THE EFFECT OF COLD WATER IMMERSION ON PREFRONTAL CORTEX OXYGENATION, SALIVARY CORTISOL LEVELS, AND EXECUTIVE FUNCTIONING

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Abstract

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PURPOSE: Cold water immersion (CWI) is a popular form of voluntary cold exposure. In an environment such as a cold-water immersion, survival becomes paramount, and the ability to engage in top-down processing and take control of our actions through Executive function (EF) becomes imperative. The difficulty associated with the imposition of EF during stress like CWI is due to an increase in circulating cortisol levels, which have been associated with considerable impairments in working memory, a component of EF. This investigation aims to examine the feasibility and the effects of CWI on brain activation in the prefrontal cortex and executive function (EF) in young, healthy adults. **METHODS**: In this study, thirty-four healthy young adults (19 M and 15 F;22.74∓2.39yrs) participated. All participants arrived fasted without any consumption of caffeine or alcohol or heavy exercise within the last 24 hours. Participants completed the Mental Fatigue Scale and an assessment of basic anthropometrics. The collection of O2Hb levels using near-infrared spectroscopy (fNIRS) in the prefrontal cortex, scores on EF-specific tests from the NIH-EXAMINER, and scores on a verbal categorization task (VCT) were assessed pre and post-CWI. After collecting baseline data, participants were immersed in a tank containing 50–60 degrees Fahrenheit water up to the base of the neck, with both hands out of the water, for 5-10 minutes. During immersion, a

verbal cold discomfort scale was administered. fNIRS data, verbal categorization task data, and NIH-EXAMINER EF tests were analyzed using repeated measures ANOVA, and post hoc t-tests were performed to examine specific differences. **RESULTS**: Examination of the fNIRS data indicated a significant decrease in prefrontal recruitment from a pre immersion resting state to post immersion resting state (Pre: 35.21∓10.36 vs. Post: 27.81∓7.83, p=0.004), as well as from pre immersion executive function testing to post immersion executive function testing (Pre: 38.69∓11.60 vs. Post: 28.58∓8.69, p=<0.001). There were no significant differences between NIH Examiner and VCT scores pre and post-CWI. **CONCLUSIONS**: This study provides evidence that acute CWI does affect O2Hb levels without negatively affecting EF.

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Forward

Chapter 1 of this thesis will be submitted to *Journal of the International Neuropsychological Society*, an international peer-reviewed journal owned by Cambridge University and published by Cambridge University Press; it has been formatted according to the style guide for that journal.

Introduction

Cold exposure elicits several physiological and psychological responses in humans. Physiologically, the primary responses are cutaneous vasoconstriction, and metabolic energy transformation in the form of thermogenesis (Falla et al., 2021; Stocks et al., 2004), as well as many other endocrine responses, such as the activation of the sympathetic nervous system. Cold water immersion (CWI) is likely the most popular form of voluntary cold exposure. Cold water immersion may be initiated in a variety of settings, including recovery, rehabilitation, and general health improvement. CWI is also a part of many professional or occupational settings, such as fire and rescue, paramedics, and military applications.

Cortisol is one of the many hormones that has been responsive to CWI (Stocks et al., 2004). Commonly referred to as the "stress hormone," cortisol's main functions are to mobilize glucose, amino acids, and fatty acids, increase vascular tone, inhibit allergic and immune reactions, and to regulate metabolism, the inflammatory response, and immune function, as well as mediation of the stress response (Pääkkönen & Leppäluoto, 2002; Thau, Gandhi, & Sharma, 2022).

The hypothalamus-pituitary-adrenal (HPA) axis is responsible for both the production and secretion of cortisol, which is a steroid hormone that is synthesized from cholesterol (Thau et al., 2022). As a glucocorticoid, cortisol is able to bind to receptors present in almost all tissues of the body, and therefore affect numerous organ systems (Thau et al., 2022). Plasma cortisol levels diffuse freely into saliva, are independent of salivary secretion rate, and are free and unbound by carrier proteins, therefore representing the biologically active component of the hormone in salivary samples (Nicolson, 2008). As a result, salivary cortisol samples are thought to be a more accurate reflection of free cortisol in plasma than blood

cortisol samples (Williams & Wilson, 1998).

From a neural standpoint, cognition relies heavily on adequate blood flow to the brain. Cognitive capabilities are associated with the prefrontal cortex (PFC), which is responsible for executive function (EF) (Kar & Jain, 2016).The exact mechanism for the regulation of cerebral blood flow has yet to be identified, but pressure autoregulation and metabolic regulation both appear to be theories with support from previous literature, due to the concepts of cerebral perfusion pressure and flow-metabolism coupling (Peterson, Wang, & Britz, 2011).

Cold exposure has been documented to have a significant effect on both circulating cortisol levels. Even a brief exposure to cold stress can lead to increased circulating levels of cortisol (Eimonte et al., 2021), which is the result of an increase in sympatho-adrenal activity (Stocks et al., 2004) to produce heat in an effort towards thermoregulation (Shida et al., 2020). This increase in cortisol has been shown to stay above baseline for anywhere between two and twelve hours post exposure (Eimonte et al., 2021). Data suggest that temperatures at or above 20-25°C have shown no functional alteration of the adrenal cortex (Wilkerson et al., n.d.), which is the production site of cortisol, so it is assumed that temperatures at or above this value would not elicit a significant response in circulating cortisol levels. Other factors that may alter cortisol levels independent of cold exposure are individual levels of acclimation to cold, or the time of day, being that cortisol levels naturally follow a diurnal pattern that is set by circadian rhythms (Leppaluoto et al., 2008; Podstawski et al., 2021).

Cold exposure also has profound effects on CBF. In the event of a cold stimulus to the skin, humans activate the diving reflex, which is characterized by peripheral limb

vasoconstriction, decreased heart rate, and a maintenance or increase in cerebral perfusion (Brown, Sanya, & Hilz, 2003). It is assumed that these changes occur to divert oxygenated blood towards the brain and contribute to survival during CWI (Brown et al., 2003). In studies that have examined the effects of the diving reflex, levels of cerebral perfusion quickly returned to baseline upon removal of the cold stimulus (Brown et al., 2003).

Cognition, which can be defined as "the sum total of mental processes that enables us to acquire knowledge and keeps us aware of our surroundings and thus enables us to arrive at appropriate judgments" (Kar & Jain, 2016), is commonly divided into six domains, or major categories of functioning. These are perceptual-motor function, language, learning and memory, social cognition, complex attention, and EF (Sachdev et al., 2014). Of these six domains, EF, which is defined as "the ability to coordinate thought and action and direct it toward obtaining goals (Miller & Wallis, 2009)," holds specific importance because it is needed to "overcome local considerations, plan and orchestrate complex sequences of behavior, and prioritize goals and subgoals." (Miller & Wallis, 2009). EF contrasts with automatic forms of brain functioning and provides us with the ability to use top-down control, or "the modulation of various cognitive, perceptual, and motor processes according to abstract goals and intentions" (Gilbert & Burgess, 2008). Testing of EF has evolved greatly over the last 40 years and has provided great insight into processing beyond the "well learned or directly cued associations between stimuli and responses" (Gilbert & Burgess, 2008), as well as some clarity on the links between paradigms that compose EF and activity in the PFC, which is theorized to play a central role in EF (Miller & Wallis, 2009). Traditionally, there has been a large obstacle in relation to quantifiable, objective evaluation of EF, but with the recent development of the National Institutes of Health- Executive

Abilities: Measures and Instruments for Neurobehavioral Evaluation and Research (NIH-EXAMINER) battery, we are presented with an option that is "modular, modifiable, efficient, appropriate for a broad range of ages and ability levels, psychometrically robust, and suitable for clinical trials and clinical research" (Kramer et al., 2014), and that reliably measures inhibition, set shifting, working memory, fluency, planning, error monitoring, insight, and social function. In addition to this, Functional Near-Infrared Spectroscopy (fNIRS) can also be used to indirectly measure the delivery of oxygenated hemoglobin to the PFC, which is thought to have a direct correlation to activation in that region of the brain.

In an environment such as a cold-water immersion, survival becomes of paramount importance, and the ability to engage in top-down processing and take control of our actions through EF becomes imperative. There is difficulty associated with the imposition of EF during a stress like CWI due to an increase in circulating cortisol levels, which have been associated with considerable impairments in working memory, a component of EF. This can suggest that stress induced impairments on working memory can at least partially be created by the specific actions of cortisol on neurons in the PFC (Schoofs, Wolf, & Smeets, 2009). Paradoxically, immersion into cold water also activates the diving response, which is characterized by an increase in global CBF and the partitioning of circulation towards the brain. Through the lens of flow-metabolism coupling, this increase in circulation is thought to be accompanied by neuronal activity, which in the PFC would likely translate to a positive effect on EF.. Therefore, the purpose of this investigation is to elucidate the effects of cold-water immersion on the oxygenation of the PFC, salivary cortisol levels, and EF testing results in young healthy adults. It is hypothesized that, upon or as a result of CWI,

oxygenation of the PFC will increase, salivary cortisol levels will increase in some and remain unaffected in others, and EF will be enhanced in some and decreased in others.

Literature Review

Executive Function

Executive function is a component of cognition at large. At the lower levels of cognition are routine, automatic processes that don't require much conscious effort to take place, such as sensory analysis, memories, motor act details, and well-learned skills (Miller & Wallis, 2009). At this level, the cognitive system flows from input to output along previously established pathways without any modification or difficulty. This is also commonly referred to as bottom-up processing. However, at any given instance, the executive system may step in, as it is able to detect if automatic processes are insufficient in leading us toward our given goal (Miller & Wallis, 2009). This interjection of the executive system and carrying out of EF is known as top-down control, or higher-level cognition, and aims toward non-routine processing. These two levels of cognition are not unidirectional and tend to work in concert with each other, with continual interaction between higher level and lower-level processes (Gilbert & Burgess, 2008). EF at its core is the ability to coordinate thought and action and direct it toward one's goals in a nonroutine situation (Banich, 2009; Miller & Wallis, 2009). It is necessary for the planning and orchestration of complex behaviors, prediction and consideration of goals and the means to achieve them, the consideration of obstacles, the prioritization of goals and sub goals, categorization of commonalities across different elements, and the handling of novel information or situations (Banich, 2009; Miller & Wallis, 2009). Specific times when we engage in EF are when we plan and order for future actions, consider information and make decisions, voluntarily

switch activities, resist temptation, or anything else that allows us to lead independent, purposeful lives (Gilbert & Burgess, 2008).

