

The influence of operational stress and ambiguity on Soldier shoot/don't-shoot performance

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ABSTRACT

Stress carries diverse implications for perceptual, cognitive, and affective functions. One population particularly susceptible to acute stress-induced cognitive changes are individuals with high-stress jobs (i.e., military personnel). These individuals are often tasked with maintaining peak cognitive performance during conditions of emotional and physical stress. Previous research demonstrates that the effects of acute emotional and physical stress often depend on the cognitive domain assessed and the type and intensity of the stressor. Moreover, Soldiers are also continually called upon to make critical decisions under uncertain conditions. One of the most critical decisions is whether to shoot or withhold fire, which often requires quickly interpreting ambiguous information to make threat versus non-threat determinations. Previous research has separately examined shoot/don't-shoot decisions under conditions of stress or uncertainty (i.e., ambiguous discrimination between friends and foes). However, questions remain about how ambiguity impacts shoot/don't-shoot decisions made during stress. To address this gap, we examined the influence of an operationally-relevant emotional (Study 1) and physical (Study 2) stressor on a novel Soldier-relevant shoot/don't-shoot cognitive task during conditions of high and low stimulus ambiguity. The first study utilized threat of shock (TOS) as a high-stakes outcome for decision errors (i.e., incorrectly orienting, missing a threat, or false-alarming to a non-threat). This emotional stress manipulation (TOS) increased sympathetic arousal, but did not affect subjective emotional or HPA responses. Further, shoot/don't-shoot performance varied by stimulus ambiguity, but did not differ between stress conditions. The second study explored whether a prolonged bout of load carriage would influence ambiguous shoot/don't-shoot decisions. Physical stress led to elevated heart rate, greater perceived exertion and less positive, more negative emotions. However, similar to Study 1, shoot/don't-shoot performance was impacted by ambiguity but not load. In sum, high levels of stress and uncertainty characterize

military operations. However, stress experienced in military contexts can be difficult to induce in laboratory settings. We discuss several avenues for future research, including methodological considerations to better assess the magnitude and specificity of emotional and physical stress-induction techniques in Soldiers.

Keywords: acute stress, threat of shock, load carriage, ambiguity, shoot/don't-shoot

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**The influence of operational stress and ambiguity on Soldier
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GENERAL INTRODUCTION

Stress occurs when circumstances (environmental or social) tax or exceed an individual's adaptive resources (Lazarus & Folkman, 1984). Stress is often elicited by the inherent novelty, uncontrollability, unpredictability, or threatening nature of human experience (Gagnon & Wagner, 2016; McEwen, 2007) and is considered a normal physiological and mental response to such challenges. Stress carries diverse neurotransmitter, hormonal, genomic, and immune implications through activation of the autonomic nervous system (ANS) and hypothalamic pituitary adrenal (HPA) axis. First is a relatively rapid catecholamine response, including the release of epinephrine and norepinephrine from the adrenal medulla, which correlates with several physiological changes such as increased heart rate and blood pressure, increased blood flow to skeletal muscles, sweating, and decreased heart rate variability (Dimsdale & Moss, 1980; Schwabe et al., 2012; Terkelsen et al., 2005; van Doornen & van Blokland, 1992). Second is a relatively slow HPA response and glucocorticoid (cortisol) release, which is known to cause genomic and nongenomic effects on the central nervous system (Sapolsky et al., 2000). These two systems produce highly diverse effects on the brain, particularly in regions such as the amygdala, hippocampus, striatum, and prefrontal cortex, which are brain regions with high catecholamine and/or glucocorticoid receptor densities (Arnsten, 1998, 2009; Kim et al., 2015; Roozendaal et al., 2009).

Stress and cognitive performance

Given that these brain regions are responsible for perceptual, cognitive, and affective functions, stress carries diverse implications for maintaining cognitive performance. Research shows that chronic or sustained stress may lead to declines in cognition, whereas acute stress may improve or impair performance depending on the cognitive domain assessed. For example,

stress can positively influence performance on relatively simple or well-rehearsed tasks (Arnsten, 2009; Broadbent, 1971) that do not demand prefrontal cortical engagement. However stress can negatively influence performance on relatively complex tasks involving high levels of executive function (Arnsten, 1998, 2009; Cerqueira et al., 2007). Executive functions, also called cognitive control processes, consist of mental set shifting (moving back and forth between tasks, mental operations, etc.), information updating (such as receiving updated intelligence on an operation), and inhibition (holding back a response, such as firing a weapon), which are thought to rely on the frontal lobe (Miyake et al., 2000; Miyake & Friedman, 2012). These executive functions are susceptible to acute stress (Shields et al., 2016, 2017, 2019; Starcke et al., 2016), which can be especially pronounced, for example, during unpredictable and ambiguous military operations (Delahaij et al., 2006).

Stress and cognitive performance in military populations

Indeed, one population particularly susceptible to acute stress-induced cognitive changes are individuals with high-stress jobs (i.e., military personnel). These individuals are often exposed to diverse stressors across both physical and emotional domains. At the same time, these individuals are tasked with maintaining peak performance under such acutely stressful conditions, such as during strenuous bouts of load carriage and physical exertion, prolonged mental states of isolation, ambiguity, danger and threat, powerlessness, and high workload (Bartone, 2006). A recent review of field studies simulating military sustained operations (SUSOPS) found significant decrements in all cognitive constructs examined (vigilance, reaction time, working memory, and reasoning) during representative combat stress (Vrijkotte et al., 2016).

Although measuring cognitive performance in the field may provide greater ecological validity, field studies inherently involve substantial variation, as well as methodological and practical limitations (Knapik et al., 1991; Mahoney et al., 2007). In turn, it is difficult to disentangle effects due to stress given that cognitive changes are likely compounded by operational realities of sleep loss, fatigue, thermal load, dehydration, resource limitations, and constantly changing mission demands (Adam et al., 2008; Parsons et al., 2019; Sullivan-Kwantes et al., 2021; Troxel et al., 2015). However, even in more controlled settings, most laboratory-grade stress- and anxiety-inductions are relatively mild (e.g., public speaking or submerging an arm in cold water) (Kirschbaum et al., 1993; Schwabe et al., 2008) and may fail to reflect what soldiers experience during training and operations. Therefore, research emulating soldier-relevant stressors in controlled laboratory settings is needed to understand better the impacts of stress on varying aspects of Soldier cognition.

Stress and Soldier-relevant cognitive performance

Understanding how stress influences cognitive performance outcomes relevant to military operations is crucial. Within the military-specific cognitive literature, various assessment approaches have been utilized. Task selection has ranged from employing “basic” non-military specific cognitive assessments, such as computer-based simple and choice reaction time, and vigilance tasks (Knapik et al., 1991; Lieberman et al., 2005, 2006; Mahoney et al., 2007) to military-specific assessments, like a military-specific auditory go/no-go task where go and no-go stimuli are various types of gun fire (Eddy et al., 2015; Giles et al., 2019). Examining changes in performance on non-military specific cognitive tasks may provide insights into how particular cognitive domains or types of executive functions are affected by stress. However, it is important to consider the external validity of such assessments and whether these tasks generalize to the

cognitive demands placed upon soldiers during military operations (Vine et al., 2021). Though soldiers must perform seemingly basic combat tasks of move, shoot, communicate, it is important to ensure that cognitive tasks selected match operational task demands. This will increase the potential for laboratory findings to transfer to Soldier performance in real-world settings (Blacker et al., 2019).

Stress and shoot/don't-shoot performance

One critical Soldier task involves deciding whether to shoot or withhold fire. At the basic level this behavior mirrors a classic go/no-go decision. However, when performed in operational settings shoot/don't-shoot decisions entail a complex series of actions that can be linked to specific cognitive processes. Indeed, locating a potential target involves visual search and attention. Determining whether someone is a friend or foe involves decision-making processes. Aiming involves perceptual estimations of distance and motion, and pulling the trigger (or withholding the shot) involves response execution (or inhibition). Within a military context, these cognitive processes fall into a framework referred to as the OODA Loop: observe-orient-decide-act (Boyd, 1995). Despite the critical life-or-death implications, these decisions are often executed rapidly and without the deliberation that accompanies a classic decision. As such, shoot/don't-shoot decisions do not necessarily fall within the classic decision-making literature (Biggs, 2022; Biggs & Pettijohn, 2022).

Interestingly, shoot/don't-shoot decisions, specifically, decision errors, have been linked to inhibitory control, see Biggs (2022) for review. Inhibitory control describes the ability to withhold a behavior or inhibit an initiated response (Aron & Verbruggen, 2008; Diamond, 2012; Bissett & Logan, 2011; Friedman & Mikaye, 2004; Logan & Cowan, 1984). For instance, previous work has demonstrated that the inability to inhibit responses in a sustained attention

task (SART) is related to a higher number of shooting errors (e.g., commission errors of shooting at a friendly target) (Wilson et al., 2014, 2015). This causal link between shoot/don't-shoot decisions, and response inhibition is further supported by evidence demonstrating that inhibitory control training can reduce the rate of unintended casualties (e.g., friendly fire or firing upon allied forces) (Biggs et al., 2015; Boyle, 2013; Garfield & Neugut, 1991; Hamilton et al., 2019; Johns & Davies, 2019; Walsh, 2015). Unintended casualties are among the most consequential shooting errors. Notably, previous work has demonstrated that various stress inductions can lead to differing effects on response inhibition (Cantelon et al., 2019; Cantelon & Giles, 2021; Shields et al., 2016; Starcke et al., 2016). However, less work has examined how operationally relevant stressors (i.e. threat, physiological strain, sleep loss, heat stress) influence inhibition and, in turn, shoot/don't-shoot decisions or shoot/don't-shoot errors.

An additional factor of relevance in real-world high-stakes contexts is uncertainty. Soldiers are continually called upon to make critical decisions under ambiguous circumstances. One of the most critical decisions is whether to shoot or withhold shooting, which necessitates threat versus non-threat determinations, often very quickly with insufficient visual information. In many cases, threats versus non-threats can be easily disambiguated and have been the focus of much of the prior work on shoot/don't-shoot decision errors (Biggs et al., 2015; Biggs & Pettijohn, 2022; Hamilton et al., 2019; Scribner, 2016; Vickers & Lewinski, 2012; Wilson et al., 2014). However, previous research suggests that unpredictability is aversive (Grillon et al., 2004; Herry et al., 2007; Weiss, 1970) and elicits heightened responses to ambiguous stimuli in brain regions associated with threat detection (Davis et al., 2016). As such, research in the affective domain has explored how stress impacts decisions about emotional ambiguity (i.e., valence bias) (Brown et al., 2017; Neta et al., 2017). Additionally, extensive laboratory research has focused

on perceptual decision-making under ambiguous circumstances, but has primarily done so using computer-based tasks whose outcome is, at best, tied merely to small monetary incentives (Clark et al., 2012; Brunyé & Gardony, 2017; Heekeren et al., 2004; Philiastides et al., 2011; Philiastides & Sajda, 2006). Questions remain about how ambiguity impacts shoot/don't-shoot decisions, especially during stressful conditions.

Questions also remain about how ecologically valid prior shoot/don't-shoot scenarios have been. There is a need to investigate how operational stressors impact shoot/don't decisions using realistic simulations to better translate the findings to military and law enforcement settings. Previous studies examining shoot/don't-shoot decisions have attempted to create realism through anxiety induction in order to simulate the stress experienced during a use-of-force encounter (Nieuwenhuys et al., 2012; Nieuwenhuys & Oudejans, 2010, 2011; Oudejans, 2008). However, a realistic environment should also replicate the perceptual judgments and cognitive processes, as well as emotional and physiological reactions involved in real-world threat assessments.

One way to mimic such real-world settings is by using virtual environments (VEs). VEs, offer the ability to present high dimensional, interactive, and ecologically valid stimuli while maintaining a high degree of internal validity (Bohil et al., 2011; Schilbach, 2015). They do this by allowing the researcher to include more natural settings and interactions with stimuli compared with static stimuli, increasing the generalizability of results (Asbee & Parsons, 2021). Additionally, interactive VEs may lead to a greater sense of presence (i.e., the subjective experience of "being there") (Beeli et al., 2008), which may produce greater emotional and physiological reactions (Asbee & Parsons, 2021; Parsons & Rizzo, 2008). This may be especially true when paired with stress induction. For instance, when stress induction (via shock following

task errors) is paired with virtual simulation, participants report feeling the highest levels of both anxiety and immersion, as compared to less or non-stressful conditions (i.e., life bar or no feedback conditions) (Patton, 2014). Thus, more immersive environments are likely to have higher ecological validity and better reflect real-life situations. This is especially important when studying threat assessment scenarios Soldiers face in operational environments. However, to date, there have been few studies leveraging controlled VEs to study shoot/don't-shoot decision making under stress and ambiguity.

