the pituitary gland and finally, cortisol from the adrenal glands (Hellhammer, Wüst, & Kudielka, 2009).

The Effects of TES on Stress, Cognition, and Performance

Stress

Only one study appears to have explored the effects of trigeminal TES on either anxiety or depression, finding no anxiolytic or anti-depressant effect in individuals with epilepsy (Gil-López et al., 2020). Studies of the effects of tVNS on depressive behavior are more common and are generally consistent; indeed, a meta-analysis reports that tVNS

decreases both clinician-reported and self-reported symptoms of depression more than sham, but has no significant effect on anxiety symptoms (Wu et al., 2018). Research suggests that this results from down-regulation of inflammation (Wang et al., 2021) and increases in functional connectivity in key emotion circuits, such as that between the amygdala and dorsolateral PFC (Liu et al., 2016).

One mechanism by which trigeminal TES may improve the anxious and depressive symptoms associated with stress by modulation of HPA axis function and, in particular, cortisol release. Only two studies have explored the influence of trigeminal TES on HPA axis functioning in healthy individuals, one finding that trigeminal TES increased cortisol levels upon awakening (Boasso, Mortimore, Silva, Aven, & Tyler, 2016) but the other finding no difference between the effects of sham and active stimulation on cortisol levels after stress (Tyler et al., 2015). It is, therefore, difficult to draw any strong conclusions as to the effect of trigeminal TES on HPA axis function.

Neurotransmitters hypothesized to account for the effects of TES on stress, anxiety, and depression include NE, GABA, 5-HT, and DA. Although very little work has been done regarding the influence of trigeminal TES on these neurotransmitters, the available literature supports the role of NE in the effects of trigeminal TES. In a rodent model of hemorrhagic shock, trigeminal TES activated both the SNS and PNS, increasing HRV and NE levels (Li et al., 2019). Meanwhile, the effects of tVNS on NE are more concrete and apparent in EEG studies. Application of tVNS has been associated with changes in the P3 wave, namely increases in its amplitude and decreases in its latency (Rufener et al., 2018; Ventura-Bort et al., 2018). Acute stimulation has been shown to

significantly increase P3b amplitude for more than 100 minutes, indicating that even short-term stimulation can induce lasting changes in NE activity, although this effect was not replicated (Lewine, Paulson, Bangera, & Simon, 2018; Warren et al., 2018). Furthermore, research has shown that application of tVNS increases salivary α-amylase levels, consistent with increases in NE release (Ventura-Bort et al., 2018; Warren et al., 2018).

The influence of TES on NE activity is supported by studies of HRV and PPG. Studies show that trigeminal TES is associated with physiological activation of the PNS, as measured by increasing HRV in rodents (Li et al., 2019) and improved parasympathetic preponderance in healthy humans (Monaco, Cattaneo, Ortu, Constantinescu, & Pietropaoli, 2017). Trigeminal TES also increases mean arterial pressure and other indicators of cardiac activity (Prodel, Barbosa, Nóbrega, & Vianna, 2018). tVNS is associated with robust decreases in heart rate and PPG amplitude in individuals with PTSD reading trauma scripts (Gurel, Huang, et al., 2020; Gurel, Wittbrodt, et al., 2020), suggesting that tVNS decreases sympathetic preponderance.

In contrast to the effects of TES on NE, studies have demonstrated conflicting results regarding the effects of TES on GABA that may be specific to the stimulation methodology. In a study of healthy humans, trigeminal TES had no effect on GABA or glutamate activity measured by magnetic resonance spectroscopy (Ritland et al., in press), suggesting that trigeminal TES may have a different mechanism of action than tVNS, which has demonstrated very consistent effects on GABA levels. Indeed, tVNS increases GABA circulation (Ben-Menachem et al., 1995), receptor density (Marrosu et

al., 2003), and activity (Capone et al., 2015; Beste et al., 2016; Keute et al., 2018). There is also evidence that the effects of tVNS on response inhibition are mediated by enhanced GABA activity (Beste et al., 2016). In particular, tVNS appears to influence activity of $GABA_A$ receptors (Capone et al., 2015), which is associated with activity of both DA and 5-HT (Goetz, Arslan, Wisden, & Wulff, 2007). Additionally, the antidepressant and anxiolytic effects of DHEA require activation of $GABA_A$ receptors, which in turn modulate 5-HT activity (Gartside, Griffith, Kaura, & Ingram, 2010).

No research, to date, has directly assessed the activity of ACh, 5-HT, or DA following trigeminal TES. The tVNS literature, however, suggests that tVNS may influence ACh activity and ultimately, efferent vagal pathways (Li et al., 2020; Rawat et al., 2019; Wang et al., 2021). tVNS is also associated with increases in 5-HT levels in humans and rodents when administered regularly for four weeks (Li et al., 2018; Shi et al., 2021). Although research has not directly explored the association between tVNS and DA activity, studies demonstrated that tVNS increases reward learning and motivation in healthy (Neuser et al., 2020) and epileptic participants (Weber et al., 2021) and enhances enjoyment of healthy food choices in non-obese individuals (Öztürk, Büning, Frangos, de Lartigue, & Veldhuizen, 2020). Neuroimaging studies show that four weeks of tVNS increases the functional connectivity between the NAc and a number of brain regions, including the PFC and anterior cingulate, suggesting that the effects of tVNS on reward functioning may result from enhanced dopaminergic function (Wang et al., 2018).

Neuroimaging studies also have described the impact of TES on the brain using electroencephalography, single photon emission computed tomography, and functional

magnetic resonance imaging. Studies of trigeminal TES have demonstrated increases in absolute alpha and delta power globally and in frontal and temporal regions in individuals with epilepsy (Ginatempo et al., 2019) and depression (Shiozawa et al., 2016). Furthermore, regular use of trigeminal TES for four weeks is associated with increases in frontal and frontal midline spectral power in association with improvement in attention-deficity/hyperactivity disorder (ADHD) symptomatology in children (Loo et al., 2021; McGough et al., 2019). Furthermore, a study of the effects of trigeminal TES on brain function in epilepsy using single photon emission computed tomography found that trigeminal TES increases global perfusion in the limbic and temporal lobes (Mercante et al., 2021). No effect of trigeminal TES, however, was found when exploring its influence on blood-oxygen-level-dependent activity using functional magnetic resonance imaging (Rubia, Westwood, Aggensteiner, & Brandeis, 2021).

Cognition and Performance

The bulk of research completed on the influence of trigeminal TES on cognition has focused on attention and executive function in ADHD and epilepsy. In children with ADHD, trigeminal TES decreases the frequency and severity of ADHD symptoms (Loo et al., 2021; McGough et al., 2015; McGough et al., 2019) and improves performance on working memory (Loo et al., 2021) and attention network tasks (McGough et al., 2015). This is an interesting contrast to studies of the effects of tVNS on attention, which have shown no benefit in healthy adults (Rufener et al., 2018; Ventura-Bort et al., 2018; Jacobs, Riphagen, Razat, Wiese, & Sack, 2015; Ridgewell et al., 2021). Similarly, tVNS has no effect in healthy or epileptic adults on working memory or immediate and delayed

word recall and recognition (Jacobs et al., 2015; Mertens et al., 2020; Ridgewell et al., 2021; Gil-López et al., 2020). However, both trigeminal TES and tVNS appear to improve executive function, most notably response inhibition (McGough et al., 2015; Ridgewell et al., 2021; Steenbergen et al., 2015; Jacobs et al., 2015; Keute et al., 2018; Jongkees et al., 2018; Kühnel et al., 2020; Beste et al., 2016), in healthy adults and children with ADHD but not in adults with epilepsy (Gil-López et al., 2020). The effect in healthy individuals is particularly pronounced when task demands (such as task difficulty or cognitive load) are high (Beste et al., 2016; Jongkees et al., 2018; Fischer, Ventura-Bort, Hamm, & Weymar, 2018) or positive mood is low (Steenbergen, Colzato, & Maraver, 2020), suggesting that some baseline decrement in performance may be required for the positive effects of TES to be seen. This is supported by the fact that studies with healthy individuals have not consistently reported effects of TES on attention, while studies in individuals with ADHD do so more consistently. To date, no studies have been published on the association between TES and marksmanship performance.

The bulk of this evidence indicates that TES may improve autonomic function and increases parasympathetic activity, thereby reducing the potential negative physiological and cognitive effects of acute stress.

Conclusion

SMs encounter significant and often overwhelming stress during training and field operations. Enhancement of the SM's resilience to stress is an important goal of the Army as it benefits the SM's cognitive and physiological performance in the face of an

ever-changing and increasingly lethal operating environment. Optimal performance by the SM is necessary in order to meet and exceed the challenges to mission success under such stressful conditions. One technique by which SMs may reduce their stress and enhance performance is TES. The literature indicates that TES may provide a safe, non-invasive means of supporting and improving performance in anticipation of and during stressful events and is both safe and scalable, with fewer side effects and better specificity compared to other interventions. However, results from studies examining the effects of TES on cognitive performance and the biological consequences of TES are inconsistent and remain unclear at this time.

CHAPTER TWO: METHODS

Specific Aims and Hypotheses

This project included three specific aims and one post-hoc aim.

Aim 1: Determine the effects of TES on markers of stress (salivary cortisol and α-amylase, heart rate variability, and self-reported indices of physical and cognitive workload) before, during, and after a challenging marksmanship task.

> **Hypothesis 1:** Active TES compared to sham stimulation will reduce stress, as indicated by decreases in salivary cortisol and α-amylase, heart rate and pulse, and self-reports of physical and cognitive workload during the marksmanship challenges.

Aim 2: Determine the effects of TES stimulation on marksmanship performance, as measured using speed of target engagement and accuracy.

> **Hypothesis 2:** Active TES compared to sham stimulation will improve marksmanship performance (as indicated by faster target engagement and greater accuracy during the friend-foe discrimination challenge).

Aim 3: Determine the effects of TES relative to sham stimulation on sustained attention (as measured by the gradCPT, a continuous performance task), working memory (as measured by an N-back task) and response inhibition (as measured by a Go/No-Go task) before, during, and after a challenging marksmanship task.

Hypothesis 3: Active TES in comparison to sham stimulation will improve both speed and accuracy on the gradCPT, N-back, and Go/No-Go tasks and attenuate decline in performance expected after a long testing period.

Exploratory Aim: Although we are aware that power is not sufficient, we aim to *explore* the impact that personality characteristics and coping skills might have on the effects of TES.

Participants

Healthy male and female civilians and members of the military between the ages of 18 and 55 who were stationed at the Natick Soldier Systems Center (NSSC) or the United States Army Research Institute of Environmental Medicine (USARIEM) or assigned to the NSSC as healthy military research volunteers were invited to participate. Potential participants were excluded for self-report of any recent illicit drug use (including marijuana) or significant alcohol (defined as greater than 7 drinks a week for women and 14 drinks a week for men) or caffeine consumption (defined as greater than four 8 ounce cups of coffee daily), a history of any brain injury or concussion within the last 18 months, a current diagnosis of anxiety disorder, any condition or injury that limits the range of motion of hand or fingers or the ability to comfortably hold an assault rifle (carbine, military model 4 [M4]), uncorrectable vision or hearing deficits, or (females only) pregnancy or breastfeeding (see **Appendix A** for a detailed list of exclusionary criteria). These exclusionary criteria were selected because of their known or suspected impact on the physiological stress response, ability to perform marksmanship tasks,

influence on biomarkers of interest, or wellbeing of the participant. All exclusionary criteria were self-reported; if participants self-identified as having a history of any exclusionary condition, they were immediately disqualified from the study.

Recruitment

Military personnel stationed at the NSSC or USARIEM were recruited using study announcements printed and posted across the installation or distributed electronically via IRB-approved email lists and social media platforms. The study was also advertised by word of mouth (e.g., via a prior study volunteer). Participants were identified via two recruitment mechanisms, first targeting community volunteers and second, targeting individuals participating in the Human Research Volunteer program at NSSC.

Potential community research volunteers (RVs) were invited to contact the principal investigator or study coordinator directly via telephone or email. Upon initial contact, the investigator provided the potential participant with a copy of the consent form and briefly outlined the study procedures. Those who self-identified as meeting study inclusion criteria completed the consent process. If apparently eligible, they were assigned a unique research identification number without personal identifiers. Participants then completed a health history questionnaire, which was reviewed by the investigator with the participant to verify eligibility. Female participants were asked by the investigator if they could be pregnant, and each female participant took a urine

pregnancy test prior to any testing activities. If a female participant identified as pregnant or the pregnancy test was positive, she was withdrawn from the study.

Briefing and consent for members of the Human Research Volunteer program was modified slightly to fulfill programmatic requirements, including those focused on avoiding coercion. RVs were briefed on study procedures in groups and received a copy of the study consent form to read independently. Interested volunteers returned to a second, private session to be consented and were subsequently assigned a research identification number. A designated Ombudsperson, who was not a part of the RV's chain of command, was present during briefing and consenting to ensure that participants were not coerced. Study eligibility was verified by the investigator as described above.

Experimental Design and Randomization

This study was a randomized, cross-sectional, single-blind experiment using a cross-over design, such that each participant received one day of active TES prior to testing and one day of sham TES prior to testing, with at least one and no more than seven days between testing sessions. This allowed each participant to act as his or her own control, thus requiring fewer participants to achieve adequate statistical power and minimizing the effects of confounders or covariates on the analyses. Prior to enrollment, randomization to receive active vs. sham TES on the first testing day was achieved using a random number generator. Counterbalancing the groups controlled for order effects.

Study procedures were carried out across five days. On Day 1, participants were screened for eligibility. After eligibility was confirmed, participants were enrolled in the

study and began familiarization procedures, during which they were exposed to the tasks involved in the study. Familiarization was repeated on Days 2 and 3, before participants underwent testing procedures on Days 4 and 5.

Transcutaneous Electrical Stimulation

The device used in this study (Thync One, Cerevast Therapeutics; Redmond, WA) has been used successfully to modify heart rate variability, salivary α-amylase, and the cortisol awakening response (Boasso et al., 2016; Tyler et al., 2015). For both active and sham stimulation, an electrode was attached to the right side of the participant's face over the ophthalmic branch of the trigeminal nerve, between the brow line and the hairline (**Figure 6**). Although the placement of this device at the ophthalmic branch of the trigeminal nerve is hypothesized to affect the trigeminal nerve, it may also influence other cranial nerves or the function of other neurons (Tyler et al., 2015); at this point, the exact effects of the Thync One device and of trigeminal TES in general are unknown (Thomas et al., 2021). The TES device was attached to the electrode and a second, connected electrode was placed horizontally across the back of the neck below the hairline. An Apple operating system (iOS) application controlled the stimulation via Bluetooth and administered 20 minutes of active or sham stimulation before self-terminating.

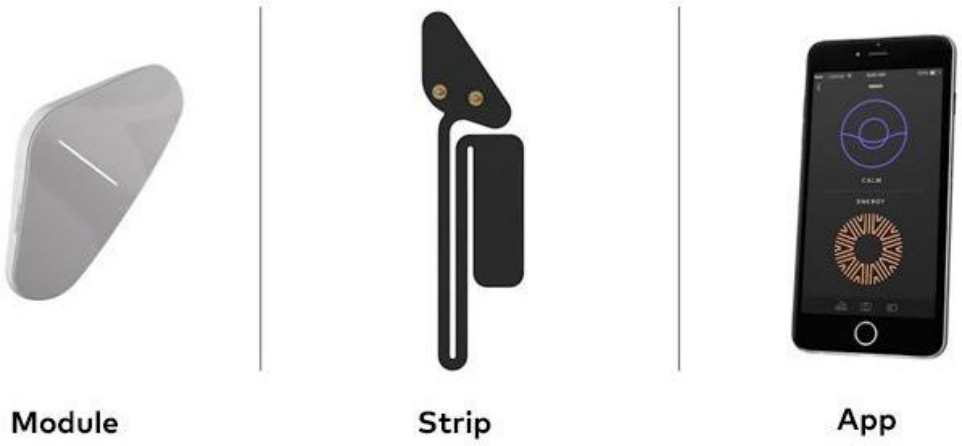


Figure 6. Components of the Thync One device.

Although specific details of the electrical waveforms delivered during stimulation are proprietary knowledge, the device documentation indicates that this device delivers a maximum peak current density of 6 mA/cm^2, an average current density of 2 mA/cm^2, a maximum direct current voltage of 53 volts, a maximum charge of 1.8 C/cm^2, and a maximum frequency of 20 kHz, with most waveforms ranging from a frequency of 0.5 to 0.75 kHz. These limits are each below the generally accepted limits established to maintain participant safety (Nitsche et al., 2008; Prausnitz, 1996; Rossi, Hallett, Rossini, Pascual-Leone, & Safety of TMS Consensus Group, 2009). These parameters also fall within the ranges of those used in other protocols using similar forms of stimulation and targeting the trigeminal nerve.

The Thync One app allows the user to adjust stimulation intensity levels within the range specified above to suit individual tolerance and comfort levels. For each participant, the maximum stimulation intensity was identified prior to experimental

sessions and then held constant across experimental sessions. To determine the maximum stimulation intensity level for each participant, the investigator fitted the device to the participant's head and neck on the first day of training and increased the stimulation intensity until the participant first indicated that he or she felt mild discomfort. The investigator then reduced the intensity by one unit and tested the participant's tolerance of stimulation at that intensity. If the participant indicated that this adjusted stimulation intensity was tolerable, this level of stimulation was recorded and used for all testing days.

For both active and sham stimulation conditions, stimulation ramped up during the first 30 seconds of the stimulation program to the participant's pre-determined maximum intensity level and ramped down in the final 30 seconds of the stimulation program. For the period of 19 minutes between the ramp up and ramp down periods, stimulation was either delivered according to a proprietary program (active stimulation) or was not delivered (sham). This method of administering sham stimulation (Paneri et al., 2016) was intended to blind the participant to the stimulation being applied and is similar to sham procedures reported in the transcranial stimulation literature (Reinhart et al., 2016). Similar sham procedures are used in other approved USARIEM TES protocols at USARIEM.

Measures

Marksmanship

All participants completed a 5-minute practice marksmanship task on each of the three training days and a 30-minute marksmanship task twice on each testing day (**Table 2**). Marksmanship tasks were conducted using the Engagement Skills Trainer (EST) 2000 (manufacturer), an electronic, laser-based system with a digital projector, sound effects, and weapon recoil that mimics real-life marksmanship using a modified M4 weapon. The EST is housed inside a sound-proofed, windowless building with black paneling in order to minimize the amount of light and sound entering or leaving the building. In addition, weapons are modified so they can no longer fire live ammunition but instead, the timing of trigger pulls relative to target appearance is recorded on an associated computer program. A laser system attached to the weapon identifies where on the projector screen the weapon has fired (i.e., on- or off-target) using a coordinate-based system. The weapon also has a small button attached to its side within reach of the trigger, which participants may press during the marksmanship task, and which is recorded by the EST computer system.

Participants fired from a simulated standing foxhole supported firing position (**Figure 7**), in which they stood next to a wooden box podium approximately five feet tall and fired with their weapons resting lightly on sandbags. Those of short stature were offered raisers to stand on in order to see above the foxhole. All participants wore

earplugs and noise-cancelling headphones, which muffled the noise generated by the EST (approximately 124.1 decibels).

Figure 7. Example of a standing foxhole supported firing position.
Image retrieved from Fitchett (2022).

Participants zeroed the sights of their weapon and practiced the marksmanship task in the training days prior to testing. “Zeroing” is the process of aligning the laser system of the EST with the participant’s perception of where he or she is firing. Because each participant’s viewpoint is different, the system must adjust to that viewpoint; it does so by first asking participants to “group” their shots and then “zero” their weapon. The process of “grouping” requires the participant to fire five out of six shots at the center of mass of a target and to place those shots in a circle with a diameter of five centimeters. Although shots are unlikely to appear in the center of mass at this point, this process identifies whether participants are able to place their shots in a tight group. Once participants have “grouped” their shots, the EST program calculates a coordinate adjustment so that shots appear at the center of mass of the target, as expected. Again,

participants must place five out of six shots within the target's center of mass in order to "zero" their weapons and be considered eligible for this study. Afterward, they are introduced to and practice a short (5 minute) version of the marksmanship task used in this study. The use of three training days to familiarize participants with the marksmanship task is intended to reduce training effects by establishing a stable baseline of performance. This EST training protocol has been successfully used in other USARIEM marksmanship protocols. Participants generally completed training and testing days within a 10-14 day period, except in situations where female participants needed to be rescheduled due to menstrual cycle phase.

The marksmanship task consisted of a friend-foe discrimination challenge (**Figure 8**) with varying levels of cognitive demand. During this task, marksmanship targets were denoted as "friend" or "foe" based on a brief (3 second) color cue (red or black) that appeared before the presentation of a new target. The color of the cue target denoted the "foe." For example, presentation of a red cue indicated that all red marksmanship targets to follow were foes and black targets were friends. Participants were instructed to shoot all foe targets (trigger pull) and press a button on the side of the weapon close to the trigger to indicate a friend. Furthermore, certain targets were designated as "high value targets" (HVTs) based on the sequence of colored targets preceding them. If two red targets followed two black targets, or two black targets followed two red targets, the final target in the sequence was considered an HVT and participants were instructed to respond with a button push prior to the action indicated by the friend-foe color criterion. Unpublished data from our laboratory indicate that this

results in slower reaction times to identify friends (approximately 37% slower) and shoot foes (approximately 16% slower) over time. Throughout the task, additional "distractor" targets appeared in blue; participants were instructed to not make any action toward them unless prompted to do so by a blue cue. Following a blue cue, participants fired at the blue targets only and made no action toward either black or red targets. This method of distraction was expected to a) achieve attentional capture due to its salient contrast to the friend and foe targets, as well as b) reduce accuracy and increase reaction times during the task (Bretherton, Eysenck, Richards, & Holmes, 2017; Laidlaw, Zhu, & Kingstone, 2016; Liesefeld, Liesefeld, Töllner, & Müller, 2017; Weichselbaum & Ansorge, 2018). Previous studies in SMs using an alternate version of this task without presentation of distractor targets demonstrated that this marksmanship task requires significant mental effort to complete (Smith et al., 2019).

Figure 8. The friend-foe discrimination challenge.

A) A cue target denotes the color of the friend or foe target (in either black or red).
B) When a target is presented, participants must decide to shoot (for foe) or press a button (for friend).

All targets were presented for up to 5 seconds with inter-target intervals between 3 and 10 seconds, and at simulated distances of between 100 and 250 meters from the shooter. From a standing foxhole supported firing position, participants fired the weapon or pressed buttons as quickly and accurately as possible, using only one shot per target. If the target was hit, it disappeared, but if a target was missed, it remained visible until the full 5 seconds had elapsed. It was assumed that identification and selection, as well as target shot placement in this task required a variety of executive processes including attention, working memory, response inhibition, choice, and set shifting in addition to perceptual, motor planning and coordination skills (Kelley et al., 2011). Participants completed four versions of the marksmanship task, with two versions completed on each

testing day. Target ratios were balanced across each version; in each marksmanship task, there were 44 friends, 60 foes, 54 distractor targets, and 10 HVTs.

Performance on the marksmanship task was quantified according to eight outcomes (**Table 1**) stratified by marksmanship session (active TES session #1, active TES session #2, sham TES session #1, sham TES session #2).

MARKSMANSHIP OUTCOME	DESCRIPTION
SHOT ACCURACY (%)	Percentage of shots fired that hit a foe target.
TARGET DETECTION ACCURACY (%)	Percentage of friend and foe targets that required a response and were responded to.
TARGET DISCRIMINATION ACCURACY (FOR ALL TARGETS PRESENTED) (%)	Percentage of correct actions taken in response to targets, including not only those that require a response (friend, foe) but also distractor targets that required a non-response.
TARGET DISCRIMINATION ACCURACY (FOR ALL TARGETS TO WHICH A RESPONSE WAS MADE) (%)	Percentage of correct actions taken in response to targets that require a response (friend, foe). This outcome does not include non-responses to distractor targets.
HIGH VALUE TARGET ACCURACY (%)	Percentage of high value targets that were correctly identified.
SHOT LATENCY (MSEC)	Amount of time after a foe target appears before the trigger is pulled.
TARGET DETECTION LATENCY (MSEC)	Amount of time after a friend target appears before the button is pressed.
DISTANCE OF SHOTS FROM THE CENTER OF MASS (M)	Proximity of shots to the center of the target.

Table 1. Definitions of marksmanship outcomes.

Questionnaires

Physical and psychological health and traits may affect stress reactivity (Mücke, Ludyga, Colledge, & Gerber, 2018; Suzuki, Poon, Papadopoulos, Kumari, & Cleare, 2014). To explore and control for any confounding effects, the following measures were

collected on the first training day to assess the participant's physical and psychological health, psychological characteristics and traits, while questionnaires on perceived workload and alertness were administered repeatedly throughout the two testing days.

Physical and Psychological Health

1. *Demographic and Health History:* All participants were asked to provide information regarding their age, sex, educational and military background, use of tobacco, and general medical/psychiatric conditions (including depression and PTSD) via the Demographic and Health History Questionnaire. This information was used to ascertain eligibility based on study inclusion criteria and inform statistical analyses. Those who met study inclusion criteria based on their responses were retained in the study and their data from the Demographic and Health History Questionnaire were used in subsequent analyses supporting study-related objectives. Those who endorsed exclusion criteria were removed from the study and their data were retained for future analysis.
2. *Menstrual Cycle Interview:* The Menstrual Cycle Interview is a series of questions commonly used to ascertain phases and sub-phases of female participants' menstrual cycle. A female staff member interviewed female participants regarding the timing of their menstrual cycle (e.g., "how often do you get your period," "on what date did your last period begin," and "are you currently using any form of contraception") in order to determine the length of their menstrual cycles. For females who were on continuous hormonal contraception (e.g., hormone-releasing IUD, oral contraceptives

without placebo, dermal/injectable contraceptives), the timing of study activities occurred as convenient to the participant and research staff. For females who were not on continuous hormonal contraception (e.g., oral contraceptives with a placebo, no contraception), study testing days were scheduled to occur during the follicular phase of the menstrual cycle (approximately days 3-11 after onset of menses) to minimize the impact of changes in hormones on salivary cortisol measurements or test performance. Female menstrual phase may also affect pain perception (de Tommaso, 2011; Ileri, Baka, Akin, Apiliogullari, & Basciftci, 2016; Piroli et al., 2019), thus scheduling participation during the follicular phase minimizes the chances that female participants will experience pain during the stimulation period.

3. *The Neurobehavioral Symptom Inventory (NSI):* The NSI (Cicerone & Kalmar, 1995) is a self-administered measure of neurological symptoms commonly associated with post-concussive syndrome. Although the scale is used as a screening measure for traumatic brain injury, scores may be elevated for other affective disorders, including PTSD, depression, and anxiety (Porter et al., 2018). Respondents were asked to rate the degree to which each of 22 symptoms (organized into four categories: somatic, cognitive, affective, and sensory) has bothered them over a two-week period of time. Symptoms were rated on a scale of 0 (rarely distressed) to 4 (severe distress), with 0 meaning that the symptom has rarely if ever been disturbing and 4 meaning that the symptom disturbance has been very severe. The NSI has demonstrated high internal consistency (total $\alpha = 0.95$; subscale $\alpha = 0.88$ to 0.92) and discriminant validity,

differentiating veterans with a history of traumatic brain injury from those without (King et al., 2012). This measure is in the public domain.