From a neurobiological perspective, the PFC is critical in EF. Various areas of the PFC have connections with other brain areas that process external information, such as sensory systems and cortical and subcortical motor structures, as well as connections to internal information systems like the limbic and midbrain structures that are involved in memory and reward, and voluntary behaviors such as limb and eye movements (Miller & Wallis, 2009). These connections put the PFC in an advantageous place anatomically to support the idea of a connection between it and the concept of EF. There are several different theories regarding the mechanisms of the PFC and EF. Dehaene and Changeux proposed a model that includes an executive layer of "rule-coding" that corresponds directly to the PFC. This layer would control the flow of information from input to output layers and vice versa, and mediate with the implicit "rules" of the "game" of life (Miller & Wallis, 2009). Shimamura created a model of PFC function that involves dynamic filtering, where "patterns of information sustained by the PFC select and reroute the flow of activity" (Miller & Wallis, 2009) in the posterior association cortex. Miller & Wallis proposed that the main function of the PFC is to "acquire and actively maintain patterns of activity that represent goals and the means to achieve them (rules) in terms of a map of the cortical pathways needed to perform the task" (Miller & Wallis, 2009). This is also referred to as the model of "Rulemaps." In this model, activation of a rulemap in the PFC engages bias signals that "propagate throughout much of the rest of the cortex, affecting sensory systems as well as systems responsible for response execution, memory retrieval, emotional evaluation, etc." (Miller & Wallis, 2009) In sum, the outcome is a guided path of neural activity that creates proper maps between input,

internal state, and output to generate optimal performance on a task within one's capability (Miller & Wallis, 2009).

Executive function is a popular topic of interest in multiple communities because it is critical for self-directed behavior. This is exemplified in studies (Hanks et al., 1999) that observed a correlation between a detriment in EF following brain damage and a poorer ability to live independently (Banich, 2009). In the case of aging, EF seems to be one of the most affected cognitive processes, with an even more severe decline in the presence of conditions like Alzheimer's disease. EF is also compromised in various psychiatric illnesses, such as "schizophrenia, bipolar disorder, depression, substance use disorders, and attention deficit hyperactivity disorder" (Banich, 2009).

Executive Function and Cold

There is a convincing body of research that establishes the impairment of cold environments on human beings. However, research highlighting the effects of cold on psychological performance seems to be conflicting. The stress response of the human body, which is the primary response to cold, has two major components: the rapid activation of the autonomic nervous system, and the slower activation of the HPA axis, which can affect cognitive functioning (Solianik et al., 2015). The PFC is the region of the brain that is most affected by the stress response, which is shown to cause measurable deficits in working memory (Solianik et al., 2015). The stress response seems to disrupt the neurochemical environment in the PFC, which is dependent on performance in working memory tasks (Solianik et al., 2015). This is due specifically to a rise in concentrations of catecholamines and glucocorticoids in the PFC (Solianik et al., 2015), so much so that specific studies have confirmed that cognitive functions like working memory were only impaired when cortisol

and adrenergic activity had shown an increase (Solianik et al., 2015). As well as adrenergic activity, noradrenergic activity has also shown to be associated with impaired PFC function in working memory tasks (Duncko et al., 2009) and is also activated by stress arousal. These changes in response to stress, specifically cold stresses in the form of the Cold Pressor Test, have been theorized to last up to 30 minutes after stress cessation (Duncko et al., 2009).

There are two hypotheses that may suggest interactions between cold and cognitive performance. The distraction hypothesis states that discomfort caused by cold produces an attentional shift from the task at hand and leads to impaired cognitive performance, and the arousal hypothesis states that the initial slight decline in core temperature associated with cold exposure is sensed as a homeostatic challenge and leads to improved performance. It is important to note that this is not theorized to be a dose-response relationship, and that extended time periods of cooling seem to degrade cognitive performance (Solianik et al., 2015).

Of the studies that support the positive effects of cold on EF, some have specified that this is only the case when circulating cortisol levels do not reach high concentrations (Duncko et al., 2009). For instance, the application of the cold pressor test was associated with an enhanced effect on learning (Duncko et al., 2009), which may point to a possible link with working memory, as well as shorter reaction time.

Previous literature has also confirmed the negative effects of cold on EF. In a review of 18 studies on cold exposure and its effect on cognitive performance (Falla et al., 2021), fifteen demonstrated an impairment of cognitive performance before the accidental onset of hypothermia. This article explored more deeply the specific domains of cognitive performance that were most or least affected by cold exposure, and even amongst the

confirmation of these categories, conflicting results were reported. Although it seemed that complex tasks, in comparison to more simple ones, were more negatively affected, specifically attention, memory, processing speed and EF. This negative effect also seemed to take place in a dose-response relationship with a decline in core temperature. In examining acute cold air exposure specifically, cognitive performance was hindered in six of eight investigations, and unaffected in two (Falla et al., 2021). There appeared to be no effect on cognitive performance in regard to whether the tests took place during the cold exposure or after. In this review, seven studies explicitly confirmed the impairment of EF or components of EF, such as working memory, attention, planning, and impulse inhibition with cold exposure (Falla et al., 2021). One study (Falla et al., 2021) specifically found that cognitive performance was hindered during both exposure to cold and the "rewarming " recovery phase, which could not be explained by the distraction hypothesis, nor the arousal hypothesis. It was theorized that possible acute changes in cerebrovasculature could be the cause of such cognitive impairment. In a study on core temperature and psychomotor performance during cold weather military training (Jones et al., 2022), it was suggested that exposure as brief as 10 minutes to 1°C water deteriorates psychomotor performance without any influence from core temperature . It is theorized that this is due to the slowing of neuronal signals, distraction due to feeling cold and shivering, loss of dexterity due to muscle temperature and decreased force production, and decreased cerebral oxygenation as a result of hyperventilation at the onset of exposure. In examining cognitive performance following CWI (Ordille, 2020), impairments in EF were also observed through decreases in reaction time and accuracy in a match-to-sample task. It was restated that it is unknown whether these effects are due to physiological factors such as core body temperature, or psychological

factors, such as distraction, although it was confirmed that some level of disruption occurred in relation to physiological homeostasis.

Evidence of the interactions between cold and cognitive performance are relevant for a variety of reasons. First, is that PFC mediated cognitive functions may be influenced by rising levels of glucocorticoids, like cortisol, during times of stress. This is exemplified through the impairment of working memory tasks requiring EF during the cold pressor test (Schoofs et al., 2009). Since the PFC is also involved in feedback and regulation of the HPA axis, the primary stress system, it may be possible that there is an aggregate effect of cold on this system that is coupled with cognitive performance. The presence of cold has been shown to disrupt both normal psychological and cognitive functioning, and as a result, performance metrics, including psychomotor skills, become quickly impaired (Jones et al., 2002). These skills are employed in a variety of settings and may contribute to potentially life preserving abilities, such as threat response in a hostile environment in the case of military personnel, responses to dynamic, high stress situations in the case of an occupational worker, or the adjustment of swimming technique in response to the changing state of open water in the case of an athlete (Jones et al., 2002). Contrary to Jones et al., Ordille proposes that CWI deteriorates cognitive performance, and can negatively impact the readiness of military personnel, specifically warfighters, and labels it as “a continuous threat” (Ordille, 2020). Exposure to the cold pressor test, an adrenergic stress, demonstrated shorter reaction times and higher false alarm rates (Duncko et al., 2009), which is a behavioral pattern consistent with streamlined information processing, and therefore may serve as an advantage in a threatening situation. Both the shorter reaction times, and higher false alarm rates are said to be linked to the impaired remembering of details, which may be theorized as

a component of EF. In the review by Falla et al., the majority of studies confirm that "a single acute exposure to cold may impair attention, speed of processing, memory and EF and these effects might depend on individual physiological responses to cold as well as the extent of the exposure in terms of duration and temperature reached." (Falla et al., 2021) This information is relevant for many occupational categories, such as soldiers, mountain rescue, fisherman, and others that are exposed to cold, due to the assumption that the cognitive decline following exposure may result in negative consequences with severity such as injuries or even fatality. It is also noted that this decline may be partially due to distraction from the task at hand induced by the discomfort of the cold.

Executive Function and CBF

For adequate processes to take place in the brain that underlie EF, a continuous supply of glucose and oxygen are required from cerebral circulation (Ogoh, 2017). This is due to the highly limited energy reserves in the brain itself. Because of the combination of limited storage for substrate and a constant requirement (Ogoh, 2017), the importance of CBF for cognitive processes such as EF then becomes paramount.

Although the human brain only comprises 2% of body weight, it consumes up to 20% of circulating oxygen and glucose at rest. This is because all ATP in the brain is produced through the oxidative metabolism of glucose, and the intra-cellular capacity for the storage of these substrates is extremely limited. To combat the limited substrate storage and high demand, the brain is equipped with extremely high vascularization and dynamic control of blood flow. In humans, it is very strongly suggested that the sympathetic nervous system provides control over cerebral vasculature, and therefore, sophisticated functional control over blood flow. Along with this, the other primary modulators of CBF are the partial

pressures of arterial blood gasses such as oxygen and carbon dioxide, and cerebral metabolism, which has been suspected to be an important factor in the determination of cognitive function. When sufficient perfusion pressure is present, the remaining factors adjust to regulate blood flow via changes in the resistance of cerebral vessels (Phillips et al., 2016).

Neurovascular coupling, which is "the close spatial and temporal relationship between neural activity and regional CBF" (Phillips et al., 2016), is the core of the intersection between physiological processes and psychological outcomes. Synaptic activity, which is the neural basis for brain function, requires an increased demand for oxygen and glucose, and the resulting lack of oxygen and glucose in the region where the synaptic activity is taking place prompts for an increase of blood flow (Ogoh, 2017). The two main vessels supplying this blood flow to the brain are the vertebral and internal carotid arteries, with the internal carotid supplying approximately 70% of total CBF and the vertebral arteries supplying the remainder (Phillips et al., 2016). These two arteries are innervated by sympathetic neurons and may regulate the flow of blood to the cerebrum through the release of norepinephrine and neuropeptide Y (Phillips et al., 2016).