Proposed studies

Previous work has demonstrated that Soldiers experience high levels of stress, which can influence cognitive performance and, in turn, operational readiness (Bartone, 2006; Vrijkotte et al., 2016). However, questions remain regarding the transferability and representativeness of military-specific cognitive research (Vine et al., 2021). External validity refers to the transferability of research findings to the target population (i.e., military personnel). In contrast, representative design refers to methodological approaches chosen to ensure that the experimental task constraints characterize those experienced during the performance (i.e., military training or operational environment) (Pinder et al., 2011). Thus, a more holistic approach to examining the effects of stress on Soldier cognition might include using ecologically valid stress manipulations, imposing operationally-relevant physical and cognitive tasks, using immersive virtual environments, and assessing individual differences in physiological and psychological responses. Thus, the purpose of the proposed studies is to examine how operationally-relevant emotional (Study 1) and physical (Study 2) stressors influence performance on a Soldier relevant shoot/don't-shoot cognitive task during conditions of ambiguity.

STUDY 1

Introduction

Military and law enforcement personnel are often tasked with maintaining peak performance under stressful and unpredictable conditions, exerting high levels of cognitive control to sustain attention and suppress task-irrelevant actions. For example, they might be required to hold fire from targets that do not pose an imminent threat (Ehrlich, 2000; Mahoney et al., 2007). This type of shoot/don't-shoot decision is critical to Soldier performance and survivability, especially in high-stakes contexts.

Previous work shows that a variety of factors, such as target race and gender (Plant et. al, 2011) and participant working memory capacity and emotionality (Kleider et al., 2010; Kleider & Parrott, 2009) can influence the decision to engage a potential target under high stakes contexts. Furthermore, environmental factors that invoke an anxiety or stress response also affect these types of decisions (Nieuwenhuys & Oudejans, 2012, 2017; Starcke & Brand, 2012, 2016). However, stress experienced in military settings is difficult to emulate in the lab. Thus, research examining how shoot/don't-shoot decisions are impacted under stressful conditions similar to those experienced during military training and operations is still somewhat limited. We designed the current study to address this knowledge gap.

Eliciting Soldier stress in the lab

Various paradigms have been used to elicit acute emotional stress in laboratory settings. These include social-evaluative threat (e.g., Trier Social Stress Test), aversive images and videos (e.g., International Affective Picture System), threat of aversive shock (e.g., finger shock, torso shock), noxious noise exposure, demanding cognitive tasks, and the cold pressor test (Dickerson

& Kemeny, 2004; Gamble et al., 2018; Giles et al., 2014; Lang et al., 1993, 2008). However, most laboratory-grade stress- and anxiety-inductions are relatively mild (e.g., public speaking, or submerging an arm in cold water) (Kirschbaum et al., 1993; Schwabe et al., 2008) and may fail to reflect what Soldiers experience during training and operations.

Military operations are characterized by physical and emotional stress. In general, the most robust and reliable subjective and physiological stress responses are induced by unpredictable physical or psychological threats. One such method for inducing acute stress in the laboratory is threat of electric shock (TOS). Interestingly, the response to anticipation of a shock is similar to receiving an actual shock (Grillon et al., 1991). However, there may be additional stress-induced when a shock is delivered and not simply anticipated (Lyby et al., 2012). The electric shock poses both a physical threat to comfort and well-being and a psychological threat given its unpredictability and resulting anticipatory anxiety. For example, relative to control conditions, moderate-intensity electric shock is associated with increased subjective anxiety ratings, hypervigilance to threat and increased aversive processing, as well as sustained cortisol responses, higher heart rate, and lower heart rate variability (Berghorst et al., 2013; de Berker et al., 2016, Dickerson & Kemeny, 2004; Gamble et al., 2018; Grillon et al., 2004; Hansen et al., 2009; Patton et al., 2013; Robinson et al., 2011).

This type of stressor may be of particular relevance to operational settings. In the most comprehensive review and meta-analysis of stress induction methods to date, TOS was considered to be one of the more intense physical and psychological stressors that can be employed in laboratory settings (Dickerson & Kemeny, 2004). Additionally, performance-contingent manipulations of TOS (i.e., the anticipation of aversive shock in the case of performance failure) may better represent stressors experienced in high-stress domains (e.g.,

military or law enforcement personnel). Previous work shows that this can emulate the anxiety experienced in response to anticipation of adverse consequences of error making, like the return of hostile fire (Oudejans & Nieuwenhuys, 2009; Patton, 2014).

Threat of shock and shoot/don't-shoot decisions

Stress is an evolutionary adaptation involving physiological and psychological changes, protecting the organism against potential harm (Cannon, 1929; McEwen & Sapolsky, 1995; McEwen, 1998). An emotional stress response to threat can cause decisive behavior to occur before a cognitive evaluation (Lupien et al., 2007) and negatively impact decision making (Starcke et al., 2008). Indeed, stress, such as that induced by TOS, enhances bottom-up defensive responses that may protect the organism from danger (e.g., heightened startle reflex; environmental scanning) but impairs top-down cognition that is less critical for immediate defense (Eysenck et al., 2007; Lang and Davis, 2006; Öhman et al., 2001; Phelps, 2006;). This impaired top-down cognition due to robust or salient threats may be context-appropriate and even adaptive for survival. In particular, impairments to response inhibition (i.e., impaired suppression of inappropriate responses) may help express defensive behaviors that protect the organism against immediate threat and subsequent harm (e.g., wartime scenarios; Nesse, 2005). Thus, when in a threatening context, it may be adaptive to adopt a liberal decision criterion and respond as though all stimuli pose a threat, as missing a threat could have dire consequences (Bateson et al., 2011; Nesse, 2005; Trimmer et al., 2013).

Indeed, previous research has demonstrated that stress-induced by TOS can impact such friend/foe discriminations. Studies have demonstrated impaired shoot/don't-shoot performance under shock, compared to no shock (vibrate) conditions when the stakes are high for decision errors (i.e., missing a foe or shooting a friendly). These declines in performance are driven by

increased false alarms and shifts in response criterion (Gamble et al., 2018; Patton et al., 2013; Patton & Gamble, 2016). More specifically, TOS induces a more liberal decision criterion, where participants correctly identify enemy targets (hits) at the expense of increased false alarms for friendlies. Thus, acute stress from TOS may impair cognitive control, yet these stress-induced changes may be adaptive in threatening environments

Threat of shock and shoot/don't-shoot under conditions of ambiguity

Individuals who operate under highly stressful conditions often face challenges of quickly interpreting ambiguous information in uncertain and threatening environments. However, most previous work examining threat discrimination using TOS paradigms included clearly distinguishable stimuli (Robinson et al., 2013b for a review). For instance, participants in one shoot/don't-shoot task distinguished foes from friends by a handheld pistol, compared to innocuous objects or even no objects (Gamble et al., 2018). We know less about how operationally-relevant stress impacts performance when stimuli are more ambiguous. Fortunately, a host of research has examined the role of ambiguity in the context of economic decision-making. For example, TOS increases risk avoidance during gambling tasks in which probabilities are unknown and thus ambiguous (Clark et al., 2012). Unfortunately, less is known about the effects of stress on decisions regarding ambiguous threats.

Executing shoot/don't-shoot decisions under conditions of uncertainty (i.e., ambiguous discrimination between friends and foes) is highly relevant to military personnel. Data from the field suggests that the saliency of friendly targets and target exposure time can increase shoot/don't-shoot errors. Specifically, shorter target exposure time and greater discrimination difficulty (due to low visual contrast) lead to higher rates of friendly engagements (Scribner, 2002). However, empirical work examining response to images of ambiguous postures (i.e.,

actor holding one hand behind the back) has demonstrated heterogeneous findings. Biggs and colleagues (2021) found that high threat ratings were not necessarily a strong predictor of behavioral threat response (i.e., identifying ambiguous images as threats in a go/no-go task). When faced with ambiguity in non-threatening contexts, there may be individual variation in how ambiguity is perceived and subsequent behavioral responses. When faced with stress, it is likely adaptive to view potentially dangerous stimuli as threatening until contextual information proves otherwise (i.e., identify a target as an enemy). However, under operationally stressful conditions, response to ambiguity and subsequent decisions to fire or withhold fire remains unexplored. This leads to our first research questions:

- 1) How does stress induced by threat of shock influence shoot/don't-shoot decisions?
- 2) How does stress induced by threat of shock influence ambiguous shoot/don't-shoot decisions?

The present study

Little work has examined how operational stressors impact responses to ambiguity in high-stakes contexts. Recent work has suggested that stress exposure alters decisions made in response to uncertainty (ambiguity) (Starcke & Brand, 2016). Further, acute environmental threats involving the anticipation of aversive states and experiences (e.g., threat of shock) can trigger a cascade of emotional and neuroendocrine stress responses (Bali & Jaggi, 2015). Such acute emotional stress is associated with increased negative affect, reduced cognitive control, and flexibility, and reduced regulation of negative emotion (Plessow et al., 2012; Raio et al., 2013; Schwabe et al., 2012). Taken together, this suggests that the stress may lead to altered shoot/don't-shoot decision making. The aim of Study 1 is to assess how stress and stimulus ambiguity impact shoot/don't-shoot decisions.

Objectives and Hypotheses

Our first objective was to replicate previous findings demonstrating changes in physiological and emotional responses during TOS. Previous work has demonstrated that TOS can lead to changes in physiological and subjective emotional responses (Berghorst et al., 2013; de Berker et al., 2016, Dickerson & Kemeny, 2004; Hansen et al., 2009; Patton et al., 2013; Patton & Gamble, 2016; Robinson et al., 2011). Therefore, we predicted higher salivary cortisol, higher state anxiety, lower positive affect, and higher negative affect following shock compared to no shock conditions. Additionally, we predicted that mean heart rate would be higher during shock than no shock conditions.

The second objective was to examine how stress induced by TOS influences shoot/don't-shoot decisions. Acute threat triggers a cascade of responses that rapidly increase attentional vigilance and promote fast stimulus-driven responding at the cost of maintaining cognitive control (Hermans et al., 2014). Although such automatic defensive responses are generally considered to be adaptive (Blanchard et al., 2001), performance may be negatively affected on tasks that rely heavily on cognitive control functions (Eysenck et al., 2007; Nieuwenhuys & Oudejans, 2012, 2017). In line with this, previous work has shown that threat of shock leads to shifts in response criterion and increased false alarms on shoot/don't-shoot tasks (Gamble et al., 2018; Patton et al., 2013). We aimed to replicate these findings, demonstrating a significant main effect of stress on response bias and d' scores. Specifically, we expected a more liberal response bias (i.e., tendency to shoot) during shock compared to no shock conditions. Additionally, we expected lower d' scores under shock than no shock. Finally, we expected that these changes in d' would primarily be driven by an increased rate of false alarms.

The third objective was to explore how stress-induced by TOS influences ambiguous shoot/don't-shoot decisions. To date, no research has examined the influence of stress on ambiguous shoot/don't-shoot decisions. Under non-stressful conditions, varying stimulus clarity in a perceptual decision-making task can lead to varying levels of accuracy (i.e., lower decision accuracy with lower image clarity) (Brunyé & Gardony, 2017). In shoot/don't-shoot tasks, greater discrimination difficulty (due to low visual contrast) leads to higher rates of friendly engagements (Scribner, 2002). Further, previous work has suggested that during stress, it may be adaptive to adopt a liberal decision criterion and respond as though all stimuli are threatening (Blanchard et al., 2001; Nesse, 2005; Shackman et al., 2011; Trimmer et al., 2013). Such criterion shifts – between liberal and conservative standards of evidence for a decision – tailor decision strategy to the situation and can be essential to avoiding potentially deadly errors, especially when decision evidence is ambiguous. This led us to predict an interaction between stress and stimulus ambiguity. In the shock condition, we expected that high stimulus ambiguity would result in more liberal decision criterion, lower d' scores, and higher hits and false alarms compared to low stimulus ambiguity. We expected stimulus ambiguity would impact these performance outcomes to a lesser extent under no shock.