4. *The Veterans RAND 12 Item Health Survey (VR-12):* The VR-12 (Kazis et al., 2006) is a brief, generic, multi-use, self-administered health survey comprised of 12 items. The instrument is primarily used to measure health-related quality of life, estimate disease burden, and evaluate disease-specific benchmarks. The 12 items in the questionnaire correspond to eight principal physical and mental health domains including general health perceptions, physical functioning, role limitations due to physical and emotional problems, bodily pain, energy-fatigue, and social functioning and mental health. The 12 items are summarized into two scores, a "Physical Health Summary Measure (PCS - physical component score)" and a "Mental Health Summary Measure (MCS-mental component score)." Although these analyses did not include outcomes from the VR-12, the VR-12 provides an important contrast between physical and psychological health status. This measure is in the public domain.
5. *Positive and Negative Affect Schedule (PANAS):* The PANAS (Watson, Clark, & Tellegen, 1988) is a widely used measure of general mood state. Respondents were asked to rate each of 20 mood-related adjectives according to how much that adjective reflects how they felt. The scale contains 10 positive affect adjectives (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and 10 negative affect adjectives (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid). Participants were asked to rate each adjective on a scale from 1 to 5, based on the strength of emotion, where 1 = "very

slightly or not at all," and 5 = "extremely." The scales have demonstrated high internal consistency (Cronbach's α ranging from 0.86 to 0.90 for the positive dimension and from 0.84 to 0.87 for the negative dimension) (Crawford & Henry, 2004; Watson et al., 1988), and remain stable over a 2-month time period (test-retest reliability of 0.68 for positive dimension and 0.71 for negative dimension) (Watson et al., 1988). The positive and negative dimensions are largely uncorrelated (α ranging from -0.12 to -0.23) (Watson et al., 1988). This measure is in the public domain.

6. *Questionnaire of Sensations Related to TES*: The Questionnaire of Sensations Related to TES was originally developed by Antal and colleagues (2017); it is in the public domain and has been adapted for use in this and other protocols administered at USARIEM. It is a 14-item questionnaire in which the participant answered questions regarding sensations experienced following TES, the severity and duration of these sensations, and their location. The participant was also asked whether they believed they had received actual TES or not (sham), or if they were uncertain. The questionnaire was administered after each testing session (Days 4 and 5) was used to monitor both the safety of TES and the study blinding.

Psychological and Personality Traits

Personality characteristics, such as resilience, have been associated with task performance under stress (Duarte et al., 2022; Sakurai, Li, Inamura, Masuoka, & Hisatsune, 2020). Moreover, positive mood is associated with differences in the effects of tVNS on cognitive performance (Steenbergen, Colzato, & Maraver, 2020), suggesting

that changes in psychological states or traits may influence cognitive outcomes following TES. Therefore, these analyses explore and control for the impact of personality characteristics and coping skills on the effects of TES.

1. *The UPPS-P Impulsive Behavior scale (UPPS-P):* The UPPS-P (Whiteside & Lynum, 2001) is a 59-item self-report questionnaire with five subscales (urgency, premeditation, perseverance, sensation-seeking and positive urgency) to measure impulsivity across dimensions of a four-factor model of personality (Whiteside & Lynam, 2001), including premeditation (lack of), urgency, sensation seeking and perseverance (lack of). Participants were asked to rate incidents occurring over the past 6 months using a 4-point scale from "Strongly Agree" (1) to "Strongly Disagree" (4). Total score, as well as subscale scores (Urgency, Premeditation, Perseverance, and Positive Urgency) were obtained, with higher scores reflecting greater impulsivity. Cyders and Smith (2008) reported average internal consistencies for the UPPS-P ranging from 0.83 to 0.94, and test–retest reliabilities across a three month period ranging from 0.62 to 0.81. The measure has demonstrated good convergent validity across methods of assessment, as well as good discriminant validity with respect to other measures of impulsivity (Cyders & Smith, 2008; Smith et al., 2007). Copyrighted survey forms were purchased for this study.
2. *Grit Scale Short Version (Grit-S):* The Grit-S (Duckworth & Quinn, 2009) is an 8-item self-report measure of grit, defined as trait-level perseverance and passion for one's longer-term goals. The Grit-S, adapted from an earlier version of the

Grit Scale (Duckworth, Peterson, Matthews, & Kelly, 2007), consists of two factors, consistency of interests and perseverance of effort. Respondents were asked to rate the extent to which each statement reflected how they feel about themselves (e.g., "Setbacks don't discourage me.") using a scale ranging from 0 ("Not at all like me") to 4 ("Very much like me"); 4 items were reverse scored. A total score was obtained by calculating the sum of all items and dividing by 8; total scores ranged from 0 to 5, with 5 reflecting higher levels of grit. Good internal consistency estimates (Cronbach's α) were reported for the overall scale (ranging from 0.73 to 0.83) and for the individual factors: Consistency of Interests (0.60 to 0.78) and Perseverance of Effort (0.73 to 0.79) (Duckworth & Quinn, 2009). Test-retest reliability across a 1-year period was adequate (r = 0.68) (Duckworth & Quinn, 2009). This scale is in the public domain.

3. *Dispositional Resilience Scale 15 (DRS-15):* The DRS-15 (Bartone, Ursano, Wright, & Ingraham, 1989) is a self-report measure of hardiness, derived from earlier 45- and 30-item versions of the measure. The DRS-15 consists of a total score as well as three subscales, Commitment (versus alienation), Control (versus powerlessness), and Challenge (versus threat), each reflecting a key facet of the hardiness construct. Participants were asked to rate 15 statements (5 items per subscale) reflecting aspects of hardiness using a scale ranging from 0 ("not at all true") to 3 ("completely true"). Six items are negatively keyed to provide balance for negative and positive items. Total scores range from 0 to 45. The DRS-15 has demonstrated good internal consistency (Cronbach's α up to 0.83 for the full scale

and ranging from 0.70-0.77 for the individual subscales) and good criterion-related validity across diverse samples (Bartone, 1999). A test-retest reliability coefficient of 0.78 has been reported for repeated measurements taken over a three-week period of time (Bartone, 2007). Copyrighted survey forms of this scale were purchased.

4. *Life Orientation Test-Revised (LOT-R):* The LOT-R (Scheier, Carver, & Bridges, 1994) is a widely used measure of dispositional optimism, and taps an individual's tendency to view the world and the future in positive ways. The LOT-R consists of a set of 10 statements (e.g., "I'm always optimistic about my future"). Participants were asked to rate their agreement or disagreement with each statement using a 5-point scale ranging from 0 ("I disagree a lot") to 4 ("I agree a lot"). Total scores (4 items are filler items) range from 0 to 24, with a higher score indicating greater degree of optimism. Adequate psychometric properties were demonstrated. Test-retest reliability for the scale ranges from 0.56 to 0.79 for time intervals of up to 28 months and internal consistency reliability has been estimated at 0.78 (Scheier et al., 1994). This measure is in the public domain.
5. *Perceived Stress Scale (PSS-10):* The PSS-10 (Cohen, Kamarck, & Mermelstein, 1983) is a 10-item self-report measure that captures the degree to which events in a person's life are perceived as stressful. Participants rated the degree to which they found their lives to be unpredictable, uncontrollable, and loaded over the past month (e.g., "In the past month, how often have you been upset because of

something that happened unexpectedly?"). Questions were scored using a 4-point scale ranging from 0 ("Never") to 4 ("Very Often"). Total scores (4 items are reverse scored) range from 0 to 40, with higher scores reflecting greater levels of perceived stress. The PSS-10 has demonstrated good internal stability for the total scale (Cronbach's $\alpha = 0.89$) and for the individual subscales (Perceived Helplessness, 0.85; Perceived Self-efficacy, 0.82; Roberti, Harrington, & Storch, 2006). A test-retest reliability of 0.85 was reported across a testing interval of 2 days, whereas a reliability estimate of 0.55 was reported across a 6-week interval (Cohen et al., 1983). This measure is in the public domain.

Workload, Effort, and Alertness

1. *Sleepiness Scale:* Participant ratings of subjective alertness were evaluated using the Sleepiness Scale from the Automated Neuropsychological Assessment Metrics Version 4 (ANAM4) cognitive assessment task battery (Cognitive Science Research Center, 2007). Participants were asked to select one of seven statements ranging from "Feeling very alert, wide awake, and energetic" to "Very sleepy and cannot stay awake much longer" to indicate current state of alertness. The ANAM4 Sleepiness Scale was adapted from the Stanford Sleepiness Scale (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973), which is in the public domain and is comparable in both scoring and interpretation.
2. *The NASA Task Load Index (NASA-TLX):* The NASA-TLX (Hart & Staveland, 1988) is a widely used (more than 1600 studies over the past 20 years)

multidimensional scale designed to assess subjective mental workload associated with task performance (Cao, Chintamani, Pandya, & Ellis, 2009; Hart, 2006). This two-part evaluation system uses a combination of "Weights" and "Ratings" to calculate overall perceived workload (Hart & Straveland, 1988). The participant's evaluation of workload is derived from a weighted average of six factors: (1) Cognitive Workload, (2) Physical Workload, (3) Temporal Demand, (4) Performance, (5) Effort, and (6) Frustration. The first three factors represent demands imposed on the subject by the task, and the latter three factors represent the interaction of the participant with the task (Hart, 2006). "Weights" are assigned based upon the participant's judgment of the relative contribution of each factor to his/her perceived workload via 15 possible pair-wise comparisons of the six factors. Participants also rate the magnitude of each factor within a given task (e.g., how mentally demanding was this task?) by placing a mark along a line divided into 20 equal intervals and anchored by the descriptors "High" and "Low" (Hart & Straveland, 1988). In this study, we used a modified version of the NASA-TLX that relies on the unweighted rankings for each of the six scale factors. Numerous studies have demonstrated strong correlations between the full and modified version of the NASA-TLX (Hart, 2006; Moroney, Biers, & Eggemeier, 1995). The modified NASA TLX has been implemented in a number of USARIEM studies (Adam & Merullo, 2006; McBride, Merullo, Johnson, Banderet, & Robinson, 2007; Smith et al., 2019). This measure is in the public domain.

3. *Task Engagement Questionnaire (TEQ*) was developed for use in this study to evaluate participants' perceived engagement in the primary study tasks. Participants were asked to rate to what degree a feeling or emotion word (e.g., "happy") describes how they felt during the task, how they felt about the task itself, and how they would feel about completing the task again in the future using a scale from 1 "Strongly disagree" to 5 "Strongly agree." Midpoint on this scale represents a neutral emotional state ("Neither agree nor disagree"). Scores for each scale were calculated by adding up the scores (1-5) for each question (with a minimum score of 10 and a maximum score of 50). Participant ratings of task engagement in this study were included in analyses to control for potential effects of participant motivation on performance outcomes.

Neuropsychological Tasks

1. *Go/No-Go:* This study used the Go/No-Go task module of the ANAM4 battery (Cognitive Science Research Center, 2007). This task provides an assessment of response inhibition and is based on a standard Go/No-Go paradigm in which the examinee is required to provide a response for a target stimulus and to not respond (to ignore) to other stimuli (Lezak, Howieson, Loring, Hannay, & Fischer, 2004). Previous work has shown that as task demands increase (either due to the complexity of instruction or duration of task), an individual is more likely to respond in error to the "no-go" stimulus (Nee, Wager, & Jonides, 2007). A meta-analysis of brain activation in individuals performing this task found

activation in the right frontal cortex, including the dorsolateral prefrontal cortex and inferior frontal gyrus, regions known for their involvement in executive function (Nee et al., 2007). The Go/No-Go task module of the ANAM4 required participants to respond by pressing a key on the computer keyboard when an "X" is presented on screen and to not respond to when an "O" is presented. Each stimulus was presented on a plain, dark background and with an inter-stimulus interval of 0.5 seconds. The Go/No-Go task has demonstrated adequate test-retest reliability (r = 0.65) (Rana & Rao, 2013; Weafer, Baggott, & de Wit, 2013; Wöstmann et al., 2013) with a moderate correlation (r = 0.46) between this task and the Stop-Signal Task, another widely used measure of response inhibition, suggesting adequate convergent validity (Rana & Rao, 2013). This task took approximately 5 minutes to complete. In the present study, we analyzed percent accuracy and reaction time (in msec) for correct responses.

2. *Gradual-Onset Continuous Performance Test (gradCPT) (Esterman, Noonan, Rosenberg, & Degutis, 2013)*: The gradCPT is a version of the commonly-used neuropsychological task, the continuous performance test, which asks participants to press a button when presented with a city scene (90% of trials) and to not respond when mountain scenes appear (10% of trials) (**Figure 9**). City and mountain scenes gradually transition into each other at a rate of approximately one per 800ms. The gradual transitioning of scenes creates uncertainty as to when the image is presented and limits the time the image is clearly in view, creating the need for increased attention. The gradCPT has demonstrated vigilance

decrements in as short a testing period as 8 minutes (Esterman et al., 2013). These results were replicated using neuroimaging and in a number of clinical validation studies, showing robust individual differences (Esterman et al., 2013; Esterman et al., 2016; Esterman, Reagan, Liu, Turner, & DeGutis, 2014; Esterman, Rosenberg, & Noonan, 2014; Esterman & Rothlein, 2019; Fortenbaugh, DeGutis, & Esterman, 2013; van den Brink, Murphy, & Nieuwenhuis, 2016). The gradCPT is administered using JavaScript and took approximately 4 minutes to complete. This task has excellent reported reliability (estimates ranging from 0.80 to 0.98) (Jun, Remington, Koutstaal, & Jiang, 2019; Rosenberg et al., 2016). In the present analyses, we analyzed overall accuracy (error rate and d'), average response times, standard deviation of response times, and the rate of commission (false positive) and omission (false negative) errors.

Figure 9. An example of the gradCPT.
City scenes (press button) transition to mountain scenes (no response) and back to city scenes (Fortenbaugh et al., 2015).

3. *N-Back:* The N-back is a widely used measure of working memory (Kirchner, 1958). During this task, the participant is presented with a series of letters or numbers and must answer "yes" or "no" if the letter or number presented is the same as the one presented *n* letters or numbers ago. For this task, if a participant

saw the letters B, P, E, and B, he or she should press the "M" button ("M" for "Memory") after seeing the second "B" because the second "B" is the same as three letters ago (making this a 3-back task). This task was administered for approximately five minutes using PsyToolkit, a web-based program designed for designing and administering psychological assessments (Stoet, 2010; Stoet, 2017). The N-back has moderately high test-retest reliability (r = 0.69) (Soveri et al., 2018) and sufficient face validity (Kane, Conway, Miura, & Colflesh, 2007). There are moderate, significant correlations between this measure and similar tasks assessing working memory, such as the memory updating task (r = 0.42), suggesting that the task has adequate convergent validity (Schmiedek, Lövdén, & Lindenberger, 2014). Percent accuracy and average reaction time for correct responses for all trials and for 3-back responses only were used for this task. This measure is in the public domain.

Stress Responsivity

1. *Electrocardiography (ECG):* SNS dominance during an acute stress response has a number of effects on heart rate. While many cardiac measures are complicated by posture and physical exertion, heart rate variability (HRV) measured using ECG is a well-studied indicator of increased SNS tone that can be separated from other influences on cardiac output. HRV is often quantified using beat-to-beat (R-R) interval variability, where R is a point corresponding to the peak of the QRS complex of the ECG wave and R-R is the interval between successive R peaks.

HRV is also associated with vagal coordination between cardiac output and respiration (Lane, Adcock, & Burnett, 1992). Variability of RR intervals across time spans in the low frequency range (LF: 0.04 to 0.15 Hz) is associated with SNS activity while variability in the high frequency range (HF: 0.15 to 0.4 Hz) is associated with PNS activity (Lane et al., 1992). Thus, changes in SNS/PNS balance can be derived by analyzing relative changes in the low and high frequency HRV components (LF/HF). Heart Rate (HR) was recorded using the BioNomadix wireless ECG module (BIOPAC Systems, Inc.: Goleta, CA). Three solid gel electrodes received electrical activity generated by the heart, and were attached to a 3-lead set, which directly connected to the BioNomadix transmitter worn by the participant via a Velcro strap around the chest. This transmitter wirelessly transmits the participant's heart rate data, at a rate of 2,000 Hz, to the RSPEC-R receiver, which connects directly to the MP150 Data Acquisition System.

HRV was analyzed by BioNomadix AcqKnowledge (BIOPAC Systems, Inc.: Goleta, CA) and Kubios (Kubios Ltd.: Kuopio, Finland) HRV software using motion artifact correction procedures, specifically, "strong" beat correction and "medium" noise corrections. "Strong" beat correction refers to returning any heart beats in which the R-R interval is less than or greater than 0.15 seconds different than the average R-R interval across that section of data to a within 0.15 seconds from the average R-R interval (Tarvainen, Lipponen, Niskanen, & Ranta-aho, 2021). Meanwhile, "medium" noise correction removes any heart beats or

artifacts which appear to be affected by abnormal movement and replaces them with NaNs (Not a Number) automatically. The data were then examined closely and any remaining artifacts were removed from the data as NaNs manually.

Finally, the Kubios software generated both time-domain data, such as mean heart rate and root mean square of successive differences between heart beats (RMSSD), and frequency-domain data (such as activity in the HF range and the LF/HF ratio) heart rate variability statistics. Although Kubios also reports the parametric Autoregressive Model (AR) Transformation technique for electrophysiological data, only results using the non-parametric Fast Fourier Transformation (FFT) technique were reported in these analyses because FFT is the more common power spectral analysis method in the literature (Silva et al., 2009). Also, Kubios automatically applies Welch estimations to frequency-series outcomes; Welch estimations break the sample into smaller pieces and average frequency spectrum data across those samples, thereby minimizing the influence of noise on the sample (Welch, 1967).

Relevant HRV metrics included in these analyses include:

a. *Heart rate (HR)*: mean heart rate.
b. *Time-domain HRV measures:*
    i. Mean R-R interval,
    ii. Standard deviation of normal R-R intervals (SDNN),
    iii. Root mean square of successive R-R interval differences (RMSSD),

iv. Percentage of successive R-R intervals that differ by more than 50 milliseconds (pNN50; Shaffer & Ginsberg, 2017).

c. Frequency-domain HRV measures:

i. *Total absolute power*: total signal energy throughout all frequency bands, measured in milliseconds squared ($msec^2$) divided by cycles per second (Hertz [Hz]),

ii. *Very low frequency (VLF) absolute power*: signal energy found within the VLF band (0.0033 – 0.04 Hz), measured in $msec^2$,

iii. *Low frequency (LF) absolute power*: signal energy found within the LF band (0.04 – 0.15 Hz), measured in $msec^2$,

iv. *High frequency (HF) absolute power*: signal energy found within the HF band (0.15 – 0.40 Hz), measured in $msec^2$,

v. *Low frequency (LF) relative power*: percentage of total HRV power found within the LF band (0.04 – 0.15 Hz), calculated by the absolute power of the LF band divided by the summed absolute power of the LF and HF bands and measured in normal units (n.u.),

vi. *High frequency (HF) relative power*: percentage of total HRV power found within the HF band (0.15 – 0.40 Hz), measured in n.u.,

vii. *Ratio of low- to high-frequency power*: ratio of absolute power found in the LF frequency band to that found in the HF frequency

band. LF/HF ratio is considered an estimate of SNS to PNS activity (Shaffer & Ginsberg, 2017).

2. *Photoplethysmography (PPG):* PPG measures changes in blood volume using an infrared light source that is often placed on the fingertip (Allen, 2007). Since TES may affect cardiac tone, PPG provides an additional method of assessing physiological changes due to trigeminal nerve stimulation. A study using vagal nerve stimulation demonstrates that it may even alter PPG amplitude to a more observable extent than HRV (Gurel, Jung, Hersek, & Inan, 2019). With each heartbeat, there are changes in blood volume throughout the body that a PPG device can detect via a light source. The movement of blood manifests as a wave-like motion that begins in the heart and moves to the extremities with a frequency of about 1 Hz (Allen, 2007; Elgendi, 2012). The PPG pulse wave is divided into anacrotic and catacrotic phases (Allen, 2007). The anacrotic phase is associated with systole and the catacrotic phase is associated with diastole (Allen, 2007). A low systole amplitude is associated with increased sympathetic activity (Allen, 2007). The pulse interval is the distance from the beginning to the end of the PPG waveform and is highly correlated with HRV R-R intervals (Jeyhani, Mahdiani, Peltokangas, & Vehkaoja, 2015; Pernice et al., 2018); thus it could be used as an alternative estimate of HRV (Lu et al., 2007).

Similarly to HRV, PPG were measured using the BioNomadix PPG/EDA module (BIOPAC Systems, Inc.: Goleta, CA), which were attached to the participant's wrist by an elastic strap, the BioNomadix MP150 Data Acquisition

System, AcqKnowledge software, and Kubios HRV software. As with HRV analyses, “strong” beat correction was used, but the noise correction algorithm used in the Kubios software is insufficient for use with PPG data and removes large portions of data mistakenly identified as artifacts. As a result, no noise correction was applied to PPG data and artifacts were removed from the data as NaNs manually. Again, time- and frequency-domain (using the Fast Fourier Transformation (FFT) technique) PPG statistics were reported.

3. *Salivary cortisol and α-amylase:* Measuring cortisol and α-amylase in saliva is a non-invasive way of measuring HPA axis and SAM axis stress responses (Laurent, Powers, and Granger, 2013). Cortisol in saliva has been shown to be proportional to the amount released by the adrenal glands during stress. The SNS influences release of α-amylase, a digestive enzyme, by the salivary glands. Saliva samples were collected using a non-invasive, passive drool saliva collection system (Salimetrics, State College, PA) before and after each firing iteration. Participants repeatedly collected saliva in their mouths before drooling into a test-tube shaped container; the container then was sealed with a lid to prevent the saliva from drying. Saliva samples were stored at -20˚C until analysis.

During low arousal states, serum cortisol is found bound to globulins with a very low percentage (0-5%) found in the free-unbound state (Fell, Shutt, & Bentley, 1985). During stress, free cortisol is released from the adrenal glands into the blood before traveling to target tissues to support stress-related activities. Generally, the stronger the response to stress, the more free cortisol released by

the adrenal glands (Fell et al., 1985). Since only free cortisol can cross into the salivary glands, the amount of cortisol measured in saliva is directly proportionate to the amount of free cortisol in plasma, and thus the physiological response to stress (Fell et al., 1985). For short duration stressors (>15 minutes), salivary cortisol typically peaks 2-10 minutes after the stressor and returns to baseline over the next 120 minutes.

α-amylase is produced by the salivary glands and is rapidly released into the saliva in response to SNS stimulation. It has been used extensively as a non-invasive marker for stress (Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). For example, acute increases of salivary α-amylase levels during stress were associated with adrenaline and noradrenaline increases in plasma, particularly during psychosocial challenge (Ditzen, Ehlert, & Nater, 2014; Thoma, Kirschbaum, Wolf, & Rohleder, 2012). Due to the quick release of α-amylase from the salivary gland in response to SNS activity, salivary levels rise quickly and return to baseline within minutes after stress initiation.

An important component of salivary stress biomarker analysis is inclusion of salivary flow rate in calculations. Because the rate of saliva secretion may affect the level of α-amylase present in saliva, salivary α-amylase results should be presented in units (e.g., pg) per time. In other words, a measured concentration of α-amylase in saliva is multiplied by the volume of saliva collected over a specified unit of time. To calculate this, the volume of saliva produced during a recorded amount of time (e.g., mL/min) is multiplied by the concentration of α-

amylase (e.g., pg/mL) (Granger, Johnson, Szanton, Out, & Schumann, 2012), statistical models including salivary α-amylase levels included the amount of saliva collected at that sample collection time point as a covariate. In addition, the ratio of resting salivary α-amylase to cortisol has been positively associated with chronic stress and depression (Ali & Pruessner, 2012; Schumacher, Kirschbaum, Fydrich, & Ströhle, 2013). Comparing the trajectories of salivary α-amylase and cortisol over time predict academic performance in children and thus may be associated with cognitive ability (Keller, El-Sheikh, Granger, & Buckhalt, 2012). Therefore, the α-amylase to cortisol ratio after an acute laboratory stressor may measure long-term stress-related dysfunction of the SNS or HPA axis and predict cognitive performance (Ali & Pruessner, 2012).

Study Procedures

Study visits were conducted according to the following schedule (**Table 2**):

<table>
<tr><td colspan="5">Screening (Brain Health and Performance Laboratory) and Training Day 1 (EST)</td></tr>
<tr><td>1. Completed Demographic & Health History Survey</td><td>2. Verified eligibility

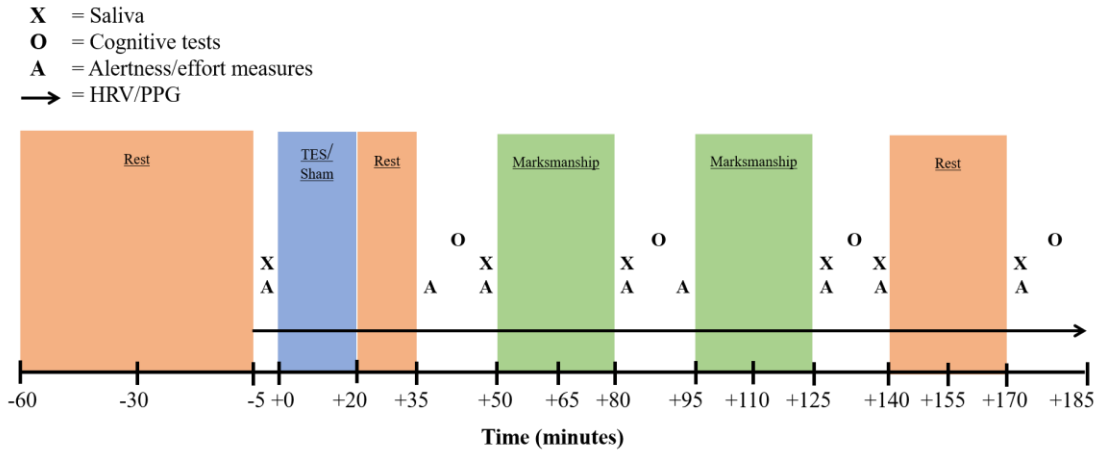
3. Psychological Trait Measures</td><td>4. Zeroed weapon (EST)</td><td>5. Cognitive Test Practice
a. N-back
b. gradCPT
c. Go/No-Go</td><td>6. Introduced TES, HRV/PPG sensors</td></tr>
<tr><td colspan="5">Training Days 2 and 3 (EST)</td></tr>
<tr><td>1. Alertness measure</td><td>2. Practiced cognitive tasks</td><td>3. TES practice</td><td>4. Marksmanship practice (friend-foe paradigm)</td><td>5. Alertness and effort measures</td></tr>
<tr><td colspan="5">Testing Days 1 and 2 (EST)* (in the early afternoon)</td></tr>
<tr><td colspan="5"> 
</td></tr>
<tr><td colspan="5">* TES (active vs. sham) were counterbalanced across testing days.</td></tr>
</table>

Table 2. Schedule of events.

On Day 1 of the study, participants came individually to the USARIEM Brain Health and Performance Laboratory at the NSSC to undergo the consent process and then complete initial eligibility screening procedures using the Demographic and Health History Questionnaire. This questionnaire also asks about physical and psychological health as well as medication use and includes the female Menstrual Cycle Interview. If eligible, participants then went to the EST, where they were introduced to the devices used in this study and trained on neuropsychological tasks and the friend-foe discrimination marksmanship challenge. These training sessions are intended to establish a relatively stable performance baseline, with minimal interference from novelty or practice effects (Smith et al., 2019). All eligible participants were introduced to the M4 weapon and asked to zero their weapons; those unable to zero their weapons after nine attempts were withdrawn from further study participation. Participants also were introduced to the HRV and PPG sensors and TES device, at which point the investigator set the level of stimulation based on participant feedback regarding comfort. Finally, participants practiced the neuropsychological tests (gradCPT, N-Back, and Go/No-Go) and completed a variety of self-report assessments measuring psychological health and personality characteristics (NSI, PANAS, UPPS-P, etc.). On training days 2 and 3, participants were fitted with the TES device and again practiced both the neuropsychological tasks and the friend-foe discrimination challenge.