Neurovascular coupling can also be altered or disrupted in various pathologies. There is an apparent relationship between cognition and neurovascular coupling, where cognition is noticeably impaired when resting CBF is also impaired, of which both are mediated by neurovascular coupling. This specific interaction can be exemplified in individuals with chronic hypotension. Inversely, cognition can be restored as a result of the pharmacological treatment of hypotension through improvements in neurovascular coupling (Phillips et al., 2016). This demonstrates that cognitive function shares a positive relationship with neuronal

oxygen delivery. One condition where this link could be particularly alarming would be in the smoking population, being that the deficit in neurovascular coupling that follows this condition does not repair after cessation. As well as chronic hypotension and smoking, dementia is another condition that is suggested to be affected by cerebrovascular dysfunction, being that it often precedes the cognitive impairment that characterizes the disease. Oxidative stress at the cerebrovascular level paired with structural and functional alterations of the cerebral microvasculature at the regional or global level are suspected to be the main cause behind the cognitive impairment that follows the disease and can also be attributed to the cognitive declines seen in conditions such as hypertension, diabetes, and normal aging. More specifically in the case of aging, the attenuation of CBF is often the cause of cognitive deterioration, and this is normally due to ischemia of the brain and subsequent metabolic energy depletion. Alternatively, conditions such as more severe spinal cord injury, autonomic dysfunction, and general hypotension also do generally see a decline in cognitive function, but this is due to insufficient cerebral perfusion pressure as a result of dysregulation of cerebrovascular tone. Some cerebral and vascular diseases, such as hypertension, atherosclerosis, diabetes, high cholesterol, atrial fibrillation, dyslipidemia, smoking, obesity, and excessive alcohol consumption also see the same cognitive deficit for a similar reason, which is the chronic alteration of the regulation of CBF, which leads to the negative effect of cognitive function. In previous studies, it has been shown that this cognitive deficit is reversible with the transient occlusion of CFB in patients with vascular disease, which further suggests the link between CFB and cognitive function (Ogoh, 2017), and similarly, it is suggested that the maintenance of ideal vascular health from young adulthood to middle age is tied to better cognitive performance later in life (Ogoh, 2017). Altogether, it is clear that

healthy CBF could very well be a key factor in preserving cognition in the face of certain conditions such as disease and aging.

CBF and Cold

It has been clearly demonstrated that a cold stimulus alone can elicit significant changes on CBF. In humans, the diving reflex, which is one of the primary responses to cold exposure, is characterized by bradycardia, increased sympathetic nervous system activity, decreased limb blood flow, and maintained or even increased blood flow to the cerebrum (Brown et al., 2003). The induced bradycardia is specifically a result of an increase in cardiac parasympathetic nerve activity, peripheral vasoconstriction of the arterial tree, and an increase in sympathetic activity in response to the cessation of respiration or the stimulation of cold receptors (Foster & Sheel, 2005). Along with these more recognized responses that characterize the diving reflex, there is also evidence of a contraction of the spleen, which serves as a dynamic reservoir for erythrocyte blood cells that are released during increased activity, diving, or in response to severe hypoxia (Foster & Sheel, 2005). In humans, the diving reflex is triggered in response to respiratory arrest or the stimulation of cold receptors, specifically on the face (Foster & Sheel, 2005). This response is theorized to prolong underwater survival and conserve oxygen for the extremely sensitive brain and heart tissue by diverting blood away from peripheral vasculature in the limbs, slowing the rate of oxygen uptake in the lungs and reducing the rate of arterial blood desaturation, and increasing blood flow towards vital organs, presumably for the bolstering of conscious and unconscious processes that preserve human life (Foster & Sheel, 2005). This has been demonstrated in research through the measurement of the mean flow velocity of the mid-cerebral artery, where either facial immersion in cold water or the application of a cold stimulus to the face

has shown to increase this metric (Brown et al., 2003; Kjeld, Pott, & Secher, 2009). Being that the diving response can be elicited from both a cold stimulus and a simple breath hold, it can be assumed that this mechanism was acquired to increase the chances of survival, specifically during CWI. It has also been shown that repetitive exposure to a cold facial stimulus will abolish the diving response in the short term, most likely due to an adaptation to the feeling of cold via the thermal receptors of the face (Sterba & Lundgren, 1988), which suggests some type of initial shock response from the cold stimulus alone. Several mechanisms play into the regulation of cerebral circulation, but they all seemingly work toward one common goal, which is to oppose changes in cerebral perfusion pressure and maintain blood flow as close to a constant as possible. Being that cerebral blood vessels have dense autonomic innervation from parasympathetic and sympathetic nerves, and various animal models have shown autonomic activity to have variable effects on cerebral hemodynamics, it is suggested that these nerves do have some influence on the mechanisms that regulate CBF. Since it is known that stresses, such as cold temperatures, can increase the activation of the sympathetic nervous system, then it is easily conceivable to establish a link between cold temperatures and the dynamic control of CBF.

Cerebral Blood Flow and Cortisol

There is a clear link between cortisol and its effect on CBF, and this is most commonly exemplified through the lens of Cushing's Disease, which is a condition characterized by excess amounts of endogenous cortisol (Zhang et al., 2021). Cushing's Disease patients also have metabolic abnormalities in the brain that are caused by this excessive exposure to cortisol (Cheng et al.,2020). It has been shown that Cushing's Disease patients had increased CBF in subcortical structures, and decreased CBF in cortical

structures, such as the PFC, and consequently suffered from altered brain metabolism (Cheng et al., 2020). These Cushing's Disease-identified patients then underwent surgery to correct the cause of Cushing's disease, and out of the 50 that were operated on, 45 reached remission. The remitted patients had restored CBF with no significant differences from control groups, and these changes were positively correlated with 24-hour urinary free cortisol levels. These results demonstrate that chronic exposure of the brain to circulating cortisol can induce changes in CBF that are negatively associated with metabolism in the PFC. It is important to note that the brain has a wide distribution of glucocorticoid receptors in both cortical and subcortical regions, and is vulnerable to the exposure of excess glucocorticoids, like cortisol, and may directly lead to the aforementioned metabolic abnormalities. In other studies involving subjects with Cushing's Disease, it was explicitly shown that an excess of cortisol was associated with disrupted CBF in regions involving cognitive processing, like the PFC (Zhang et al., 2021). Moreover, these regions with disrupted CBF were associated with cortisol dosage and cognitive decline (Zhang et al., 2021). This disrupted CBF due to excess cortisol is reflective of poorer brain metabolism, substrate utilization and delivery, such as glucose and oxygen, and removal of potentially deleterious by-products of cerebral metabolism. Similar pathologies involving neurovascular decoupling are found in subjects with schizophrenia, Alzheimer's Disease, and major depressive disorder, and are shown to play an important role in the mechanism of brain dysfunction (Zhang et al., 2021). The majority of research examining the interactions between CBF and cortisol have taken place in chronic conditions, like Cushing's disease, and not in acute settings, like a voluntary CWI. It has also been shown by Hodkinson, et al., that the diurnal pattern that cortisol follows can also affect regional CBF. The results of this study demonstrate that morning cortisol levels,

which tend to be the highest, are negatively correlated with regional CFB, and could be related to the actions of cortisol on glucocorticoid receptors in the brain.

Executive Function and Cortisol

The effects of circulating cortisol on EF and similar cognitive domains have been confirmed in multiple peer-reviewed studies. Working memory, which is a sub-category of EF, has shown to be specifically affected by glucocorticoids like cortisol (Schoofs et al., 2009). It is believed that this is mainly because there are high amounts of glucocorticoid receptors distributed through the PFC in humans, which is the area of the brain where elements of EF take place (Schoofs et al., 2009). The PFC is very dense with glucocorticoid receptors and presents a target for glucocorticoid-mediated actions that can have an effect on neuronal metabolism, morphology and survival (Lee et al., 2007). The reason that glucocorticoid receptors are located in the PFC is believed to be that this area serves as feedback regulation for the hypothalamic-pituitary-adrenal axis, which is the collection of physiological processes that occur in response to stress (Schoofs et al., 2009). This suggests that PFC-mediated functions can be influenced by a rise in glucocorticoid levels (Schoofs et al., 2009). It has also been noted that the stress responses of the HPA axis can cause adverse effects on the brain, where an elevated level of cortisol can be a risk factor for cognitive dysfunction as well as cognitive decline over the long-term process of aging (Ogoh, 2017). It is important to note that cortisol has also shown to be a primary mediator in studies of adverse health consequences of exposure to stress, more specifically, negatively impacted cognition (Lee et al., 2007). Furthermore, to support the point of cortisol's negative impact on cognition, it has been shown that elevated basal cortisol levels are associated with cognitive impairment in subjects with conditions like Cushing syndrome, depression, and

Alzheimer's disease (Lee et al., 2007). Even in subjects without these diseases, elevated basal cortisol levels were still associated with poorer cognitive function across a wide range of domains (Lee et al., 2007). Additionally,, "higher levels of pretest and mean cortisol as well as the area under the curve of cortisol over the study visit were associated with worse performance in 6 domains" (Lee et al., 2007), including EF. Although the rise in cortisol can't necessarily be attributed to a lab-induced stressor, this is another clear indicator that elevated cortisol levels can negatively impact cognition.

Furthermore, acute stress induced by a cold stimulus can impair working memory performance for tasks requiring executive functions that operate on stored information, but not necessarily for tasks that only require the maintenance of information (Schoofs et al., 2009). In this study, cortisol response over time and scores on the O-span task and the digit-span task were both paired and revealed significant negative correlations, and higher individual cortisol increases were even associated with poorer individual performances. These results are in line with past studies that have explored the acute influence of cortisol elevations on working memory (Scholz et al., 2009), indicated by longer reaction times and more incorrect responses during working memory tests following a rise in glucocorticoids. The digit span forward and backwards task, which were assessed in this study, both assess working memory, but the backwards test represents a capacity to temporarily store and manipulate information, while the forwards task represents the capacity to passively maintain information (Schoofs et al., 2009). In this study, when both the backward and forward subtests were given, it seemed that only the backward test was affected by the cold, cortisol-elevating stimulus. This suggests that cortisol may only have a detrimental effect on the manipulation and associated executive component of working memory. When data from

these two tests were synthesized together, it seemed that cortisol only diminishes working memory at higher concentrations, or when the working memory task was particularly demanding. Physiological or psychological stress both have similar effects on the HPA axis and affect the sympathetic nervous system and dopaminergic system similarly, and both also have tight associations with working memory functions (Schoofs et al., 2009). This, in conjunction with the observed correlations between cortisol and working memory performance, supports the idea that working memory impairments after stress are at least partially caused by the activation of the HPA axis (Schoofs et al., 2009), and can further be suggested by evidence that shows a modulation of PFC activity from the induction of stress and/or cortisol. As well as this evidence, the indication that higher cortisol responses were associated with stronger working memory impairments suggest that this detriment is at least partially brought on by the specific effects of cortisol on neurons in the PFC (Schoofs et al., 2009).