Methods

Note that because this study was accomplished immediately upon the post-SARS-CoV-2 reopening of Tufts University, all experimenters and participants followed approved (by safety committees at Tufts University and U.S. Army) safety protocols including procedures for personal protective equipment (e.g., masks, gloves, glasses, gowns, face shields), biospecimen handling, hand sanitizing, social distancing, and equipment and space sanitization.

Participants

This study used a repeated-measures design, with threat (shock, vibrate) and stimulus ambiguity (low, high) as the within-participants factor. Data from 79 Soldiers are reported herein (79 men, 0 woman). The participants represented a healthy Soldier population. Sample characteristics are listed in Table 1.1. This study was part of a larger body of work exploring predictors of Soldier performance. As such, sample size estimation was based on effect sizes from Aminoff and colleagues (2012), who examined the impact of individual differences and target probability on criterion shifting during decision-making. In their study, they analyzed data from 95 participants and found an effect size (Cohen's d) of 1.59 when comparing the criterion adopted during low versus high target probability conditions (Aminoff et al., 2012).

Written informed consent was obtained, and the U.S. Army Combat Capabilities Development Command Armaments Center Institutional Review Board, Tufts University Institutional Review Board, and the Army Human Research Protections Office approved all procedures.

Stimuli and Paradigm

Decision Making under Uncertainty and Stress Task

The Decision Making under Uncertainty and Stress (DeMUS) task was inspired by research tasks used to probe perceptual decision-making and elicit variable uncertainty (Brunyé & Gardony, 2017; Green & Heekeren, 2009; Heekeren et al., 2004, 2008). In this task, participants were asked to discriminate friendly versus enemy targets based upon the camouflage pattern displayed on a virtual avatar. An animated Soldier-style avatar walked towards the participant in the virtual environment, and the participant decided to shoot the target (enemy) or let it pass (friendly).

DeMUS Stimuli: To develop the camouflage patterns, we used a publicly-available binary space partitioning algorithm (Åström, 2011) and then manually modified pattern features in Photoshop (Adobe Inc., San Jose, CA, USA) to create two distinct friendly and enemy patterns. We conducted a pilot study (Brunyé & Giles, *under review*) to develop four sets of camouflage patterns (6 patterns per set; 3 enemy, 3 friendly) that would reliably elicit high uncertainty, moderate uncertainty, and low uncertainty to be used in the DeMUS task. To introduce uncertainty, we used Photoshop to layer friendly and enemy camouflage patterns atop one another and vary the top layer opacity. Importantly, even if the patterns may be indistinguishable at a 51% opacity (i.e., lowest clarity), there was always an objectively correct answer (e.g., *friendly* if the pattern is 51% friendly and 49% enemy) (see Figure 1.1 for example). For each of the four sets of patterns, we used the Unity 3D gaming engine to wrap a virtual avatar (of a military member) with the pattern, adorning the uniform and helmet (see Figure 1.1).

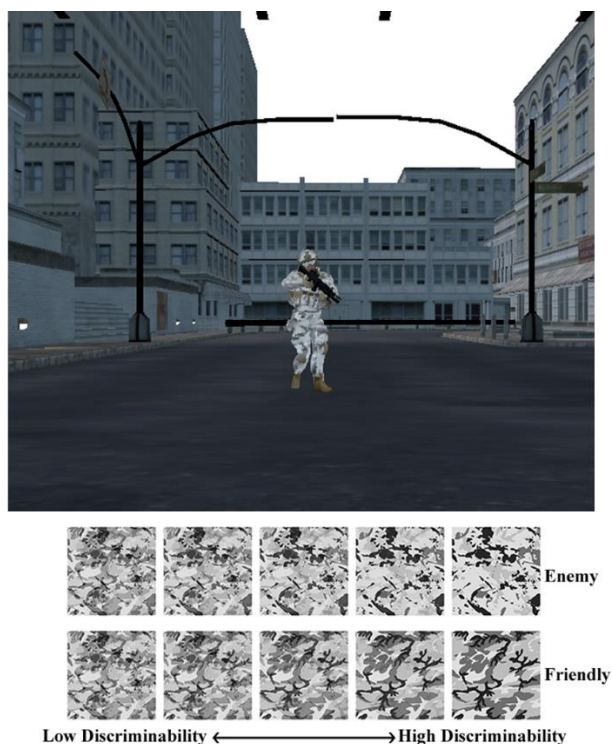


Figure 1.1 Example of camouflage patterns for Friendly and Enemy targets ranging from low discriminability (51% clarity) to high discriminability (100% clarity)

Criterion Learning Test (CLT): The CLT was designed to ensure that participants learned the required stimuli before beginning the DeMUS task in the virtual environment. We developed the CLT study and testing materials in Unity 3D, which provided a framework for presenting stimuli, recording and evaluating test responses, and re-administering tests as necessary to reach the task-specific criterion. For this task, participants were presented with the two canonical (100%) versions of the friendly and enemy camouflage patterns; each of the four versions of the shoot/don't-shoot task used a unique pairing of camouflage patterns, as described previously. The presented enemy-friendly pair was labeled and presented in the center of the computer monitor for 3 minutes. Participants then completed a 30-second arithmetic filler task and tested their memory for the camouflage pairing. During the test, the low uncertainty (always 100% clarity) and moderate uncertainty (typically 60-70% clarity) versions of the patterns were presented, one at a time on the computer monitor, in random order. Participants were instructed to *“as quickly and accurately as you can, choose whether the camouflage pattern you see more closely resembles that of a friendly or enemy.”* Responses were provided by clicking a labeled button corresponding to one of the two options (enemy, friendly). A total of 16 trials were presented, repeating each of the four patterns four times. Over the sequence of 16 successive trials, participants were required to meet the 0.8 accuracy criterion by correctly categorizing at least 13 of the 16 stimuli. If they did not reach this accuracy level, they repeated the study-test cycle until their performance reached criterion. Participants were never exposed to the high uncertainty stimulus (51% clarity) versions during the criterion learning test. The CLT was performed each test day on a standard desktop computer and monitor before entering the virtual reality system, whereas the DeMUS task was performed in an immersive virtual reality system.

Virtual Reality System: The task was executed in a large-scale rear-projection virtual reality system with approximately 220 horizontal and 90 vertical fields of view, made possible by an array of five viewing panels. The virtual reality system used the Unity 3D gaming engine to display dynamic virtual content across the five panels, each running at 1920×1080 resolution. Participants stood on a rumble platform that used an array of low-frequency audio transducers to vibrate the platform in response to low-frequency sound events (e.g., explosions, vehicles). They held an airsoft rifle with electric recoil (model M4A1, KWA, Inc., City of Industry, CA) modified to use a Logitech (Laussane, Switzerland) wireless game controller mounted to the handrail of the rifle; this controller had a thumb joystick (to use with the thumb), and we configured (soldered and wired) two response buttons on the weapon: one mounted to the handrail (to use with the index finger), and one mounted behind the trigger (to send a button press command when the trigger was pulled). The Bluetooth controller communicated with the computer running Unity 3D to recognize and log participant responses (button presses, thumb joystick inputs, trigger pulls). An example of the virtual scenario is displayed in Figure 1.2.



Figure 1.2 Example of the virtual reality system used to execute Decision Making under Uncertainty and Stress task.

Decision Making under Uncertainty and Stress (DeMUS) Task: The virtual scenario was centered around a fictitious military scenario in which participants needed to scan their local environment for potential threats (i.e., find the objects they learned for a Recognition Memory Task (RMT)), direct coalition forces around the environment by pointing towards waypoints (i.e., point towards the landmarks they learned for a Spatial Orienting Task (SOT)), and guard an entry control point where they needed to decide whether to let approaching personnel pass or engage (fire upon) them with a replica airsoft rifle (i.e., distinguish friendly versus enemy using the pattern distinctions learned for the DeMUS Task). In the present study, we focused on moderate (65%) and high clarity (100%) patterns since a pilot study revealed chance performance for low clarity (51%) patterns (Brunyé & Giles, *under review*). The scenario began with ten trials of the RMT and SOT tasks (data from these tasks were not used in the present study). Participants then began a series of 15 DeMUS trials. There were two trials for each of the camouflage patterns (12 total: 3 enemy, 3 friendly) and three additional trials with three randomly chosen patterns (15 total). The three additional trials were included to avoid an exact 50% proportion of friend/foe trials, reducing the likelihood of probability matching and strategic response shifts towards the end of the task (Edwards, 1956; Meyers, 2014).

Participants were virtually located in a relatively open urban scene, standing on a road that continued forward from their position, with an intersecting road about 50 meters ahead. The participant's view of the scene was fixed, and their pitch, yaw, and translational movement were restricted. On a given trial, a potential target would emerge (begin walking) from one of three locations in the scene: from the left or right side of the intersecting road or from straight ahead in the distance (about 100 meters ahead). The potential target would emerge, walk to the central road (if emerged from left or right), and begin walking towards the participant's position at a

walking pace of about 1 m/s. The potential target was holding a rifle in the relatively unthreatening “low-ready” position (pointing down towards the ground) and wearing one of the six camouflage patterns (three friendly, three enemy). The participant was asked to examine the camouflage pattern and decide whether the avatar was more likely a friendly or enemy; based on their perceived evidence, they would indicate a decision by either allowing the avatar to “pass” (by pressing the button on the weapon’s handgrip) or firing upon the avatar (by aiming the rifle and pulling the trigger). They were asked to make a *pass* or *shoot* decision before the avatar reached the horizontal red line overlaid onto the scene (see Figure 1.3).

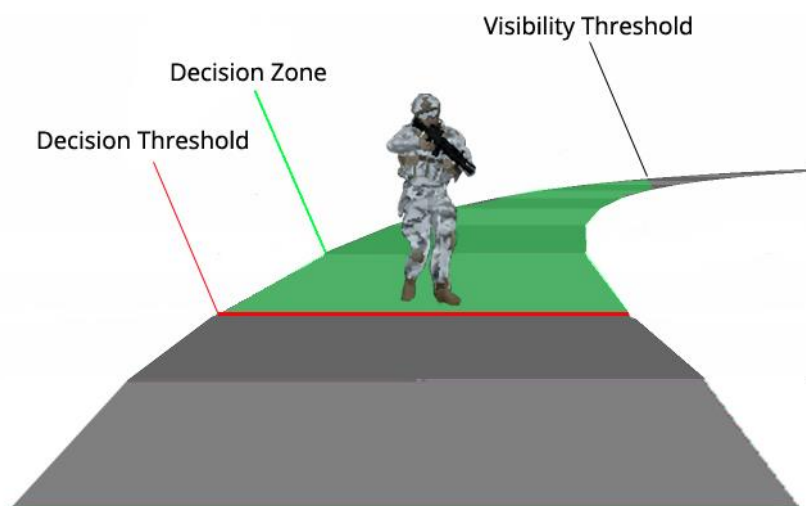


Figure 1.3 Example of virtual avatar approaching shoot/don't-shoot decision zone

If the participant chose to let the avatar pass, they would press the button, and a message *told to pass* would appear on the screen. If the participant chose to shoot the avatar, they would fire rounds with the airsoft rifle until the avatar responded by falling to the ground. Any shot placement on the body of the avatar was sufficient to make the avatar fall, and typically only one shot was required to defeat the target (unless the rifle was inadequately aimed). After a *pass* or *shoot* decision was made, if the participant’s response was incorrect (i.e., a miss or

false alarm), the Unity 3D software would send a trigger to wirelessly activate the torso shock at this time (described in the Threat of Shock section below). This process was repeated for each of the 15 DeMUS trials, which were randomly paired with an entrance location (scene center, left, right) and presented in random order.

Threat of Shock

To induce anxiety and stress in participants, electric shock was delivered using a StressX PRO Belt (StressVest, Winnipeg, Canada) worn around the torso. This belt can deliver up to 4500 volts at less than 1mA over a maximum discharge duration of 150ms (maximum joules 0.092 or 92mJ). The belt used 5 amperage-modulating intensity settings ranging from lowest (1) to highest (5) shock intensity. The device was triggered wirelessly by a base station connected to the virtual reality computer.