On testing days 1 and 2, participants adhered to a schedule in which they did not eat, drink anything other than water, brush their teeth, or exercise for two hours prior to testing, which was scheduled for the early afternoon. Regular tobacco/nicotine users were

asked to use tobacco/nicotine product to satisfaction 45 minutes before testing (unless on a continuous delivery patch), as verified by self-report. Female participants were scheduled within the follicular phase of their menstrual cycle. These procedures were intended to reduce variability in salivary biomarkers associated with idiosyncratic participant characteristics and diurnal variation, thus allowing measurement of a baseline state without confounding by individually variable exposure to stress, exercise, hormones, or other factors. Upon arriving at the EST, participants rested comfortably for approximately 30 minutes and then drank some grape juice, thus guaranteeing maximal glucose availability as is necessary for optimal cortisol reactivity (Gonzalez-Bono, Rohleder, Hellhammer, Salvador, & Kirschbaum, 2002; Kirschbaum et al., 1997). Participants remained seated and rested for an additional 30 minutes. After the rest period was complete, participants were fitted with the HRV and PPG sensors and recording began. Participants also completed the first baseline saliva collection of the day and an alertness measure (Sleepiness Scale). Next, participants received 20 minutes of active or sham stimulation by the TES device (with the order of stimulation on Testing Days 1 and 2 determined by randomization).

After the neurostimulation period ended, the TES device was removed and participants rested for fifteen minutes, during which time they were allowed to engage in calming activities (e.g., read a book, color in a coloring book, play Sudoku) but not potentially stressful activities such as watching videos on their phones. After this brief rest period, participants completed the gradCPT, N-back, and Go/No-Go tasks, provided another saliva sample, and completed the alertness and effort scales (in order: Sleepiness

Scale, NASA-TLX, TEQ). Participants then underwent two iterations of the friend-foe discrimination marksmanship challenge. After the first friend-foe discrimination challenge, participants completed the gradCPT, N-back, and Go/No-Go tasks, provided a saliva sample, and completed measures of alertness and effort. At the completion of this testing (which lasted ~20 minutes), participants repeated the friend-foe discrimination task, followed by collection of saliva samples and measures of alertness and effort. Together the two marksmanship and other task periods lasted about 80 minutes. (Of note, previous unpublished work from our laboratory shows that completion of marksmanship tasks for an hour or more produces changes in HRV indicative of SNS activation.)

Saliva samples were collected twice again at 15 and 45 minutes during the 45-minute recovery period following the second marksmanship task. Neuropsychological tasks (gradCPT, N-back, Go/No-Go) and the alertness and effort measures were completed during the first and third 15-minute block of the recovery period. At the end of the recovery period, HRV and PPG sensors were removed. The total time to complete all study procedures on each testing day was five hours.

Statistical Analyses

Sample Size and Power

We planned for a total sample size of 30 participants (15 randomized to receive sham stimulation and 15 randomized to receive active stimulation on the first day of testing; stimulation type then was reversed for the second day of testing). We also

planned to recruit an additional 15 participants to account for potential attrition. The sample size was calculated based on previous studies (**Table 3**) that found differences in salivary stress biomarkers across 9 to 12 participants exposed to active or sham TES (Boasso et al., 2016; Tyler et al., 2015) and studies that demonstrated differences in performance on the N-back and Go/No-Go tasks from before and after stress (Dierolf et al., 2018; Luettgau, Schlagenhauf, & Sjoerds, 2018). Moreover, marksmanship data suggest that under acute sleep restriction, friend-foe HVT detections decreased from 68% to 50%. Assuming that TES improves detection rates by 15%, a total sample size of 30 was sufficient to reach 80% statistical power.

PUBLICATION	SAMPLE SIZE	MEASURE	COHEN'S *D*	A	POWER
TYLER ET AL., 2015	Active = 9 Sham = 10	α-amylase	-1.50	0.05	0.92
BOASSO ET AL., 2016	Active = 12 Sham = 10	α-amylase	-1.74	0.05	0.97
LUETTGAU ET AL., 2018	N = 32 (within subjects)	N-back d'	-2.5	0.05	0.99
DIEROLF ET AL., 2018	Control = 14 Stress = 16	Go/No-Go false alarms	2.41	0.05	0.99

Table 3. Representative sample sizes of relevant previous studies.

However, data collection was placed on hold for one year due to prohibitions against in-person research activities during the COVID-19 pandemic. As a result, the following analyses include data collected from 23 participants. Because the intended sample size was not reached, sample power was recalculated using a sample size of 23 (**Table 4**).

Data Preparation

Prior to analysis, all measured variables were plotted using histograms to determine their distributions; if non-normal, non-parametric statistical methods were used. In addition, residual plots were generated and Breusch-Pagan tests (conducted using *lmtest* package in R; Zeileis & Hothorn, 2002) were completed to identify the variance structure of the data.

Demographic Characteristics

Demographic characteristics were compared using t-tests and Chi-square analyses for those receiving sham TES first versus active TES first. Effects of demographic features on the outcome variables also were analyzed using simple linear regressions. Those characteristics found to differ significantly across these groups or to have an effect on the outcomes of interest were included in statistical models to control for their potential confounding effects.

Tolerability of Stimulation

The Questionnaire of Sensations Related to TES asks participants to rate types of sensations experienced during the stimulation period and how significant (or unpleasant) those sensations were. The percent of participants reporting sensations following TES was calculated by the severity of the reported sensations.

Stimulation Blinding

The extent to which blinding of the stimulation condition (active versus sham) was achieved was evaluated by calculating the percent accuracy of the participants' reports of what type stimulation they thought they had received, as captured by the Questionnaire of Sensations Related to TES. Pearson correlations were completed between guess accuracy and the time, duration, and location of side effects reported by participants during the stimulation period.

Sensitivity Analyses

Data points falling greater than or equal to three standard deviations above or below the mean were considered outliers and Winsorized (changed to equal the value of the closest three standard deviations above or below the mean) (Blaine, 2018). Although this method alters the data's variance structure, it minimizes the impact of removing outliers from a dataset gathered from a small sample; therefore, sensitivity analyses were run to compare results from the original dataset to results from the Winsorized dataset. Sensitivity analyses also were run comparing outcomes including and then excluding participants with results suggesting poor motivation. For example, based on procedures published for ANAM4 task data (Vincent, Roebuck-Spencer, Gilliland, & Schlegel, 2012), accuracy scores less than 56% on the Go/No-Go task were flagged for sensitivity analysis. Accuracy scores from the N-back were not used to indicate motivation due to the very high frequency of low accuracy scores, which would have removed data from 9 to 11 participants each time the N-back was administered. The sensitivity model that best fit the data was identified according to the Akaike Information Criterion (AIC) for which a score closer to zero indicates better model fitness.

Aim 1: Effects of TES on markers of stress

The data used to address Aim 1 included salivary cortisol and α-amylase levels, as well as ECG and PPG variability. Both ECG and PPG heart rate variability metrics, as well as salivary cortisol and α-amylase values from each of the time points gathered on testing days were analyzed between stimulation conditions to assess the effects of TES on stress response. Furthermore, the ratio of α-amylase-to-cortisol and the ratio of low frequency-to-high frequency heart rate and pulse were analyzed at each time point. Means and standard deviations for each outcome were calculated by stimulation condition (active versus sham).

Data were analyzed using linear mixed models, with measurement time point nested under participant in order to account for the repeated measures study design (using *nlme* package in R; Pinheiro, Bates, DebRoy, Sarkar, & R Core Team, 2021). In mixed models, random slopes may be included in order to account for expected individual differences resulting from the independent variable (in this case, stimulation condition), thereby reducing Type I error rate (Schielzeth & Forstmeier, 2009; Barr, Levy, Scheepers, & Tily, 2013). Because we expected stimulation to affect individuals differently, all models included random slopes of stimulation condition, except when convergence could not be achieved due to very high correlations between the random intercept and the slope. These analyses also specified a correlation structure in order to minimize Type I error rate because the data were expected to be correlated within participants and between measurement time points. Additionally, if the data variance

structure was heteroscedastic, weights were included in the model to account for heterogeneity or non-normality of residuals across different values of the independent variable (in this case, stimulation condition). Finally, in each model, missing observations (i.e., that were not a number [NaN] in the dataset) were excluded from analysis.

Multiple comparisons corrections on all maximum likelihood ratio *p*-values were made using the Benjamini-Hochberg correction (using *multcomp* package in R; Hothorn, Bretz, & Westfall, 2008). The Benjamini-Hochberg correction, also referred to as the False Discovery Rate correction, is a less conservative correction method than the Bonferroni correction and adjusts *p*-values based on their size (Chen, Feng, & Yi, 2017). Because the variance in multilevel modeling stems from many fixed and random predictors, it can be difficult to compute effect sizes with confidence. Nonetheless, Cohen's *d* effect sizes were calculated by dividing the regression estimate (β) from the mixed model by the model's standard deviation (calculated as the standard error multiplied by the square root of the number of observations included in the model). The resulting effect size is classified according to standard guidelines, i.e., $d < 0.20$ is "small," $d >= 0.50$ is "medium," and $d >= 0.80$ is "large."

Aim 2: Effects of TES on marksmanship performance

The data used to address Aim 2 included speed of target engagement and accuracy in discriminating friend from foe. Again, nested linear mixed models were completed to explore the overall effects of TES on the outcome variables during each trial of the marksmanship task. Model conditions otherwise were the same as reported for

Aim 1 and multiple comparisons corrections were made using the Benjamini-Hochberg correction.

Aim 3: Effects of TES on cognitive performance

The data used to address Aim 3 included accuracy and mean response times on the gradCPT, the N-back, and the Go/No-Go tasks. Comparisons of performance (accuracy, response times) in response to the active and sham TES conditions were conducted for each neuropsychological task across testing time points. Again, linear mixed models used nesting for repeated measures analyses. Corrections for multiple comparisons were made using the Benjamini-Hochberg correction.

Post-hoc: Effects of personality characteristics and coping skills

Post-hoc linear regressions were used to analyze the effects of personality characteristics and coping skills on the associations between stimulation and physiological, cognitive, and marksmanship outcomes of interest. Relevant covariates were then included in the original linear mixed models to identify their effects on model results. The results of maximum likelihood ratio tests (using *lmtest* package in R; Zeileis & Hothorn, 2002) were compared between the models with and without personality characteristics to see which model better fit the data. If the addition of a personality characteristic improved model fit, the personality characteristic was quantified according to levels predefined by the scale developers or by quartiles (lowest 25%, middle 50%, and highest 25%). Analysis of variance (ANOVA) with post-hoc linear regression then

was used to assess the effects of a particular personality characteristic on the association between the stimulation and outcomes; the association then was depicted using a regression plot.

For all analyses, a significance level of $\alpha = 0.05$ was used. All analyses were conducted using R software, version 4.0.3 (R Core Team, 2020), and plotted using the *ggplot2* (Wickham, 2016) and *ggpubr* (Kassambara, 2020) packages in R.

CHAPTER THREE: RESULTS

Sample Power

Although sample size was calculated *a priori*, the intended sample size (N = 30) could not be reached due to events outside of the control of the research team. In order to define how much credence should be given to these analyses, statistical power was calculated for the actual sample size of 23 (**Table 4**). Sample power was sufficient (greater than or equal to 80% with α set at 0.05) for α-amylase, cognitive workload, most ECG, PPG, and marksmanship outcomes, gradCPT d' and accuracy, overall accuracy for the N-back, and response times for 3-backs on the N-back task. However, sample size was insufficient to reach 80% power for cortisol, the α-amylase to cortisol ratio, both ECG and PPG pNN50, ECG high frequency absolute and relative powers, ECG low-to high-frequency ratio, PPG RMSSD and low frequency relative power, physical workload, high value target accuracy, target detection latency, gradCPT response times and both

commission and omission error rates, Go/No-Go accuracy and response times, overall response times for the N-back, and accuracy for 3-backs on the N-back task. Therefore, these analyses should be interpreted with caution.

OUTCOME	POWER (%)
CORTISOL	9.97
A-AMYLASE	98.67
A-AMYLASE TO CORTISOL RATIO	17.55
ECG MEAN HEART RATE	100.00
ECG MEAN R-R INTERVAL	100.00
ECG SDNN	100.00
ECG RMSSD	100.00
ECG PNN50	9.37
ECG TOTAL ABSOLUTE POWER	100.00
ECG VLF ABSOLUTE POWER	100.00
ECG LF ABSOLUTE POWER	100.00
ECG HF ABSOLUTE POWER	6.65
ECG LF RELATIVE POWER	100.00
ECG HF RELATIVE POWER	6.65
ECG LOW- TO HIGH-FREQUENCY RATIO	44.62
PPG MEAN HEART RATE	100.00
PPG MEAN R-R INTERVAL	100.00
PPG SDNN	100.00
PPG RMSSD	16.09
PPG PNN50	5.00
PPG TOTAL ABSOLUTE POWER	100.00
PPG VLF ABSOLUTE POWER	100.00
PPG LF ABSOLUTE POWER	100.00
PPG HF ABSOLUTE POWER	100.00
PPG LF RELATIVE POWER	38.17
PPG HF RELATIVE POWER	100.00
PPG LOW- TO HIGH-FREQUENCY RATIO	100.00
COGNITIVE WORKLOAD	100.00
PHYSICAL WORKLOAD	63.13
TARGET DISCRIMINATION ACCURACY FOR ALL TARGETS	100.00

TARGET DISCRIMINATION ACCURACY FOR TARGETS TO WHICH A RESPONSE WAS MADE	100.00
SHOT ACCURACY	100.00
HIGH VALUE TARGET ACCURACY	57.51
TARGET DETECTION ACCURACY	100.00
TARGET DETECTION LATENCY	15.78
SHOT LATENCY	100.00
SHOT DISTANCE FROM THE CENTER OF MASS	100.00
GRADCPT D'	88.05
GRADCPT ERROR RATE	97.10
GRADCPT COMMISSION ERROR RATE	24.28
GRADCPT OMISSION ERROR RATE	8.29
GRADCPT MEAN RESPONSE TIMES	36.67
GRADCPT STANDARD DEVIATION OF RESPONSE TIMES	100.00
GO/NO-GO ACCURACY	37.42
GO/NO-GO MEAN RESPONSE TIMES	73.90
N-BACK ACCURACY FOR 3-BACKS	7.80
N-BACK MEAN RESPONSE TIMES FOR 3-BACKS	100.00
N-BACK ACCURACY OVERALL	91.35
N-BACK OVERALL MEAN RESPONSE TIMES	5.07

Table 4. Study statistical power, recalculated with a sample size of 23.

Data Preparation

Task data for participants whose accuracy on the Go/No-Go and gradCPT tasks was lower than 56% were removed, leaving 95.65% and 98.37% of the data for analysis, respectively (**Table 5**). There were also several missing data points for the N-back task due to computer errors while saving the data, leaving between 97.82% and 98.91% of the data for analysis. For the marksmanship task, data points were removed if there were methodological or technological errors during the task, resulting in 1 data point (1.09% of the data) being removed from the firing latency dataset and ten data points (10.87% of the data) from the target discrimination latency dataset. No data were missing from the other

marksmanship outcomes or from the cognitive or physical workload outcomes. Of the 276 saliva samples analyzed (12 samples per participant), four were insufficient for analysis due to poor saliva production; therefore, data from 1.45% of the cortisol and α-amylase samples are missing. Finally, data from 63 ECG blocks (collected across 5 participants) and 26 PPG blocks (collected across 4 participants) could not be analyzed due to large amounts of noise or motion artifacts in the block; therefore, 11.41% and 4.71% of the ECG and PPG data are missing.

OUTCOME	MISSING %	PRESENT %
CORTISOL	1.5	98.5
A-AMYLASE	1.5	98.5
A-AMYLASE TO CORTISOL RATIO	1.5	98.5
ELECTROCARDIOGRAPHY OUTCOMES	11.4	88.6
PHOTOPLETHYSMOGRAPHY OUTCOMES	4.7	95.3
COGNITIVE WORKLOAD	0.0	100.0
PHYSICAL WORKLOAD	0.0	100.0
TARGET DISCRIMINATION ACCURACY FOR ALL TARGETS	0.0	100.0
TARGET DISCRIMINATION ACCURACY FOR TARGETS TO WHICH A RESPONSE WAS MADE	0.0	100.0
SHOT ACCURACY	0.0	100.0
HIGH VALUE TARGET ACCURACY	0.0	100.0
TARGET DETECTION ACCURACY	0.0	100.0
TARGET DETECTION LATENCY	10.9	89.1
SHOT LATENCY	1.1	98.9
GRADCPT D'	1.6	98.4
GRADCPT ACCURACY	1.6	98.4
GRADCPT MEAN RESPONSE TIMES	1.6	98.4
GO/NO-GO ACCURACY	4.4	95.6
GO/NO-GO MEAN RESPONSE TIMES	4.4	95.6
N-BACK ACCURACY FOR 3-BACKS	1.1	98.9
N-BACK MEAN RESPONSE TIMES FOR 3-BACKS	2.2	97.8

N-BACK ACCURACY OVERALL	1.1	98.9
N-BACK OVERALL MEAN RESPONSE TIMES	1.6	98.4

Table 5. Rates of missing outcome data.

Histograms of data distributions indicate that these data were not normally distributed, even when log transformations were applied; therefore, non-parametric statistical methods were used. Breusch-Pagan tests concluded that most outcome variables in these analyses had homoscedastic variances, except for marksmanship target discrimination latency, marksmanship target detection, marksmanship target discrimination, marksmanship high value target detection, and salivary cortisol and α-amylase levels. As a result, variance weights were used in the relevant mixed models.

Sample Demographic Characteristics

Among the sample of 23 individuals, 12 were assigned to receive active TES on the first testing day and sham stimulation on the second testing day, and 11 were assigned to receive sham TES on the first testing day and active TES on the second testing day (**Table 6**). The sample was 78.3% male and 73.9% Caucasian, with a mean age of 24.0 ± 5.6 years and mean education of 13.0 ± 1.8 years. Four individuals (17.4%) were civilians while 18 individuals (78.3%) were enlisted Soldiers and one individual (4.35%) was an officer. Of the military sample, 69.6% were infantry. The average time in service was 3.3 ± 6.0 years and most participants were qualified sharpshooters (record fire score of 30-35) or expert marksmen (record fire score greater than or equal to 36), with a mean highest record fire score of 35.7 ± 2.4 out of a maximum score of 40. Approximately

30.4% of the sample identified as tobacco users. Those participants assigned to the active versus sham stimulation group on the first testing day did not differ significantly with regard to any demographic characteristics (*p*-value range 0.05 – 0.98).

	ALL PARTICIPANTS (N = 23)		ACTIVE / SHAM (N = 12)		SHAM / ACTIVE (N = 11)		T / X^2	*P*
AGE (M, SD)	24.00	5.65	22.42	4.85	25.73	6.17	-1.42	0.17
SEX (MALE; N, %)	18.00	78.26	10.00	83.33	8.00	72.73	0.38	0.54
ETHNICITY (N, %)							1.35	0.72
CAUCASIAN	17.00	73.91	8.00	66.67	9.00	81.82	--	--
BLACK/AFRICAN AMERICAN	0.00	0.00	0.00	0.00	0.00	0.00	--	--
ASIAN/PACIFIC ISLANDER	1.00	4.35	1.00	8.33	0.00	0.00	--	--
HISPANIC/LATIN	3.00	13.04	2.00	16.67	1.00	9.09	--	--
NATIVE AMERICAN	0.00	0.00	0.00	0.00	0.00	0.00	--	--
OTHER	2.00	8.70	1.00	8.33	1.00	9.09	--	--
ENGLISH AS FIRST LANGUAGE (YES; N, %)	21.00	91.30	12.00	100.00	9.00	81.82	2.39	0.12
EDUCATION (YEARS; M, SD)	13.04	1.85	12.58	1.24	13.55	2.30	-1.23	0.24
HANDEDNESS (N, %)							2.01	0.37
RIGHT	21.00	91.30	11.00	91.67	10.00	90.91	--	--
LEFT	1.00	4.35	1.00	8.33	0.00	0.00	--	--
AMBIDEXTROUS	1.00	4.35	0.00	0.00	1.00	9.09	--	--
TOBACCO USER (YES; N, %)	7.00	30.43	3.00	25.00	4.00	36.36	1.03	0.31
MILITARY OCCUPATIONAL SPECIALTY (N, %)							8.74	0.19
INFANTRY	16.00	69.57	10.00	83.33	6.00	54.55	--	--
NON-INFANTRY	3.00	13.04	1.00	8.33	2.00	18.18	--	--
MILITARY RANK (N, %)							4.94	0.42
CIVILIAN	4.00	17.39	1.00	8.33	3.00	27.27	--	--
PRIVATE II	14.00	60.87	8.00	66.67	6.00	54.55	--	--

PRIVATE FIRST CLASS	1.00	4.35	1.00	8.33	0.00	0.00	--	--	
SPECIALIST	1.00	4.35	1.00	8.33	0.00	0.00	--	--	
STAFF SERGEANT	1.00	4.35	0.00	0.00	1.00	9.09	--	--	
FIRST/MASTER SERGEANT	1.00	4.35	1.00	8.33	0.00	0.00	--	--	
CAPTAIN	1.00	4.35	0.00	0.00	1.00	9.09	--	--	
TIME IN SERVICE (YEARS; M, SD)	3.25	6.00	2.43	5.15	4.38	7.23	-0.65		0.53
HIGHEST RECORD FIRE (MAXIMUM: 40; M, SD)	35.67	2.38	36.55	2.16	34.29	2.14	2.18		0.05
STIMULATION GUESS ACCURACY (M, SD)	45.65	29.82	45.83	33.43	45.45	26.97	0.03		0.98

Table 6. Sample demographic characteristics.

Abbreviations and Symbols: M = mean; N or n = number of individuals in group; p = p-value; SD = standard deviation; t = t-statistic; X^2 = chi-square statistic.

Tolerability of Stimulation

Both active and sham stimulation patterns were well-tolerated by participants, with 60.87% of participants reporting only mild symptoms of itching, pain, burning, heat, and fatigue at the site and time of the stimulation. Only two participants reported moderate heat or itching and only one participant reported severe fatigue during or after the stimulation. These symptoms were anticipated and were not determined to increase risk to the participant or require a cessation or interruption in scheduled testing procedures. There was no correlation between the type of stimulation received and the time at which side effects were noted ($p = 0.56$), duration of side effects ($p = 0.90$), how much the side effects influenced mental state ($p = 0.51$), or the location of the side effects ($p = 0.70$) (**Table 7-top**).

IV	DV	T	DF	R	*P*	
STIMULATION TYPE	Time	0.59	44	0.09	0.56	
	Duration	0.12	44	0.02	0.90	
	Influence on mental state	-0.67	44	-0.10	0.51	
	Location	0.38	44	0.38	0.70	
GUESS ACCURACY	Time	1.34	44	0.20	0.19	
	Duration	1.54	44	0.23	0.13	
	Influence on mental state	3.42	44	0.46	< 0.01	*
	Location	1.30	44	0.19	0.20	

Table 7. Correlations indicating stimulation tolerability and blinding.
Abbreviations and Symbols: * = $\alpha < 0.05$; *df* = degrees of freedom; DV = dependent variable; IV = independent variable.

Stimulation Blinding

Participants were 45% accurate in their guesses of the type of stimulation received on each of the testing days (**Table 6**). There was no correlation between guess accuracy and the time at which side effects of stimulation were noted ($p = 0.19$), duration of side effects ($p = 0.13$), or the location of the side effects ($p = 0.20$). However, there was a significant, positive correlation between guess accuracy and the reported extent to which side effects influenced mental state ($p < 0.01$), as measured by the question "How much did these sensations affect your general state?" (**Table 7-bottom**).

Sensitivity Analyses

Sensitivity analyses of cognitive, marksmanship, and saliva data using full datasets versus datasets that were "Winsorized" and from which data were removed for unreliable motivation (indicated by less than 56% accuracy). Therefore, in order to preserve variance in the original data as much as possible, the models reported are those for which data points reflecting unreliable motivation were removed but not Winsorized.

Specific Aims

Aim 1: Effects of TES on markers of stress

Salivary cortisol and α-amylase. As expected, there was a significant effect of measurement time point on salivary cortisol levels (p_{unadj} range < 0.01 – 0.01) wherein salivary cortisol decreased gradually across the marksmanship and post-stress periods (**Table 8**). There were no significant effects of stimulation condition or the interaction between stimulation condition and measurement time point on salivary cortisol (p_{unadj} range < 0.17 – 0.78; **Appendix B**).

In contrast, there were no significant effects of measurement time point, stimulation condition, or their interaction on raw salivary α-amylase levels (p_{unadj} range 0.17 – 0.78) or salivary α-amylase levels adjusted for salivary flow rate (p_{unadj} range 0.16 – 0.94). There was, however, a significant effect of measurement time point on the ratio of α-amylase to cortisol during the final cognitive task (p_{unadj} = 0.01), at which point the ratio was higher than at any other point in time. There were no significant effects of stimulation condition or the interaction between condition and time (p_{unadj} range 0.12 – 0.93) on the ratio of salivary α-amylase to cortisol.

OUTCOME	TIME POINT	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
SALIVARY CORTISOL (UG/DL)	Baseline	0.18	0.15	0.22	0.17	0.13	0.20
	Cognitive test 1	0.15	0.12	0.19	0.13	0.10	0.16
	Marksmanship 1	0.13	0.10	0.18	0.13	0.10	0.17
	Marksmanship 2	0.13	0.10	0.17	0.15	0.12	0.18
	Rest 2	0.13	0.09	0.17	0.12	0.09	0.16
	Cognitive test 4	0.12	0.08	0.15	0.11	0.08	0.14
SALIVARY A-AMYLASE (U/ML)	Baseline	129.10	94.10	164.00	112.50	83.00	142.00
	Cognitive test 1	115.70	80.80	151.00	99.50	70.00	129.00
	Marksmanship 1	113.10	78.20	148.00	99.10	69.70	129.00
	Marksmanship 2	102.20	66.90	138.00	88.60	59.20	118.00
	Rest 2	99.70	64.10	135.00	106.70	77.20	136.00
	Cognitive test 4	129.50	94.20	165.00	118.80	89.30	148.00
SALIVARY A-AMYLASE, ADJUSTED BY SALIVARY FLOW RATE (U/MIN)	Baseline	190.00	85.10	294.00	114.00	22.50	190.00
	Cognitive test 1	192.00	87.40	297.00	110.00	18.40	192.00
	Marksmanship 1	158.00	52.90	262.00	110.00	18.20	158.00
	Marksmanship 2	182.00	75.00	288.00	180.00	88.10	182.00
	Rest 2	216.00	107.30	324.00	203.00	111.00	216.00
	Cognitive test 4	217.00	110.30	323.00	253.00	161.30	217.00
RATIO OF A-AMYLASE TO CORTISOL	Baseline	802.00	376.00	1228.00	824.00	305.00	802.00
	Cognitive test 1	910.00	484.00	1336.00	906.00	387.00	910.00
	Marksmanship 1	1003.00	577.00	1428.00	774.00	256.00	1003.00
	Marksmanship 2	835.00	405.00	1264.00	654.00	135.00	835.00
	Rest 2	958.00	523.00	1394.00	1109.00	590.00	958.00
	Cognitive test 4	1262.00	832.00	1692.00	1720.00	1200.00	1262.00

Table 8. Means and 95% CIs for salivary outcome measures.
Abbreviations and Symbols: 95% CI = 95% confidence interval.

Electrocardiography and photoplethysmography.