Cold and Cortisol

Any extreme thermal environment that the human body is presented with provides a considerable amount of stress. Cold provides a specific stress and consequent set of challenges for the body to thermoregulate and preserve life. As thermoreceptors sense a drop in body temperature, the hypothalamus, which is the body's regulatory center in the brain, sets off a series of mechanisms that produce heat and prevent heat loss. Heat is produced through muscular contractions, more commonly known as shivering, and are accompanied by a series of chemical reactions that liberate heat energy for bodily warming, such as oxidative phosphorylation of carbohydrates and fats (Stocks et al., 2004). Thyroid and adrenal hormones, along with the sympathetic nervous system, control the oxidative

phosphorylation of brown fat, white fat, and skeletal muscle, so much to the point that animals that do not have their thyroid or adrenal glands are unable to tolerate the cold (Pääkkönen & Leppäluoto, 2002). These processes that aim to conserve body heat are a small part of the neurological and endocrinological cascade of events that are activated in the face of a cold stimulus, which together are called the Hypothalamic-Pituitary-Adrenal (HPA) Axis (Smith & Vale, 2006). More broadly, the HPA axis can also be defined as the collection of structures that mediate the stress response and are found in both CNS and peripheral tissues (Smith & Vale, 2006).

Steroid hormones are the group of hormones that regulate physiological processes during thermal stress, and these include cortisol, testosterone, and dehydroepiandrosterone sulfate (Podstawski et al., 2021). Steroid hormones are fat soluble and can easily penetrate cell membranes and bind to the cell's nucleus (Podstawski et al., 2021). Once the hormone is bound to the receptor, the complex then binds to DNA and activates specific genes that produce the necessary proteins and enzymes to carry out physiological processes (Podstawski et al., 2021). One of the more specific effects of the HPA axis in response to the stimulus of cold temperatures is the secretion of stress response hormones, with cortisol being the foremost (Pääkkönen & Leppäluoto, 2002). Cortisol is a glucocorticoid released from the adrenal cortex that travels through the bloodstream and binds to glucocorticoid receptors on the brain. Cortisol's main metabolic function is to keep blood glucose levels sufficiently high during physiological stress, and it does this through the mobilization of amino acids, lipids, and fatty acids in skeletal muscle and adipose tissue (Pääkkönen & Leppäluoto, 2002; Podstawski et al., 2021). The pituitary hormone ACTH stimulates the secretion of cortisol, which is primarily produced in the zona fasciculata of the adrenal gland, via a feedback

mechanism within the HPA axis (Pääkkönen & Leppäluoto, 2002; Shida et al., 2020). Along with catecholamines like epinephrine and norepinephrine, cortisol levels are raised in response to cold exposure and denote increased activity of the HPA axis. Cortisol is necessary to produce heat in the body because it is directly involved with the mobilization of glucose and lipids, in which the chemical reaction frees usable heat energy, which is then used to keep body temperatures stable in the face of a cold external stimulus (Solianik et al., 2015). During thermal stresses resulting from a cold stimulus, hormone production alters to match the demand for energy and responds to volumes of body water and body temperature. These two measures are specifically important due to the need for sufficient circulation and a stable core body temperature to combat dehydration and hypothermia, as well as many other tertiary physiological processes, such as vasoconstriction and an increase in blood pressure (Pääkkönen & Leppäluoto, 2002; Podstawski et al., 2021). Cortisol affects these measures by prompting renal free water absorption in conjunction with the metabolic effects mentioned above (Wilkerson et al., n.d.).

Cold water immersion particularly stimulates intense activity of the HPA axis (Stocks et al., 2004). In studies conducted by Pääkkönen and Leppäluoto and Eimonte, cold water exposures of as little as 10-minutes led to an increase in serum cortisol levels. Furthermore, data from a postmortem analysis showed that cortisol levels of subjects that died due to hypothermia were three times that of any other case of death (Shida et al., 2020). This demonstrates the immense stimulus that cold can provide in modulating circulating cortisol levels. Shida et al. also demonstrated that along with cortisol being secreted in large quantities in response to cold exposure, that there also may even be a reuptake mechanism that occurs, and these results suggest that cold exposure may lead to an increased secretion of

cortisol independent of signaling from ACTH (Shida et al., 2020). To further support the point of intensity within the cortisol response to cold, Wilkerson et al. observed that a near maximal cortisol secretion rate may have been reached after exposure to ambient temperatures of less than 15℃. It is also necessary to note that outside factors of everyday life may affect cortisol levels, such as psychological stress, anxiety, intense exercise, or even dietary modulations such as carbohydrate or hydration state (Izawa et al., 2009).

Cortisol and its Measurement

In the face of psychological or physiological stress, the sympathetic nervous system activates the fight or flight response, which consists of various hormonal and physiological responses (Thau et al., 2022). In the human brain, the amygdala processes fear, arousal, and emotion to determine an appropriate response, and if necessary, the amygdala communicates a stress signal with the hypothalamus (Thau et al., 2022). The hypothalamus will then activate the sympathetic nervous system, which results in an immediate rush of catecholamines released by the adrenal glands which cause an increased heart rate and ventilation rate (Thau et al., 2022). As the stimuli is further perceived as a threat to the organism's wellbeing, the hypothalamus will activate the HPA axis, and cortisol is secreted from the adrenal cortex. Cortisol allows the organism to continually stay alert while its catabolic effects mobilize substrates the organism can use for energy (Thau et al., 2022).

Cortisol has the ability to affect almost every tissue in the body due to the presence of glucocorticoid receptors in specific systems such as the nervous, immune, cardiovascular, respiratory, reproductive, musculoskeletal, and integumentary systems (Thau et al., 2022). Cortisol belongs to the class of steroid hormones, which are primary messengers and have

the ability to cross cytoplasmic membranes due to fat solubility (Thau et al., 2022). Once it enters the cell, cortisol binds to glucocorticoid receptors in the cytoplasm, and the newly formed complex subsequently enters the cell nucleus and exerts its effect on gene transcription (Thau et al., 2022).

Activation of the HPA axis follows a diurnal rhythm based on the internal circadian rhythm, which is prompted by light, the sun, and sleep-wake cycles (Thau et al., 2022) (Williams & Wilson, 1998). ACTH is secreted in brief episodic bursts, which causes sharp rises in plasma concentrations of both ACTH and cortisol, followed by a slow decline in plasma levels of cortisol (Williams & Wilson, 1998). This is due to the slower plasma clearing rate of cortisol from the bloodstream (Williams & Wilson, 1998). Due to these episodic bursts, cortisol levels tend to be higher in the morning upon waking, relatively low in the late afternoon, and lowest at nighttime about an hour or two into sleep (Williams & Wilson, 1998). After the first 30-40 minutes of consciousness in the morning, salivary cortisol levels can rise between 60-150%, and return to baseline within 60-75 minutes of waking with a gradual decrease following (Nicolson, 2008). In people with normal nocturnal sleep habits and sufficient daytime activity, cortisol levels tend to be lowest between 10pm and 4am (Nicolson, 2008). Eating can also influence plasma cortisol levels, with blood glucose and protein content of a meal being two modulating factors (Williams & Wilson, 1998). It is interesting to note that in the absence of external time cues, HPA rhythmicity and sleep-wake cycles tend to revert to a similar, synchronized circadian rhythm (Williams & Wilson, 1998).

Physiological and psychological stress both activate the HPA axis which leads to an increase in plasma cortisol (Williams & Wilson, 1998). The cortisol response to acute stressors is relatively slow compared to that of catecholamines with a similar stimulus. Within minutes of the onset of the stimulus, cortisol levels slowly rise and gradually decrease back to the pre-stress levels, and this return to baseline can take up to an hour or two (Nicolson, 2008). This is partially representative of the approximate one-hour half-life of cortisol in saliva (Nicolson, 2008). In addition to the mobilization of substrates for energy, cortisol blunts the initial fight or flight response of the sympathetic nervous system in an effort to prevent over stimulation and subsequent damage to the organism, making glucocorticoid secretion during stress primarily a response of self-preservation (Nicolson, 2008). In smaller stresses like exercise, a fever, or a burn, the HPA response tends to be proportionate to the extent of the stress, and the same goes for acute psychological stresses like competitions, the anticipation of a surgery, or the anticipation of a challenging mental task (Williams & Wilson, 1998). Hypercortisolemia is apparent in people with various psychological conditions such as severe chronic depression and anorexia nervosa, in which sleep is usually affected in both cases (Williams & Wilson, 1998).

Cortisol is commonly measured in a laboratory setting through blood or saliva analysis. In addition to the ease and non-invasive nature of salivary collection, salivary cortisol also better represents free cortisol levels, or the biologically active component of the hormone (Nicolson, 2008). This is because salivary cortisol is unbound by corticosteroid-binding globulin (CBG) and diffuses freely into saliva independent of secretion rate, and therefore correlates highly with free cortisol in blood (Nicolson, 2008) (Williams & Wilson, 1998). Because of the partial conversion of cortisol to corticosterone during the passage

through the salivary glands, the absolute amount of cortisol in blood and saliva do differ, but changes in plasma cortisol and salivary cortisol levels are still very tightly synchronized (Nicolson, 2008). Since salivary cortisol is a very stable compound, it makes it ideal for the assessment of acute stress responses (Nicolson, 2008) (Williams & Wilson, 1998). Samples of salivary cortisol have a half-life of about 80 minutes and can be kept in short term room temperature environments without denaturing, and should be refrigerated or frozen for long term storage (Williams & Wilson, 1998).

Functional Near-Infrared Spectroscopy

Functional Near-Infrared Spectroscopy (fNIRS) is a non-invasive imaging tool that investigates the change of blood oxygenation levels in the human brain utilizing the transillumination technique (Rahman et al., 2020), which has proven to be a viable tool for the measurement of brain activation (Wilcox & Biondi, 2015). FNIRS monitors in real time, gives off no harmful radiation, is very portable, and extremely patient friendly (Rahman et al., 2020). FNIRS projects near-infrared light, which is almost completely transparent to skin, bone, and brain tissue (Rahman et al., 2020), through the scalp and skull and into the brain, and the intensity of the light that is refracted is measured (Wilcox & Biondi, 2015). We can indirectly receive brain activation information by analyzing an increasing amount of oxygenated hemoglobin (HbO2) or decreasing amount of deoxygenated hemoglobin (Hb) in an area of the brain, and this is because the neurons of that area utilize oxygen to metabolize glucose and use it as a substrate for synaptic activity (Rahman et al., 2020). Through this principle, also referred to as neurovascular coupling or the hemodynamic response, synaptic activity prompts fluctuations in regional CBF (Rahman et al., 2020), and increased regional blood flow volume is assessable through measurement of local concentrations of HbO2 and

Hb, or the summed total of both (Wilcox & Biondi, 2015). HbO2 and HB are both chromophores that exist in blood and have the ability to absorb near infrared light, but the absorption coefficients of these two compounds are different (Rahman et al., 2020). Therefore, an fNIRS unit can examine the refracted light and indicate the concentrations of these two compounds individually because it emits two separate wavelengths of light, simultaneously (Rahman et al., 2020).