Because participants likely varied in pain tolerance and would respond differently to varying levels of shock intensity, they performed a shock belt calibration. During the calibration, participants were asked to don the StressX PRO shock belt around the torso and administer a shock to themselves (using a provided button). They were asked to self-deliver increasing shocks, starting at the lowest intensity (1) and stopping when they were no longer comfortable increasing the intensity; they then removed the belt. The experimenter recorded the highest intensity achieved by the participant (e.g., 3), and one level below this value was used (e.g., 2) for the DeMUS task, in accordance with our approved safety protocol. If a participant never tried above level 1, that level was used in the DeMUS task. In the shock condition, participants received a shock upon making an incorrect response (i.e., a miss or false alarm). In the no shock condition, participants donned the belt and received a vibration from the belt upon making an error. Thus, task-related feedback was maintained in both conditions.

Bioharness

The induction of stress or the presence of threat increases heart rate (Suess et al., 1980). Continuous heart rate monitoring was collected using a Zephyr BioModule device and chest strap (Medtronic, Boulder, CO) as a manipulation check manipulation check to determine if the threat of shock procedure reliably induced anxiety in participants (Lavric et al., 2003; Shackman et al., 2006). Heart rate was collected during a 5-minute seated baseline period, prior to beginning the DeMUS task (while the experimenter set up the testing computers) and during shock and vibrate conditions. Heart rate was measured in beats per minute at 1Hz (derived from an 18Hz R-R interval extracted from contiguous 250ms blocks of electrocardiogram/ECG data) and averaged across baseline, shock and vibrate conditions. Heart rate data from 55 participants are reported herein due to technical error with Bioharness equipment.

Questionnaires

State-Trait Anxiety Inventory- State (STAI-S): The STAI-S is a 10-item subscale that measures state anxiety: how participants feel at that particular moment, ranging from “not at all” (1) to “very much so” (4) (Spielberger et al., 1983). Scores were computed by summing items. Scores can range from 20-80, with higher scores indicating greater anxiety. The STAI-S has demonstrated good internal consistency (Cronbach’s $\alpha = 0.65 - 0.75$ over a two month period) and good test-retest reliability [ICC’s = 0.69 – 0.89, (Spielberger, 1989)]. Participants completed the STAI-S pre and 5 minutes post the virtual DeMUS scenario.

The Positive and Negative Affect Schedule (PANAS): The PANAS is a 20-item scale with two subscales measuring positive affect (PA) and negative affect (NA). The PA and NA subscales each consist of 10 items (Watson et al., 1988). Items are rated using a 5-point Likert scale (1= “very slightly or not at all”; 5= “extremely”). Scores were computed by summing items

within each subscale. Scores can range from 10-50, with higher scores indicating higher levels of PA or NA. The PANAS has demonstrated good internal consistency (PA Cronbach's α s > .89; NA Cronbach's α s > .84; Crawford & Henry, 2004) and good test-retest reliability [PA ICC's = .86 - .90; NA ICC's = .84 - .87 (Watson & Clark, 1988)] over a 1 year period. Participants completed the PANAS pre and 5 minutes post the virtual DeMUS scenario.

Procedure

Participants were consented in accordance with ethics approvals prior to arrival at the laboratory. Participant came to the laboratory on two separate occasions, separated by 2 days. Both sessions took place between approximately 7:00 am and 11:00 am. Upon arrival for their session, participants were seated in a private testing room. During their first session they completed a demographics instrument (e.g., age, sex, education, military occupational specialty) and in both sessions completed pre-task surveys on a computer monitor (24" at 1920×1080 resolution, with standard keyboard and mouse). These surveys included the state-trait anxiety inventory (STAI) (Spielberger et al., 1983), the positive and negative affect schedule (PANAS) (Watson, 1988), and the simulator sickness questionnaire (SSQ) (Kennedy et al., 1993). They then provided their first of several saliva samples. All saliva samples were taken using the SalivaBio Oral Swab method (Salimetrics, LLC, Carlsbad, CA), which involves placing a swab under the tongue for 2 minutes to collect approximately 2 mL saliva volume, placing the swab into a storage tube, and storing the sample in a -20° C freezer until analysis.

Participants completed the shock calibration (described above) and then began the CLT phase to ensure adequate memory for camouflage patterns. On average, this phase took about 15 minutes to complete, depending upon how many learning iterations were required to reach criterion.

Participants were then walked to the virtual reality system, where they donned the Bioharness chest strap. They were provided with instructions on using the rifle and attached controller (button press, thumb joystick). They then stepped onto a rumble platform positioned in the center of the VR system, surrounded by five display panels.

Participants then sat in a chair for five minutes to gather baseline physiological data. Finally, the StressX PRO Belt was donned and set to the appropriate condition (shock or no shock) and the participant's predetermined comfort level. Shock conditions were counterbalanced across participants.

Participants were then guided through a brief practice session for the DeMUS task. Written and verbal instructions were provided, and participants practiced 2-3 trials, using environments and stimuli that were not previously learned (i.e., one of the two other task versions that were not used). No shock was administered during practice. Once participants were familiar with the task, we started the experimental session. One of the four developed scenario versions was randomly chosen for use in each of the two experimental sessions without replacement. First, participants performed a sequence of 10 trials of a ROT and SOT task (reported elsewhere), which took about 20 minutes. They then performed a series of 15 DeMUS trials, which took about 10 minutes. The virtual scenario took about 35 minutes to complete from start to finish. When the participant finished the task, the experimenter removed the Bioharness and shock belt and then walked the participant back to the private seated testing room.

Participants completed the STAI and PANAS 5 minutes after completing the DeMUS task. They then provided an additional saliva sample at 25 minutes post. The saliva sampling and questionnaire administration allowed us to confirm that our stress manipulation induced increased cortisol responses and changes in self-report mood and anxiety. After providing their

final saliva sample, participants were thanked for their time and excused for the day. See Figure 1.4 for a schematic of the experimental sessions.



Figure 1.4 Schematic of Experimental Sessions, where participants first completed questionnaires, provided a baseline saliva sample and completed shock calibration, followed by the Critical Learning Task (CLT). They then donned physiological monitoring devices and the shock belt and completed the Decision Making under Uncertainty and Stress (DeMUS) task. Following the DeMUS task they completed questionnaires at 5 minutes and provided a saliva sample at 25 minutes.

Statistical Methods

Data processing

For the DeMUS task, we calculated several primary measures. Shoot/don't-shoot performance was analyzed using signal detection theory (Green & Swets 1966). One of the main advantages of signal detection theory (SDT) is that it yields independent measures of sensitivity (i.e., the pure ability to discriminate between “signal” and “noise”) and response bias (i.e., the general tendency to identify stimuli as signal versus noise). In the context of the current study, a signal trial is one in which an enemy camouflage pattern is worn, and a noise trial is one in which a friendly camouflage pattern is worn. As a reminder, participants were asked to decide whether approaching avatars were friends or foes. In SDT, a liberal response bias refers to the tendency to identify camo patterns as an enemy more frequently than friendly; a conservative response bias refers to the tendency to identify camo patterns as friendly more frequently than an enemy. The combination of target type (enemy, friendly) and response (shoot, don't-shoot) results in four different performance classifications:

- 1) Hit, in which an enemy is correctly shot
- 2) Miss, in which an enemy is misidentified and not shot

- 3) False alarm, in which a friendly is misidentified and shot
- 4) Correct rejection, in which a friendly is correctly identified and not shot

For each participant, measures of sensitivity and response bias were calculated. Sensitivity (d') was then calculated using the following formula: $d' = z(\text{hits}) - z(\text{false alarms})$, and response bias or (criterion (c)) was calculated as $c = -0.5 (z(\text{hits}) + z(\text{false alarms}))$. A value of $d' = 3$ is close to perfect performance; a value of $d' = 0$ is chance (“guessing”) performance. For criterion, negative values indicated a bias toward responding yes (i.e., shooting). Each measure was determined separately for each participant and each stress (shock, vibrate) and stimulus ambiguity condition (low, high).

Physiological responses included heart rate and cortisol. For heart rate, we calculated mean beats per minute (BPM) in 10-second increments measured in beats per minute at 1Hz averaged across baseline, shock and vibrate conditions. Salivary analysis for cortisol was done using the Enzyme-Linked Immunosorbent Assay (ELISA) kit in triplicate. Because cortisol data were positively skewed (Fisher’s skewness = 3.42), we performed a fourth-root transformation $X' = \sqrt[4]{X} = X^{.25}$ to reduce skewness (Fisher’s skewness = 1.21), as recommended in previous research (Miller & Plessow, 2013), to ascertain that they met the requirements of general linear model-based statistics in terms of Gaussian distribution. This method has been shown to be superior in terms of meeting normality and homoscedasticity (indicated by skewness and kurtosis) compared to the log-transformation commonly used on times-series endocrine data (Miller & Plessow, 2013).

Data Analysis

To assess differences in shoot/don’t-shoot performance due to emotional stress and ambiguity, we conducted an omnibus 2 (stress condition: shock, vibrate) x 2 (stimulus

ambiguity: low, high) repeated measures ANOVA's. Analyses were done separately for hits, false alarms, d' , and criterion c .

To confirm that our stress manipulation produced significant differences in physiological and emotional responses between conditions, we conducted omnibus 2 (stress condition: shock, vibrate) x 2 (time: before, after) repeated measures ANOVA's to evaluate changes in positive and negative affect, state anxiety, and cortisol responses following shock and vibrate conditions. We also conducted an omnibus 2 (stress condition: shock, vibrate) x 2 (time: before, during) repeated measures ANOVA to assess differences in HR during shock and vibrate conditions.

All statistical analyses were performed using Jamovi v 2.2.5. (<https://www.jamovi.org/>), which is a free and open-source statistical platform. Data were assessed for normality using Shapiro-Wilk tests. When sphericity was violated, Greenhouse-Geisser corrections were used. When an ANOVA yielded a significant main effect or interaction ($p < .05$), post-hoc tests using the Bonferroni corrections were conducted. Effect sizes are provided for all analyses, using η_p^2 for ANOVAs, and Cohen's d for t -tests.

Results

Emotional and physiological responses (manipulation checks)

Positive and Negative Affect Schedule

There were no effects of Stress or Time, nor interactions for positive affect ($ps > .14$). There was a main effect of Time for negative affect, $F(1, 74) = 8.15$, $p < .006$, $\eta_p^2 = .10$, in which negative affect was higher after both shock and vibrate conditions than before (see Table 1.2). There were no effects of Stress, nor interactions for negative affect ($ps > .21$).

State Trait Anxiety Index (State)

There was a main effect of Time, $F(1, 76) = 10.13, p < .002, \eta_p^2 = .12$, in which state anxiety was higher after both shock and vibrate conditions than before (see Table 1.2). There were no effects of Stress nor Stress by Time interactions ($ps > .29$).

Salivary Cortisol

There was a main effect of Time, $F(1, 77) = 118.71, p < .001, \eta_p^2 = .61$, in which salivary cortisol levels were lower after both shock and vibrate conditions than before (see Table 1.3). There were no effects of Emotional Stress nor Emotional Stress by Time interactions ($ps > .32$).

Heart Rate

There was a main effect of Stress, $F(1, 54) = 5.70, p = .028, \eta_p^2 = .09$, and a main effect of Time, $F(1, 54) = 147.56, p < .001, \eta_p^2 = .73$, but no Emotional Stress by Time interaction ($p = .399$). Average heart rate (bpm) was higher during the DeMUS Task compared to baseline and higher during shock than vibrate conditions (see Table 1.4)

Shoot/don't-shoot performance***Hits***

There was a main effect of Stimulus Ambiguity, $F(1, 78) = 11.12, p < .001, \eta_p^2 = .14$, in which hits were higher in the high ambiguity than low ambiguity condition (see Table 1.5). There were no effects of Emotional Stress nor Emotional Stress by Stimulus Ambiguity interactions ($ps > .084$).

False Alarms

There was a main effect of Stimulus Ambiguity, $F(1, 78) = 29.41, p < .001, \eta_p^2 = .27$, in which false alarms were higher in the high ambiguity than low ambiguity condition (see Table 1.5). There were no effects of Emotional Stress nor Emotional Stress by Stimulus Ambiguity interactions ($ps > .64$).