Time-series data. Significant effects of measurement time point were noted on ECG mean heart rate (p_{unadj} range < 0.01 – 0.04), mean R-R interval (p_{unadj} range < 0.01 – 0.01), RMSSD (p_{unadj} range 0.01 – 0.03), and pNN50 (p_{unadj} < 0.01 for all). Marksmanship stress decreased HRV while rest periods and periods of cognitive testing were associated with increased HRV (**Table 9**). There were no significant overall effects of stimulation condition on any time-series index of HRV when using ECG (p_{unadj} range 0.10 – 0.96; **Appendix C**). However, the interaction between measurement time point and stimulation condition was significant for each time-series index of HRV. There were significant effects of stimulation on mean heart rate during the second half of the first marksmanship task (β = -2.64, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01), the first (β = -3.19, p_{unadj} = 0.01, p_{adj} = n.s., d < 0.01) and second halves of the second marksmanship task (β = -3.76, p_{unadj} < 0.01, p_{adj} = 0.03, d < 0.01), the third cognitive task (β = -3.39, p_{unadj} = 0.01, p_{adj} = n.s., d < 0.01), and the fourth cognitive task (β = -2.87, p_{unadj} = 0.02, p_{adj} = n.s., d < 0.01). At each time point, active TES was associated with higher mean heart rates. There were also significant effects of stimulation on ECG mean R-R interval during the first (β = 27.71, p_{unadj} = 0.048, p_{adj} = n.s., d = 0.01) and second halves of the second marksmanship task (β = 38.64, p_{unadj} = 0.01, p_{adj} = n.s., d = 0.01), the third cognitive task (β = 41.59, p_{unadj} < 0.01, p_{adj} = 0.03, d = 0.01), and the fourth cognitive task (β = 35.19, p_{unadj} = 0.01, p_{adj} = n.s., d = 0.01). Active TES was associated with lower mean R-R intervals at each significant point.

Furthermore, there was a significant effect of stimulation on ECG SDNN (β = 6.57, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01) and RMSSD (β = 9.92, p_{unadj} = 0.02, p_{adj} = n.s., d < 0.01) during the third cognitive task, in which active TES was associated with lower SDNN and lower RMSSD. Finally, there was a significant time by condition effect on ECG pNN50 during the first (β = 6.72, p_{unadj} = 0.02, p_{adj} = n.s., d < 0.01) and second halves of the first marksmanship task (β = 6.47, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01), the first (β = 6.44, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01) and second halves of the second marksmanship task (β = 7.55, p_{unadj} = 0.01, p_{adj} = n.s., d < 0.01), the third cognitive task (β = 8.09, p_{unadj} = 0.01, p_{adj} = n.s., d < 0.01), the final rest period (β = 6.41, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01), and the fourth cognitive task (β = 6.04, p_{unadj} = 0.04, p_{adj} = n.s., d < 0.01). At each time point, active TES was associated with lower pNN50.

Time-series results from the PPG data largely support those from the ECG data (**Appendix D**). There were significant effects of measurement time point on mean heart rate (p_{unadj} range < 0.01 – 0.01), mean R-R interval (p_{unadj} range < 0.01 – 0.046), RMSSD (p_{unadj} = 0.04), and pNN50 (p_{unadj} range 0.01 – 0.04). These effects indicated that heart rate increased while R-R interval and pNN50 decreased during the marksmanship stress sessions while RMSSD increased during the final rest session (**Table 10**). Unlike when using ECG, there was a significant effect of stimulation condition on pNN50 when using PPG (β = --4.97, p_{unadj} = 0.04, p_{adj} = n.s., d < 0.01) in which active TES was associated with a higher pNN50. However, there were no other PPG time-series outcomes in which there was a significant effect of stimulation condition (p_{unadj} range 0.21 – 0.34).

In addition, there were no significant effects of the interaction between stimulation condition and measurement time point for SDNN or RMSSD when using PPG (p_{unadj} range 0.07 – 0.90). There were, however, significant time by condition effects for mean heart rate during the second half of the first marksmanship task (β = -3.33, p_{unadj} = 0.046, p_{adj} = n.s., d < 0.01), the first (β = -6.24, p_{unadj} < 0.01, p_{adj} < 0.01, d < 0.01) and second halves of the second marksmanship task (β = -5.73, p_{unadj} < 0.01, p_{adj} = 0.01, d < 0.01), and the fourth cognitive task (β = -3.57, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01). In each case, active TES was associated with higher mean heart rates. Active TES also was associated with lower mean R-R intervals the first (β = 69.27, p_{unadj} < 0.01, p_{adj} = 0.01., d = 0.02) and second halves of the second marksmanship task (β = 52.22, p_{unadj} = 0.01, p_{adj} = n.s., d = 0.02), and the fourth cognitive task (β = 45.84, p_{unadj} = 0.03, p_{adj} = n.s., d = 0.02). There was also a significant time by condition effect for pNN50 in which active TES lowered pNN50 during the first marksmanship task (β = 7.31, p_{unadj} = 0.02, p_{adj} = n.s., d < 0.01), the second half of the second marksmanship task (β = 6.37, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01), and the third (β = 7.01, p_{unadj} = 0.02, p_{adj} = n.s., d < 0.01) and fourth (β = 6.69, p_{unadj} = 0.03, p_{adj} = n.s., d < 0.01) cognitive tasks.

Frequency-series data. Significant effects of measurement time point were noted on ECG low (p_{unadj} range 0.01 - 0.03) and high frequency relative powers (p_{unadj} range < 0.01 - 0.03), as well as high frequency absolute power (p_{unadj} range < 0.01 - 0.03) and the ratio of low- to high-frequency power (p_{unadj} range < 0.01 - 0.01). In general, marksmanship stress was associated with increased indices of SNS activity while rest periods were associated with decreased SNS activity (**Table 11**). There were significant, overall effects

of stimulation condition on ECG high frequency absolute power (β = 8.50, $p_{unadj} < 0.01$, $p_{adj} = 0.01.$, $d < 0.01$; **Appendix E**) and relative power (β = -8.54, $p_{unadj} < 0.01$, $p_{adj} = 0.01.$, $d < 0.01$) in which active TES was associated with an increase in high frequency absolute power and a decrease in high frequency relative power compared to sham TES. There was also a small significant effect of stimulation condition on the ratio of low- to high-frequency power in which active TES increased the ratio (β = 0.89, $p_{unadj} = 0.04$, p_{adj} =n.s., $d < 0.01$). However, there were no effects of stimulation condition on ECG total absolute power, very low frequency absolute power, low frequency absolute power, or low frequency relative power (p_{unadj} range 0.07 - 0.96).

In contrast, there were significant interaction effects of stimulation condition and measurement time point for ECG total absolute power during the first (β = 1153.18, $p_{unadj} = 0.03$, p_{adj} = n.s., $d = 0.52$) and third (β = 1421.58, $p_{unadj} = 0.01$, p_{adj} = n.s., $d = 0.52$) cognitive tasks. Similarly, there were significant interaction effects on ECG low frequency relative power during the first (β = 493.32, $p_{unadj} = 0.02$, p_{adj} = n.s., $d = 0.21$) and third (β = 717.17, $p_{unadj} = 0.01$, p_{adj} = n.s., $d = 0.21$) cognitive tasks. There were also significant effects of the interaction between condition and time on ECG high frequency absolute and relative powers during all blocks (p_{unadj} range < 0.01 – 0.03, $d < 0.01$ for all) except during the stimulation procedure (p_{unadj} 0.08 and 0.07, respectively). Active TES was associated with an increase in high frequency absolute power but decreases in high and low frequency relative powers. There were no significant effects of the interaction between stimulation condition and measurement time point on ECG very low or low frequency absolute power at any time point (p_{unadj} range 0.07 – 0.96). However, there was

a significant effect of the interaction between stimulation condition and measurement time point for the ratio of low- to high-frequency power during all blocks (p_{unadj} range < 0.01 – 0.045, d < 0.01 for all) except during the stimulation procedure (p_{unadj} = 0.51) and the first (p_{unadj} = 0.28) and second (p_{unadj} = 0.07) cognitive tests. Active TES was associated with higher ratios of low- to high-frequency power.

Effects of measurement time point on PPG frequency-series outcomes mirrored those using ECG (**Table 12**). There were significant effects of time on very low frequency absolute power (p_{unadj} range < 0.01 – 0.04), high frequency absolute and relative powers (p_{unadj} range 0.01 - 0.02), low frequency relative power during the final rest period (p_{unadj} = 0.02), and the ratio of low- to high-frequency power during final cognitive test (p_{unadj} = 0.03). However, there were no significant effects of stimulation condition or the interaction of condition and time on total absolute power (p_{unadj} range 0.19 – 0.52), low (p_{unadj} range 0.16 – 0.85) or high frequency absolute powers (p_{unadj} range 0.09 – 0.95), low (p_{unadj} range 0.07 – 0.97) or high frequency relative powers (p_{unadj} range 0.09 – 0.95), or on the ratio of low- to high-frequency power (p_{unadj} range 0.07 – 0.99). In contrast, there was a small, significant effect of stimulation condition on PPG very low frequency absolute power (β = -367.98, p_{unadj} = 0.01, p_{adj} = 0.01., d = 0.12; **Appendix F**). There were also significant effects of stimulation on PPG very low frequency absolute power during all blocks (p_{unadj} range < 0.01 – 0.048, d = 0.16 for all; **Figure 10**) except the first half of the first marksmanship task (p_{unadj} = 0.19) and the final rest period (p_{unadj} = 0.63). During each block, active TES was associated with lower very low frequency power.

OUTCOME	TIME POINT (MINUTES)	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
MEAN HEART RATE (BEATS/MIN)	Baseline	74.10	68.70	79.60	75.80	69.90	81.70
	Stimulation	73.40	68.00	78.80	75.50	69.60	81.40
	Rest 1	73.80	68.40	79.20	75.00	69.10	80.90
	Cognitive test 1	74.30	68.90	79.70	75.00	69.10	80.90
	1st half of marksmanship session 1	79.40	74.00	84.90	79.10	73.20	85.00
	2nd half of marksmanship session 1	80.00	74.60	85.40	79.00	73.10	84.80
	Cognitive test 2	70.10	64.70	75.50	69.70	63.80	75.60
	1st half of marksmanship session 2	77.90	72.40	83.30	76.30	70.40	82.20
	2nd half of marksmanship session 2	79.20	73.80	84.60	77.00	71.10	82.90
	Cognitive test 3	69.90	64.50	75.30	68.10	62.20	74.00
	Rest 2	67.30	61.90	72.70	66.60	60.70	72.50
	Cognitive test 4	68.90	63.50	74.40	67.70	61.80	73.60
MEAN R-R INTERVAL (MS)	Baseline	826.00	765.00	887.00	812.00	751.00	872.00
	Stimulation	835.00	774.00	895.00	810.00	750.00	871.00
	Rest 1	827.00	766.00	888.00	819.00	758.00	880.00
	Cognitive test 1	823.00	762.00	884.00	821.00	760.00	881.00
	1st half of marksmanship session 1	771.00	711.00	832.00	772.00	712.00	833.00
	2nd half of marksmanship session 1	768.00	707.00	829.00	773.00	712.00	833.00
	Cognitive test 2	874.00	813.00	934.00	885.00	824.00	945.00
	1st half of marksmanship session 2	790.00	729.00	850.00	803.00	742.00	863.00
	2nd half of marksmanship session 2	774.00	713.00	835.00	798.00	737.00	859.00
	Cognitive test 3	878.00	817.00	939.00	905.00	844.00	966.00
	Rest 2	909.00	848.00	970.00	919.00	858.00	980.00
	Cognitive test 4	885.00	825.00	946.00	906.00	845.00	967.00
SDNN (MS)	Baseline	52.80	42.10	63.40	52.60	40.20	65.10
	Stimulation	51.90	41.20	62.60	49.60	37.20	62.00
	Rest 1	54.30	43.60	64.90	52.60	40.20	65.00
	Cognitive test 1	54.60	44.00	65.30	57.20	44.80	69.70
	1st half of marksmanship session 1	50.90	40.30	61.60	53.70	41.20	66.10
	2nd half of marksmanship session 1	53.00	42.40	63.60	55.80	43.40	68.20
	Cognitive test 2	60.80	50.20	71.50	65.60	53.20	78.10
	1st half of marksmanship session 2	54.90	44.30	65.50	59.30	46.90	71.80

	2nd half of marksmanship session 2	56.10	45.40	66.70	58.80	46.30	71.20
	Cognitive test 3	61.00	50.40	71.60	67.40	55.00	79.90
	Rest 2	61.70	51.00	72.40	62.40	50.00	74.90
	Cognitive test 4	65.70	55.00	76.40	68.00	55.50	80.40
	Baseline	49.60	36.20	63.00	48.10	31.70	64.50
	Stimulation	46.60	33.20	60.00	42.40	26.00	58.80
	Rest 1	45.80	32.40	59.20	46.50	30.10	62.90
	Cognitive test 1	48.90	35.50	62.30	51.00	34.60	67.40
	1st half of marksmanship session 1	35.00	21.60	48.40	37.70	21.30	54.10
	2nd half of marksmanship session 1	35.60	22.30	49.00	38.30	21.90	54.70
RMSSD (MS)	Cognitive test 2	58.40	45.00	71.80	62.90	46.50	79.30
	1st half of marksmanship session 2	39.10	25.70	52.40	43.20	26.80	59.70
	2nd half of marksmanship session 2	38.60	25.20	52.00	42.20	25.70	58.60
	Cognitive test 3	58.50	45.10	71.90	66.90	50.50	83.30
	Rest 2	58.30	44.80	71.70	61.40	45.00	77.80
	Cognitive test 4	59.90	46.40	73.40	64.80	48.30	81.20
	Baseline	25.40	18.67	32.20	20.80	13.37	28.30
	Stimulation	22.80	16.07	29.60	18.00	10.57	25.40
	Rest 1	21.00	14.25	27.80	20.90	13.47	28.30
	Cognitive test 1	22.50	15.68	29.20	22.10	14.67	29.50
	1st half of marksmanship session 1	12.00	5.19	18.70	14.00	6.61	21.50
	2nd half of marksmanship session 1	12.20	5.43	19.00	14.00	6.60	21.50
PNN50 (%)	Cognitive test 2	27.00	20.13	33.80	27.80	20.39	35.20
	1st half of marksmanship session 2	15.50	8.75	22.30	17.30	9.89	24.70
	2nd half of marksmanship session 2	14.30	7.49	21.00	17.20	9.74	24.60
	Cognitive test 3	26.50	19.72	33.20	29.90	22.51	37.40
	Rest 2	28.70	21.84	35.50	30.40	22.98	37.90
	Cognitive test 4	28.00	21.15	34.90	29.40	21.97	36.90

Table 9. Least squares means. Means and 95% CIs for time-series ECG outcome measures.

Abbreviations and Symbols: 95% CI = 95% confidence interval; HR = heart rate; pNN50 = proportion of successive R-R intervals that differ by more than 50 msec, divided by the total number of R-R intervals; RMSSD = root mean square of the successive differences; SDNN = standard deviation of normal R-R intervals.

OUTCOME	TIME POINT (MINUTES)	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
MEAN HEART RATE (BEATS/MIN)	Baseline	70.00	65.30	74.70	72.00	67.20	76.90
	Stimulation	69.10	64.50	73.80	70.80	66.00	75.70
	Rest 1	69.70	65.00	74.30	70.60	65.80	75.50
	Cognitive test 1	70.40	65.80	75.10	70.50	65.60	75.30
	1st half of marksmanship session 1	75.70	71.00	80.30	76.60	71.80	81.40
	2nd half of marksmanship session 1	76.70	72.10	81.40	75.40	70.60	80.30
	Cognitive test 2	66.80	62.10	71.40	67.00	62.20	71.80
	1st half of marksmanship session 2	75.40	70.80	80.10	71.20	66.40	76.10
	2nd half of marksmanship session 2	76.30	71.60	80.90	72.60	67.70	77.40
	Cognitive test 3	66.50	61.80	71.10	67.20	62.40	72.10
	Rest 2	65.30	60.60	69.90	65.50	60.70	70.40
	Cognitive test 4	66.20	61.50	70.80	64.60	59.80	69.50
MEAN R-R INTERVAL (MSEC)	Baseline	881.00	825.00	937.00	858.00	804.00	911.00
	Stimulation	890.00	834.00	945.00	876.00	823.00	930.00
	Rest 1	880.00	825.00	935.00	882.00	828.00	935.00
	Cognitive test 1	870.00	815.00	925.00	881.00	828.00	935.00
	1st half of marksmanship session 1	824.00	769.00	879.00	807.00	754.00	861.00
	2nd half of marksmanship session 1	816.00	761.00	871.00	824.00	771.00	878.00
	Cognitive test 2	925.00	870.00	980.00	928.00	875.00	982.00
	1st half of marksmanship session 2	827.00	772.00	882.00	873.00	819.00	927.00
	2nd half of marksmanship session 2	820.00	765.00	875.00	849.00	795.00	903.00
	Cognitive test 3	926.00	871.00	981.00	920.00	867.00	974.00
	Rest 2	939.00	884.00	994.00	936.00	882.00	989.00
	Cognitive test 4	933.00	878.00	988.00	956.00	902.00	1009.00
SDNN (MSEC)	Baseline	64.30	56.80	71.80	60.90	56.60	65.20
	Stimulation	63.40	56.20	70.50	62.10	57.80	66.30
	Rest 1	63.80	56.70	70.90	64.90	60.60	69.10
	Cognitive test 1	63.40	56.20	70.50	66.40	62.10	70.60
	1st half of marksmanship session 1	61.10	54.00	68.20	62.90	58.60	67.10
	2nd half of marksmanship session 1	64.10	57.00	71.20	61.70	57.50	66.00
	Cognitive test 2	64.70	57.60	71.80	61.90	57.70	66.20
	1st half of marksmanship session 2	60.90	53.80	68.00	65.30	61.00	69.60

	2nd half of marksmanship session 2	62.50	55.40	69.60	68.00	63.70	72.30
	Cognitive test 3	64.30	57.20	71.40	66.40	62.20	70.70
	Rest 2	75.70	68.60	82.80	67.40	63.20	71.70
	Cognitive test 4	64.50	57.40	71.60	67.80	63.60	72.10
	Baseline	53.10	43.60	62.50	49.00	43.40	54.50
	Stimulation	51.40	42.30	60.50	48.60	43.00	54.10
	Rest 1	50.80	41.70	59.80	50.30	44.70	55.80
	Cognitive test 1	52.10	43.10	61.20	52.50	46.90	58.00
	1st half of marksmanship session 1	47.60	38.60	56.60	50.20	44.70	55.80
RMSSD (MSEC)	2nd half of marksmanship session 1	49.20	40.20	58.30	48.80	43.20	54.30
	Cognitive test 2	54.80	45.80	63.80	51.70	46.20	57.20
	1st half of marksmanship session 2	49.30	40.20	58.30	51.10	45.50	56.60
	2nd half of marksmanship session 2	50.60	41.50	59.60	52.60	47.10	58.20
	Cognitive test 3	54.60	45.50	63.60	56.60	51.10	62.20
	Rest 2	70.50	61.50	79.60	60.90	55.30	66.40
	Cognitive test 4	58.90	49.90	68.00	59.70	54.10	65.20
	Baseline	30.30	25.10	35.50	25.30	20.30	30.30
	Stimulation	28.50	23.40	33.60	24.30	19.30	29.30
	Rest 1	27.60	22.50	32.60	26.40	21.40	31.40
	Cognitive test 1	27.60	22.50	32.60	27.60	22.60	32.60
	1st half of marksmanship session 1	23.10	18.10	28.10	25.50	20.50	30.40
PNN50 (%)	2nd half of marksmanship session 1	24.60	19.60	29.60	24.70	19.70	29.70
	Cognitive test 2	28.90	23.90	34.00	27.00	22.10	32.00
	1st half of marksmanship session 2	24.70	19.70	29.80	25.60	20.60	30.70
	2nd half of marksmanship session 2	25.40	20.40	30.40	26.80	21.80	31.80
	Cognitive test 3	28.80	23.80	33.80	30.80	25.80	35.80
	Rest 2	35.20	30.20	40.20	35.10	30.10	40.10
	Cognitive test 4	32.20	27.10	37.20	33.90	28.90	38.90

Table 10. Means and 95% CIs for time-series PPG outcome measures.

Abbreviations and Symbols: 95% CI = 95% confidence interval; HR = heart rate; msec = milliseconds; pNN50 = proportion of successive R-R intervals that differ by more than 50 msec, divided by the total number of R-R intervals; RMSSD = root mean square of the successive differences; SDNN = standard deviation of normal R-R intervals.

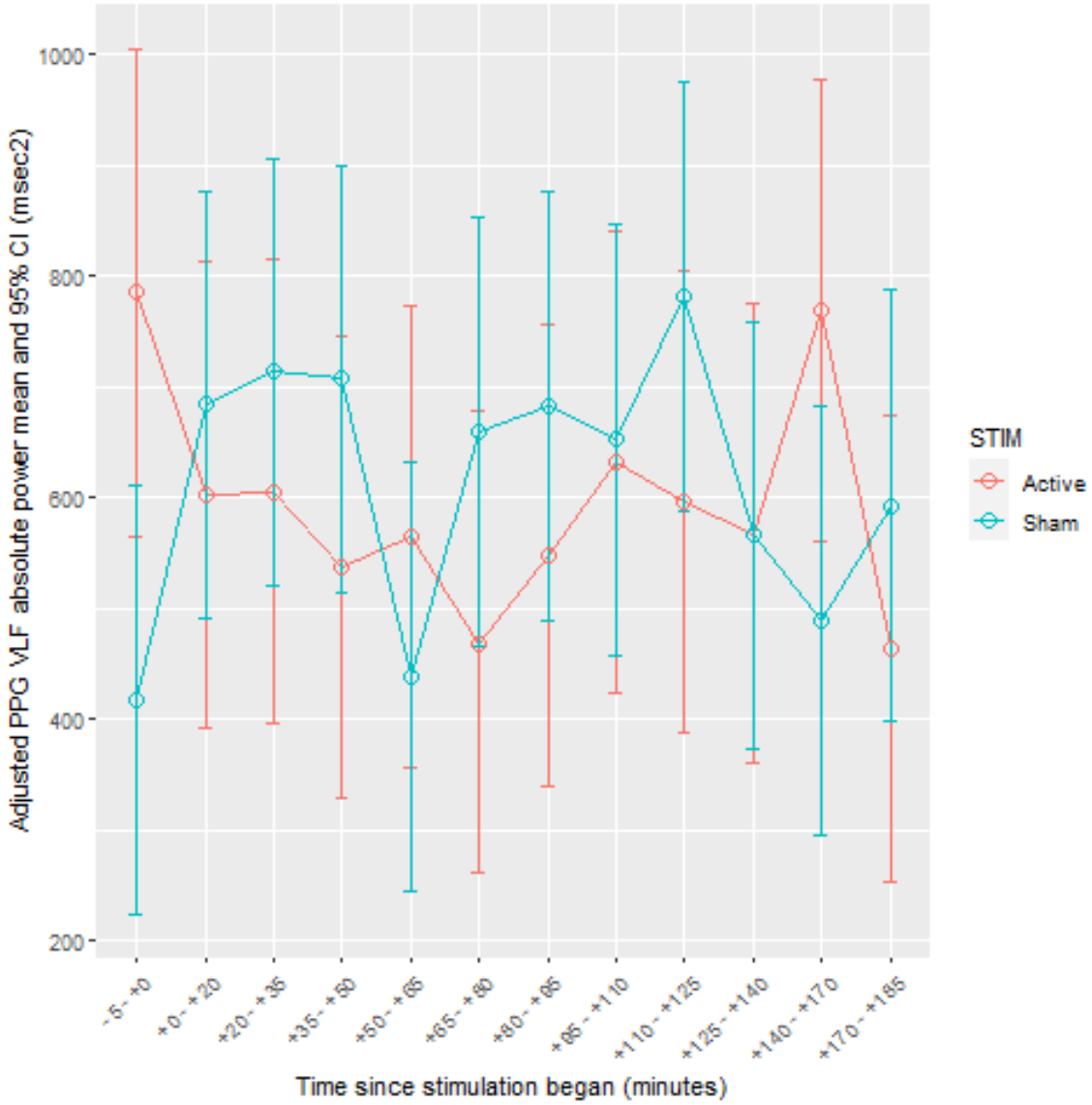


Figure 10. Trajectory of very low frequency absolute power showing the effect of stimulation condition.
Abbreviations: 95% CI = 95% confidence interval; msec2 = milliseconds squared; STIM = stimulation condition; VLF = very low frequency.