One of the many advantages of utilizing fNIRS imaging is the localization and specialization of neural responses, which makes it simple to identify the cortical structures that are responding to the selected stimulus (Wilcox & Biondi, 2015). Effects that are measured by fNIRS are localized to a 1-2 cm radius within the area of activation, which allows for highly accurate identification of the cortical structures being activated (Wilcox & Biondi, 2015). In the case of EF, it would be most sensical to measure blood flow volume and concentrations of HbO2 and Hb in the PFC, being that this is the area of the brain that is theorized to house the synaptic activity behind these psychological processes. If the theory of neurovascular coupling is applied, it would be expected to see a rise of HbO2 and a fall of Hb in the PFC of a subject that is prompted with a stimulus requiring some form of EF.

Methods

Participants

Participants between the ages of 20 and 35, in good health, were recruited for this study. Participants were excluded if they had any condition, such as Raynaud's Disease, that would provide any unnecessarily adverse reactions to cold exposure. Participants with diagnosed abnormalities in cortisol levels, or any conditions affecting the circulatory system were excluded. Participants with type 2 diabetes, high blood pressure, obesity, depression, little or no mental activity, little or no physical activity, or active smokers were excluded. Additionally, participants did not have any known injury to or dysfunction of the PFC, such as diagnosed neurological conditions including, but not limited to, attention-deficit/hyperactivity disorder, a traumatic brain injury to the PFC, or any form of cognitive impairment. The study was approved prior to the investigation by the university's Institutional Review Board. Written consent was obtained from each participant prior to testing. Participants were recruited from Appalachian State University and the surrounding area of Boone, North Carolina through direct contact, email, and flier.

Protocols

Participants completed a single session intervention-based study, and were asked to arrive fasted without any consumption of caffeine or alcohol or participation in heavy exercise 24- hours prior to collection. Data collection started with a collection of demographic variables, including age, sex, education level and handedness. Participants were then asked to dress in full length compression tops and bottoms to standardize the amount of skin exposed to the cold water. After dressing, baseline levels of oxygenated hemoglobin in the PFC, baseline

salivary cortisol levels, baseline scores on the NIH-EXAMINER, and baseline scores on a verbal categorization task (VCT) were collected. Participants were then immersed in a tank containing water that was kept between 50-60°F up to the base of the neck, with both hands kept out of the water, for 5-10 minutes. During immersion, a verbal cold tolerance scale and VCT were assessed at the two-minute mark. After immersion, the participant immediately put the fNIRS unit back on, salivary cortisol was collected via assay, followed by the administration of the NIH-EXAMINER and VCT without drying or re-warming. Once the second VCT was completed, participants were provided towels to dry and re-warm themselves. Additional salivary cortisol assays were taken 30 minutes post-immersion and 60 minutes post-immersion. After the last salivary cortisol sample, the participant was directed to change back into their normal clothes, height and mass were collected, and the participant was dismissed.

Measures

In order to measure EF, two tests from the NIH EXAMINER (Kramer et al., 2014) were administered to participants on a portable tablet before and directly after immersion. Only the Flanker Inhibitory Control and Attention Test and the Dimensional Change Card Sort Test were used for analysis. Additionally, scores on a 30 second VCT were recorded pre-immersion, two minutes into immersion, and post-immersion. The exact prompt of the VCT was “You have 30 seconds to name as many objects as you can that start with the letter [randomized letter]. Go.”

Participant concentrations of oxygenated hemoglobin in the PFC were measured (Hawkins et al., 2018) during a non-active baseline, the administration of the Flanker

Inhibitory Control and Attention Test and the Dimensional Change Card Sort Test, and a VCT, both before and directly after immersion. These measurements were taken with an Octamon fNIRS unit that was placed on the participant's forehead.

Salivary cortisol assays were collected pre-immersion, post immersion, 30 minutes post-immersion, and 60 minutes post immersion through direct saliva deposition into a sealed centrifuge tube. Samples were frozen at -20°C immediately after collection and until the time of analysis (Rozen, Feldman & de L. Horne, 2007).

Analysis

Raw scores from both the Flanker Inhibitory Control and Attention Test and the Dimensional Change Card Sort Test assessments were calculated and fully corrected for various demographics such as age, sex, race, ethnicity, education level, mother's education level, and handedness by the NIH Toolbox application downloaded onto the mobile tablet. Corrected scores were then exported and statistically analyzed using IBM SPSS Statistics Software v. 28.0.0.0.

Concentrations of O2Hb were recorded with an Artinis Medical Systems (Elst, Gelderland, Netherlands) Octamon fNIRS headset on Artinis Medical Systems OxySoft software v. 3.2.51.4. Raw values of O2Hb, per second, were averaged across time points that corresponded with resting states and testing events in both pre and post immersion conditions. Averaged values of O2Hb per event were then exported and statistically analyzed using IBM SPSS Statistics Software v. 28.0.0.0.

Salivary cortisol samples were analyzed using a Salivary Cortisol ELISA kit produced by DRG International (Springfield, New Jersey), which is a solid phase enzyme-linked immunosorbent assay based on the principle of competitive binding. Following collection, samples were stored at -20 °C and thawed directly before analysis. After thawing, samples were centrifuged and pipetted into wells on a microtiter plate that were coated with an anti-cortisol monoclonal antibody directed towards an antigenic site on the cortisol molecule. Endogenous cortisol competes with a cortisol-horseradish peroxidase conjugate for binding sites on the coated antibody. An enzyme conjugate was then dispensed into each well and the contents were thoroughly mixed for ten seconds. Plates were then incubated for 60 minutes at room temperature on a shaker set at 300 rpm. After incubation, the contents of the wells were briskly shaken out and wells were washed 5 times with a water-diluted wash solution that was included in the kit. This is to remove all unbound conjugate from the plate. After removal of remnant droplets, a substrate solution was added to each well and incubated for 30 minutes at room temperature. The amount of bound peroxidase conjugate is inversely proportional to cortisol concentrations in the subject's samples. The substrate solution develops intensity of color, which is inversely proportional to cortisol concentrations in the sample. After incubation, a stop solution was added to each well. Absorbances of each well were read with a BioTek (Winooski, Vermont) Synergy Neo2 Reader at 450 ± 10 nm and analyzed against an included standard curve and control samples.

Statistics

Scores from the Dimensional Change Card Sort Test and The Flanker Inhibitory Control and Attention Test were separately analyzed with paired T-tests, with an independent variable of testing time and an independent variable of test scores. VCT scores were analyzed

using a one-way repeated measures ANOVA, with an independent variable of assessment time on three levels and a dependent variable of testing scores. fNIRS data were analyzed using a one-way repeated measures ANOVA, with an independent variable of task assessment time on six levels and a dependent variable of oxygenated hemoglobin concentrations. Salivary cortisol samples were analyzed using a one-way repeated measures ANOVA, with an independent variable of sample timing on four levels and a dependent variable of sample concentrations.

Results

19 males and 15 females participated in this study. Average height of participants was 67.83 ± 3.47 inches, average mass was 166.76 ± 30.30 lbs, and average age was 22.74 ± 2.39 years. Statistical analysis revealed that there was no significant difference between pre-immersion and post-immersion mean scores on both the Dimensional Change Card Sort Test (PRE: 55.36 ± 10.968, POST: 56.26 ±10.661, $p = 0.502$) and the Flanker Inhibitory Control and Attention Test (PRE: 49.88 ± 10.682, POST: 51.9091 ± 10.62239, $p = 0.502$) from the NIH-EXAMINER, although the Flanker Inhibitory Control and Attention pre and post tests did show a trend toward significance. Results are displayed in figure 1 below.

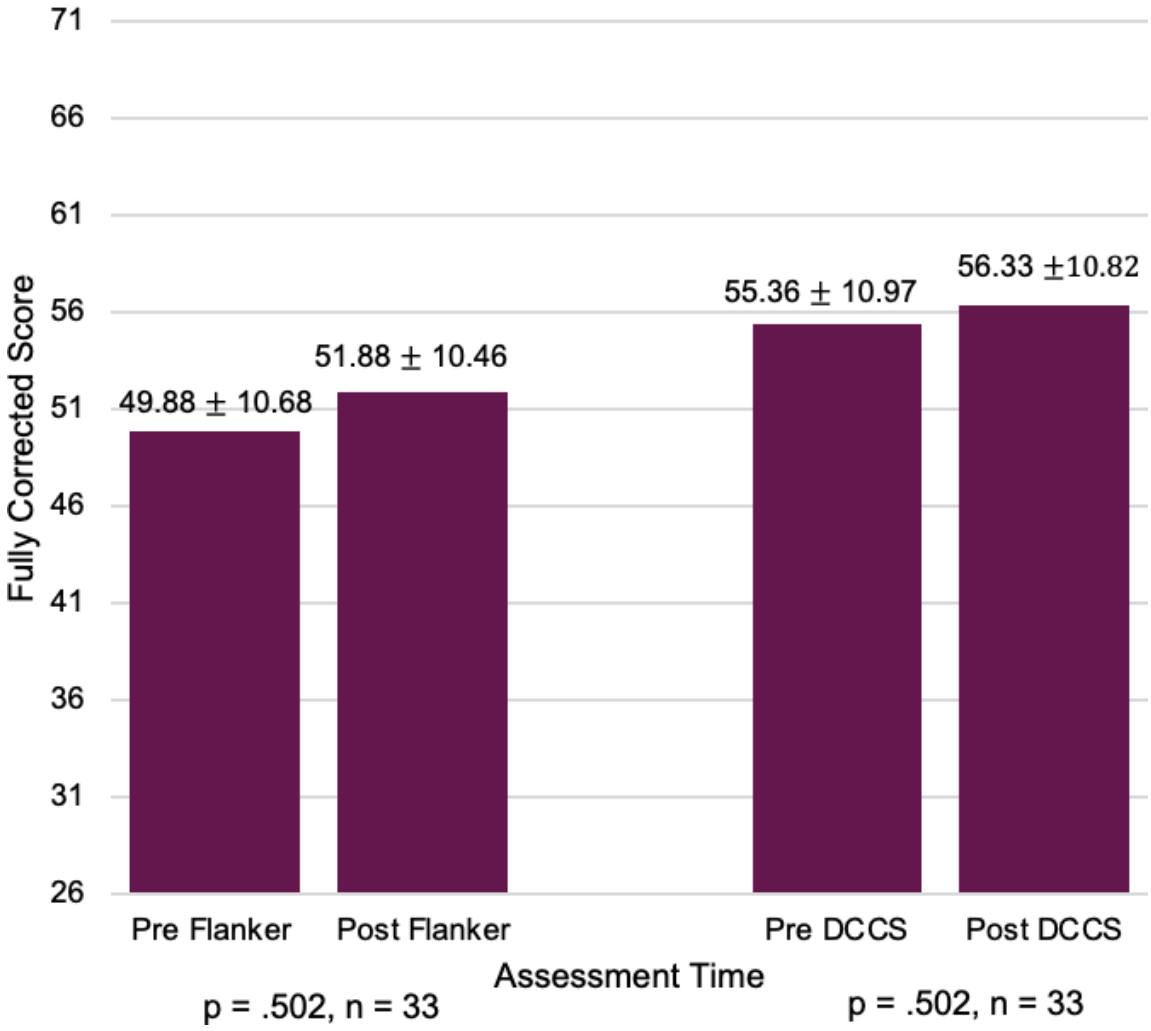

Figure 1, NIH-EXAMINER Testing Scores.