Sensitivity (d')

There was a main effect of Stimulus Ambiguity, $F(1, 78) = 4.79, p < .032, \eta_p^2 = .06$, in which participants were less sensitive to the difference between signal (foe) and noise (friendly) in the high ambiguity compared to low ambiguity condition (see Figure 1.5). There were no effects of Emotional Stress nor Emotional Stress by Stimulus Ambiguity interactions ($ps > .213$).

Response Bias (Criterion c)

There was a main effect of Stimulus Ambiguity, $F(1, 78) = 32.91, p < .001, \eta_p^2 = .30$, in which participants were more biased towards responding yes (i.e. shooting) in the high ambiguity than low ambiguity condition (see Figure 1.6). This likely contributed to higher hit rates observed under high compared to low ambiguity conditions. There were no effects of Emotional Stress nor Emotional Stress by Stimulus Ambiguity interactions ($ps > .170$).

Response Time (RT)

When examining RT for hits there was a main effect of Stress, $F(1, 62) = 4.56, p = .037, \eta_p^2 = .07$, a main effect of Stimulus Ambiguity, $F(1, 62) = 5.30, p = .025, \eta_p^2 = .08$ and an Emotional Stress by Stimulus Ambiguity interaction, $F(1, 62) = 4.15, p = .046, \eta_p^2 = .09$. Bonferroni post-hoc analyses revealed this interaction was driven by slower RT for high

ambiguity than low ambiguity stimuli in the vibrate condition ($p = .021$) and faster RT for high ambiguity stimuli in the vibrate compared to shock condition ($p = .020$), but no difference in RT for high compared to low ambiguity stimuli during shock ($p > .10$) (see Table 1.5).

When examining RT for false alarms there was a main effect of Stimulus Ambiguity, $F(1, 66) = 37.44$, $p < .001$, $\eta_p^2 = .36$, in which RT was slower in the high ambiguity than low ambiguity condition (see Table 1.5). There were no effects of Emotional Stress or Emotional Stress by Stimulus Ambiguity interactions ($ps > .11$).

Study 1 Discussion

In the present study, we evaluated whether stress induced by threat of shock impacted shoot/don't-shoot decisions and ambiguous shoot/don't-shoot decisions. Participants made friend/foe discriminations using camouflage patterns presented on avatars in a virtual environment, while under shock or vibrate conditions. In addition, we collected measures of positive and negative affect, state anxiety and salivary cortisol before and after, as well as heart rate during both conditions.

How does threat of shock influence physiological and emotional responses?

We found that threat of shock impacted some physiological responses, but not others. Results showed that heart rate was higher during the shock than vibrate condition. This aligns with previous research demonstrating threat-induced increases in sympathetic autonomic nervous system (ANS) activation (Lavric et al., 2003; Roxburgh et al., 2019; Shackman et al., 2006). Yet, we found no differences in salivary free cortisol between conditions. Previous research has demonstrated mixed findings with regards to the effects of shock on HPA axis response and glucocorticoid release. One study found significant differences in cortisol responses between

shock and safe conditions (de Berker et al., 2016), whereas another study found differences between conditions only after dividing participants into “stress reactive” and “no stress” groups based on differing levels of physiological (cortisol levels) and self-reported experiential stress (STAI scores) (Berghorst et al., 2013). Thus, it is possible that threat of shock may only be stressful for a sub-group of participants. However, using a validated classification proxy to distinguish between cortisol responders and non-responders within our sample only yielded 1% of participants who would classify as “responders” (i.e., 1.5 nmol/l or 15.5% cortisol increase under shock, relative to baseline) (Miller et al., 2013). Taken together, we believe our pattern of biochemical and physiological data suggests that threat of shock reliably activates relatively early (i.e., noradrenergic) SAM responses but does not appear to reliably influence relatively latent HPA axis-related (i.e., glucocorticoid) stress responses.

Furthermore, we did not observe changes in positive affect, negative affect or state anxiety in response to stress exposure. Rather, negative affect and anxiety increased over time following both shock and vibrate conditions. However, although statistically significant, these changes were relatively subtle (see Table 1.). For instance, mean state anxiety scores fell within the “no or low anxiety” (20-37) classification range following both shock and vibrate conditions (Spielberger et al., 1983). These findings differ from previous research demonstrating significantly higher levels of self-reported negative affect and anxiety under shock (Berghorst et al., 2013; Bogdan and Pizzagalli, 2006; Hansen et al., 2009; Robinson et al., 2013a, 2015). Results indicate that the intensity of negative emotions induced by our stress procedure were relatively low, possibly contributing to the lack of HPA axis-related physiological differences between conditions.

In this study, participants received shocks following task errors. This was thought to emulate the anxiety experienced in response to anticipation of adverse consequences of error making in operational settings, like the return of hostile fire (Oudejans & Nieuwenhuys, 2009; Patton, 2014). However, it is possible that our method of shock delivery reduced the stressfulness of the “threat-of-shock” by increasing the perceived predictability of the stressor. In fact, predictable stressors typically elicit smaller physiological stress responses and are experienced as less aversive than unpredictable stressors (Anisman & Matheson, 2005). Given that trials alternated between high and low levels of stimulus ambiguity, this likely led to varying ability among participants to predict whether shocks would be received. This variability in predictability of threat could have limited the extent to which stress was sustained across all trials, diminishing the overall effect of our stress manipulation.

Additionally, it is important to note that previous work showing emotional and physiological stress reactivity in response to threat of shock have sampled from populations of healthy young adults. Previous studies using threat of shock in military populations have not included measures of subjective emotional responses or HPA axis response. In contrast, these studies have relied on heart rate and heart rate variability (HRV) as a physiological index of stress (Gamble et al., 2018; Hansen et al., 2009; Patton & Gamble, 2016). Sympathetic-adrenal-medullary (SAM) activity pre, during and post-stressor is a critical component of the overall ANS stress response (Allen et al., 2014). However, although sympathetic activation and parasympathetic withdrawal have been linked to psychological stress and cognitive function, non-stressful arousal manipulations can also produce significant increases in ANS and SAM activity (i.e. changes in heart rate, HRV or salivary α -amylase) (Nielson et al., 2005; Shields et al., 2020; Wiemers et al., 2013). Indeed, SAM activation has been shown to be associated with

feelings of alertness or action proneness and can increase in response to pleasurable stimuli or eustress, as well as during stress (Cool & Zappetti, 2019; Gordis et al., 2021). In contrast, the HPA axis is particularly activated in response to stressful situations associated with factors such as unpredictability, which leads to negative feelings of distress (Dickerson & Kemeny, 2004; Lundberg & Frankenhaeuser, 1980). Taken together, it remains unclear whether the threat of shock paradigm reliability induces stress in our population of interest, or whether previous results are merely due to arousal manipulation. Future work should seek to include both subjective emotional responses and stress-specific biomarkers, such as glucocorticoid (e.g., cortisol) or proinflammatory cytokine increases (Dickerson & Kemeny, 2004; Marsland et al., 2017; Shields et al., 2020). This will allow us to better assess the magnitude and specificity of this stress-induction technique in Soldiers.

How does threat of shock influence shoot/don't-shoot decisions?

Contrary to our predictions, we did not observe any differences in shoot/don't, accuracy, sensitivity or bias measures between shock and vibrate conditions. These findings are inconsistent with previous work demonstrating a negative impact of shock on shoot/don't-shoot decisions (Gamble et al., 2018; Patton, 2014; Spangler et al., 2018). It is important to note that Patton and colleagues (2014) compared shock to no-feedback and life-bar feedback conditions, making it challenging to compare the effects observed under shock and vibrate conditions examined here. Nonetheless, this body of work has shown that threat of shock leads to a more liberal decision criterion and increased false alarms on shoot/don't-shoot tasks. We did find differences in response time due to stress (i.e., total time taken to identify and fire at a target once perceptible). Results indicated that participants were slower at responding when under shock. This slowed response time was evident across both low and high ambiguity trials,

whereas participants were quicker to respond during low ambiguity than high ambiguity trials in vibrate conditions. Previous research has demonstrated mixed results with regards to effects of threat of shock on reaction time, or this measure is often unreported (Gamble et al., 2018; Patton et al., 2014; Robinson et al., 2013b; Spangler et al., 2018). Here, participants were likely more cautious when making decisions, i.e., taking longer, when the outcome of their decision would entail an aversive shock. However, this did not appear to impact the accuracy of their shoot or don't-shoot decisions.

As mentioned above, one methodological factor that could have contributed to the discrepancy in results observed here is the predictability of threat. Studies employing predictable threat of shock as a relatively high-stakes outcome for decision errors (i.e., incorrectly orienting, missing a threat, or false-alarming to a non-threat) have demonstrated decrements in shoot/don't-shoot performance (Gamble et al., 2018; Patton et al., 2014; Spangler et al., 2018). Interestingly, unpredictable threat of shock can lead to improvements on inhibitory control tasks, similar to a shoot/don't-shoot task (i.e., go/no-go task). In these studies, participants are better able to withhold responses to infrequently-presented no-go stimuli when under shock compared to safe conditions. Furthermore, this result has been replicated several times (Grillon et al., 2017; Mkrtchian et al., 2017; Torrisi et al., 2016). Given that our stimuli varied in level of ambiguity they also likely varied in subsequent predictability of threat. Thus, effects of predictable and unpredictable threat may have nullified any changes in shoot/don't-shoot performance that may have been observed under each threat type alone. On the other hand, in real world settings there are rarely cues that predict the onset of threat with 100% accuracy. Nevertheless, future work could compare how the relative predictability of threat impacts inhibitory control and shoot don't/shoot decisions.

However, it is worth noting that the relationship between stress-induced anxiety states and inhibitory control is not yet fully understood. While earlier theories argue that anxiety will facilitate stimulus driven responding at the expense of inhibitory control (Cornwell et al., 2012; Eysenck et al., 2007), others argue anxiety will lead to cautious responding and thus improved inhibition (Robinson et al., 2013a). As we did not observe a reliable stress response, nor stress-induced changes to inhibitory control (as measured in our shoot/don't-shoot task), it remains unclear the extent to which acute threat facilitates adaptive or maladaptive responses in applied contexts.

How does threat of shock influence ambiguous shoot/don't-shoot decisions?

Shoot/don't-shoot performance varied by stimulus ambiguity, but did not differ between stress conditions. Consistent with previous work, we found that participants were slower and less sensitive to the difference between friends (noise) and foes (signal) under conditions of high compared to low stimulus ambiguity (as demonstrated by greater rates of false alarm and lower d' scores) (Brunyé & Gardony, 2017; Scriber et al., 2002). Interestingly, hit rates were lower under conditions of low ambiguity rather than high ambiguity, as hypothesized. However, this result was likely driven by a shift in response bias where participants were more biased towards responding yes (i.e., shooting) in the high than low ambiguity condition. This shift in response strategy led to more correct identifications of enemy targets, but also more friendly engagements.

We predicted that when faced with stress, it is likely adaptive to view potentially dangerous stimuli as threatening until contextual information proves otherwise (i.e., identify targets as enemies). However, it appears that when under conditions of high uncertainty (i.e., ambiguous discrimination between friends and foes) participants adopted a more liberal bias for identifying targets as threatening, regardless of stress condition. Research has shown that humans

often use heuristics, or biases, to make decisions when certainty is low and ambiguity is high (Epstein et al., 1996; Kahneman, 2003; Stanovich, 2012). Here, it appears Soldiers' may have been relying on certain cognitive biases to guide decisions under both safe and threatening contexts. Although ambiguity has been shown to be aversive, previous research has also demonstrated a greater tendency to "shoot", even under unambiguous conditions. Indeed, during out-brief interviews, Patton and colleagues (2014) noted that many Soldier's in their study reported shooting at all targets because they felt they had a better chance of not being "shot". It is possible that certain response patterns may emerge as a result of training or performing in high-stakes environments. Thus, it is important to understand whether individual difference factors specific to the military population may impact response biases and cognitive performance that guide shoot/don't-shoot decisions.