OUTCOME	TIME POINT (MINUTES)	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
VERY LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	145.00	70.80	220.00	144.00	54.40	234.00
	Stimulation	147.00	72.20	221.00	150.00	60.30	239.00
	Rest 1	180.00	105.90	254.00	199.00	109.20	288.00
	Cognitive test 1	202.00	127.70	276.00	238.00	148.80	327.00
	1^{st} half of marksmanship session 1	198.00	124.00	273.00	199.00	109.90	289.00
	2^{nd} half of marksmanship session 1	202.00	127.80	276.00	235.00	146.00	325.00
	Cognitive test 2	223.00	148.30	298.00	248.00	158.30	337.00
	1^{st} half of marksmanship session 2	217.00	142.80	291.00	295.00	205.50	384.00
	2^{nd} half of marksmanship session 2	253.00	178.40	327.00	319.00	229.40	408.00
	Cognitive test 3	275.00	200.70	349.00	288.00	198.50	377.00
	Rest 2	218.00	142.80	293.00	222.00	132.60	312.00
	Cognitive test 4	240.00	164.50	315.00	293.00	204.00	383.00
LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	1815.00	965.00	2666.00	1770.00	707.00	2834.00
	Stimulation	1897.00	1047.00	2747.00	1773.00	709.00	2836.00
	Rest 1	2195.00	1348.00	3043.00	1834.00	772.00	2897.00
	Cognitive test 1	1997.00	1148.00	2845.00	2197.00	1136.00	3259.00
	1^{st} half of marksmanship session 1	2018.00	1170.00	2866.00	2327.00	1264.00	3389.00
	2^{nd} half of marksmanship session 1	2271.00	1423.00	3118.00	2547.00	1486.00	3608.00
	Cognitive test 2	2477.00	1625.00	3329.00	3050.00	1989.00	4112.00
	1^{st} half of marksmanship session 2	2336.00	1489.00	3184.00	2735.00	1674.00	3797.00
	2^{nd} half of marksmanship session 2	2443.00	1596.00	3290.00	2559.00	1497.00	3620.00
	Cognitive test 3	2389.00	1542.00	3236.00	3038.00	1977.00	4100.00
	Rest 2	2459.00	1607.00	3310.00	2494.00	1431.00	3556.00
	Cognitive test 4	2867.00	2011.00	3722.00	3085.00	2023.00	4148.00
HIGH FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	61.10	55.40	66.80	69.60	63.80	75.40
	Stimulation	67.10	61.40	72.70	70.70	64.90	76.50
	Rest 1	70.20	64.50	75.80	68.20	62.40	74.00
	Cognitive test 1	65.60	59.90	71.30	68.20	62.40	74.00
	1^{st} half of marksmanship session 1	81.40	75.80	87.10	79.20	73.50	85.00
	2^{nd} half of marksmanship session 1	82.80	77.20	88.50	79.90	74.10	85.60
	Cognitive test 2	65.80	60.10	71.50	65.70	59.90	71.50
	1^{st} half of marksmanship session 2	81.20	75.50	86.90	79.00	73.30	84.80

	2nd half of marksmanship session 2	81.50	75.80	87.10	79.80	74.00	85.60
	Cognitive test 3	65.60	59.90	71.20	63.60	57.80	69.30
	Rest 2	64.90	59.20	70.60	61.70	55.90	67.50
	Cognitive test 4	67.60	61.90	73.40	65.50	59.70	71.30
	Baseline	1234.00	590.30	1878.00	1064.00	212.90	1916.00
	Stimulation	1021.00	376.70	1664.00	866.00	15.10	1718.00
	Rest 1	1004.00	361.90	1647.00	1003.00	152.20	1854.00
	Cognitive test 1	1172.00	528.50	1815.00	1397.00	546.70	2247.00
LOW FREQUENCY RELATIVE POWER (N.U.)	1st half of marksmanship session 1	618.00	-24.90	1260.00	758.00	-92.50	1609.00
	2nd half of marksmanship session 1	653.00	10.50	1295.00	755.00	-95.00	1605.00
	Cognitive test 2	1615.00	970.20	2259.00	1938.00	1087.80	2788.00
	1st half of marksmanship session 2	725.00	82.70	1368.00	912.00	62.00	1762.00
	2nd half of marksmanship session 2	715.00	72.60	1357.00	824.00	-26.20	1674.00
	Cognitive test 3	1608.00	965.80	2250.00	2155.00	1305.00	3005.00
	Rest 2	1446.00	802.00	2091.00	1615.00	764.30	2466.00
	Cognitive test 4	1679.00	1032.70	2326.00	1819.00	967.90	2669.00
	Baseline	38.80	33.10	44.50	30.30	24.50	36.10
	Stimulation	32.90	27.20	38.60	29.30	23.50	35.00
	Rest 1	29.80	24.10	35.50	31.70	26.00	37.50
	Cognitive test 1	34.40	28.70	40.00	31.70	26.00	37.50
HIGH FREQUENCY RELATIVE POWER (N.U.)	1st half of marksmanship session 1	18.50	12.90	24.20	20.70	15.00	26.50
	2nd half of marksmanship session 1	17.10	11.50	22.80	20.10	14.40	25.90
	Cognitive test 2	34.20	28.50	39.90	34.30	28.50	40.10
	1st half of marksmanship session 2	18.80	13.10	24.40	20.90	15.20	26.70
	2nd half of marksmanship session 2	18.50	12.90	24.20	20.20	14.40	25.90
	Cognitive test 3	34.40	28.80	40.10	36.40	30.60	42.20
	Rest 2	35.00	29.30	40.70	38.20	32.50	44.00
	Cognitive test 4	32.40	26.60	38.10	34.50	28.70	40.30
	Baseline	2.22	1.27	3.17	3.12	2.11	4.13
RATIO OF LOW- TO HIGH-FREQUENCY POWER	Stimulation	2.51	1.56	3.46	3.07	2.06	4.08
	Rest 1	2.93	1.98	3.87	2.48	1.47	3.49
	Cognitive test 1	2.51	1.56	3.46	2.86	1.85	3.87
	1st half of marksmanship session 1	4.91	3.96	5.86	4.79	3.78	5.80
	2nd half of marksmanship session 1	5.37	4.42	6.31	4.79	3.79	5.80

Cognitive test 2	2.39	1.43	3.34	2.36	1.35	3.37
1st half of marksmanship session 2	5.41	4.46	6.36	4.84	3.83	5.85
2nd half of marksmanship session 2	5.33	4.39	6.28	5.23	4.22	6.24
Cognitive test 3	2.30	1.36	3.25	2.08	1.07	3.09
Rest 2	2.20	1.24	3.15	1.82	0.81	2.83
Cognitive test 4	2.53	1.57	3.49	2.29	1.27	3.30

Table 11. Least squares means and 95% CIs for frequency-series ECG outcome measures.

Abbreviations and Symbols: 95% CI = 95% confidence interval; ECG = electrocardiography; $msec^2$ = milliseconds squared; n.u. = normal units.

OUTCOME	TIME POINT (MINUTES)	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
VERY LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	785.00	565.00	1005.00	417.00	224.00	610.00
	Stimulation	602.00	392.00	812.00	684.00	491.00	876.00
	Rest 1	605.00	396.00	814.00	713.00	520.00	905.00
	Cognitive test 1	537.00	329.00	745.00	708.00	515.00	900.00
	1^{st} half of marksmanship session 1	564.00	356.00	773.00	438.00	246.00	631.00
	2^{nd} half of marksmanship session 1	469.00	261.00	678.00	660.00	466.00	853.00
	Cognitive test 2	547.00	339.00	755.00	682.00	490.00	875.00
	1^{st} half of marksmanship session 2	633.00	424.00	841.00	652.00	458.00	846.00
	2^{nd} half of marksmanship session 2	597.00	388.00	805.00	781.00	587.00	975.00
	Cognitive test 3	567.00	360.00	775.00	566.00	374.00	759.00
	Rest 2	769.00	561.00	977.00	490.00	296.00	683.00
	Cognitive test 4	464.00	254.00	674.00	593.00	399.00	787.00
LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	2675.00	1502.00	3849.00	2112.00	1698.00	2526.00
	Stimulation	2651.00	1531.00	3771.00	2769.00	2357.00	3181.00
	Rest 1	2775.00	1657.00	3892.00	2978.00	2566.00	3391.00
	Cognitive test 1	2413.00	1296.00	3529.00	2704.00	2293.00	3116.00
	1^{st} half of marksmanship session 1	2561.00	1446.00	3677.00	2765.00	2353.00	3176.00
	2^{nd} half of marksmanship session 1	3011.00	1896.00	4126.00	2598.00	2184.00	3012.00
	Cognitive test 2	2761.00	1646.00	3877.00	2510.00	2099.00	2921.00
	1^{st} half of marksmanship session 2	2400.00	1285.00	3516.00	3003.00	2587.00	3420.00
	2^{nd} half of marksmanship session 2	2665.00	1549.00	3780.00	3212.00	2797.00	3628.00
	Cognitive test 3	2724.00	1609.00	3839.00	2792.00	2381.00	3204.00
	Rest 2	4500.00	3385.00	5615.00	2926.00	2513.00	3338.00
	Cognitive test 4	2418.00	1300.00	3535.00	2903.00	2488.00	3318.00
HIGH FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Baseline	67.80	63.50	72.00	69.80	65.80	73.90
	Stimulation	70.10	66.00	74.30	73.00	69.00	77.10
	Rest 1	71.90	67.80	76.00	73.80	69.70	77.80
	Cognitive test 1	69.00	64.90	73.10	73.30	69.20	77.30
	1^{st} half of marksmanship session 1	76.00	71.90	80.10	74.70	70.70	78.80
	2^{nd} half of marksmanship session 1	76.60	72.50	80.70	74.40	70.40	78.50
	Cognitive test 2	70.50	66.40	74.60	72.20	68.10	76.30

	1st half of marksmanship session 2	74.40	70.30	78.50	75.40	71.30	79.50
	2nd half of marksmanship session 2	75.50	71.40	79.60	77.00	72.90	81.10
	Cognitive test 3	71.90	67.80	76.00	68.90	64.90	73.00
	Rest 2	69.70	65.60	73.80	66.50	62.50	70.60
	Cognitive test 4	69.10	64.90	73.20	69.90	65.80	74.00
	Baseline	798.00	-63.20	1659.00	677.00	446.00	909.00
	Stimulation	788.00	-35.30	1612.00	733.00	502.50	964.00
	Rest 1	795.00	-27.70	1618.00	797.00	565.80	1028.00
	Cognitive test 1	804.00	-18.20	1627.00	877.00	646.90	1108.00
LOW FREQUENCY RELATIVE POWER (N.U.)	1st half of marksmanship session 1	683.00	-139.10	1506.00	760.00	528.70	991.00
	2nd half of marksmanship session 1	750.00	-72.00	1573.00	773.00	541.30	1005.00
	Cognitive test 2	870.00	47.50	1692.00	729.00	498.50	960.00
	1st half of marksmanship session 2	745.00	-77.20	1568.00	795.00	561.60	1028.00
	2nd half of marksmanship session 2	786.00	-36.70	1608.00	794.00	561.20	1027.00
	Cognitive test 3	851.00	28.40	1673.00	984.00	753.60	1215.00
	Rest 2	2345.00	1523.00	3168.00	1210.00	978.90	1442.00
	Cognitive test 4	1061.00	238.20	1885.00	1053.00	820.40	1285.00
	Baseline	32.10	27.90	36.40	30.10	26.00	34.20
	Stimulation	29.80	25.70	34.00	26.90	22.90	31.00
	Rest 1	28.10	24.00	32.20	26.20	22.10	30.30
	Cognitive test 1	30.90	26.80	35.00	26.70	22.70	30.80
HIGH FREQUENCY RELATIVE POWER (N.U.)	1st half of marksmanship session 1	24.00	19.90	28.10	25.20	21.20	29.30
	2nd half of marksmanship session 1	23.40	19.30	27.50	25.50	21.40	29.60
	Cognitive test 2	29.50	25.40	33.60	27.80	23.70	31.80
	1st half of marksmanship session 2	25.60	21.50	29.70	24.50	20.40	28.60
	2nd half of marksmanship session 2	24.40	20.30	28.50	23.00	18.90	27.10
	Cognitive test 3	28.10	24.00	32.20	31.00	27.00	35.10
	Rest 2	30.30	26.20	34.40	33.40	29.30	37.50
	Cognitive test 4	30.90	26.80	35.00	30.00	25.90	34.10
RATIO OF LOW- TO HIGH-FREQUENCY POWER	Baseline	3.61	2.88	4.34	3.61	2.70	4.52
	Stimulation	3.40	2.70	4.11	4.08	3.17	4.99
	Rest 1	3.55	2.86	4.25	3.83	2.92	4.75
	Cognitive test 1	3.13	2.44	3.83	3.60	2.69	4.51
	1st half of marksmanship session 1	4.42	3.73	5.11	3.92	3.01	4.83

2nd half of marksmanship session 1	4.28	3.59	4.98	4.32	3.41	5.24
Cognitive test 2	3.04	2.35	3.73	3.84	2.93	4.75
1st half of marksmanship session 2	3.41	2.72	4.10	4.57	3.65	5.49
2nd half of marksmanship session 2	3.64	2.95	4.34	4.44	3.53	5.36
Cognitive test 3	3.28	2.59	3.97	2.88	1.97	3.79
Rest 2	2.71	2.02	3.40	2.45	1.54	3.36
Cognitive test 4	2.41	1.71	3.11	3.24	2.32	4.15

Table 12. Means and 95% CIs for frequency-series PPG outcome measures.
Abbreviations and Symbols: 95% CI = 95% confidence interval; $msec^2$ = milliseconds squared; n.u. = normal units; PPG = photoplethysmography.

Self-reported physical and cognitive workload. There were no effects of stimulation condition, measurement time point, or their interaction on perceived cognitive workload (p_{unadj} range 0.25 – 0.92; **Appendix G**). In contrast, there was a significant effect of measurement time point on physical workload ($p_{unadj} < 0.01$), in which participants reported greater perceived physical workload following the marksmanship sessions (**Table 13**). There were no significant effects of stimulation condition or the interaction of condition and time on physical workload (p_{unadj} range 0.31 – 0.79).

		ACTIVE			SHAM		
OUTCOME	TIME POINT	**Mean**	**95% CI**		**Mean**	**95% CI**	
COGNITIVE WORKLOAD	Cognitive test 1	36.80	-13.69	87.30	32.40	-18.04	82.80
	Marksmanship session 1	42.10	-8.41	92.60	36.80	-13.59	87.20
	Cognitive test 2	42.10	-8.41	92.60	41.30	-9.15	91.70
	Marksmanship session 2	42.60	-7.85	93.10	31.50	-18.87	81.90
	Cognitive test 3	44.00	-6.46	94.50	39.00	-11.37	89.40
	Cognitive test 4	38.80	-11.74	89.30	37.90	-12.48	88.30
PHYSICAL WORKLOAD	Cognitive test 1	51.20	12.60	89.80	52.00	13.50	90.60
	Marksmanship session 1	70.10	31.50	108.70	66.80	28.20	105.40
	Cognitive test 2	56.20	17.60	94.80	55.70	17.10	94.20
	Marksmanship session 2	69.00	30.40	107.60	65.70	27.10	104.20
	Cognitive test 3	57.90	19.30	96.50	55.40	16.80	94.00
	Cognitive test 4	57.60	19.00	96.20	57.30	18.70	95.90

Table 13. Means and 95% CIs for cognitive and physical workload.
Abbreviations and Symbols: 95% CI = 95% confidence interval.

Aim 2: *Effects of TES on marksmanship performance*

Linear mixed models identified significant effects of measurement time point on shot accuracy (p_{unadj} = 0.046) and target detection latency (p_{unadj} = 0.02) but not on any other measures of marksmanship performance (p_{unadj} range 0.12 – 0.64). These effects demonstrated that shot accuracy and target detection latency each improved from the first to the second marksmanship session (**Table 14**). At the same time, there were medium-sized significant effects of stimulation condition on shot accuracy (β = 0.14, p_{unadj} = 0.01, p_{adj} = n.s., d = 0.60; **Figure 11**) and shot distance from the target's center of mass (β = -0.08, p_{unadj} = 0.02, p_{adj} = n.s., d = 0.56; **Figure 12**), with accuracy decreasing and distance from the center of mass increasing after active TES compared to sham (**Appendix H**). There was no significant effect of stimulation condition on target detection latency (p_{unadj} = 0.07), but there was a significant condition by time effect on target detection latency (β = 220.46, p_{unadj} = 0.04, p_{adj} =n.s., d = 0.49), in which latency was lower after active TES than after sham stimulation during the second marksmanship session. There were no other time by condition interaction effects on marksmanship outcomes (p_{unadj} range 0.06 – 0.97).

OUTCOME	TIME POINT	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
SHOT ACCURACY (%)	Session 1	57.50	47.50	67.50	71.90	66.00	77.80
	Session 2	70.00	60.00	80.10	73.10	67.10	79.00
TARGET DETECTION ACCURACY (%)	Session 1	84.00	72.80	95.20	77.00	64.20	89.90
	Session 2	88.70	77.60	99.90	80.70	67.80	93.50
TARGET DISCRIMINATION ACCURACY (FOR ALL TARGETS PRESENTED) (%)	Session 1	83.00	72.00	94.00	75.80	63.40	88.20
	Session 2	87.40	76.40	98.40	79.90	67.50	92.30
TARGET DISCRIMINATION ACCURACY (FOR ALL TARGETS RESPONDED TO) (%)	Session 1	88.40	80.30	96.60	93.70	90.90	96.40
	Session 2	93.00	84.80	100.20	94.60	91.90	97.40
HIGH VALUE TARGET ACCURACY (%)	Session 1	89.80	83.30	96.30	94.50	92.50	96.50
	Session 2	93.90	87.40	100.40	95.00	93.00	97.00
SHOT LATENCY (MSEC)	Session 1	3197.00	2964.00	3431.00	3074.00	2855.00	3292.00
	Session 2	3105.00	2874.00	3336.00	3084.00	2865.00	3303.00
TARGET DETECTION LATENCY (MSEC)	Session 1	2311.00	1366.00	3255.00	2155.00	1219.00	3091.00
	Session 2	2103.00	1160.00	3046.00	2168.00	1233.00	3103.00
DISTANCE FROM CENTER OF MASS (M)	Session 1	0.31	0.21	0.40	0.23	0.15	0.31
	Session 2	0.27	0.17	0.36	0.23	0.16	0.31

Table 14. Means and 95% CIs for marksmanship outcome measures.
Abbreviations and Symbols: m = meters; msec = milliseconds.

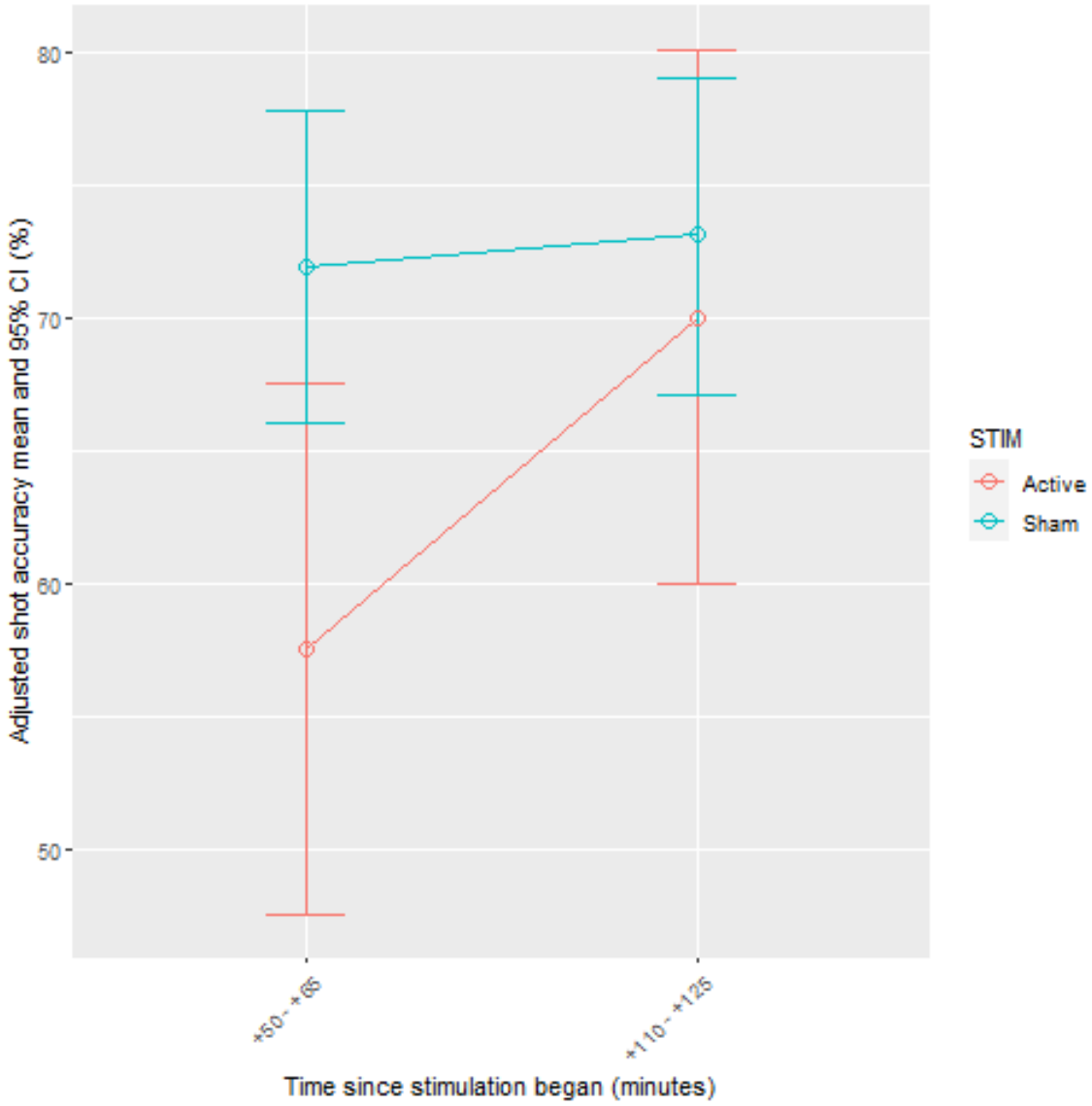


Figure 11. Trajectory of shot accuracy showing the effect of stimulation condition.
Abbreviations and Symbols: 95% CI = 95% confidence interval; STIM = stimulation condition.

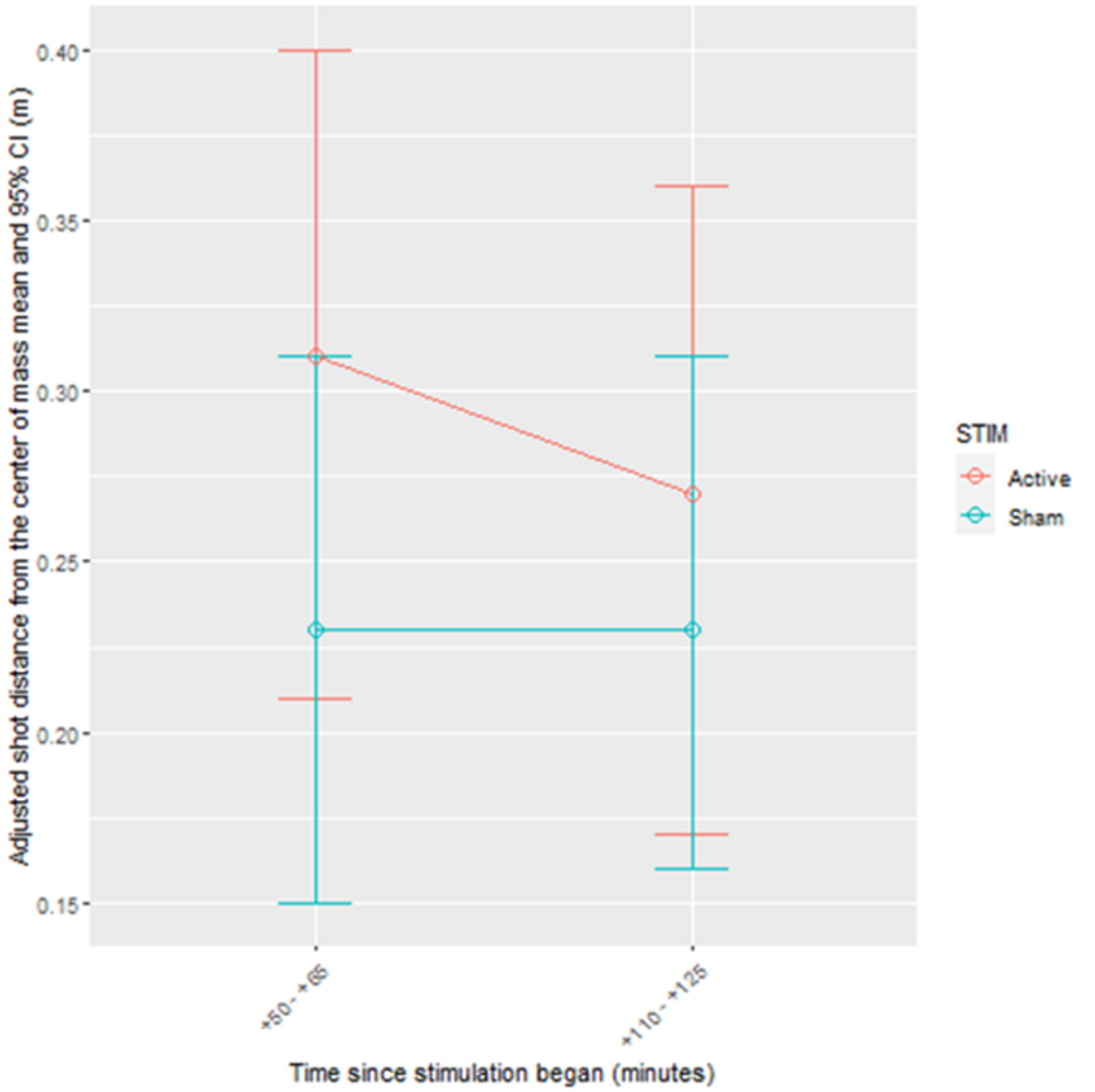


Figure 12. Trajectory of shot distance from the target's center of mass showing the effect of stimulation condition.
Abbreviations and Symbols: 95% CI = 95% confidence interval; STIM = stimulation condition.

Aim 3: *Effects of TES on cognitive performance*

There were no significant effects of stimulation condition, measurement time point, or their interaction on Go/No-Go accuracy or mean response times (p_{unadj} range 0.13 – 0.99; **Table 15, Appendix I**). There were also no significant effects of measurement time point on gradCPT accuracy or response time outcomes (p_{unadj} range 0.15 – 0.99; **Table 16**), but there was a significant effect of stimulation condition (β = 16.29, p_{unadj} = 0.045, p_{adj} = n.s., d = 0.43) on the standard deviation of response times for the gradCPT (**Appendix J**). Active TES was associated with smaller standard deviations of response times compared to sham TES. Additionally, there were no significant effects of stimulation condition or the interaction between condition and time on N-back accuracy (**Table 17, Appendix K**; p_{unadj} range 0.23 – 0.95). However, there were significant effects of time on N-back response times during the third and fourth cognitive test blocks (p_{unadj} range 0.01 – 0.02). At these time points, mean response times decreased compared to those for the previous cognitive test blocks.

Post-hoc: *Effects of personality characteristics and coping skills*

There were no models for which adding personality characteristics or coping skills significantly changed the model results, as measured using Maximum Likelihood Ratio Tests.

OUTCOME	TIME POINT	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
GO/NO-GO ACCURACY (%)	Test 1	88.40	85.40	91.50	89.10	85.70	92.40
	Test 2	88.30	85.30	91.40	88.90	85.40	92.30
	Test 3	88.90	85.90	91.90	86.50	83.10	89.90
	Test 4	87.30	84.30	90.40	86.20	82.80	89.60
GO/NO-GO MEAN RESPONSE TIMES FOR CORRECT RESPONSES (MSEC)	Test 1	341.00	324.00	357.00	345.00	330.00	360.00
	Test 2	341.00	324.00	357.00	341.00	326.00	357.00
	Test 3	343.00	327.00	360.00	335.00	320.00	350.00
	Test 4	343.00	326.00	359.00	334.00	319.00	350.00

Table 15. Means and 95% CIs for Go/No-Go outcome measures.
Abbreviations and Symbols: 95% CI = 95% confidence interval; msec = milliseconds.

OUTCOME	TIME POINT	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
GRADCPT ACCURACY (ERROR RATE, %)	Test 1	8.10	5.15	11.05	7.46	5.14	9.78
	Test 2	7.89	4.94	10.84	8.36	6.07	10.65
	Test 3	7.87	4.89	10.86	9.31	7.02	11.59
	Test 4	7.45	4.47	10.44	7.55	5.26	9.84
GRADCPT COMMISSION ERRORS (ERROR RATE, %)	Test 1	8.10	5.15	11.05	7.46	5.14	9.78
	Test 2	7.89	4.94	10.84	8.36	6.07	10.65
	Test 3	7.87	4.89	10.86	9.31	7.02	11.59
	Test 4	7.45	4.47	10.44	7.55	5.26	9.84
GRADCPT OMISSION ERRORS (ERROR RATE, %)	Test 1	6.88	2.20	11.56	6.33	2.21	10.53
	Test 2	5.90	1.23	10.58	6.15	1.96	10.33
	Test 3	5.77	1.07	10.48	6.81	2.63	11.00
	Test 4	5.01	0.30	9.72	5.27	1.08	9.45
GRADCPT D'	Test 1	2.53	2.00	3.06	2.52	2.05	2.98
	Test 2	2.44	1.91	2.97	2.38	1.92	2.85
	Test 3	2.35	1.82	2.88	2.17	1.71	2.63
	Test 4	2.48	1.95	3.02	2.45	1.99	2.91
GRADCPT MEAN RESPONSE TIMES (MSEC)	Test 1	772.00	725.00	820.00	785.00	732.00	838.00
	Test 2	760.00	712.00	808.00	769.00	716.00	821.00
	Test 3	751.00	704.00	799.00	743.00	690.00	796.00
	Test 4	742.00	694.00	789.00	751.00	699.00	804.00
GRADCPT SD OF RESPONSE TIMES (MSEC)	Test 1	117.00	84.40	149.00	133.00	98.90	168.00
	Test 2	126.00	93.30	158.00	127.00	93.30	162.00
	Test 3	119.00	86.50	151.00	129.00	94.50	163.00
	Test 4	118.00	85.00	150.00	131.00	96.40	165.00

Table 16. Means and 95% CIs for gradCPT outcome measures.
Abbreviations and Symbols: 95% CI = 95% confidence interval; msec = milliseconds.