Flanker: Flanker Attention and Inhibitory Control Test

DCCS: Dimensional Change Card Sort Test

Analysis of VCT data revealed that mean VCT scores did not differ significantly across three time points ($F(2,66) = 0.562$, $p= 0.547$). Results are displayed in figure 2 below.

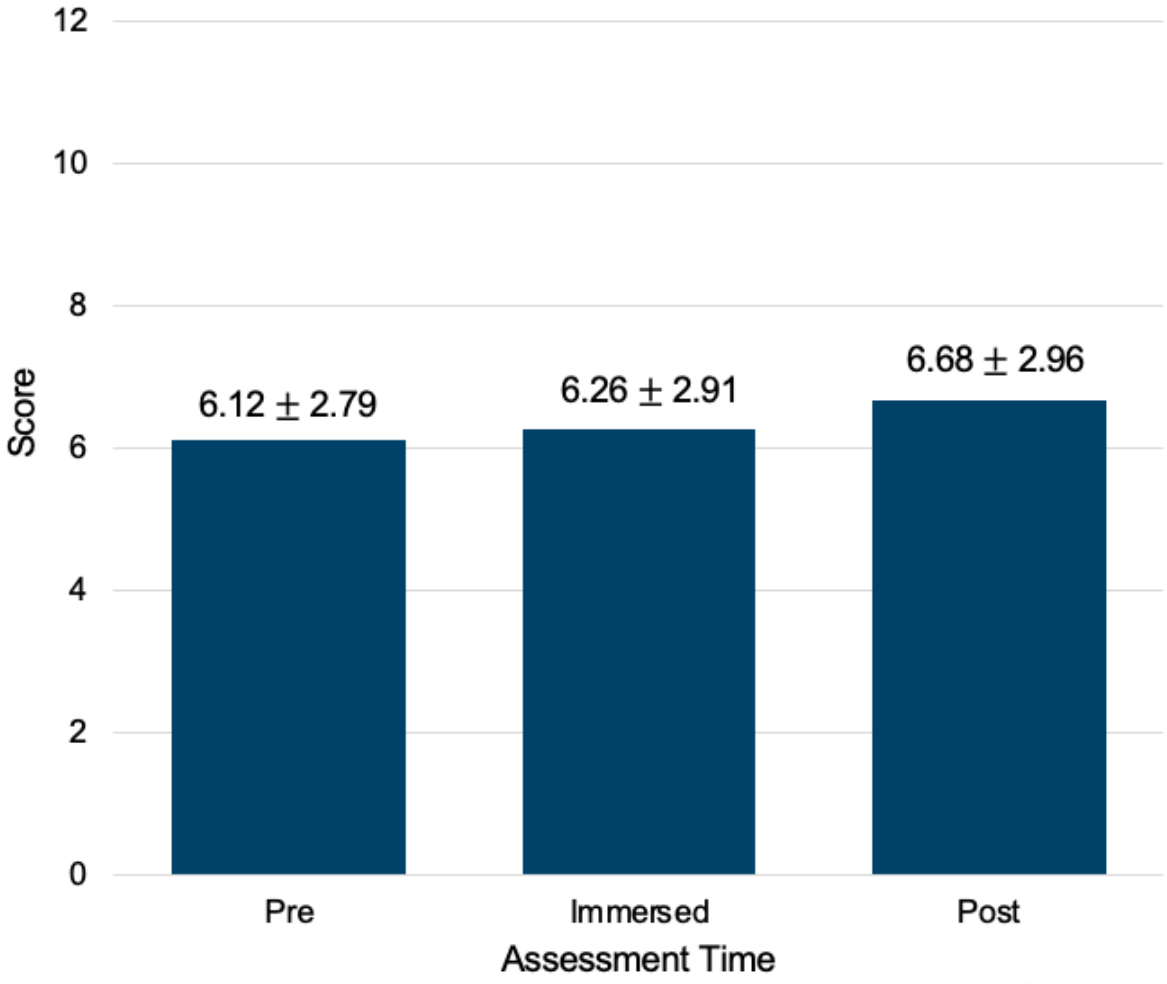

Figure 2, Verbal Categorization Task Scores.

Analysis of mean salivary cortisol concentrations showed that concentrations did not differ significantly across samples pre, post or 30 minutes post-immersion, but did differ significantly between samples 30 minutes post-immersion and 60 minutes post-immersion ($F(3,96) = 9.295$, $p = <.001$). Results are displayed in figure 3 below.

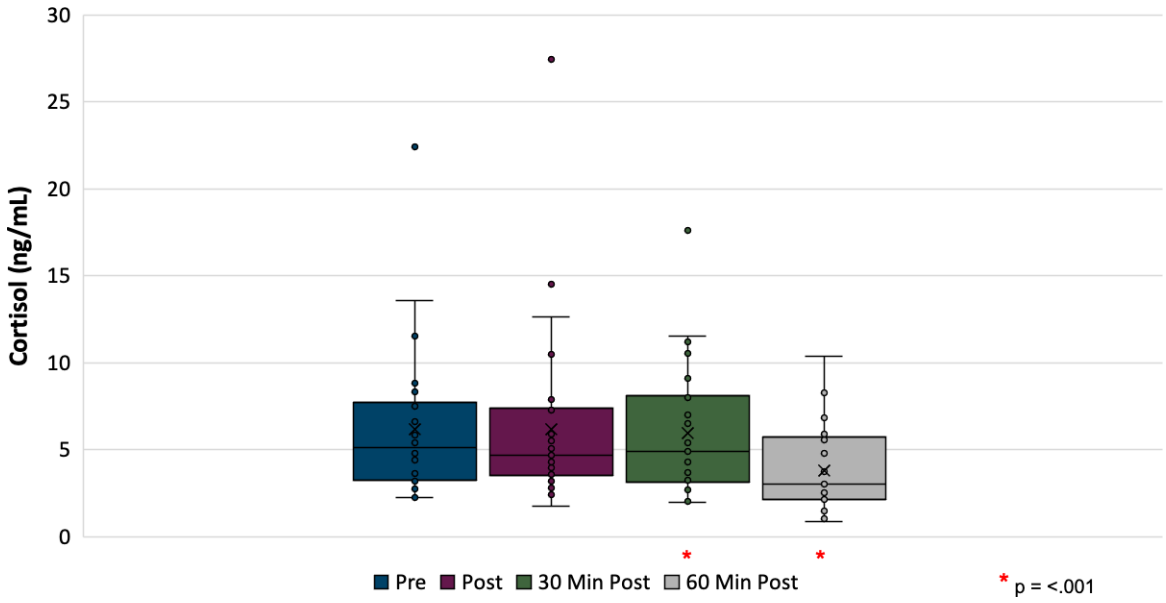

Figure 3, Salivary Cortisol Sample Concentrations.

Analysis of fNIRS data revealed significant differences in concentrations of oxygenated hemoglobin in the PFC across six time points ($F(5,160) = 24.657$, $p = <.001$): a pre-immersion resting baseline (PRE Resting), the pre-immersion assessment of the Dimensional Change Card Sort Test and the Flanker Inhibitory Control and Attention Test (PRE EXAMINER), the pre-immersion assessment of a verbal categorization task (PRE VCT), a post-immersion resting baseline (POST Resting), the post-immersion assessment of the Dimensional Change Card Sort Test and the Flanker Inhibitory Control and Attention Test (Post Examiner), the post-immersion assessment of a verbal categorization task (Post VCT). A post hoc pairwise comparison using the Bonferroni correction showed significant differences from time point time point 1 to 4 ($p = 0.004$), 2 to 5 ($p < .001$), 3 to 6 ($p < .001$), 1 to 2 ($p = <.001$), time point 1 to 3 ($p = <.001$), and time point 4 to 6 ($p = 0.010$). Mean concentrations across all six time points are displayed in figure 4 below:

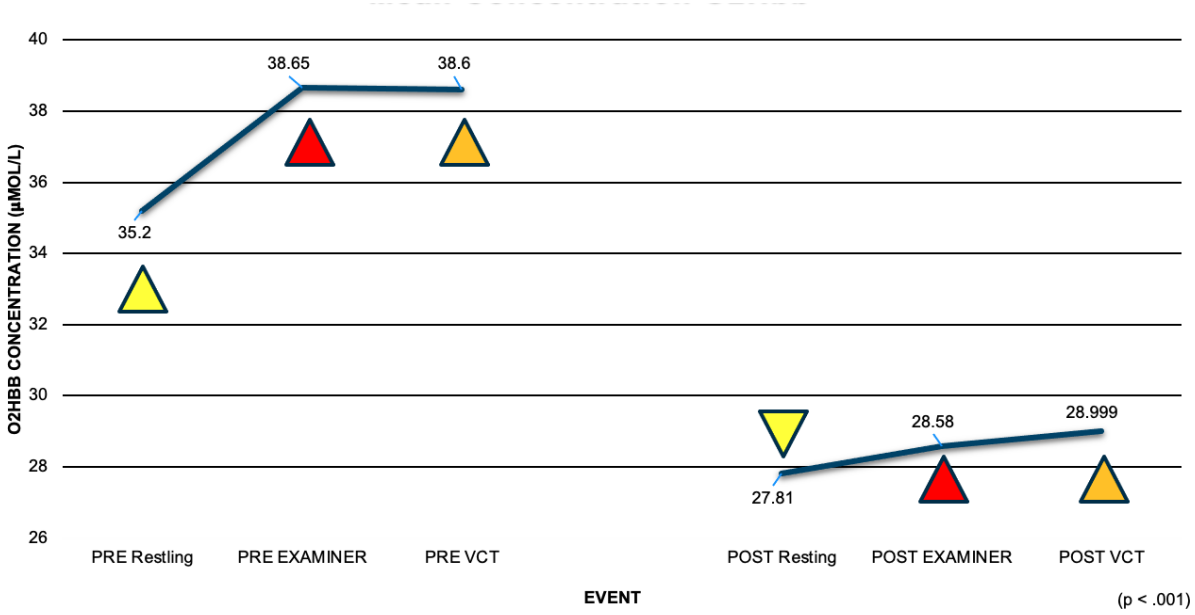

Figure 4, Mean O2Hbb Concentrations

Discussion

Cortisol

In previous studies, cold immersion has had a significant effect on circulating concentrations of cortisol, more specifically, a rise and fall coinciding with the onset and removal of a stress, such as cold-water immersion (Eimonte et al., 2021). In this study, this effect is presumed to be masked by the natural rhythm of cortisol secretions (ZRT Laboratory, n.d.), which peak early in the day upon waking, and is almost precisely when samples were collected: between 7:00am and 8:30am. Data from our study were following the natural rhythm of cortisol and there was no additional elevation above the natural secretion curve.