Study 1 Limitations and Future Directions

In the present study we found limited evidence of successful stress manipulation, and therefore, did not find differences in shoot/don't-shoot performance between stress conditions. To further understand the relationships between emotional stress, ambiguity and shoot/don't-shoot decision making, future research could expand upon these findings in several ways.

First, a number of design differences may account for the inconsistent findings within the threat of shock literature. A key advantage of the threat of shock paradigm is that it provides a well-controlled manipulation of stress and state anxiety in a within-subjects design. However, there is no "standard" paradigm (Robinson et al., 2013b). Some studies manipulate threat between participants and some within, some utilize performance-contingent shocks and others administer unpredictable shocks which are unrelated to task performance. We chose performance-based shock because of its relevance to high stakes military contexts (i.e. failing to

identify and eliminate an enemy threat could have imminent life or death consequences).

However, a clearer picture may be achievable if more variables (e.g., block-length, frequency of shock delivery) were held consistent across studies. Yet, to date, there are still few studies utilizing threat of shock, leaving a large number of gaps in the literature and much to be understood about how threat either does, or does not, induce cognitive changes. Additionally, future work may look to manipulate and explore not only the predictability of when threat (i.e. shock) will occur, but also the temporal proximity, likelihood of occurrence and control over shock exposure. Further examining how factors of uncertainty and controllability over perceived stressors influences decision making, and decision making in response to ambiguity, will help to generalize findings to real-world high-stakes contexts.

Second, the timing of our subjective and physiological sampling may have reduced our ability to detect any stress-induced changes. Due to safety protocols for collecting human subject's data and bio samples during the ongoing SARS-CoV-2 pandemic, we were limited in several ways. First, the additional need to don personal protective equipment (e.g., masks, gloves, glasses, gowns, face shields), carefully handle biospecimen, sanitize hands, social distance, and sanitize lab spaces and equipment resulted in lengthy study sessions. To limit the time burden placed on both researchers and participants, all study sessions were scheduled between 7:00 and 11:00 am. However, research shows that among healthy individuals, cortisol follows a diurnal rhythm in which levels peak roughly 30 minutes after waking and then steadily drop throughout the day, reaching nadir around bedtime. (Adam et al., 2017; Schmidt-Reinwald et al., 1999). To control for diurnal rhythms in cortisol, test sessions are ideally scheduled between the hours of 1:00 and 6:00 pm because it is easier to elevate levels when cortisol is relatively low in the afternoon than when it is high in the morning (Dickerson and Kemeny,

2004). With our sessions scheduled in the morning, cortisol levels were likely at ceiling given the post-awakening surge, or cortisol awakening response, making it challenging to further elevate cortisol levels post stress. Since previous work using threat of shock in military populations has not included measurement of HPA axis response, the effective magnitude of this particular stressor remains unclear.

Additionally, although we assessed affect and state anxiety before and after completion of shock and vibrate sessions, these were administered roughly 5 minutes following the stress cessation. Affect is notoriously fleeting, particularly when it is of low intensity, and can be suppressed by engagement in distracting tasks (Erber & Erber, 2000; Garrett & Maddock, 2001; Gross, 1998). Thus, the short walk from the virtual reality cave to an isolated testing room could have served to distract participants and diminish any negative affect or anxiety experienced. Thus, the timing of these assessments may have limited the sensitivity to detect effects of our stress manipulation. Despite this, previous research using other stress manipulation techniques (TSST) have demonstrated a relationship between cortisol responses and emotional responses (Campbell & Elhert, 2012). It will be useful for future work to consider inclusion and timing of such measures to expand our understanding of the correspondence of HPA axis and emotional responses when using the threat of shock paradigm. This will also help to establish the efficacy and generalizability of this paradigm for inducing stress across various populations of interest.

Finally, a growing body of work highlights the importance of individual difference factors in driving friend/foe decisions. This leads to several avenues for future research in the realm of shoot/don't-shoot decision making. For example, use of force errors (firing upon a friendly) has been shown to be predicted by individual differences in inhibitory control, specifically, stopping ability (i.e. no-go accuracy in a go/no-go task or stop-signal response time

in a stop signal task) (Biggs & Pettijohn, 2022). Additionally, higher rank, often indicative of military experience, is associated with fewer errors in shoot/don't-shoot scenarios (Scribner, 2016). However, the current sample had on average two and a half years military service, with zero deployments. This limits our ability to explore the extent to which certain factors related to military experience could have impacted decision making under stress. Finally, previous work has suggested that differences in autonomic balance between low and high stress conditions, or the ratio of low frequency to high frequency HRV, can predict shifts in decision criterion under stress (Gamble et al., 2018). The extent to which HRV frequency domain analysis corresponds to cardiac sympathetic and cardiac parasympathetic neural activity has been debated (Billman et al., 2013). However, results highlight that differences in an individual's physiological profiles may impact behavioral responses during stress. Thus, future studies should determine the extent to which individual differences in training, physiological or stress reactivity, or cognitive control may also impact interpretations of ambiguity during stress.

Study 1 Conclusions

In conclusion, while the lack of support for our hypotheses may indicate that our hypotheses were unfounded, it is also possible that methodological decisions made it less likely to observe effects of emotional stress on behavior in our laboratory setting, or that threat of shock is a relatively mild emotional stressor limited in its ability to significantly impact cognitive performance within a military population.

However, stress can be both physical and emotional in nature. Stress is a state of threatened homeostasis during which a variety of adaptive processes are activated to produce physiological and behavioral changes. As such, similar physiological components of the neuroendocrine "stress response" are produced during physical activity (e.g., exercise) and are

generally larger than responses elicited when reacting to (aversive) stressful emotional stimuli (Calvo & Gutierrez-Garcia, 2016; Dickerson & Kemeny, 2004, Joëls, 2006). Given that Soldiers operate in environments that can be extremely physically demanding, we sought to understand whether and how this type of stress may lead to changes in behavior in response to ambiguity. Moreover, less work has explored how operationally relevant physical stressors, such as load carriage, impact shoot/don't-shoot decisions or ambiguous shoot/don't-shoot decisions. This will be the aim of Study 2.

STUDY 1 TABLES**Table 1.1** Sample Characteristics (n = 79 men, 0 woman).

	Mean	SD	Minimum	Maximum
Age (years)	23.1	3.8	18	35
Years in Military	2.6	2.2	0	11
Number of Deployments	0.3	0.5	0	2

Table 1.2 Positive and negative affect and state anxiety means $\pm$ SD before and after shock and vibrate conditions (n = 77).

		Time Relative to Stress	
		Before	After
Positive Affect	Shock	28.8 $\pm$ 8.6	29.2 $\pm$ 8.4
	Vibrate	28.1 $\pm$ 8.8	28.8 $\pm$ 8.8
Negative Affect	Shock	11.4 $\pm$ 3.0	13.1 $\pm$ 4.8
	Vibrate	11.9 $\pm$ 3.5	12.5 $\pm$ 4.6
State Anxiety	Shock	31.2 $\pm$ 8.4	33.0 $\pm$ 9.1
	Vibrate	31.2 $\pm$ 9.3	32.0 $\pm$ 8.9

Table 1.3 Salivary cortisol concentration ($\mu\text{g/dL}$) means $\pm$ SD sampled before and 20 minutes after shock and vibrate conditions ($n = 78$).

	Time Relative to Stress	
	Before	After
Shock	0.41 ± 0.19	0.23 ± 0.10
Vibrate	0.42 ± 0.19	0.25 ± 0.17

Table 1.4 Heart rate (bpm) means $\pm$ SD sampled during a 5-minute seated baseline period before, and during shock and vibrate conditions (n = 55).

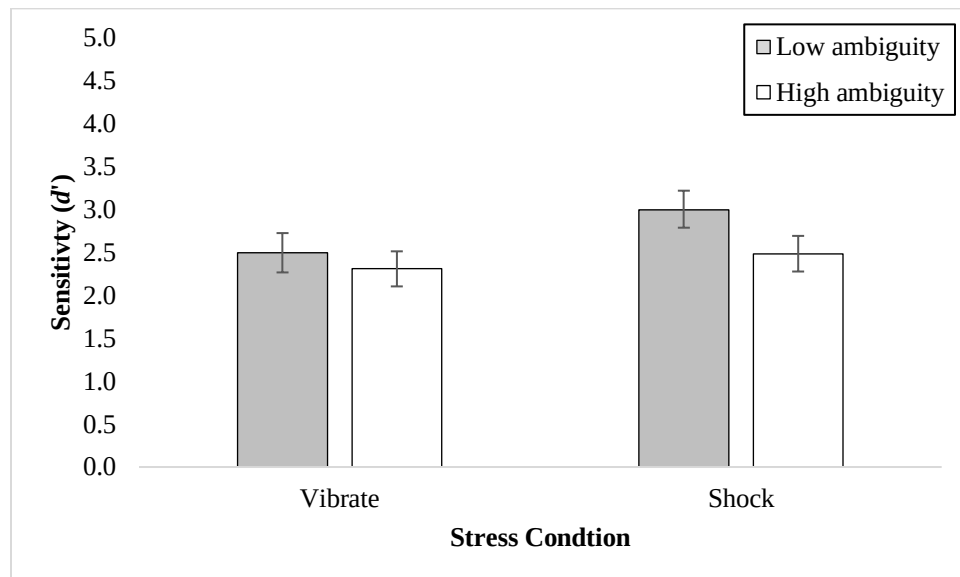
	Time Relative to Stress	
	Before	During
Shock	75.4 $\pm$ 24.5	88.4 $\pm$ 13.6
Vibrate	72.2 $\pm$ 10.0	82.4 $\pm$ 13.6

Table 1.5 Shoot/don't-shoot hit rate, false alarm rate, and response time means $\pm$ SD by Emotional Stress and Stimulus Ambiguity (n = 79).

Stress Condition	Stimulus Ambiguity	Hit Rate		False Alarm Rate		Response Time (Sec)	
		M	SD	M	SD	M	SD
Shock	Low	0.82	0.29	0.12	0.29	21.48	3.47
	High	0.77	0.27	0.27	0.24	23.28	3.69
Vibrate	Low	0.64	0.35	0.11	0.23	19.96	4.57
	High	0.81	0.24	0.29	0.29	22.50	3.42

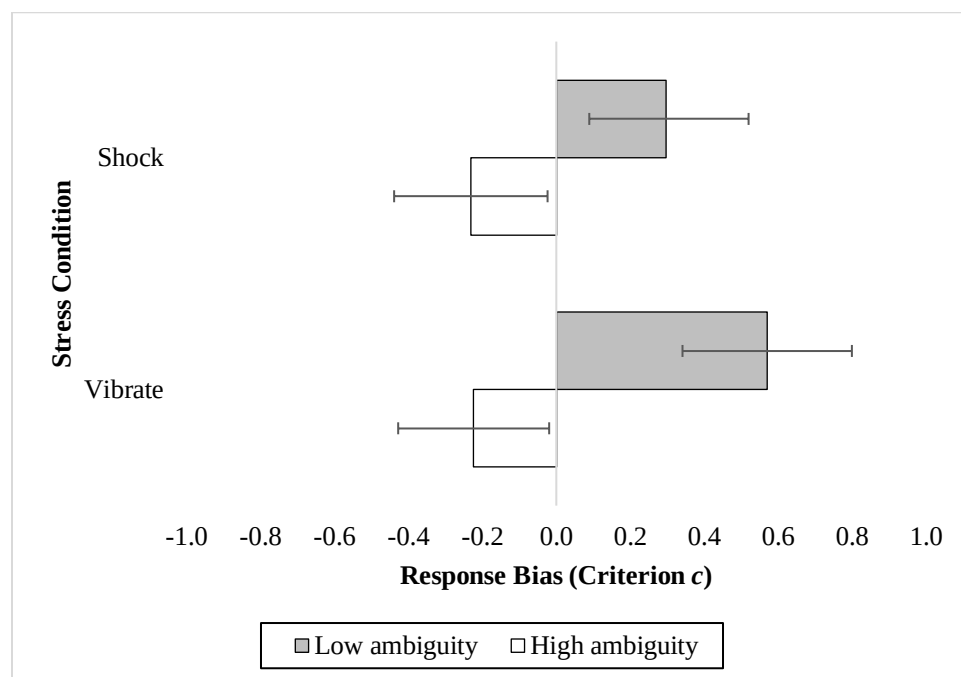
STUDY 1 FIGURES

Figure 1.5 Shoot/don't-shoot Sensitivity Means (SEM) by Emotional Stress and Stimulus Ambiguity.