OUTCOME	TIME POINT	ACTIVE			SHAM		
		Mean	**95% CI**		**Mean**	**95% CI**	
N-BACK ACCURACY FOR 3-BACK RESPONSES (%)	Test 1	85.60	60.90	110.00	85.80	61.10	111.00
	Test 2	87.80	63.10	113.00	86.10	61.40	111.00
	Test 3	87.20	62.50	112.00	85.00	60.30	110.00
	Test 4	83.90	59.20	109.00	84.80	60.10	109.00
N-BACK MEAN RESPONSE TIMES FOR CORRECT RESPONSES ON 3-BACK RESPONSES (MSEC)	Test 1	680.00	608.00	751.00	748.00	650.00	846.00
	Test 2	647.00	574.00	720.00	664.00	567.00	760.00
	Test 3	643.00	571.00	715.00	683.00	587.00	780.00
	Test 4	589.00	518.00	661.00	643.00	547.00	740.00
N-BACK OVERALL ACCURACY (%)	Test 1	80.50	54.50	107.00	80.40	54.30	106.00
	Test 2	84.00	57.90	110.00	82.30	56.30	108.00
	Test 3	84.20	58.20	110.00	85.50	59.50	112.00
	Test 4	81.90	55.80	108.00	82.60	56.50	109.00
N-BACK OVERALL MEAN RESPONSE TIMES FOR CORRECT RESPONSES (MSEC)	Test 1	668.00	582.00	754.00	655.00	567.00	743.00
	Test 2	662.00	574.00	750.00	627.00	541.00	712.00
	Test 3	600.00	514.00	687.00	630.00	545.00	687.00
	Test 4	591.00	504.00	677.00	620.00	634.00	706.00

Table 17. Means and 95% CIs for N-back outcome measures.
Abbreviations and Symbols: 95% CI = 95% confidence interval; msec = milliseconds.

CHAPTER FOUR: DISCUSSION

This study explored the impact of trigeminal TES, applied using the Thync One neurostimulation device, on chemical and physiological markers of the stress response and on cognitive and military marksmanship performance. Analyses found that there was no effect of TES on salivary biomarkers of stress, but that TES is associated with increases in physiological markers of SNS activity, impaired shot accuracy and improved target detection latency. This study is among the first to report the impact of trigeminal TES on SNS activity and is the first to do so in a military population. It is also the first to analyze the effects of TES on marksmanship performance. As such, the results of this work, while preliminary, may provide useful direction for future TES research in this context, as well as military applications of TES more broadly.

Aim 1: Effects of TES on markers of stress

These analyses found that trigeminal TES was not associated with changes in cortisol or α-amylase that would have been representative of HPA and SAM axis modulation, respectively. Although a preprint study demonstrated that trigeminal TES influenced the cortisol awakening response (Boasso et al., 2016), a published study showed that trigeminal TES had no effect on cortisol following an acute laboratory stressor (Tyler et al., 2015). The results from the current study are, therefore, in line with previous findings suggesting that trigeminal TES does not induce decreases in cortisol, as characterized by salivary free cortisol levels, either under stress or during periods of rest. Furthermore, previous studies showed that trigeminal TES increased NE in rodents (Li et

al., 2019) and decreases salivary α-amylase in humans (Tyler et al., 2015). However, the current study found no effect of TES on salivary α-amylase. Because the trigeminal nerve partially innervates the LC, which is the primary source of NE in the brain (Breton-Provencher, Drummond, & Sur, 2021; Contreras et al., 1982), it was hypothesized that TES exerts its effects on cognitive functions (Loo et al., 2019) by directly modulating NE system activity. Nonetheless, the current study's findings suggest that trigeminal TES does not change NE activity in a way that is measurable in saliva.

Previous studies have demonstrated that trigeminal TES influences on the parasympathetic nervous system may also be measured using heart rate variability. TES is associated with improvements in parasympathetic activity in healthy humans (Monaco et al., 2017), but within the context of) indicated by decreases in time- and frequency-series HRV indices after stress (Tyler et al., 2015). In the current study, HRV was measured using both ECG and PPG because there is some suggestion that PPG is more sensitive to the effects of TES than ECG (Gurel, Huang, et al., 2020). Significant effects of trigeminal TES were noted on both time- and frequency-series HRV indices across the testing days using both ECG and PPG, suggesting that TES increased heart rate and decreased HRV indices such as mean R-R interval, RMSSD, and LF and HF relative power. However, TES also increased the LF/HF ratio compared to sham.

While increases in heart rate and the LF/HF power ratio, along with decreases in mean R-R interval, are indicative of increased sympathetic nervous system function, decreases in LF relative power indicate improvements in parasympathetic nervous system function.

Although the observed improvements in PNS function are not significant enough to induce parasympathetic preponderance, as would be indicated by LF/HF ratios less than one, they suggest that PNS activity increased alongside SNS activity in response to TES. Increases in PNS activity may be less dramatic than increased in SNS activity in response to stress, leading to greater sympathetic preponderance in the LF/HF ratio. The finding that trigeminal TES is significantly associated with increases in SNS activity is in direct contrast to the two previously published studies concluding that trigeminal TES improved PNS function (Monaco et al., 2017) and suppressed SNS activity in response to stress (Tyler et al., 2015) in healthy humans. While the two salivary biomarkers of stress explored in this study were unaffected by TES, there are a number of other neurotransmitters that may be affected by TES, including glutamate, which is associated with cardiovascular control (Holstein, Friedrich, & Martinelli, 2016; Neckel et al., 2012) and is released from the NTS (Yamamoto & Mifflin, 2018; Zoccal, Furuya, Bassi, Colombari, & Colombari, 2014) along the trigeminal nerve pathway. Therefore, it is possible that trigeminal TES in the current study influenced cardiovascular function by increasing glutamatergic, rather than noradrenergic, transmission.

As both ECG and PPG measure heart rate variability, it was expected ECG and PPG results obtained from each measure would be similar. HoweverTherefore, it is notable that significant effects of stimulation were noted on pNN50 when measured using PPG but not ECG, and on the LF/HF ratio when measured using ECG but not PPG. Although it is possible that one method of heart rate variability measurement is more sensitive than another, this finding more likely reflects low sample power and/or

considerable variability in the sample data for the affected outcomes. In particular, post-hoc power analyses indicated that the LF/HF ratio was underpowered in the ECG, but not PPG analyses, highlighting the potential for Type I error, as a significant result was found only in the underpowered ECG data and not the sufficiently-powered PPG data. Similarly, pNN50 was underpowered in both the ECG and PPG data and usually correlates with RMSSD, in which there were no significant findings. These findings, therefore, should be interpreted with caution as they potentially reflect Type I error.

Finally, these analyses found no significant effects of trigeminal TES on either cognitive or physical workload, suggesting that TES does not improve or impair perceived effort. However, it is possible that the parasympathetic effects observed in this study after trigeminal TES, combined with the stress induced by the marksmanship task, biased any influence on workload to the null.

Together, these findings do not support our hypotheses that trigeminal stimulation applied using the Thync One device would modulate the HPA and SAM axes. Instead, the current study indicated that stimulation using the Thync One device had no effect on the activity of NE (as indicated by salivary α-amylase measures) or free cortisol levels. However, stimulation with the Thync One device did increase both SNS and PNS activity, pointing to the potential utility of trigeminal TES in increasing PNS activity when used under different circumstances (e.g., during more severe stress, in participants with medical or psychiatric conditions, or when using alternative stimulation parameters).

Aim 2: Effects of TES on marksmanship performance

To date, no studies have explored the effects of TES on marksmanship performance. However, at least one study has demonstrated enhancing effects of TES on attention and response inhibition (McGough et al., 2015), which may influence marksmanship performance. This study is the first to demonstrate that trigeminal TES is associated with impairments in shot accuracy as well as improved target detection latency. The findings regarding target detection latency are remarkable because latency was influenced by time and an interaction between stimulation and time. However, the observed effect on latency may be the result of strong effects of time rather than an improvement due to stimulation condition because latency was significantly greater in the active TES condition compared to sham during the first marksmanship session but not the second session. The direction of the relationship between the two groups suggests that active TES improved target detection latency while sham TES did not. Target detection latency was stable across both marksmanship sessions during the sham condition. During the active TES condition, target detection latency was relatively long compared to sham during the first marksmanship session but normalized by the second session. These findings suggest that active TES initially impaired response speed. This finding may be due to three possible reasons: first, that performance improved as a function of a) practice effects, as supported by the finding of a significant effect of time on target detection latency, b) habituation or compensation for the negative effects of TES by the second marksmanship session, or c) wearing off of the negative effects of TES by the second marksmanship session. Notably though, the effects of TES on SNS activity were still

present during the second marksmanship session, providing evidence that at least some TES effects on physiological stress were still present. Additionally, these findings may be the result of increased glutamatergic or noradrenergic activity along with decreased activity of LC GABAergic interneurons (Jin et al., 2016; Breton-Provencher & Sur, 2019). GABA levels in the anterior cingulate cortex and dorsolateral PFC are associated with better decision making and slower responses (Durazzo & Meyerhoff, 2020), while activity of glutamate in rodents and of NE genes is associated with faster, but not necessarily more accurate, choices (Floresco, Tse, & Ghods-Sharifi, 2007; Kim et al., 2013). By extension, it is possible that TES may increase the release of NE from the LC to increase response speed; however, the mechanisms underlying these findings is currently unknown.

Aim 3: Effects of TES on cognitive performance

Studies have demonstrated that trigeminal TES is associated with improvements in working memory (Loo et al., 2021), attention, and response inhibition (McGough et al., 2015) in individuals with ADHD. However, the tVNS literature suggests that some level of impairment may be necessary at baseline to see such effects. The current study sought to evaluate the effects of trigeminal TES on healthy cognition and revealed that across the testing day, TES had no significant effects on accuracy or response speeds on tasks of sustained attention (gradCPT), working memory (N-back), and response inhibition (Go/No-Go). Only the standard deviation of response times for the gradCPT was affected by stimulation; response times were less variable after trigeminal TES

compared to sham stimulation. The lack of effects of TES on response speed-related outcomes parallels the lack of effect of TES on salivary α-amylase. This supports the idea that effects of TES may not depend on NE exclusively, as activation of the LC might be expected to produce an increase in NE and an increase in arousal in association with improved response times. The findings instead suggest that arousal was unaffected by TES. Other neurotransmitter systems, including GABAergic and glutamatergic systems, might be involved given that LC activity is influenced both by GABAergic interneurons (Jin et al., 2016; Breton-Provencher & Sur, 2019) and glutamatergic inputs (Fung, Reddy, Liu, Wang, & Barnes, 1994) that can inhibit or disinhibit LC noradrenergic neurons. In the present study, GABA activity was not evaluated, thus we are unable to test this hypothesis directly.

Additionally, the unexpected absence of TES effects on attention may be the result of high levels of tonic, rather than phasic, activity of the LC because tonic LC activity is associated with poor attentional performance, while phasic LC activity is associated with good attentional performance (Aston-Jones et al., 1999). Phasic activity of the LC is associated with release of NE from LC terminals (Janitzky, 2020) and increased information salience (Vazey, Moorman, & Aston-Jones, 2018). A balance between tonic and phasic activity is required to optimize attention and prevent under- or over-arousal at any given point in time (Howells, Stein, & Russell, 2012). If TES increases tonic activity, arousal and attention may be impaired, resulting in increased target detection latency as seen in these analyses. As with GABA, measures of tonic and phasic activity of the LC were not directly assessed in this study.

Post-hoc Analyses: Effects of personality characteristics and coping skills

Personality factors, such as hardiness and coping skills, were evaluated in this study for their potential to modulate participant's response to TES during a stressful marksmanship task. Previous studies have shown that the effect of tVNS on reward discounting is modulated by level of positive mood (low positive mood or high positive mood; Steenbergen, Colzato, & Maraver, 2020), suggesting that mood states may alter the effects of TES on cognitive functions. Results from post-hoc analyses found that adding personality traits into statistical models had no significant effect on the study outcomes. Thus, it appears that personality characteristics may not play a significant role in modulating the effects of trigeminal TES.

Findings in the context of the military

The military is exploring the use of trigeminal TES using the Thync One device for performance enhancement and recovery from stress in largely healthy individuals. However, the current study found that trigeminal stimulation applied by the Thync One device was not associated with changes in HPA or SAM axis activity or improvement in marksmanship or cognitive performance. This study did indicate that trigeminal TES compared to sham stimulation was associated with increases in SNS and PNS activity in response to stress.

The SNS response is intended to enable a human to respond quickly and efficiently to a stressor. Based on data from the current study, the utility of the Thync

One device specifically, and for TES more generally, for applications related to performance enhancement may be limited given observed increases in SNS response without coinciding improvements in performance. Impairments in shot accuracy seen in the context of increases in SNS activity in the current study would not be favorable in operational (combat) contexts.

Nevertheless, the Thync One device is only one of several commercially available devices by which trigeminal TES might be applied. Further evaluation of the device's effects on performance and stress response is significantly limited by the fact that the specific stimulation parameters delivered by the device are proprietary. Moreover, this study evaluated only a small number of the potential mechanisms of the effects of trigeminal TES on performance and stress response. Further research is required to identify the exact neuronal impact that stimulation using this device might have using methods such as magnetic resonance spectroscopy and other measures of functional brain activity. The positive effects on PNS function observed in the current study, for example, suggest that vagus nerve activity was increased by stimulation. Therefore, it is possible that the Thync One device not only activated the trigeminal nerve but also the vagus nerve. Finally, these effects were noted under moderate stress and do not represent the effects that the Thync One device, or trigeminal TES more generally, might have under more or less stressful conditions, such as rest. It is also important to note that the proprietary Thync One program used in this study was originally developed and marketed to increase a sense of calm and reduce sleep latency. Thus, it is possible that under a different stress scenario, the effects of TES using this device might have been different.

Therefore, it is critical that the results of this study be viewed as preliminary and specific to this particular marksmanship scenario.

Strengths, limitations, and future directions

This study had several limitations, including a smaller sample size than anticipated and as a result, possible low statistical power that may obscure true effects of TES. In addition, the electrical stimulation parameters of the Thync One device are proprietary. Therefore, one cannot speculate about the impact of using different levels of TES intensity or frequency on the outcomes analyzed. Furthermore, the findings may not be generalizable to the greater population, including the larger military, as it is overwhelmingly young, male, and neither racially nor ethnically diverse. In addition, the use of self-report questionnaires can introduce bias due to participant motivation, emotional state or other factors. Finally, these analyses identified significant effects using marksmanship as a performance measure but were largely unable to identify effects on neuropsychological tests that measure comparable cognitive functions. This suggests that complex military tasks, such as marksmanship, may be more sensitive to the effects of TES and that this study may have profited from the inclusion of more military-relevant tasks as performance outcomes. Also, experimental paradigms that more closely mimic the stress that SMs experience, the tasks that they perform, and the consequences that they face if errors are made may be more valid representations of military performance.

Nevertheless, this study also has a number of strengths. Stimulation blinding was achieved, as guesses about the type of stimulation received were 45% accurate (i.e., no better than chance). Sham stimulation was administered by giving participants brief (30

second) bursts of stimulation at the beginning and end of the 20-minute stimulation period and no stimulation in between. This method is intended to blind the participant to the type of stimulation being received and appears to have been effective. It also minimizes the likelihood that sham stimulation will activate the trigeminal nerve and confound the results, as reported previously in studies of tVNS using different methods of sham stimulation, such as earlobe stimulation (Borges et al., 2021). Additionally, the combination of salivary biomarker, physiological, cognitive, and military-relevant performance outcomes provides a more holistic picture of the effects of TES than has previously been described in the literature. The use of traditional neuropsychological tasks to compare and contrast with the marksmanship task also helps in understanding the mechanisms of TES. Moreover, the addition of personality and coping characteristics to statistical models to predict outcomes is novel and presents a unique perspective on the effects of TES, which may be stronger or weaker in certain individuals depending on their pre-existing characteristics. Finally, the rigor of the study's exclusion criteria, statistical methods, and restrictions on pre-study activities (e.g., no food within two hours of initiating study activities; controlling time of tobacco use) minimize the effects of adventitious physiological perturbations on biomarkers and performance while maximizing the study's ability to identify the true effects of TES in a "clean" sample.

In summary, the current study found that trigeminal TES using the Thync One device influenced physiological markers of stress and marksmanship performance but had no effect on chemical biomarkers or cognitive performance under high cognitive load. TES increased the physiological stress response as measured by heart rate and heart

rate variability, but impaired shot accuracy while improving target detection latency. Results from this preliminary investigation of the effects of trigeminal TES on military-relevant task performance (marksmanship) underscore the need for further evaluation of the biological basis of trigeminal TES, as well as the influence of trigeminal nerve stimulation on the stress response and marksmanship. In addition, future studies involving research-grade stimulation devices that permit greater control over stimulation parameters, timing, and frequency of stimulation are needed. Finally, future studies may wish to use additional military-relevant tasks and diverse operational scenarios to further examine a potential role for TES in military performance enhancement.

APPENDIX A: Exclusionary Criteria

- A history of neurological disorders such as stroke or seizures;
- A history of persistent headaches, migraines, or chronic pain disorders that require regular use of pain medications;
- A history of Temporomandibular Joint Disorder, Bell's Palsy, impaired cranial nerve function, or facial pain;
- A history of cardiovascular disease including hypertension;
- A history of immune or autoimmune conditions including asthma, dermatitis, psoriasis, and cancer;
- A recent history of brain injury or concussion (within the 18 months preceding enrollment);
- A recent history of fever caused by infection (within the 30 days preceding enrollment);
- Any recent illicit drug use (including marijuana);
- A 6-month history of average alcohol consumption greater than 3 drinks per day/7 drinks per week for females or 4 drinks per day/14 drinks per week for males;
- A 6-month history of average caffeine consumption greater than 450mg per day (i.e., more than 4-8 oz. cups of coffee or 4-12 oz. cans of Red Bull);
- For females, a recent history of abnormal or irregular menstrual cycles (not between 26-32 days in duration or not between 5-6 menstrual cycles within the past 6 months preceding enrollment) not due to contraceptive use, or those who have had an IUD placed in the last month or removed in the last 3 months preceding enrollment;
- Susceptibility to fainting or Reflex Syncope as a result of donating blood, receiving a shot, or due to anxiety or panic attacks;
- Any indication of current oral disease;
- A current diagnosis of anxiety disorder, Major Depressive Disorder, or metabolic disorder such as diabetes;

- Metal in their bodies;
- A cardiac pacemaker, implanted defibrillator, implanted neurostimulator, or other implanted metallic or electronic device;
- A condition or injury that limits range of hand or finger motions on either hand;
- A condition or injury that would limit ability to comfortably hold an M4 weapon;
- Cuts, lesions, or other breaks in the skin of the head and neck that interfere with the placement of the TES device;
- Uncorrectable vision or hearing;
- For females of childbearing potential, a reasonable suspicion or confirmation of pregnancy and/or breastfeeding.

APPENDIX B: Mixed Model Results for Saliva Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}		$\|D\|$
SALIVARY CORTISOL (UG/DL)	Stimulation	-0.02	0.02	128	-0.96	0.34				
	Cognitive test 1	-0.03	0.02	110	-1.66	0.10				
	2nd half of marksmanship 1	-0.05	0.02	110	-2.85	0.01	*	n.s.		0.59
	2nd half of marksmanship 2	-0.05	0.02	110	-2.71	0.01	*	n.s.		0.57
	Cognitive test 3	-0.05	0.02	110	-2.90	0.00	*	n.s.		0.60
	Rest 2	-0.06	0.02	110	-3.66	0.00	*	0.04	*	0.76
	Stimulation : Cognitive test 1	-0.01	0.02	128	-0.28	0.78				
	Stimulation : 2nd half of marksmanship 1	0.03	0.02	128	1.25	0.21				
	Stimulation : 2nd half of marksmanship 2	0.03	0.02	128	1.39	0.17				
	Stimulation: Cognitive test 3	0.01	0.02	128	0.47	0.64				
	Stimulation: Rest 2	0.01	0.02	128	0.42	0.67				
SALIVARY A-AMYLASE (U/ML)	Stimulation	-16.56	13.03	127	-1.27	0.21				
	Cognitive test 1	-13.39	15.40	110	-0.87	0.39				
	2nd half of marksmanship 1	-15.99	15.40	110	-1.04	0.30				
	2nd half of marksmanship 2	-26.89	15.58	110	-1.73	0.09				
	Cognitive test 3	-29.35	15.80	110	-1.86	0.07				
	Rest 2	0.40	15.58	110	0.03	0.98				
	Stimulation : Cognitive test 1	0.36	17.33	127	0.02	0.98				
	Stimulation : 2nd half of marksmanship 1	2.60	17.32	127	0.15	0.88				
	Stimulation : 2nd half of marksmanship 2	3.00	17.56	127	0.17	0.86				
	Stimulation: Cognitive test 3	23.48	17.67	127	1.33	0.19				
	Stimulation: Rest 2	5.87	17.56	127	0.33	0.74				
SALIVARY A-AMYLASE (U/MIN)	Stimulation	-75.54	60.67	128	-1.25	0.22				
	Cognitive test 1	2.30	59.79	110	0.04	0.97				
	2nd half of marksmanship 1	-32.13	59.79	110	-0.54	0.59				
	2nd half of marksmanship 2	-8.14	60.57	110	-0.13	0.89				

	Cognitive test 3	26.23	61.39	110	0.43	0.67				
	Rest 2	27.16	60.57	110	0.45	0.65				
	Stimulation : Cognitive test 1	-6.36	79.34	128	-0.08	0.94				
	Stimulation : 2nd half of marksmanship 1	27.84	79.34	128	0.35	0.73				
	Stimulation : 2nd half of marksmanship 2	73.75	79.94	128	0.92	0.36				
	Stimulation: Cognitive test 3	62.29	80.56	128	0.77	0.44				
	Stimulation: Rest 2	111.66	79.94	128	1.40	0.16				
RATIO OF A-AMYLASE TO CORTISOL	Stimulation	21.76	228.28	127	0.10	0.92				
	Cognitive test 1	107.82	178.59	110	0.60	0.55				
	2nd half of marksmanship 1	200.61	178.44	110	1.12	0.26				
	2nd half of marksmanship 2	32.33	180.85	110	0.18	0.86				
	Cognitive test 3	156.24	183.94	110	0.85	0.40				
	Rest 2	459.91	180.87	110	2.54	0.01	*	n.s.	0.53	
	Stimulation : Cognitive test 1	-26.05	278.08	127	-0.09	0.93				
	Stimulation : 2nd half of marksmanship 1	-250.59	277.99	127	-0.90	0.37				
	Stimulation : 2nd half of marksmanship 2	-202.08	280.73	127	-0.72	0.47				
	Stimulation: Cognitive test 3	128.90	281.34	127	0.46	0.65				
	Stimulation: Rest 2	435.91	280.66	127	1.55	0.12				

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; pg/mL = picograms per milliliter; SE = standard error; U/min = units per minute; U/mL = units per milliliter.

APPENDIX C: Mixed Model Results for ECG Time-Series HRV Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}		$\|D\|$
ECG MEAN HR (BEATS/MIN)	Stimulation	1.61	1.57	212	1.03	0.30				
	TES	-1.27	2.11	231	-0.60	0.55				
	Rest 1	0.12	2.12	231	0.05	0.96				
	Cognitive test 1	1.12	2.11	231	0.53	0.60				
	1st half of marksmanship 1	7.29	2.11	231	3.45	< 0.01	*	0.01	*	< 0.01
	2nd half of marksmanship 1	8.46	2.11	231	4.01	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 2	-2.04	2.15	231	-0.95	0.34				
	1st half of marksmanship 2	6.90	2.11	231	3.27	< 0.01	*	0.02	*	< 0.01
	2nd half of marksmanship 2	8.80	2.12	231	4.16	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 3	-0.85	2.12	231	-0.40	0.69				
	Rest 2	-4.52	2.14	231	-2.11	0.04	*	n.s.		< 0.01
	Cognitive test 4	-2.34	2.16	231	-1.08	0.28				
	Stimulation: TES	0.53	1.23	212	0.43	0.67				
	Stimulation: Rest 1	-0.44	1.23	212	-0.36	0.72				
	Stimulation: Cognitive test 1	-0.96	1.23	212	-0.78	0.44				
	Stimulation: 1st half of marksmanship 1	-1.99	1.23	212	-1.62	0.11				
	Stimulation: 2nd half of marksmanship 1	-2.64	1.23	212	-2.14	0.03	*	n.s.		< 0.01
	Stimulation: Cognitive test 2	-2.01	1.24	212	-1.61	0.11				
	Stimulation: 1st half of marksmanship 2	-3.19	1.23	212	-2.58	0.01	*	n.s.		< 0.01
	Stimulation: 2nd half of marksmanship 2	-3.76	1.23	212	-3.06	< 0.01	*	0.03	*	< 0.01
	Stimulation: Cognitive test 3	-3.39	1.23	212	-2.75	0.01	*	n.s.		< 0.01
	Stimulation: Rest 2	-2.32	1.25	212	-1.86	0.06				
	Stimulation: Cognitive test 4	-2.87	1.25	212	-2.31	0.02	*	n.s.		< 0.01
ECG MEAN R-R INTERVAL (MSEC)	Stimulation	-14.51	15.94	212	-0.91	0.36				
	TES	18.21	22.79	231	0.80	0.43				
	Rest 1	-5.74	22.83	231.00	-0.25	0.80				

	Cognitive test 1	-15.18	22.79	231.00	-0.67	0.51				
	1st half of marksmanship 1	-70.16	22.81	231.00	-3.08	< 0.01	*	0.03	*	0.02
	2nd half of marksmanship 1	-77.72	22.80	231.00	-3.41	< 0.01	*	0.01	*	0.02
	Cognitive test 2	22.12	23.21	231.00	0.95	0.34				
	1st half of marksmanship 2	-64.33	22.79	231.00	-2.82	0.01	*	n.s.		0.02
	2nd half of marksmanship 2	-90.83	22.85	231.00	-3.98	< 0.01	*	< 0.01	*	0.02
	Cognitive test 3	10.24	22.84	231.00	0.45	0.65				
	Rest 2	58.58	23.12	231.00	2.53	0.01	*	n.s.		0.02
	Cognitive test 4	24.08	23.38	231.00	1.03	0.30				
	Stimulation: TES	-9.71	13.89	212	-0.70	0.49				
	Stimulation: Rest 1	6.58	13.90	212	0.47	0.64				
	Stimulation: Cognitive test 1	12.08	13.89	212	0.87	0.39				
	Stimulation: 1st half of marksmanship 1	15.47	13.89	212	1.11	0.27				
	Stimulation: 2nd half of marksmanship 1	19.38	13.89	212	1.40	0.16				
	Stimulation: Cognitive test 2	25.40	14.03	212	1.81	0.07				
	Stimulation: 1st half of marksmanship 2	27.71	13.91	212	1.99	0.05	*	n.s.		0.01
	Stimulation: 2nd half of marksmanship 2	38.64	13.89	212	2.78	0.01	*	n.s.		0.01
	Stimulation: Cognitive test 3	41.59	13.89	212	2.99	< 0.01	*	0.03	*	0.01
	Stimulation: Rest 2	24.30	14.04	212	1.73	0.08				
	Stimulation: Cognitive test 4	35.19	14.04	212	2.51	0.01	*	n.s.		0.01
ECG SDNN (MSEC)	Stimulation	-0.16	2.95	212	-0.06	0.96				
	TES	1.27	5.49	231	0.23	0.82				
	Rest 1	2.99	5.50	231	0.54	0.59				
	Cognitive test 1	-0.90	5.49	231	-0.16	0.87				
	1st half of marksmanship 1	-4.77	5.49	231	-0.87	0.39				
	2nd half of marksmanship 1	-2.77	5.49	231	-0.50	0.61				
	Cognitive test 2	3.09	5.59	231	0.55	0.58				
	1st half of marksmanship 2	-2.47	5.49	231	-0.45	0.65				
	2nd half of marksmanship 2	0.38	5.50	231	0.07	0.94				
	Cognitive test 3	1.66	5.50	231	0.30	0.76				