Direct Testing of Executive Function

Within all three measures of direct EF testing (Dimensional Change Card Sort Test, Flanker Inhibitory Control and Attention Test, VCT) there were no significant differences in test scores across time points. This indicates that EF is preserved in the face of an acute bout of CWI. Although previous studies have confirmed a degradation of EF with longer bouts of CWI (Falla et al., 2021), there has been little to no previous research regarding EF and acute bouts of CWI. To our knowledge, this study is the first of its kind to confirm the effects of an acute bout of cold-water immersion on EF.

The data from this study deny both the distraction hypothesis and the arousal hypothesis surrounding CWI and EF. The distraction hypothesis states that discomfort caused by cold produces an attentional shift from the task at hand and leads to impaired cognitive performance (Solianik et al., 2015). This is clearly not the case in our data, as there were no

significant decreases between pre-immersion and post-immersion testing of EF. Conversely, the arousal hypothesis states that the initial slight decline in core temperature associated with cold exposure is sensed as a homeostatic challenge and leads to improved performance (Solianik et al., 2015). We were also not able to observe this trend in our data, as there were no significant improvements between pre-immersion and post-immersion testing of EF.

fNIRS

In comparing pre-immersion levels of O2Hb with post immersion levels, it is clear that the PFC is receiving lesser amounts of O2Hb directly after exiting the cold water, which is indicative of a smaller amount of blood flow to the PFC in the post-immersion condition. This contrasts the concept of neurovascular coupling, which is defined as "the close spatial and temporal relationship between neural activity and regional CBF" (Phillips et al., 2016). In previous studies, synaptic activity and regional CBF have been shown to be tightly synchronized (Phillips et al., 2016), which raises a cause for concern among these results. According to our data, although O2Hb levels are significantly lower through the entire duration of the post-immersion testing battery in comparison to the same pre-immersion testing battery, EF, a cognitive capability, is preserved in the face of a CWI. This directly violates the concept of neurovascular coupling, where we would expect to see a similar detriment to EF in the post-immersion testing battery to follow along with the decrease of O2Hb delivery to the PFC (Phillips et al., 2016). This phenomenon is possibly indicative of an alteration of synaptic activity and, consequently, the dynamics of neurovascular coupling in the PFC directly after acute CWI.

It is assumed that the alteration in cerebral hemodynamics after an acute CWI is at least, in part, a consequential effect of peripheral vasoconstriction via the diving reflex in humans (Brown et al., 2003). After exiting the cold water, peripheral vasoconstriction reverses within seconds (Brown et al., 2003), resulting in a reallocation of blood and consequentially, O2Hb, to the tissues of the arms and legs, assumedly for the purpose of re-warming. The theory of re-warming can be further supported by observed shivering in some participants after exiting the cold water, which is an involuntary muscle contraction made to produce heat and deliver blood to tissues exposed to cold (Stocks et al., 2004). Since a larger volume of blood is being reallocated to peripheral tissues in the post-immersion testing condition rather than the pre-immersion testing condition, it is assumed that less blood volume containing O2Hb is available for the brain to utilize during the utilization of EF, which is reflected in data from fNIRS measurements.

In comparing the change of O2Hb concentrations between specific time points in fNIRS data, we see a significant increase between time points 1 and 2, representing the onset of EF testing in the pre-immersion battery, and no significant change between time points 4 and 5, representing the onset of EF testing in the post-immersion battery. This is presumably due to a smaller volume of blood and O2Hb being shunted to the PFC in response to the same cognitive demand in the post-immersion battery compared to the pre-immersion battery. From this, we can assume that an acute CWI alters the body's ability to provide O2Hb to the brain and that the brain still preserves its executive functioning in a less oxygenated state.

In comparing the post-immersion testing time points, there is no change from post-immersion resting state to post-immersion, and a significant increase from the post-immersion resting state to the post-immersion VCT. This difference demonstrates the body's re-warming effect on cerebral hemodynamics in real time. As time passes from the resting state to the NIH-EXAMINER tests, some re-warming has already taken place, leaving peripheral tissues and vasculature to require less blood supply, making more blood volume available to be recruited by the PFC. This can be compared to the difference in values between the pre-immersion EXAMINER testing and the pre-immersion VCT, where we see a much flatter slope between the two points, representing a more equal availability and supply of O2Hb for both EF tasks in the pre-immersion battery, rather than the upward trending line between the post-immersion resting state and the post immersion EXAMINER, where we see an unequal supply of O2Hb between both EF tasks.

Limitations

The foremost limitation experienced in this study was the time of day that data were collected. The laboratory being used for this study only had availability for data collection sessions before 8:30 am, so the overwhelming majority of participants' data were collected between 6:30 and 8:30am. This window of time correlates directly with the peak of cortisol's circadian cycle, and therefore, any potential increases in salivary cortisol concentrations were likely masked by the body's natural rhythm. Participants were also asked to come in fasted so as not to interfere with salivary cortisol samples, so to be as considerate to them as possible, it was decided to have data collections take place in the mornings rather than afternoons or nights. Height and mass values were taken for each participant in order to calculate body mass index values that were intended to be investigated for correlation with cortisol response

volume after the CWI, but due to the nature of the data we collected, no such correlation would be possible. Additionally, body fat percentages were not examined, which have the potential to insulate against a cold water immersion. According to study results, there seems to be a preservation of synaptic activity in the PFC between pre- and post-immersion tests of EF. A quantifiable measure of this activity would have strengthened the conclusion of these data.

Clinical Relevance

In an elective, rehabilitative or performance setting, such as physical therapy, muscular recovery, or voluntary immersion, it is important to consider the apparent time-sensitive cognitive decline while immersed in cold water. This is supported by past research that has concluded cognitive decline with longer immersions of 20-60 minutes in equally cold or similar temperature water to that used in this study (Falla et al., 2021). In a meta analysis that examined CWI as a recovery modality (Moore et al., 2023), the vast majority of included studies immersed participants for 10-15 minutes in water between 50-59°F . Within these parameters, it is possible that the time-sensitive effect on cognitive decline could be reached, and result in a detriment to the subject. Ideally, future research should identify the time point at which we could maximize the physical recovery benefits of CWI while minimizing the negative cognitive impact of prolonged immersion. In an environmental, occupational setting, such as Fire & Rescue, Lifeguarding, Military, or competitive open-water swimming, the time sensitive cognitive-decline should be of great concern. This cognitive decline seems to onset around the 20 minute mark of immersion (Falla et al., 2021), and this is important to consider when these populations are either training for or performing their occupations, as decision making and the imposition of EF could save their lives, the lives of others, or impact

strategic decisions for victory. This research also adds psychological focus to the quickly emerging body of research surrounding CWI that mainly examines physiological benefits, such as prolonged release of dopamine post immersion, increased metabolism, and the conversion of white fat to brown fat.

Future Directions

In future research, a possible consideration would be to investigate cold water immersion's effect on synaptic activity in the PFC. Considering the concept of neurovascular coupling, results from this study suggest that there is an unknown mechanism of synaptic activity in the PFC that contributes to the preservation of EF after CWI. Additionally, participants in this study were screened for psychological conditions such as depression, attention- deficit/hyperactivity disorder, a traumatic brain injury to the PFC, or any form of cognitive impairment, in an attempt to normalize data. In future research, the inclusion of individuals with these conditions, or research involving these conditions specifically, would possibly be beneficial, considering the conclusion of a more positive effect on EF than that has been concluded in the past.

Conclusion

Acute bouts of CWI significantly decrease PFC O2Hb availability and utility during a task that involves EF. Acute bouts of CWI also preserve, and do not degrade, EF from pre-immersion tasks in healthy, young adults, regardless of a significant decrease in PFC oxygenation. This study is the first of its kind, to our knowledge, that directly investigates the effects of an acute bout of CWI on EF. This study's results contrast the results of similar studies that have investigated the effects of a more prolonged CWI, which concluded a significant impairment in EF. The results of this study potentially point to an unknown mechanism of synaptic activity that may positively affect neurovascular coupling in order to preserve EF. This adds to the emerging body of research around CWI, further supporting the idea that it is usually of limited cost, requires little to no physical effort, is accessible to most people worldwide, and has many positive effects on overall health for a wide variety of user.