Sensitivity did not differ between shock and vibrate conditions ($p > .213$), but participants were better at distinguishing between enemy and friendly targets in the low than high ambiguity condition ($p < .001$).

Figure 1.6 Shoot/don't-shoot Response Bias Means (SEM) by Emotional Stress and Stimulus Ambiguity.



Response bias did not differ between shock and vibrate conditions ($p > .170$), but participants had a more liberal response bias and were more likely identify targets as an enemy than friendly in the high than low ambiguity condition ($p < .001$).

STUDY 2

Introduction

Exercise as a physical stressor

Though often deemed beneficial to health (Penedo & Dahn, 2005; Ströhle, 2009), physical exercise can be considered acutely stressful as it disrupts bodily homeostasis and can lead to dehydration, heat stress, metabolic depletion, and central fatigue (Blomstrand et al., 1991; Brisswalter et al., 2002; Cheuvront, & Haymes, 2001; Grego et al., 2004; Hancock, 1986). In addition to inducing such fatigue symptoms, when performed at high intensities (above the ventilatory threshold) or for extended durations (exceeding 1 hour), exercise-induced increases in physiological arousal and changes in the allocation of neural resources can compromise cognitive performance (Audiffren, 2016; Dietrich, 2003; Dietrich & Audiffren, 2011; Lambourne & Tomporowski, 2010; McMorris et al., 2011). Individuals with high-stress occupations, such as law enforcement and military personnel, often operate under such fatiguing and dangerous conditions, making critical split-second decisions to engage in or suppress actions. Thus, it is vital to understand how conditions of high physical stress may impact one's ability to differentiate between a friend and foe.

Cognitive performance during acute aerobic exercise

Research demonstrates that acute aerobic exercise influences cognition, particularly cognitive control, and the direction and magnitude of these effects vary with exercise intensity (see Cantelon & Giles, 2021 for review). The Transient Hypofrontality Theory (THT) and Reticular-Activating Hypofrontality (RAH) theories suggest that increasing exercise intensity or duration enhances or sustains performance on sensory and motor tasks, but it impairs cognitive

control processes. Empirical work supports this notion, demonstrating that at relatively low intensities (or low levels of physical stress), exercise does not influence cognitive control (Davranche et al., 2009; John et al., 2009). However, exercise at higher intensities (i.e., high physical stress) can impair set-shifting and inhibition (Davranche & McMorris, 2009; Del Giorno et al., 2010).

Effects of exercise intensity are often explored at shorter durations (30 minutes or less), with less work exploring how cognition is impacted during prolonged bouts of exercise (durations over 60 minutes). The few studies that have explored endurance exercise demonstrate decrements in working memory, preservation, set-shifting, and sustained attention at 40 and 65 minutes of running (Dietrich & Sparling, 2004), as well as improved attentional control (as measured by P300 latency and amplitude) during the first hour, followed by impairments in the second hour of cycling (Grego et al., 2004). This suggests that prolonged duration exercise (or sustained physical stress) may also lead to impairments in cognitive control.

Cognitive performance during load carriage

The carrying of heavy loads is an essential aspect of military operations and is often mission essential (Knapnik & Reynolds, 2012). Research previously discussed demonstrating that physical exertion affects cognitive control under unloaded conditions is informative for athletes. However, research on physical exertion under conditions of heavy load carriage is necessary to understand cognition in the physically demanding scenarios commonly undertaken by military personnel and first responders.

To date, few laboratory studies have explored how cognitive control more broadly, or friend/foe discrimination more specifically, are impacted during this military-specific physical stressor. For instance, previous research has found that load carriage for shorter durations (40 kg

for 30 minutes) impaired vigilance performance, particularly when walking over and around obstacles, relative to standing (Mahoney et al., 2007). Kobus and colleagues (2010) assessed the influence of a 0, 44.5, or 61.2 kg combat load on cognition during two hours of simulated dismounted patrol: treadmill walking at 0.9 kilometers per hour (kph), 0% grade. They found that carrying the heaviest load (61.2 kg) impaired target detection and spatial and verbal memory (Kobus et al., 2010). In recent work from our lab, Soldiers walked on a treadmill for two hours at a constant or variable incline while wearing 0 or 40 kg loads. They completed an auditory go/no-go response inhibition task and a visual target detection task. During this prolonged duration, response inhibition performance declined in the loaded condition, evidenced by an increased proportion of false alarms and slowed response times. Visual target detection response time also slowed in the loaded condition, but accuracy did not change (Eddy et al., 2015). Thus, laboratory work suggests that load carriage influences cognitive processes across several load configurations, exertion intensities, and durations.

Load carriage and cognitive performance on Soldier relevant tasks

Finally, to increase the translation of military-specific exercise and cognition research, performance should be examined under load carriage conditions and examined using operationally relevant cognitive tasks (Vine et al., 2021). To date, this applied work is still minimal. Among the few, Haas and colleagues (2014) examined the effects of carrying a 24 kg fighting load on navigation and radio check tests during a warehouse navigation task (lasting ~10-11 minutes). Under loaded conditions, Soldiers exhibited slower navigation time but faster radio check response times, with no concurrent effects on accuracy (Haas et al., 2014). Thus, performance on lower level sensory and motor tasks may improve, whereas higher order cognitive processes, such as learning and memory, may be impaired by short duration load

carriage. However, further work is needed to understand how prolonged duration load carriage may also impact cognitive control processes required for shoot/don't-shoot decision-making, especially under conditions of ambiguity. This leads to our research questions:

- 1) How does physical stress induced by load carriage influence shoot/don't-shoot decisions?
- 2) How does physical stress induced by load carriage influence ambiguous shoot/don't-shoot decisions?

The present study

We will address this gap by investigating how operationally relevant cognitive performance is impacted during prolonged duration load carriage (120 min). Previous work has demonstrated that load carriage can impact cognitive control processes critical in friend/foe discrimination. Specifically, the ability to selectively inhibit prepotent responding to task-irrelevant stimuli deteriorates as physical stress increases under high exercise intensities alone (Davranche & McMorris, 2009; Smith et al., 2016; Wohlwend et al., 2017), and during extended durations of load carriage simulating the physical stress conditions under which military personnel operate (Eddy et al., 2015). However, the influence of military-specific exercise on shoot/don't-shoot decisions is less well understood. The proposed study will explore how the physical stress of load carriage impacts shoot/don't-shoot decisions during loaded and unloaded conditions and conditions of uncertainty (i.e., ambiguous discrimination between friends and foes).

Objective and Hypotheses

Our first objective was to replicate extant literature demonstrating higher physiological and subjective (RPE) exertion levels under loaded compared to unloaded conditions (Ekkekakis et al., 2002; Garber et al., 2011; Giles et al., 2019). Additionally, we predicted load carriage

would reduce affective valence (FS) and increase affective arousal (FAS) relative to the unloaded foot march (Ekkekakis & Acevedo, 2006).

The second objective was to examine how physical stress induced by load carriage influenced shoot/don't-shoot decisions. Previous work has demonstrated that load carriage can impact cognitive control processes critical in friend/foe discrimination. Specifically, the ability to selectively inhibit prepotent responding to task-irrelevant stimuli deteriorates as physical stress increases under high exercise intensities alone (Davranche & McMorris, 2009; Smith et al., 2016; Wohlwend et al., 2017), and under prolonged durations of load carriage (Eddy et al., 2015; Giles et al., 2019). In contrast, light intensity exercise has shown mixed or negligible effects on cognitive control (Cantelon & Giles, 2021). Therefore, we expected a main effect of physical stress, where performance would be worst in the loaded condition (lower d' scores with greater false alarms), but would not differ between rest and unloaded conditions.

The third objective was to explore how physical stress induced by load carriage influences ambiguous shoot/don't-shoot decisions. Less is known about how physical stress impacts responses to ambiguity in general and specifically for shoot/don't-shoot decisions. Previous research in the emotional domain has demonstrated that increased arousal can lead to shifts in valence bias (i.e., tendency to rate an ambiguous facial expression as negative vs. positive) (Neta et al., 2017). Using findings from adjacent fields might lead us to predict that the significant physiological perturbations due to physical stress, such as heightened sympathetic arousal, may also shift friend/foe interpretations. This may lead to an interaction between stress and stimulus ambiguity. In the loaded condition, we expected that high stimulus ambiguity would result in more liberal decision criterion and lower d' scores compared to low stimulus ambiguity. We expected stimulus ambiguity would impact these performance outcomes to a

lesser extent under unloaded and rest conditions. However, it is to be noted that this research question is still somewhat exploratory.

Methods

Participants

Of the sixteen participants who began study activities, only thirteen men and one woman were able to complete all conditions (see Table 2.1). Session withdrawals were related to self-reported discomfort caused by the load carried (i.e., shoulder, back discomfort) or muscular discomfort. The participants represented a healthy Soldier population. In order to enroll, individuals were required to exercise regularly according to the American College of Sports Medicine's (ACSM) recommendations (Garber et al., 2011), have completed basic and advanced infantry training, have experience with load carriage and treadmill walking, have healthy vision, and have passed their most recent Army Combat Fitness Test or Army Fitness Test. Individuals could not enroll if they were left-handed, used medications for anxiety, depression, seizures, high cholesterol, diabetes, hypertension, or asthma, used illicit drugs, excessive alcohol and/or performance enhancing drugs, were pregnant or nursing, had a sensitive scalp, had any current orthopedic injuries, were on profile, or were receiving behavioral health care (to minimize interruptions to behavioral health care during travel for study participation). Participants were also excluded if they had a history of neurological or psychological disorder, metal in the head, lower back, hip, knee, or ankle problems, cardiac or respiratory problems related to exercise, lung or heart problems, or diabetes. Sample characteristics are listed in Table 2.1.

Sample size estimation was based on effect sizes from Eddy and colleagues (2015), who found that go/no-go sensitivity (d') decreased during 120 minutes of load carriage under loaded, relative to unloaded conditions ($\eta p^2 = 0.513$). Using G*Power 3.1 (Faul et al., 2007), the total

sample size was estimated to be 18 with an α level of $p = .05$ and a power of 0.95, using repeated-measures analysis of variance (ANOVA) with 1 df . This study was part of a larger body of work exploring predictors of Soldier performance. As such, we initially aimed to recruit 70 active-duty Soldiers using formal troop request processes. However, only 16 Soldiers traveled to participate in the study. Therefore, participants in this study primarily represented a convenience sample. Practical constraints limiting participant recruitment were due to several considerations. First, it is difficult to get a large number of Soldiers to take time out of their regular duty schedule to participate in studies. Second, study participation required interstate travel during an ongoing SARS-CoV-2 pandemic.

Written informed consent was obtained, and the U.S. Army Combat Capabilities Development Command Armaments Center Institutional Review Board, Tufts University Institutional Review Board, and the Army Human Research Protections Office approved all procedures

Research Design

This study used a repeated measures design, with load carriage (resting control, loaded, and unloaded) and stimulus ambiguity (low, high) as within-participants factors. The load carriage levels included unloaded condition (7.4 lbs) and operationally-relevant load condition (50% body weight), as well as a resting control. The unloaded condition was comprised of ancillary equipment, consisting of a Modular Lightweight Load-carrying Equipment (MOLLE) rucksack and mock M4 carbine. The loaded condition comprised the Soldier Plate Carrier System body armor with front, back and side plates, in addition to the same ancillary equipment as the unloaded condition, loaded to 50% of Soldiers' body weight.

Decision Making under Uncertainty and Stress task

The Decision Making under Uncertainty and Stress task (DeMUS) mirrored that described above (see *Study 1 Methods*) with the exclusion of electric shock. First, participants completed a criterial learning task where they were trained to distinguish camouflage patterns as a friend versus foe. Participants made responses using a button press affixed to the weapon. During the foot march, the task was presented on a flat monitor in front of the treadmill (see Figure 2.1). Due to technical error, 10 participants were able to complete the DeMUS task in all physical stress conditions.