	Rest 2	7.97	5.57	231	1.43	0.15			
	Cognitive test 4	10.51	5.63	231	1.87	0.06			
	Stimulation: TES	-2.14	3.08	212	-0.70	0.49			
	Stimulation: Rest 1	-1.50	3.08	212	-0.49	0.63			
	Stimulation: Cognitive test 1	2.76	3.08	212	0.90	0.37			
	Stimulation: 1st half of marksmanship 1	2.92	3.08	212	0.95	0.34			
	Stimulation: 2nd half of marksmanship 1	2.98	3.08	212	0.97	0.33			
	Stimulation: Cognitive test 2	4.97	3.11	212	1.60	0.11			
	Stimulation: 1st half of marksmanship 2	4.60	3.09	212	1.49	0.14			
	Stimulation: 2nd half of marksmanship 2	2.89	3.08	212	0.94	0.35			
	Stimulation: Cognitive test 3	6.57	3.08	212	2.14	0.03	*	n.s.	< 0.01
	Stimulation: Rest 2	0.93	3.12	212	0.30	0.76			
	Stimulation: Cognitive test 4	2.42	3.12	212	0.77	0.44			
ECG RMSSD (MSEC)	Stimulation	-1.47	4.36	212	-0.34	0.74			
	TES	-0.19	7.51	231	-0.03	0.98			
	Rest 1	-5.94	7.52	231	-0.79	0.43			
	Cognitive test 1	-4.24	7.51	231	-0.56	0.57			
	1st half of marksmanship 1	-18.78	7.52	231	-2.50	0.01	*	n.s.	0.01
	2nd half of marksmanship 1	-18.08	7.51	231	-2.41	0.02	*	n.s.	0.01
	Cognitive test 2	2.82	7.65	231	0.37	0.71			
	1st half of marksmanship 2	-16.19	7.51	231	-2.16	0.03	*	n.s.	0.01
	2nd half of marksmanship 2	-16.03	7.53	231	-2.13	0.03	*	n.s.	0.01
	Cognitive test 3	-1.03	7.53	231	-0.14	0.89			
	Rest 2	4.06	7.62	231	0.53	0.59			
	Cognitive test 4	4.01	7.71	231	0.52	0.60			
	Stimulation: TES	-2.75	4.37	212	-0.63	0.53			
	Stimulation: Rest 1	2.17	4.37	212	0.50	0.62			
	Stimulation: Cognitive test 1	3.58	4.37	212	0.82	0.41			
	Stimulation: 1st half of marksmanship 1	4.18	4.37	212	0.96	0.34			

	Stimulation: 2nd half of marksmanship 1	4.13	4.37	212	0.94	0.35				
	Stimulation: Cognitive test 2	6.00	4.42	212	1.36	0.18				
	Stimulation: 1st half of marksmanship 2	5.66	4.38	212	1.29	0.20				
	Stimulation: 2nd half of marksmanship 2	5.04	4.37	212	1.15	0.25				
	Stimulation: Cognitive test 3	9.92	4.37	212	2.27	0.02	*	n.s.		< 0.01
	Stimulation: Rest 2	4.63	4.42	212	1.05	0.30				
	Stimulation: Cognitive test 4	6.32	4.43	212	1.43	0.15				
ECG PNN50 (%)	Stimulation	-4.64	2.81	212	-1.65	0.10				
	TES	-2.39	5.03	231	-0.47	0.64				
	Rest 1	-8.97	5.04	231	-1.78	0.08				
	Cognitive test 1	-7.27	5.03	231	-1.45	0.15				
	1st half of marksmanship 1	-20.21	5.03	231	-4.02	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 1	-19.72	5.03	231	-3.92	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 2	-3.99	5.11	231	-0.78	0.44				
	1st half of marksmanship 2	-16.37	5.03	231	-3.26	< 0.01	*	0.02	*	< 0.01
	2nd half of marksmanship 2	-18.74	5.04	231	-3.72	< 0.01	*	0.01	*	< 0.01
	Cognitive test 3	-7.06	5.04	231	-1.40	0.16				
	Rest 2	-3.20	5.10	231	-0.63	0.53				
	Cognitive test 4	-3.48	5.15	231	-0.68	0.50				
	Stimulation: TE	-0.21	2.95	212	-0.07	0.94				
	Stimulation: Rest 1	4.53	2.95	212	1.53	0.13				
	Stimulation: Cognitive test 1	4.27	2.95	212	1.45	0.15				
	Stimulation: 1st half of marksmanship 1	6.72	2.95	212	2.28	0.02	*	n.s.		< 0.01
	Stimulation: 2nd half of marksmanship 1	6.47	2.95	212	2.19	0.03	*	n.s.		< 0.01
	Stimulation: Cognitive test 2	5.50	2.98	212	1.84	0.07				
	Stimulation: 1st half of marksmanship 2	6.44	2.96	212	2.18	0.03	*	n.s.		< 0.01
	Stimulation: 2nd half of marksmanship 2	7.55	2.95	212	2.56	0.01	*	n.s.		< 0.01

	Stimulation: Cognitive test 3	8.09	2.95	212	2.74	0.01	*	n.s.	< 0.01
	Stimulation: Rest 2	6.41	2.98	212	2.15	0.03	*	n.s.	< 0.01
	Stimulation: Cognitive test 4	6.04	2.98	212	2.03	0.04	*	n.s.	< 0.01

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; min = minutes; msec = milliseconds; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX D: Mixed Model Results for PPG Time-Series HRV Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}		$\|D\|$
PPG MEAN HR (BEATS/MIN)	Stimulation	2.02	1.61	237	1.25	0.21				
	TES	-0.54	2.69	242	-0.20	0.84				
	Rest 1	0.73	2.69	242	0.27	0.79				
	Cognitive test 1	2.36	2.69	242	0.88	0.38				
	1st half of marksmanship 1	6.71	2.72	242	2.46	0.01	*	n.s.		< 0.01
	2nd half of marksmanship 1	10.04	2.71	242	3.71	< 0.01	*	0.01	*	< 0.01
	Cognitive test 2	-1.46	2.69	242	-0.54	0.59				
	1st half of marksmanship 2	11.66	2.71	242	4.30	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 2	11.99	2.71	242	4.42	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 3	-2.31	2.70	242	-0.86	0.39				
	Rest 2	-2.94	2.71	242	-1.08	0.28				
	Cognitive test 4	-0.29	2.75	242	-0.11	0.92				
	Stimulation: TES	-0.34	1.66	237	-0.20	0.84				
	Stimulation: Rest 1	-1.07	1.66	237	-0.65	0.52				
	Stimulation: Cognitive test 1	-1.96	1.66	237	-1.18	0.24				
	Stimulation: 1st half of marksmanship 1	-1.07	1.66	237	-0.64	0.52				
	Stimulation: 2nd half of marksmanship 1	-3.33	1.66	237	-2.01	0.05	*	n.s.		< 0.01
	Stimulation: Cognitive test 2	-1.79	1.66	237	-1.08	0.28				
	Stimulation: 1st half of marksmanship 2	-6.24	1.66	237	-3.76	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: 2nd half of marksmanship 2	-5.73	1.66	237	-3.45	< 0.01	*	0.01	*	< 0.01
	Stimulation: Cognitive test 3	-1.25	1.66	237	-0.76	0.45				
	Stimulation: Rest 2	-1.79	1.66	237	-1.08	0.28				
	Stimulation: Cognitive test 4	-3.57	1.67	237	-2.14	0.03	*	n.s.		< 0.01
PPG MEAN R-R INTERVAL (MSEC)	Stimulation	-23.41	19.64	237	-1.19	0.23				
	TES	-1.63	32.73	242	-0.05	0.96				
	Rest 1	-25.97	32.74	242	-0.79	0.43				

	Cognitive test 1	-44.94	32.78	242	-1.37	0.17				
	1st half of marksmanship 1	-62.83	33.19	242	-1.89	0.06				
	2nd half of marksmanship 1	-97.36	33.00	242	-2.95	< 0.01	*	0.04	*	0.03
	Cognitive test 2	16.54	32.80	242	0.50	0.61				
	1st half of marksmanship 2	-123.46	33.02	242	-3.74	< 0.01	*	< 0.01	*	0.03
	2nd half of marksmanship 2	-113.30	33.04	242	-3.43	< 0.01	*	0.01	*	0.03
	Cognitive test 3	27.96	32.83	242	0.85	0.40				
	Rest 2	37.70	32.97	242	1.14	0.25				
	Cognitive test 4	6.08	33.50	242	0.18	0.86				
	Stimulation: TES	10.09	20.47	237	0.49	0.62				
	Stimulation: Rest 1	24.88	20.47	237	1.22	0.23				
	Stimulation: Cognitive test 1	34.10	20.47	237	1.67	0.10				
	Stimulation: 1st half of marksmanship 1	6.03	20.53	237	0.29	0.77				
	Stimulation: 2nd half of marksmanship 1	31.98	20.47	237	1.56	0.12				
	Stimulation: Cognitive test 2	26.86	20.46	237	1.31	0.19				
	Stimulation: 1st half of marksmanship 2	69.27	20.47	237	3.38	< 0.01	*	0.01	*	0.02
	Stimulation: 2nd half of marksmanship 2	52.22	20.47	237	2.55	0.01	*	n.s.		0.02
	Stimulation: Cognitive test 3	17.24	20.46	237	0.84	0.40				
	Stimulation: Rest 2	20.02	20.48	237	0.98	0.33				
	Stimulation: Cognitive test 4	45.84	20.56	237	2.23	0.03	*	n.s.		0.02
PPG SDNN (MSEC)	Stimulation	-3.41	3.60	237	-0.95	0.34				
	TES	-3.05	9.21	242	-0.33	0.74				
	Rest 1	-4.98	9.21	242	-0.54	0.59				
	Cognitive test 1	-7.36	9.22	242	-0.80	0.43				
	1st half of marksmanship 1	-8.36	9.26	242	-0.90	0.37				
	2nd half of marksmanship 1	-1.29	9.24	242	-0.14	0.89				
	Cognitive test 2	-0.20	9.22	242	-0.02	0.98				
	1st half of marksmanship 2	-11.18	9.24	242	-1.21	0.23				
	2nd half of marksmanship 2	-10.69	9.24	242	-1.16	0.25				
	Cognitive test 3	-5.50	9.22	242	-0.60	0.55				

	Rest 2	16.22	9.24	242	1.76	0.08			
	Cognitive test 4	-6.54	9.28	242	-0.70	0.48			
	Stimulation: TES	2.11	4.90	237	0.43	0.67			
	Stimulation: Rest 1	4.48	4.90	237	0.91	0.36			
	Stimulation: Cognitive test 1	6.43	4.91	237	1.31	0.19			
	Stimulation: 1st half of marksmanship 1	5.18	4.91	237	1.05	0.29			
	Stimulation: 2nd half of marksmanship 1	1.08	4.91	237	0.22	0.83			
	Stimulation: Cognitive test 2	0.63	4.90	237	0.13	0.90			
	Stimulation: 1st half of marksmanship 2	7.82	4.90	237	1.59	0.11			
	Stimulation: 2nd half of marksmanship 2	8.92	4.91	237	1.82	0.07			
	Stimulation: Cognitive test 3	5.53	4.90	237	1.13	0.26			
	Stimulation: Rest 2	-4.84	4.91	237	-0.99	0.33			
	Stimulation: Cognitive test 4	6.74	4.92	237	1.37	0.17			
PPG RMSSD (MSEC)	Stimulation	-4.07	4.25	237	-0.96	0.34			
	TES	-2.88	11.25	242	-0.26	0.80			
	Rest 1	-5.80	11.25	242	-0.52	0.61			
	Cognitive test 1	-5.30	11.26	242	-0.47	0.64			
	1st half of marksmanship 1	-12.11	11.29	242	-1.07	0.28			
	2nd half of marksmanship 1	-7.44	11.28	242	-0.66	0.51			
	Cognitive test 2	0.77	11.26	242	0.07	0.95			
	1st half of marksmanship 2	-9.67	11.28	242	-0.86	0.39			
	2nd half of marksmanship 2	-8.63	11.28	242	-0.77	0.45			
	Cognitive test 3	-4.62	11.26	242	-0.41	0.68			
	Rest 2	23.10	11.27	242	2.05	0.04	*	n.s.	0.01
	Cognitive test 4	1.08	11.32	242	0.10	0.92			
	Stimulation: TES	1.23	5.76	237	0.21	0.83			
	Stimulation: Rest 1	3.54	5.76	237	0.61	0.54			
	Stimulation: Cognitive test 1	4.39	5.76	237	0.76	0.45			
	Stimulation: 1st half of marksmanship 1	6.68	5.77	237	1.16	0.25			

	Stimulation: 2nd half of marksmanship 1	3.62	5.76	237	0.63	0.53			
	Stimulation: Cognitive test 2	0.97	5.76	237	0.17	0.87			
	Stimulation: 1st half of marksmanship 2	5.88	5.76	237	1.02	0.31			
	Stimulation: 2nd half of marksmanship 2	6.14	5.76	237	1.07	0.29			
	Stimulation: Cognitive test 3	6.13	5.76	237	1.06	0.29			
	Stimulation: Rest 2	-5.61	5.76	237	-0.97	0.33			
	Stimulation: Cognitive test 4	4.80	5.77	237	0.83	0.41			
PPG PNN50 (%)	Stimulation	-4.97	2.37	237	-2.10	0.04	*	n.s.	< 0.01
	TES	-2.59	5.17	242	-0.50	0.62			
	Rest 1	-6.49	5.17	242	-1.25	0.21			
	Cognitive test 1	-7.75	5.18	242	-1.50	0.14			
	1st half of marksmanship 1	-14.48	5.22	242	-2.77	0.01	*	n.s.	< 0.01
	2nd half of marksmanship 1	-10.75	5.20	242	-2.07	0.04	*	n.s.	< 0.01
	Cognitive test 2	-4.41	5.18	242	-0.85	0.40			
	1st half of marksmanship 2	-11.42	5.20	242	-2.19	0.03	*	n.s.	< 0.01
	2nd half of marksmanship 2	-11.24	5.21	242	-2.16	0.03	*	n.s.	< 0.01
	Cognitive test 3	-8.50	5.18	242	-1.64	0.10			
	Rest 2	0.02	5.20	242	< 0.01	1.00			
	Cognitive test 4	-4.81	5.26	242	-0.92	0.36			
	Stimulation: TES	0.79	3.00	237	0.26	0.79			
	Stimulation: Rest 1	3.78	3.00	237	1.26	0.21			
	Stimulation: Cognitive test 1	5.03	3.00	237	1.67	0.10			
	Stimulation: 1st half of marksmanship 1	7.31	3.01	237	2.43	0.02	*	n.s.	< 0.01
	Stimulation: 2nd half of marksmanship 1	5.09	3.00	237	1.69	0.09			
	Stimulation: Cognitive test 2	3.08	3.00	237	1.03	0.31			
	Stimulation: 1st half of marksmanship 2	5.88	3.00	237	1.96	0.05			
	Stimulation: 2nd half of marksmanship 2	6.37	3.00	237	2.12	0.03	*	n.s.	< 0.01

	Stimulation: Cognitive test 3	7.01	3.00	237	2.34	0.02	*	n.s.	< 0.01
	Stimulation: Rest 2	4.89	3.00	237	1.63	0.10			
	Stimulation: Cognitive test 4	6.69	3.01	237	2.22	0.03	*	n.s.	< 0.01

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; min = minutes; msec = milliseconds; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX E: Mixed Model Results for ECG Frequency-Series HRV Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}	$\|D\|$
ECG TOTAL ABSOLUTE POWER ($MSEC^2$)	Stimulation	-212.96	444.91	212	-0.48	0.63			
	TES	-70.22	848.80	231	-0.08	0.93			
	Rest 1	345.72	850.35	231	0.41	0.68			
	Cognitive test 1	-511.88	848.81	231	-0.60	0.55			
	1st half of marksmanship 1	-1009.12	849.62	231	-1.19	0.24			
	2nd half of marksmanship 1	-689.10	849.25	231	-0.81	0.42			
	Cognitive test 2	-9.80	864.40	231	-0.01	0.99			
	1st half of marksmanship 2	-82< 0.01	848.87	231	-0.97	0.34			
	2nd half of marksmanship 2	-252.58	851.15	231	-0.30	0.77			
	Cognitive test 3	-321.93	850.92	231	-0.38	0.71			
	Rest 2	543.88	860.94	231	0.63	0.53			
	Cognitive test 4	1064.09	872.39	231	1.22	0.22			
	Stimulation: TES	-64.06	512.25	212	-0.13	0.90			
	Stimulation: Rest 1	-144.12	512.61	212	-0.28	0.78			
	Stimulation: Cognitive test 1	689.89	512.64	212	1.35	0.18			
	Stimulation: 1st half of marksmanship 1	659.10	512.38	212	1.29	0.20			
	Stimulation: 2nd half of marksmanship 1	635.22	512.30	212	1.24	0.22			
	Stimulation: Cognitive test 2	1153.18	517.72	212	2.23	0.03	*	n.s.	0.52
	Stimulation: 1st half of marksmanship 2	911.64	513.68	212	1.77	0.08			
	Stimulation: 2nd half of marksmanship 2	503.30	512.25	212	0.98	0.33			
	Stimulation: Cognitive test 3	1421.58	512.30	212	2.77	0.01	*	n.s.	0.52
	Stimulation: Rest 2	430.44	518.15	212	0.83	0.41			
	Stimulation: Cognitive test 4	599.71	518.28	212	1.16	0.25			
ECG VERY LOW FREQUENCY	Stimulation	-1.43	31.79	212	-0.04	0.96			
	TES	-3.18	67.13	231	-0.05	0.96			
	Rest 1	14.79	67.23	231	0.22	0.83			

ABSOLUTE POWER ($MSEC^2$)	Cognitive test 1	19.19	67.13	231	0.29	0.78
	1st half of marksmanship 1	50.52	67.18	231	0.75	0.45
	2nd half of marksmanship 1	22.12	67.16	231	0.33	0.74
	Cognitive test 2	52.07	68.21	231	0.76	0.45
	1st half of marksmanship 2	-7.49	67.13	231	-0.11	0.91
	2nd half of marksmanship 2	39.67	67.27	231	0.59	0.56
	Cognitive test 3	115.27	67.24	231	1.71	0.09
	Rest 2	66.69	68.01	231	0.98	0.33
	Cognitive test 4	39.65	68.62	231	0.58	0.56
	Stimulation: TES	4.49	41.96	212	0.11	0.91
	Stimulation: Rest 1	19.97	41.98	212	0.48	0.63
	Stimulation: Cognitive test 1	37.48	41.97	212	0.89	0.37
	Stimulation: 1st half of marksmanship 1	2.43	41.96	212	0.06	0.95
	Stimulation: 2nd half of marksmanship 1	34.59	41.96	212	0.82	0.41
	Stimulation: Cognitive test 2	25.78	42.32	212	0.61	0.54
	Stimulation: 1st half of marksmanship 2	79.16	42.02	212	1.88	0.06
	Stimulation: 2nd half of marksmanship 2	67.51	41.96	212	1.61	0.11
	Stimulation: Cognitive test 3	14.26	41.96	212	0.34	0.73
	Stimulation: Rest 2	5.69	42.35	212	0.13	0.89
	Stimulation: Cognitive test 4	54.90	42.35	212	1.30	0.20
ECG LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Stimulation	-45.28	292.58	212	-0.15	0.88
	TES	160.67	572.44	231	0.28	0.78
	Rest 1	695.95	573.45	231	1.21	0.23
	Cognitive test 1	-64.86	572.44	231	-0.11	0.91
	1st half of marksmanship 1	-151.36	572.95	231	-0.26	0.79
	2nd half of marksmanship 1	133.82	572.76	231	0.23	0.82
	Cognitive test 2	43.66	582.51	231	0.07	0.94
	1st half of marksmanship 2	76.91	572.46	231	0.13	0.89
	2nd half of marksmanship 2	467.33	573.93	231	0.81	0.42
	Cognitive test 3	-120.92	573.69	231	-0.21	0.83

	Rest 2	562.40	580.38	231	0.97	0.33				
	Cognitive test 4	787.56	587.11	231	1.34	0.18				
	Stimulation: TES	-79.06	380.66	212	-0.21	0.84				
	Stimulation: Rest 1	-315.89	380.89	212	-0.83	0.41				
	Stimulation: Cognitive test 1	245.97	380.86	212	0.65	0.52				
	Stimulation: 1st half of marksmanship 1	354.03	380.74	212	0.93	0.35				
	Stimulation: 2nd half of marksmanship 1	321.54	380.68	212	0.84	0.40				
	Stimulation: Cognitive test 2	618.31	383.85	212	1.61	0.11				
	Stimulation: 1st half of marksmanship 2	444.18	381.36	212	1.16	0.25				
	Stimulation: 2nd half of marksmanship 2	160.66	380.66	212	0.42	0.67				
	Stimulation: Cognitive test 3	694.63	380.68	212	1.82	0.07				
	Stimulation: Rest 2	80.71	384.10	212	0.21	0.83				
	Stimulation: Cognitive test 4	263.81	384.11	212	0.69	0.49				
ECG HIGH FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Stimulation	8.50	2.45	212	3.47	< 0.01	*	0.01	*	< 0.01
	TES	10.82	4.70	231	2.30	0.02	*	n.s.		< 0.01
	Rest 1	19.49	4.71	231	4.14	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 1	10.35	4.70	231	2.20	0.03	*	n.s.		< 0.01
	1st half of marksmanship 1	31.02	4.71	231	6.59	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 1	33.23	4.71	231	7.06	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 2	13.26	4.78	231	2.77	0.01	*	n.s.		< 0.01
	1st half of marksmanship 2	30.73	4.70	231	6.53	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 2	30.52	4.71	231	6.47	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 3	14.96	4.71	231	3.17	< 0.01	*	0.02	*	< 0.01
	Rest 2	15.54	4.77	231	3.26	< 0.01	*	0.02	*	< 0.01
	Cognitive test 4	17.15	4.81	231	3.56	< 0.01	*	0.01	*	< 0.01
	Stimulation: TES	-4.86	2.75	212	-1.77	0.08				
	Stimulation: Rest 1	-10.43	2.75	212	-3.79	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 1	-5.86	2.75	212	-2.13	0.03	*	n.s.		< 0.01
	Stimulation: 1st half of marksmanship 1	-10.68	2.75	212	-3.89	< 0.01	*	< 0.01	*	< 0.01

	Stimulation: 2nd half of marksmanship 1	-11.49	2.75	212	-4.18	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 2	-8.59	2.78	212	-3.09	< 0.01	*	0.03	*	< 0.01
	Stimulation: 1st half of marksmanship 2	-10.64	2.75	212	-3.86	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: 2nd half of marksmanship 2	-10.14	2.75	212	-3.69	< 0.01	*	0.01	*	< 0.01
	Stimulation: Cognitive test 3	-10.50	2.75	212	-3.82	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Rest 2	-11.71	2.78	212	-4.21	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 4	-10.64	2.78	212	-3.83	< 0.01	*	< 0.01	*	< 0.01
ECG LOW FREQUENCY RELATIVE POWER (N.U.)	Stimulation	-169.89	196.87	212	-0.86	0.39				
	TES	-229.45	356.95	231	-0.64	0.52				
	Rest 1	-398.36	357.56	231	-1.11	0.27				
	Cognitive test 1	-457.95	356.95	231	-1.28	0.20				
	1st half of marksmanship 1	-926.97	357.29	231	-2.59	0.01	*	n.s.		0.33
	2nd half of marksmanship 1	-853.50	357.12	231	-2.39	0.02	*	n.s.		0.33
	Cognitive test 2	-112.93	363.02	231	-0.31	0.76				
	1st half of marksmanship 2	-865.78	356.98	231	-2.43	0.02	*	n.s.		0.33
	2nd half of marksmanship 2	-798.51	357.90	231	-2.23	0.03	*	n.s.		0.33
	Cognitive test 3	-343.47	357.84	231	-0.96	0.34				
	Rest 2	-126.35	361.59	231	-0.35	0.73				
	Cognitive test 4	135.56	366.42	231	0.37	0.71				
	Stimulation: TES	15.73	207.08	212	0.08	0.94				
	Stimulation: Rest 1	168.52	207.22	212	0.81	0.42				
	Stimulation: Cognitive test 1	395.28	207.26	212	1.91	0.06				
	Stimulation: 1st half of marksmanship 1	310.48	207.14	212	1.50	0.14				
	Stimulation: 2nd half of marksmanship 1	272.15	207.11	212	1.31	0.19				
	Stimulation: Cognitive test 2	493.32	209.28	212	2.36	0.02	*	n.s.		0.21
	Stimulation: 1st half of marksmanship 2	356.83	207.73	212	1.72	0.09				
	Stimulation: 2nd half of marksmanship 2	279.06	207.08	212	1.35	0.18				

	Stimulation: Cognitive test 3	717.17	207.10	212	3.46	< 0.01	*	0.01	*	0.21
	Stimulation: Rest 2	338.55	209.45	212	1.62	0.11				
	Stimulation: Cognitive test 4	309.34	209.55	212	1.48	0.14				
ECG HIGH FREQUENCY RELATIVE POWER (N.U.)	Stimulation	-8.54	2.44	212	-3.50	< 0.01	*	0.01	*	< 0.01
	TES	-10.82	4.69	231	-2.31	0.02	*	n.s.		< 0.01
	Rest 1	-19.47	4.70	231	-4.14	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 1	-10.38	4.69	231	-2.21	0.03	*	n.s.		< 0.01
	1st half of marksmanship 1	-30.99	4.70	231	-6.60	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 1	-33.20	4.69	231	-7.07	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 2	-13.24	4.77	231	-2.78	0.01	*	n.s.		< 0.01
	1st half of marksmanship 2	-30.70	4.69	231	-6.54	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 2	-30.49	4.70	231	-6.48	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 3	-14.94	4.70	231	-3.18	< 0.01	*	0.02	*	< 0.01
	Rest 2	-15.56	4.76	231	-3.27	< 0.01	*	0.02	*	< 0.01
	Cognitive test 4	-17.14	4.80	231	-3.57	< 0.01	*	0.01	*	< 0.01
	Stimulation: TES	4.90	2.74	212	1.79	0.07				
	Stimulation: Rest 1	10.47	2.74	212	3.82	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 1	5.93	2.74	212	2.17	0.03	*	n.s.		< 0.01
	Stimulation: 1st half of marksmanship 1	10.73	2.74	212	3.92	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: 2nd half of marksmanship 1	11.53	2.74	212	4.21	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 2	8.64	2.77	212	3.12	< 0.01	*	0.02	*	< 0.01
	Stimulation: 1st half of marksmanship 2	10.68	2.74	212	3.90	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: 2nd half of marksmanship 2	10.19	2.74	212	3.72	< 0.01	*	0.01	*	< 0.01
	Stimulation: Cognitive test 3	10.54	2.74	212	3.85	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Rest 2	11.77	2.77	212	4.25	< 0.01	*	< 0.01	*	< 0.01
	Stimulation: Cognitive test 4	10.69	2.77	212	3.86	< 0.01	*	< 0.01	*	< 0.01
ECG LOW- TO HIGH-FREQUENCY RATIO	Stimulation	0.89	0.43	212	2.08	0.04	*	n.s.		< 0.01
	TES	0.62	0.80	231	0.77	0.44				
	Rest 1	2.04	0.80	231	2.55	0.01	*	n.s.		< 0.01
	Cognitive test 1	0.83	0.80	231	1.04	0.30				