References

Banich, M. T. (2009). Executive Function: The Search for an Integrated Account. *Current Directions in Psychological Science*, *18*(2), 89–94. https://doi.org/10.1111/j.1467-8721.2009.01615.x

Brown, C. M., Sanya, E. O., & Hilz, M. J. (2003). Effect of cold face stimulation on cerebral blood flow in humans. *Brain Research Bulletin*, *61*(1), 81–86. https://doi.org/10.1016/S0361-9230(03)00065-0

Cheng, H., Gao, L., Hou, B., Feng, F., Guo, X., Wang, Z., Feng, M., Xing, B., & Fan, Y. (2020). Reversibility of cerebral blood flow in patients with Cushing's disease after surgery treatment. *Metabolism*, *104*, 154050. https://doi.org/10.1016/j.metabol.2019.154050

Diurnal cortisol curves – saliva vs urine. ZRT Laboratory. (n.d.). Retrieved April 2, 2023, from https://www.zrtlab.com/landing-pages/diurnal-cortisol-curves-saliva-vs-urine/

Duncko, R., Johnson, L., Merikangas, K., & Grillon, C. (2009). Working memory performance after acute exposure to the cold pressor stress in healthy volunteers. *Neurobiology of Learning and Memory*, *91*(4), 377–381. https://doi.org/10.1016/j.nlm.2009.01.006

Eimonte, M., Paulauskas, H., Daniuseviciute, L., Eimantas, N., Vitkauskiene, A., Dauksaite, G., Solianik, R., & Brazaitis, M. (2021). Residual effects of short-term whole-body cold-water immersion on the cytokine profile, white blood cell count, and blood markers of stress. *International Journal of Hyperthermia*, *38*(1), 696–707. https://doi.org/10.1080/02656736.2021.1915504

Falla, M., Micarelli, A., Hüfner, K., & Strapazzon, G. (2021). The effect of cold exposure on cognitive performance in healthy adults: A systematic review. *International Journal of Environmental Research and Public Health*, *18*(18), 9725. https://doi.org/10.3390/ijerph18189725

Foster, G. E., & Sheel, A. W. (2005). The human diving response, its function, and its control. *Scandinavian Journal of Medicine and Science in Sports*, *15*(1), 3–12. https://doi.org/10.1111/j.1600-0838.2005.00440.x

Gilbert, S. J., & Burgess, P. W. (2008). Executive function. *Current Biology: CB*, *18*(3), R110-114. https://doi.org/10.1016/j.cub.2007.12.014

Hanks, R. A., Rapport, L. J., Millis, S. R., & Deshpande, S. A. (1999). Measures of executive functioning as predictors of functional ability and social integration in a rehabilitation sample. *Archives of Physical Medicine and Rehabilitation*, *80*(9), 1030–1037. https://doi.org/10.1016/S0003-9993(99)90056-4

Hawkins, K. A., Fox, E. J., Daly, J. J., Rose, D. K., Christou, E. A., McGuirk, T. E., Otzel, D. M., Butera, K. A., Chatterjee, S. A., & Clark, D. J. (2018). Prefrontal over-activation during walking in people with mobility deficits: Interpretation and functional implications. *Human Movement Science*, *59*, 46–55. https://doi.org/10.1016/j.humov.2018.03.010

Hodkinson, D. J., O'Daly, O., Zunszain, P. A., Pariante, C. M., Lazurenko, V., Zelaya, F. O., Howard, M. A., & Williams, S. C. (2014). Circadian and homeostatic modulation of functional connectivity and regional cerebral blood flow in humans under normal entrained conditions. *Journal of Cerebral Blood Flow & Metabolism*, *34*(9), 1493–1499. https://doi.org/10.1038/jcbfm.2014.109

Izawa, S., Kim, K., Akimoto, T., Ahn, N., Lee, H., & Suzuki, K. (2009). Effects of cold environment exposure and cold acclimatization on exercise-induced salivary cortisol response. *Wilderness & Environmental Medicine*, *20*(3), 239–243. https://doi.org/10.1580/07-WEME-OR-123R2.1

Jones, D., Weller, R., Eggan, S., Roberts, N., Zheng, W., Heaney, J., & Dunn, T. (2022). Influence of core temperature on psychomotor performance during cold weather military training. *Journal of Human Performance in Extreme Environments*, *17*(1). https://doi.org/10.7771/2327-2937.1149

Kar, S. K., & Jain, M. (2016). Current understandings about cognition and the neurobiological correlates in schizophrenia. *Journal of Neurosciences in Rural Practice*, *07*(03), 412–418. https://doi.org/10.4103/0976-3147.176185

Kjeld, T., Pott, F. C., & Secher, N. H. (2009). Facial immersion in cold water enhances cerebral blood velocity during breath-hold exercise in humans. *Journal of Applied Physiology*, *106*(4), 1243–1248. https://doi.org/10.1152/japplphysiol.90370.2008

Kramer, J. H., Mungas, D., Possin, K. L., Rankin, K. P., Boxer, A. L., Rosen, H. J., Bostrom, A., Sinha, L., Berhel, A., & Widmeyer, M. (2014). NIH EXAMINER: Conceptualization and development of an executive function battery. *Journal of the International Neuropsychological Society*, *20*(1), 11–19. https://doi.org/10.1017/S1355617713001094

Lee, B. K., Glass, T. A., McAtee, M. J., Wand, G. S., Bandeen-Roche, K., Bolla, K. I., & Schwartz, B. S. (2007). Associations of salivary cortisol with cognitive function in the Baltimore memory study. *Archives of General Psychiatry*, *64*(7), 810. https://doi.org/10.1001/archpsyc.64.7.810

Leppäluoto, J., Westerlund, T., Huttunen, P., Oksa, J., Smolander, J., Dugué, B., & Mikkelsson, M. (2008). Effects of long-term whole-body cold exposures on plasma concentrations of ACTH, beta-endorphin, cortisol, catecholamines and cytokines in healthy females. *Scandinavian Journal of Clinical and Laboratory Investigation*, *68*(2), 145–153. https://doi.org/10.1080/00365510701516350

Miller, E. K., & Wallis, J. D. (2009). Executive function and higher-order cognition: definition and neural substrates. In *Encyclopedia of neuroscience* (pp. 99–104). Elsevier. https://doi.org/10.1016/B978-008045046-9.00418-6

Moore, E., Fuller, J. T., Bellenger, C. R., Saunders, S., Halson, S. L., Broatch, J. R., & Buckley, J. D. (2023). Effects of cold-water immersion compared with other recovery modalities on athletic performance following acute strenuous exercise in physically active participants: a systematic review, meta-analysis, and meta-regression. *Sports Medicine*, *53*(3), 687–705. https://doi.org/10.1007/s40279-022-01800-1

Nicolson, N. A. (2008). Measurement of cortisol. In *Handbook of physiological research methods in health psychology* (pp. 37–74). SAGE Publications, Inc. https://doi.org/10.4135/9781412976244.n3

Ogoh, S. (2017). Relationship between cognitive function and regulation of cerebral blood flow. *The Journal of Physiological Sciences*, *67*(3), 345–351. https://doi.org/10.1007/s12576-017-0525-0

Ordille, A. (2020). *Commercial Heat Packs do not Influence Cognitive Performance Following Cold Water Immersion.*Naval Health Research Center. https://apps.dtic.mil/sti/citations/AD1138308

Pääkkönen, T., & Leppäluoto, J. (2002). Cold exposure and hormonal secretion: A review. *International Journal of Circumpolar Health*, *61*(3), 265–276. https://doi.org/10.3402/ijch.v61i3.17474

Peterson, E. C., Wang, Z., & Britz, G. (2011). Regulation of cerebral blood flow. *International Journal of Vascular Medicine*, *2011*, 1–8. https://doi.org/10.1155/2011/823525

Phillips, A. A., Chan, F. H., Zheng, M. M. Z., Krassioukov, A. V., & Ainslie, P. N. (2016). Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. *Journal of Cerebral Blood Flow & Metabolism*, *36*(4), 647–664. https://doi.org/10.1177/0271678X15617954

Podstawski, R., Borysławski, K., Pomianowski, A., Krystkiewicz, W., & Żurek, P. (2021). Endocrine effects of repeated hot thermal stress and cold water immersion in young adult men. *American Journal of Men's Health*, *15*(2), 155798832110083. https://doi.org/10.1177/15579883211008339

Rahman, Md. A., Siddik, A. B., Ghosh, T. K., Khanam, F., & Ahmad, M. (2020). A narrative review on clinical applications of fNIRS. *Journal of Digital Imaging*, *33*(5), 1167–1184. https://doi.org/10.1007/s10278-020-00387-1

Rozen, W. M., Feldman, G., & de L. Horne, D. J. (2007). Salivary Cortisol as a Measure of Stress in Australian Football League Footballers. Individual Differences Research, 5(3), 221–229.

Sachdev, P. S., Blacker, D., Blazer, D. G., Ganguli, M., Jeste, D. V., Paulsen, J. S., & Petersen, R. C. (2014). Classifying neurocognitive disorders: The DSM-5 approach.

Nature Reviews Neurology, *10*(11), 634–642. https://doi.org/10.1038/nrneurol.2014.181

Scholz, U., La Marca, R., Nater, U. M., Aberle, I., Ehlert, U., Hornung, R., Martin, M., & Kliegel, M. (2009). Go no-go performance under psychosocial stress: Beneficial effects of implementation intentions. *Neurobiology of Learning and Memory*, *91*(1), 89–92. https://doi.org/10.1016/j.nlm.2008.09.002

Schoofs, D., Wolf, O. T., & Smeets, T. (2009). Cold pressor stress impairs performance on working memory tasks requiring executive functions in healthy young men. *Behavioral Neuroscience*, *123*(5), 1066–1075. https://doi.org/10.1037/a0016980

Shida, A., Ikeda, T., Tani, N., Morioka, F., Aoki, Y., Ikeda, K., Watanabe, M., & Ishikawa, T. (2020). Cortisol levels after cold exposure are independent of adrenocorticotropic hormone stimulation. *PLOS ONE*, *15*(2), e0218910. https://doi.org/10.1371/journal.pone.0218910

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8*(4), 383–395.

Solianik, R., Skurvydas, A., Urboniene, D., Eimantas, N., Daniuseviciute, L., & Brazaitis, M. (2015). Similar cold stress induces sex-specific neuroendocrine and working memory responses. *Cryo Letters*, *36*(2), 120–127.

Sterba, J. A., & Lundgren, C. E. (1988). Breath-hold duration in man and the diving response induced by face immersion. *Undersea Biomedical Research*, *15*(5), 361–375.

Stocks, J. M., Taylor, N. A. S., Tipton, M. J., & Greenleaf, J. E. (2004). Human physiological responses to cold exposure. *Aviation, Space, and Environmental Medicine*, *75*(5), 444–457.

Thau, L., Gandhi, J., & Sharma, S. (2022). Physiology, Cortisol. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK538239/

Wilcox, T., & Biondi, M. (2015). fNIRS in the developmental sciences. *WIREs Cognitive Science*, *6*(3), 263–283. https://doi.org/10.1002/wcs.1343

Wilkerson J.E., Raven P.B., Bolduan N.W., & Horvath S.M. (n.d.). Adaptations in man's adrenal function in response to acute cold stress. *J Appl Physiol 1974; 36:183–9.*

Williams, R. H., & Wilson, J. D. (Eds.). (1998). *Williams textbook of endocrinology* (9th ed). Saunders.

Zhang, Y., Zhou, T., Feng, S., Wang, W., Liu, H., Wang, P., Sha, Z., & Yu, X. (2021). The chronic effect of cortisol on orchestrating cerebral blood flow and brain functional connectivity: Evidence from Cushing's disease. *Metabolism*, *115*, 154432. https://doi.org/10.1016/j.metabol.2020.154432

Vita

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