	1st half of marksmanship 1	3.70	0.80	231	4.62	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 1	4.61	0.80	231	5.77	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 2	1.09	0.81	231	1.33	0.18				
	1st half of marksmanship 2	4.65	0.80	231	5.81	< 0.01	*	< 0.01	*	< 0.01
	2nd half of marksmanship 2	4.11	0.80	231	5.13	< 0.01	*	< 0.01	*	< 0.01
	Cognitive test 3	1.20	0.80	231	1.49	0.14				
	Rest 2	1.25	0.81	231	1.54	0.13				
	Cognitive test 4	1.44	0.82	231	1.76	0.08				
	Stimulation: TES	-0.33	0.50	212	-0.66	0.51				
	Stimulation: Rest 1	-1.34	0.50	212	-2.66	0.01	*	n.s.		< 0.01
	Stimulation: Cognitive test 1	-0.55	0.50	212	-1.08	0.28				
	Stimulation: 1st half of marksmanship 1	-1.01	0.50	212	-2.01	0.05	*	n.s.		< 0.01
	Stimulation: 2nd half of marksmanship 1	-1.47	0.50	212	-2.91	< 0.01	*	0.04	*	< 0.01
	Stimulation: Cognitive test 2	-0.92	0.51	212	-1.81	0.07				
	Stimulation: 1st half of marksmanship 2	-1.46	0.50	212	-2.90	< 0.01	*	0.04	*	< 0.01
	Stimulation: 2nd half of marksmanship 2	-1.00	0.50	212	-1.99	0.05	*	n.s.		< 0.01
	Stimulation: Cognitive test 3	-1.12	0.50	212	-2.22	0.03	*	n.s.		< 0.01
	Stimulation: Rest 2	-1.27	0.51	212	-2.50	0.01	*	n.s.		< 0.01
	Stimulation: Cognitive test 4	-1.14	0.51	212	-2.24	0.03	*	n.s.		< 0.01

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX F: Mixed Model Results for PPG Frequency-Series HRV Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}	$\lvert D \rvert$
PPG TOTAL ABSOLUTE POWER ($MSEC^2$)	Stimulation	-1139.33	1037.03	237	-1.10	0.27			
	Stimulation	-1526.99	2805.65	242	-0.54	0.59			
	Rest 1	-1589.82	2805.93	242	-0.57	0.57			
	Cognitive test 1	-2237.86	2806.21	242	-0.80	0.43			
	1st half of marksmanship 1	-1902.94	2809.06	242	-0.68	0.50			
	2nd half of marksmanship 1	-1091.95	2807.78	242	-0.39	0.70			
	Cognitive test 2	-1040.62	2806.40	242	-0.37	0.71			
	1st half of marksmanship 2	-2424.53	2807.89	242	-0.86	0.39			
	2nd half of marksmanship 2	-2246.71	2808.06	242	-0.80	0.42			
	Cognitive test 3	-1530.50	2806.62	242	-0.55	0.59			
	Rest 2	5102.35	2807.61	242	1.82	0.07			
	Cognitive test 4	-2244.18	2811.18	242	-0.80	0.43			
	Stimulation: TES	1246.22	1421.80	237	0.88	0.38			
	Stimulation: Rest 1	1432.27	1421.86	237	1.01	0.31			
	Stimulation: Cognitive test 1	1651.71	1421.89	237	1.16	0.25			
	Stimulation: 1st half of marksmanship 1	1306.39	1422.47	237	0.92	0.36			
	Stimulation: 2nd half of marksmanship 1	926.60	1421.92	237	0.65	0.52			
	Stimulation: Cognitive test 2	857.11	1421.81	237	0.60	0.55			
	Stimulation: 1st half of marksmanship 2	1796.26	1421.86	237	1.26	0.21			
	Stimulation: 2nd half of marksmanship 2	1877.98	1421.91	237	1.32	0.19			
	Stimulation: Cognitive test 3	1311.81	1421.83	237	0.92	0.36			
	Stimulation: Rest 2	-1868.92	1421.95	237	-1.31	0.19			
	Stimulation: Cognitive test 4	1759.94	1422.72	237	1.24	0.22			
PPG VERY LOW FREQUENCY	Stimulation	-367.98	134.71	237	-2.73	0.01	*	n.s.	0.12
	Stimulation	-632.57	301.27	242	-2.10	0.04	*	n.s.	0.26
	Rest 1	-655.39	301.39	242	-2.17	0.03	*	n.s.	0.26

ABSOLUTE POWER ($MSEC^2$)	Cognitive test 1	-786.30	301.55	242	-2.61	0.01	*	n.s.		0.26
	1st half of marksmanship 1	-462.08	303.21	242	-1.52	0.13				
	2nd half of marksmanship 1	-873.63	302.49	242	-2.89	< 0.01	*	0.04	*	0.26
	Cognitive test 2	-741.42	301.67	242	-2.46	0.01	*	n.s.		0.26
	1st half of marksmanship 2	-538.90	302.55	242	-1.78	0.08				
	2nd half of marksmanship 2	-740.22	302.64	242	-2.45	0.02	*	n.s.		0.26
	Cognitive test 3	-584.39	301.79	242	-1.94	0.05				
	Rest 2	-104.18	302.37	242	-0.34	0.73				
	Cognitive test 4	-817.28	304.49	242	-2.68	0.01	*	n.s.		0.26
	Stimulation: TES	449.82	184.31	237	2.44	0.02	*	n.s.		0.16
	Stimulation: Rest 1	475.62	184.32	237	2.58	0.01	*	n.s.		0.16
	Stimulation: Cognitive test 1	538.54	184.33	237	2.92	< 0.01	*	0.04	*	0.16
	Stimulation: 1st half of marksmanship 1	241.77	184.60	237	1.31	0.19				
	Stimulation: 2nd half of marksmanship 1	558.33	184.35	237	3.03	< 0.01	*	0.03	*	0.16
	Stimulation: Cognitive test 2	503.37	184.30	237	2.73	0.01	*	n.s.		0.16
	Stimulation: 1st half of marksmanship 2	386.99	184.32	237	2.10	0.04	*	n.s.		0.16
	Stimulation: 2nd half of marksmanship 2	552.13	184.34	237	3.00	< 0.01	*	0.03	*	0.16
	Stimulation: Cognitive test 3	366.90	184.31	237	1.99	0.05	*	n.s.		0.16
	Stimulation: Rest 2	88.46	184.36	237	0.48	0.63				
	Stimulation: Cognitive test 4	496.87	184.73	237	2.69	0.01	*	n.s.		0.16
PPG LOW FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Stimulation	-563.85	574.85	237	-0.98	0.33				
	Stimulation	-706.64	1519.00	242	-0.47	0.64				
	Rest 1	-668.07	1519.23	242	-0.44	0.66				
	Cognitive test 1	-1118.02	1519.49	242	-0.74	0.46				
	1st half of marksmanship 1	-881.28	1522.17	242	-0.58	0.56				
	2nd half of marksmanship 1	184.70	1520.98	242	0.12	0.90				
	Cognitive test 2	-226.59	1519.68	242	-0.15	0.88				
	1st half of marksmanship 2	-1442.25	1521.08	242	-0.95	0.34				
	2nd half of marksmanship 2	-1122.07	1521.24	242	-0.74	0.46				
	Cognitive test 3	-583.90	1519.88	242	-0.38	0.70				

	Rest 2	2834.94	1520.80	242	1.86	0.06			
	Cognitive test 4	-1307.29	1524.19	242	-0.86	0.39			
	Stimulation: TES	682.05	782.90	237	0.87	0.38			
	Stimulation: Rest 1	767.46	782.94	237	0.98	0.33			
	Stimulation: Cognitive test 1	855.28	782.96	237	1.09	0.28			
	Stimulation: 1st half of marksmanship 1	767.19	783.49	237	0.98	0.33			
	Stimulation: 2nd half of marksmanship 1	150.90	782.99	237	0.19	0.85			
	Stimulation: Cognitive test 2	312.39	782.89	237	0.40	0.69			
	Stimulation: 1st half of marksmanship 2	1166.90	782.94	237	1.49	0.14			
	Stimulation: 2nd half of marksmanship 2	1111.41	782.98	237	1.42	0.16			
	Stimulation: Cognitive test 3	632.40	782.91	237	0.81	0.42			
	Stimulation: Rest 2	-1010.48	783.02	237	-1.29	0.20			
	Stimulation: Cognitive test 4	1049.42	783.72	237	1.34	0.18			
PPG HIGH FREQUENCY ABSOLUTE POWER ($MSEC^2$)	Stimulation	2.08	2.34	237	0.89	0.37			
	Stimulation	1.55	4.97	242	0.31	0.76			
	Rest 1	4.33	4.97	242	0.87	0.39			
	Cognitive test 1	-0.86	4.97	242	-0.17	0.86			
	1st half of marksmanship 1	11.57	5.02	242	2.31	0.02	*	n.s.	< 0.01
	2nd half of marksmanship 1	13.10	5.00	242	2.62	0.01	*	n.s.	< 0.01
	Cognitive test 2	3.07	4.98	242	0.62	0.54			
	1st half of marksmanship 2	7.62	5.00	242	1.52	0.13			
	2nd half of marksmanship 2	8.37	5.00	242	1.67	0.10			
	Cognitive test 3	9.18	4.98	242	1.84	0.07			
	Rest 2	7.13	4.99	242	1.43	0.15			
	Cognitive test 4	2.54	5.05	242	0.50	0.62			
	Stimulation: TES	0.83	3.05	237	0.27	0.79			
	Stimulation: Rest 1	-0.19	3.05	237	-0.06	0.95			
	Stimulation: Cognitive test 1	2.13	3.05	237	0.70	0.48			
	Stimulation: 1st half of marksmanship 1	-3.35	3.06	237	-1.10	0.27			

	Stimulation: 2nd half of marksmanship 1	-4.25	3.05	237	-1.39	0.16			
	Stimulation: Cognitive test 2	-0.35	3.05	237	-0.12	0.91			
	Stimulation: 1st half of marksmanship 2	-1.01	3.05	237	-0.33	0.74			
	Stimulation: 2nd half of marksmanship 2	-0.60	3.05	237	-0.20	0.84			
	Stimulation: Cognitive test 3	-5.04	3.05	237	-1.65	0.10			
	Stimulation: Rest 2	-5.21	3.05	237	-1.71	0.09			
	Stimulation: Cognitive test 4	-1.23	3.06	237	-0.40	0.69			
	Stimulation	-120.87	403.31	237	-0.30	0.76			
	Stimulation	-75.76	1114.40	242	-0.07	0.95			
	Rest 1	-125.28	1114.51	242	-0.11	0.91			
	Cognitive test 1	-187.74	1114.60	242	-0.17	0.87			
	1st half of marksmanship 1	-311.72	1115.46	242	-0.28	0.78			
	2nd half of marksmanship 1	-191.45	1115.05	242	-0.17	0.86			
	Cognitive test 2	91.50	1114.65	242	0.08	0.93			
	1st half of marksmanship 2	-223.31	1115.08	242	-0.20	0.84			
	2nd half of marksmanship 2	-141.36	1115.14	242	-0.13	0.90			
	Cognitive test 3	-201.90	1114.72	242	-0.18	0.86			
PPG LOW FREQUENCY RELATIVE POWER (N.U.)	Rest 2	2561.37	1115.02	242	2.30	0.02	*	n.s.	0.96
	Cognitive test 4	150.85	1116.06	242	0.14	0.89			
	Stimulation: TES	65.79	554.90	237	0.12	0.91			
	Stimulation: Rest 1	122.38	554.93	237	0.22	0.83			
	Stimulation: Cognitive test 1	193.96	554.94	237	0.35	0.73			
	Stimulation: 1st half of marksmanship 1	197.03	555.13	237	0.35	0.72			
	Stimulation: 2nd half of marksmanship 1	143.77	554.95	237	0.26	0.80			
	Stimulation: Cognitive test 2	-19.79	554.91	237	-0.04	0.97			
	Stimulation: 1st half of marksmanship 2	170.48	554.93	237	0.31	0.76			
	Stimulation: 2nd half of marksmanship 2	129.07	554.95	237	0.23	0.82			

	Stimulation: Cognitive test 3	254.52	554.92	237	0.46	0.65			
	Stimulation: Rest 2	-1014.13	554.96	237	-1.83	0.07			
	Stimulation: Cognitive test 4	112.42	555.20	237	0.20	0.84			
PPG HIGH FREQUENCY RELATIVE POWER (N.U.)	Stimulation	-2.05	2.34	237	-0.88	0.38			
	Stimulation	-1.49	4.96	242	-0.30	0.76			
	Rest 1	-4.24	4.97	242	-0.85	0.39			
	Cognitive test 1	0.91	4.97	242	0.18	0.85			
	1st half of marksmanship 1	-11.48	5.01	242	-2.29	0.02	*	n.s.	< 0.01
	2nd half of marksmanship 1	-12.99	4.99	242	-2.60	0.01	*	n.s.	< 0.01
	Cognitive test 2	-2.99	4.97	242	-0.60	0.55			
	1st half of marksmanship 2	-7.54	4.99	242	-1.51	0.13			
	2nd half of marksmanship 2	-8.30	5.00	242	-1.66	0.10			
	Cognitive test 3	-9.08	4.98	242	-1.82	0.07			
	Rest 2	-7.04	4.99	242	-1.41	0.16			
	Cognitive test 4	-2.45	5.04	242	-0.49	0.63			
	Stimulation: TES	-0.84	3.05	237	-0.28	0.78			
	Stimulation: Rest 1	0.17	3.05	237	0.06	0.95			
	Stimulation: Cognitive test 1	-2.14	3.05	237	-0.70	0.48			
	Stimulation: 1st half of marksmanship 1	3.32	3.05	237	1.09	0.28			
	Stimulation: 2nd half of marksmanship 1	4.21	3.05	237	1.38	0.17			
	Stimulation: Cognitive test 2	0.33	3.05	237	0.11	0.91			
	Stimulation: 1st half of marksmanship 2	0.98	3.05	237	0.32	0.75			
	Stimulation: 2nd half of marksmanship 2	0.58	3.05	237	0.19	0.85			
	Stimulation: Cognitive test 3	5.01	3.05	237	1.64	0.10			
	Stimulation: Rest 2	5.18	3.05	237	1.70	0.09			
	Stimulation: Cognitive test 4	1.19	3.06	237	0.39	0.70			
PPG LOW- TO HIGH-FREQUENCY RATIO	Stimulation	-0.01	0.49	237	-0.01	0.99			
	Stimulation	-0.89	0.92	242	-0.97	0.33			
	Rest 1	-0.35	0.92	242	-0.38	0.70			
	Cognitive test 1	-0.95	0.92	242	-1.03	0.30			

	1st half of marksmanship 1	1.30	0.93	242	1.40	0.16			
	2nd half of marksmanship 1	0.63	0.93	242	0.68	0.50			
	Cognitive test 2	-1.38	0.92	242	-1.50	0.13			
	1st half of marksmanship 2	-1.37	0.93	242	-1.48	0.14			
	2nd half of marksmanship 2	-0.78	0.93	242	-0.84	0.40			
	Cognitive test 3	0.06	0.92	242	0.06	0.95			
	Rest 2	-0.65	0.93	242	-0.70	0.48			
	Cognitive test 4	-2.04	0.94	242	-2.18	0.03	*	n.s.	< 0.01
	Stimulation: TES	0.69	0.64	237	1.07	0.28			
	Stimulation: Rest 1	0.29	0.64	237	0.45	0.65			
	Stimulation: Cognitive test 1	0.47	0.64	237	0.73	0.46			
	Stimulation: 1st half of marksmanship 1	-0.49	0.64	237	-0.77	0.44			
	Stimulation: 2nd half of marksmanship 1	0.05	0.64	237	0.07	0.94			
	Stimulation: Cognitive test 2	0.81	0.64	237	1.27	0.21			
	Stimulation: 1st half of marksmanship 2	1.16	0.64	237	1.82	0.07			
	Stimulation: 2nd half of marksmanship 2	0.81	0.64	237	1.26	0.21			
	Stimulation: Cognitive test 3	-0.39	0.64	237	-0.61	0.54			
	Stimulation: Rest 2	-0.25	0.64	237	-0.40	0.69			
	Stimulation: Cognitive test 4	0.84	0.64	237	1.31	0.19			

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX G: Mixed Model Results for Workload Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}		$\|D\|$
COGNITIVE WORKLOAD	Stimulation	-4.44	4.57	102	-0.97	0.33				
	2nd half of marksmanship 1	6.11	8.90	85	0.69	0.49				
	2nd half of marksmanship 2	1.67	8.90	85	0.19	0.85				
	Cognitive test 3	12.50	8.90	85	1.40	0.16				
	Rest 2	7.78	8.90	85	0.87	0.38				
	Cognitive test 4	-1.67	8.90	85	-0.19	0.85				
	Stimulation : 2nd half of marksmanship 1	-0.83	5.70	102	-0.15	0.88				
	Stimulation : 2nd half of marksmanship 2	3.61	5.70	102	0.63	0.53				
	Stimulation: Cognitive test 3	-6.67	5.70	102	-1.17	0.25				
	Stimulation: Rest 2	-0.56	5.70	102	-0.10	0.92				
	Stimulation: Cognitive test 4	3.61	5.70	102	0.63	0.53				
PHYSICAL WORKLOAD	Stimulation	0.83	2.87	102	0.29	0.77				
	2nd half of marksmanship 1	23.06	6.89	85	3.34	0.00	*	0.02	*	0.79
	2nd half of marksmanship 2	6.39	6.89	85	0.93	0.36				
	Cognitive test 3	21.94	6.89	85	3.18	0.00	*	0.02	*	0.75
	Rest 2	10.00	6.89	85	1.45	0.15				
	Cognitive test 4	7.50	6.89	85	1.09	0.28				
	Stimulation : 2nd half of marksmanship 1	-4.17	4.06	102	-1.03	0.31				
	Stimulation : 2nd half of marksmanship 2	-1.39	4.06	102	-0.34	0.73				
	Stimulation: Cognitive test 3	-4.17	4.06	102	-1.03	0.31				
	Stimulation: Rest 2	-3.33	4.06	102	-0.82	0.41				
	Stimulation: Cognitive test 4	-1.11	4.06	102	-0.27	0.79				

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX H: Mixed Model Results for Marksmanship Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}	$\|D\|$
SHOT ACCURACY (%)	Stimulation	0.14	0.05	44	2.88	0.01	*	n.s.	0.60
	2nd half of marksmanship 2	0.24	0.11	22	2.11	0.05	*	n.s.	0.44
	Stimulation: 2nd half of marksmanship 2	-0.11	0.06	44	-1.91	0.06			
TARGET DETECTION ACCURACY (%)	Stimulation	-0.07	0.07	44	-0.97	0.34			
	2nd half of marksmanship 2	0.06	0.10	22	0.57	0.57			
	Stimulation: 2nd half of marksmanship 2	-0.01	0.06	44	-0.17	0.87			
TARGET DISCRIMINATION ACCURACY (ALL TARGETS) (%)	Stimulation	-0.07	0.07	44	-1.04	0.30			
	2nd half of marksmanship 2	0.05	0.10	22	0.47	0.64			
	Stimulation: 2nd half of marksmanship 2	0.00	0.06	44	-0.04	0.97			
HIGH VALUE TARGET ACCURACY (%)	Stimulation	0.05	0.03	44	1.44	0.16			
	2nd half of marksmanship 2	0.08	0.09	22	0.87	0.39			
	Stimulation: 2nd half of marksmanship 2	-0.04	0.05	44	-0.79	0.43			
SHOT LATENCY (MSEC)	Stimulation	-123.80	65.18	35	-1.90	0.07			
	2nd half of marksmanship 2	-194.86	120.21	18	-1.62	0.12			
	Stimulation: 2nd half of marksmanship 2	102.57	77.65	35	1.32	0.20			
TARGET DETECTION LATENCY (MSEC)	Stimulation	-155.71	81.90	28	-1.90	0.07			
	2nd half of marksmanship 2	-427.93	164.44	17	-2.60	0.02	*	n.s.	0.60
	Stimulation: 2nd half of marksmanship 2	220.46	104.20	28	2.12	0.04	*	n.s.	0.49
SHOT DISTANCE FROM THE CENTER OF MASS (M)	Stimulation	-0.08	0.03	33	-2.38	0.02	*	n.s.	0.56
	2nd half of marksmanship 2	-0.08	0.08	17	-1.06	0.30			
	Stimulation: 2nd half of marksmanship 2	0.04	0.04	33	1.08	0.29			

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX I: Mixed Model Results for Go/No-Go Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}	P_{ADJ}	$\|D\|$
GO/NO-GO ACCURACY (%)	Stimulation	0.62	1.68	63	0.37	0.71		
	Cognitive test 2	-0.06	3.45	48	-0.02	0.99		
	Cognitive test 3	3.47	3.44	48	1.01	0.32		
	Cognitive test 4	0.67	3.44	48	0.20	0.85		
	Stimulation: Cognitive test 2	-0.06	2.07	63	-0.03	0.98		
	Stimulation: Cognitive test 3	-3.00	2.05	63	-1.46	0.15		
	Stimulation: Cognitive test 4	-1.77	2.05	63	-0.87	0.39		
GO/NO-GO MEAN RESPONSE TIMES (MSEC)	Stimulation	4.04	6.69	83	0.60	0.55		
	Cognitive test 2	3.38	13.33	63	0.25	0.80		
	Cognitive test 3	15.16	13.30	63	1.14	0.26		
	Cognitive test 4	14.40	13.30	63	1.08	0.28		
	Stimulation: Cognitive test 2	-3.30	8.28	83	-0.40	0.69		
	Stimulation: Cognitive test 3	-12.52	8.23	83	-1.52	0.13		
	Stimulation: Cognitive test 4	-12.31	8.23	83	-1.50	0.14		

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX J: Mixed Model Results for gradCPT Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}	P_{ADJ}	$\|D\|$
GRADCPT ERROR RATE (%)	Stimulation	-0.01	0.01	85	-0.55	0.58		
	Cognitive test 2	-0.01	0.03	66	-0.50	0.62		
	Cognitive test 3	-0.02	0.03	66	-0.86	0.39		
	Cognitive test 4	-0.01	0.03	66	-0.51	0.61		
	Stimulation: Cognitive test 2	0.01	0.01	85	0.76	0.45		
	Stimulation: Cognitive test 3	0.02	0.01	85	1.40	0.17		
	Stimulation: Cognitive test 4	0.01	0.01	85	0.49	0.62		
GRADCPT COMMISSION ERROR RATE (%)	Stimulation	-0.01	0.01	85	-0.55	0.58		
	Cognitive test 2	-0.01	0.03	66	-0.50	0.62		
	Cognitive test 3	-0.02	0.03	66	-0.86	0.39		
	Cognitive test 4	-0.01	0.03	66	-0.51	0.61		
	Stimulation: Cognitive test 2	0.01	0.01	85	0.76	0.45		
	Stimulation: Cognitive test 3	0.02	0.01	85	1.40	0.17		
	Stimulation: Cognitive test 4	0.01	0.01	85	0.49	0.62		
GRADCPT OMISSION ERROR RATE (%)	Stimulation	-0.01	0.01	85	-0.40	0.69		
	Cognitive test 2	-0.18	0.03	66	-0.55	0.58		
	Cognitive test 3	-0.03	0.03	66	-0.83	0.41		
	Cognitive test 4	-0.03	0.03	66	-0.82	0.41		
	Stimulation: Cognitive test 2	0.01	0.02	85	0.44	0.66		
	Stimulation: Cognitive test 3	0.02	0.02	85	0.88	0.38		
	Stimulation: Cognitive test 4	0.01	0.02	85	0.44	0.66		
GRADCPT D'	Stimulation	-0.01	0.18	70	-0.05	0.96		
	Cognitive test 2	-0.03	0.34	54	-0.10	0.92		
	Cognitive test 3	0.00	0.34	54	-0.01	0.99		
	Cognitive test 4	-0.02	0.35	54	-0.06	0.96		
	Stimulation: Cognitive test 2	-0.05	0.22	70	-0.23	0.82		
	Stimulation: Cognitive test 3	-0.17	0.22	70	-0.78	0.44		
	Stimulation: Cognitive test 4	-0.02	0.22	70	-0.11	0.92		
GRADCPT MEAN	Stimulation	13.07	12.36	85	1.06	0.29		
	Cognitive test 2	-8.04	18.01	66	-0.04	0.66		

RESPONSE TIMES (MSEC)	Cognitive test 3	0.43	18.18	66	0.02	0.98			
	Cognitive test 4	-26.76	18.18	66	-1.47	0.15			
	Stimulation: Cognitive test 2	-4.30	12.46	85	-0.35	0.73			
	Stimulation: Cognitive test 3	-21.33	12.52	85	-1.70	0.09			
	Stimulation: Cognitive test 4	-3.65	12.52	85	-0.29	0.77			
	Stimulation	16.29	7.99	70	2.04	0.05	*	n.s.	0.43
GRADCPT STANDARD DEVIATION OF RESPONSE TIMES (MSEC)	Cognitive test 2	23.55	14.89	54	1.58	0.12			
	Cognitive test 3	8.62	14.89	54	0.58	0.56			
	Cognitive test 4	3.98	15.09	54	0.26	0.79			
	Stimulation: Cognitive test 2	-14.65	9.93	70	-1.48	0.14			
	Stimulation: Cognitive test 3	-6.58	9.93	70	-0.66	0.51			
	Stimulation: Cognitive test 4	-3.31	10.00	70	-0.33	0.74			

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

APPENDIX K: Mixed Model Results for N-back Measures

OUTCOME	FIXED EFFECT	B	SE	DF	T	P_{UNADJ}		P_{ADJ}	$\|D\|$
N-BACK ACCURACY FOR 3-BACKS (%)	Stimulation	0.20	3.26	67	0.06	0.95			
	Cognitive test 2	4.12	7.50	51	0.55	0.59			
	Cognitive test 3	4.03	7.39	51	0.54	0.59			
	Cognitive test 4	-2.41	7.39	51	-0.33	0.75			
	Stimulation: Cognitive test 2	-1.93	4.65	67	-0.41	0.68			
	Stimulation: Cognitive test 3	-2.43	4.61	67	-0.53	0.60			
	Stimulation: Cognitive test 4	0.68	4.61	67	0.15	0.88			
N-BACK ACCURACY OVERALL (%)	Stimulation	-0.14	1.79	67	-0.08	0.94			
	Cognitive test 2	4.96	4.11	51	1.21	0.23			
	Cognitive test 3	2.20	4.05	51	0.54	0.59			
	Cognitive test 4	0.53	4.05	51	0.13	0.90			
	Stimulation: Cognitive test 2	-1.50	2.56	67	-0.59	0.56			
	Stimulation: Cognitive test 3	1.48	2.53	67	0.58	0.56			
	Stimulation: Cognitive test 4	0.83	2.53	67	0.33	0.74			
N-BACK MEAN RESPONSE TIMES 3-BACKS (MSEC)	Stimulation	68.35	48.35	84	1.41	0.16			
	Cognitive test 2	-32.83	34.59	66	-0.95	0.35			
	Cognitive test 3	-36.27	34.14	66	-1.06	0.29			
	Cognitive test 4	-90.53	33.71	66	-2.69	0.01	*	n.s.	0.56
	Stimulation: Cognitive test 2	-51.45	67.27	84	-0.76	0.45			
	Stimulation: Cognitive test 3	-28.33	67.04	84	-0.42	0.67			
	Stimulation: Cognitive test 4	-14.26	66.83	84	-0.21	0.83			

Abbreviations and Symbols: * = $\alpha < 0.05$; |d| = absolute value of Cohen's d; n.s. = not significant; p_{adj} = *p*-value adjusted for multiple comparisons; p_{unadj} = *p*-value unadjusted for multiple comparisons; SE = standard error.

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