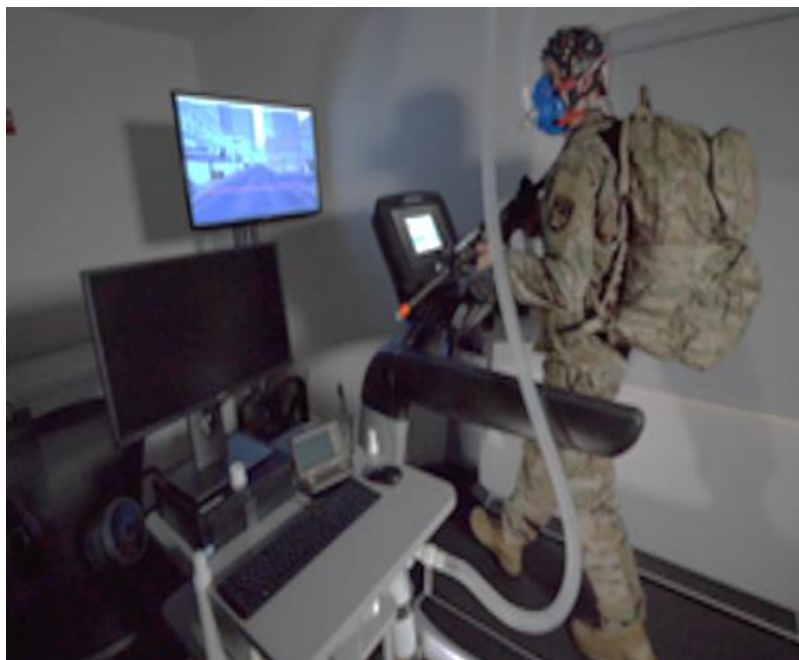


Figure 2.1 Example of Soldier donned with MOLLE rucksack and mock M4 carbine weapon completing DeMUS task during treadmill foot march.

Questionnaires

Participants completed the Feeling Scale (FS), Felt Arousal Scale (FAS), and Borg Rated Perceived Exertion Scale (RPE) immediately before, at 20, 40, 60, 80 and 120 minutes during, and immediately following load carriage.

Feeling Scale (FS): This one-item inventory measures the extent to which participants feel pleasant or unpleasant (Hardy & Rejeski, 1989). Participants were told, “while participating

in exercise, it is common to experience changes in mood. Some individuals find exercise pleasurable, whereas others find it to be unpleasant. Additionally, feelings may fluctuate across time. That is, one might feel good and bad several times during exercise. Scientists have developed this scale to measure such responses.” The scale ranged from “very good” (+5) to “very bad” (-5).

Felt Arousal Scale (FAS): This one-item inventory measures feelings of arousal (Svebak & Murgatroyd, 1985). Participants were told, “Estimate here how aroused you actually feel. Do this by selecting the appropriate number. By “arousal,” we mean how “worked-up” you feel. You might experience high arousal in one of a variety of ways- for example, as excitement, anxiety, or anger. Low arousal might also be experienced in several different ways- for example, as relaxation or boredom or calmness.” The scale ranged from “low arousal” (1) to “high arousal” (6).

Borg Rated Perceived Exertion Scale (RPE): This commonly used one-item self-report measures perceived physical exertion (Borg, 1982). Participants were told, “choose the number that best describes your level of exertion,” on a scale ranging from “no exertion at all” (6) to “maximal exertion” (20).

Treadmill Foot March

Each participant donned a weighted pack with or without plate carriers loaded with approximately 50% body mass, including a modified M4 rifle. A relative load rather than an absolute load was used to minimize potential confounds due to body size. Participants were weighed to verify that body-borne load was within $\pm 2\%$ of the goal weight. Once prepared, participants walked on the treadmill at a fixed pace (1.35 m/s, ~ 3.0 mph) which amounted to 9.7 km (6.0 mi) of distance covered. This speed was chosen based on standard forced march paces

recommended in Army doctrine (Foot marches (Field Manual 21-18), 1990) and has been previously used in load carriage studies (Hasselquist et al., 2018; Loverro et al., 2019). For the first ten minutes, participants walked at a 1% grade; this period served as a baseline walking pattern period, after which time the incline increased to 4%. This incline was sustained until the 110-minute mark, where the incline returned to a 1% grade for the final ten minutes. During the final hour of the foot march, participants completed the DeMUS task (at approximately minutes 105-110).

Procedure

In order to ensure participants were physically and mentally prepared to participate, following consent and prior to enrolling, participants completed medical release and study-specific clearance forms. A dedicated medical person authorized to review medical records reviewed these forms and participants' medical records to ensure medical readiness before giving the principal investigator a "go" or "no-go" for each participant. Prior to data collection, the principal or associate investigator reviewed the clearance and protocol, particularly the loaded foot march, to ensure participants understood study methods and expectations.

Testing Day 1

Participants first completed a checklist of questions to ascertain changes in health status, recent injuries, amount of sleep, and diet. They were then fitted with a heart rate monitor. They then completed the criterial learning task and the shoot/don't-shoot task at rest.

Participants completed a maximal exercise test using a continuous, uphill, stepwise treadmill protocol. Participants wore a lightweight mask covering the oronasal portion of the face connected by a flexible hose to the COSMED Quark CPET metabolic cart (COSMED, Rome, Italy), which will monitor oxygen uptake and carbon dioxide production. To induce maximum exhaustion, participants began running at a constant speed for 6 mph, which incrementally

increased by 0.4 mph and/or grade inclination between 1 to 2% every 1 to 2 min of exercise similar to the traditional Astrand treadmill protocol (Åstrand et al., 1960) and more recent (Jones & Doust, 1996) protocols which is extensively used and follows the continuous and constant increments in load suggested by the literature (Kang et al., 2001). The criteria for reaching VO_2max included a plateau in $\text{VO}_2 < 150 \text{ ml/min}$ with an increase in workload, surpassing 1.05 respiratory exchange ratio, achieving within 10 beats per minute of maximum heart rate, when participants can no longer physically continue, or achieving the maximum rate of perceived exertion (20 on the Borg scale (6-20)). When VO_2max was achieved, participants volitionally terminated the testing (Thompson et al., 2013). The treadmill speed was reduced to a slow walk, and the grade returned to zero. Prior to exercise testing, participants were permitted to conduct self-stretching and dynamic warm-up, and all participants performed a low-intensity cool-down as they deemed necessary. Participants were then released for the day. This initial session lasted approximately three hours (see Figure 2.2 for Day 1 schedule).



Figure 2.2 Schematic of Day 1, where participants completed questionnaires, donned physiological monitoring devices, completed the Critical Learning (CLT) and Decision Making under Uncertainty and Stress (DeMUS) tasks at rest, and completed a maximal exercise test.

Testing Days 2 and 3

Participants returned to the lab for two sessions in which they completed a two-hour treadmill foot march at one of two loads (loaded with 50% body weight, unloaded), in counterbalanced order. All other aspects of the two sessions were identical. See Figure 2.3 for Day 2 and 3 schedules.

Participants first completed the daily participant checklist to ensure they were prepared to exercise (mentioned previously). They donned the heart rate monitor and were weighed to confirm the appropriate load (50% body weight on the loaded day). They then completed the Rating of Perceived Exertion Scale (RPE), Feeling Scale (FS), Felt Arousal Scale (FAS) to assess baseline differences between conditions. They also completed the criterial learning components of the DeMUS Task. Following the baseline measures, they completed the treadmill foot march, during which they completed the RPE, FS, FAS every 20 minutes. They completed the DeMUS Task during the final hour of the treadmill foot march (at approximately 105 minutes). Following the foot march, participants completed the RPE, FS and FAS and were released for the day 60 minutes post-foot march (upon completion of additional measures not described here).

The first and second sessions were separated by at least 24 hours. The second and third (foot march) sessions were separated by at least 48 hours, during which time participants were asked to refrain from strenuous physical activity beyond their regular training. Additionally, they were asked to refrain from all strenuous exercise 24 hours prior to their sessions.

To reduce diurnal variation in cognitive and physical performance, we scheduled test sessions at the approximate same time within participants (± 1 hour). To reduce the influence of hydration status on cognitive and physical performance, we asked participants to consume 1/2 liter of water the night before a test session and 1/2 liter of water the morning of a test session. Participants were also encouraged to eat what they usually would before a ruck march.

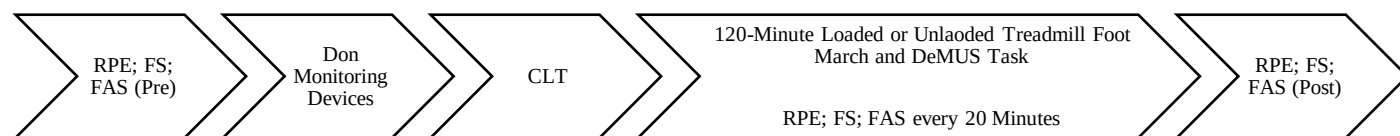


Figure 2.3 Schematic of Days 2 and 3, where participants completed questionnaires, donned physiological monitoring devices, completed the criterial learning task (CLT), completed a two-hour treadmill foot march with the Decision Making under Uncertainty and Stress (DeMUS) task performed during the final hour. Questionnaires were completed every 20 minutes during and immediately following the march.

Statistical Methods

Data processing

For the DeMUS task, we calculated several primary measures (described above; see *Study 1 Data Processing*), including hit rate, false alarm rate, miss rate, correct rejections, sensitivity (d'), and response bias (criterion c). Each measure was determined separately for each participant, physical stress condition (rest, loaded, and unloaded) and ambiguity condition (low, high).

Heart rate was measured in beats per minute (bpm) and averaged across time points within the loaded and unloaded conditions. Heart rate was not collected during the resting control condition.

Data Analysis

We conducted several analyses to confirm that our physical stress manipulation produced significant differences in physiological and emotional responses. To evaluate differences in heart rate (HR), perceived exertion (RPE), valence (FS) and arousal (FAS) between loaded and unloaded conditions, we conducted an omnibus 2 (load condition: loaded, unloaded) x 6 (time: 0, 20, 40, 60, 80, 120 min) repeated measures ANOVA for each dependent measure.

To assess differences in shoot/don't-shoot performance due to physical stress and ambiguity, we conducted an omnibus 3 (physical stress condition: rest, loaded, unloaded) x 2

(stimulus ambiguity: low, high) repeated measures ANOVA. Analyses were done separately for hits, false alarms, d' , and criterion c .

All statistical analyses were performed using Jamovi v 2.2.5. (<https://www.jamovi.org/>), which is a free and open-source statistical platform. Data were assessed for normality using Shapiro-Wilk tests. When sphericity was violated, Greenhouse-Geisser corrections were used. When an ANOVA yielded a significant main effect or interaction ($p < .05$), post-hoc tests using the Bonferroni corrections were conducted. Effect sizes are provided for all analyses, using η^2 for ANOVAs, and Cohen's d for t -tests.

Results

Physiological and emotional responses (manipulation checks)

Rated Perceived Exertion Scale

There was a main effect of Physical Stress $F(1, 12) = 80.09, p < .001, \eta_p^2 = .87$, Time, $F(5, 60) = 15.69, p < .001, \eta_p^2 = .57$, and a Physical Stress by Time interaction, $F(5, 60) = 4.81, p = .001, \eta_p^2 = .29$ (see Figure 2.4A). These effects were characterized by higher perceived exertion ratings during the loaded than unloaded foot march at minutes 20, 40, 60, 80 and 120 ($ps < .007$; $ds > .71$), but no difference at 0 minutes.

Feeling Scale

There was a main effect of Physical Stress, $F(1, 12) = 7.55, p = .018, \eta_p^2 = .39$, Time, $F(5, 60) = 3.14, p = .0014, \eta_p^2 = .21$, and a Physical Stress by Time interaction, $F(5, 60) = 5.31, p < .001, \eta_p^2 = .31$ (see Figure 2.4B). These effects were characterized by lower (more negative) emotional valence during the loaded than unloaded foot march at minutes 20, 40, 60, 80 and 120 ($ps < .032$; $ds > .67$), but no difference at 0 minutes.

Felt Arousal

There were no effects of Physical Stress, Time, nor a Physical Stress by Time interaction, ($ps > .49$; see Figure 2.4C).

Heart Rate

There was a main effect of Physical Stress, $F(1, 10) = 37.26, p < .001, \eta_p^2 = .79$, Time, $F(5, 50) = 145.73, p < .001, \eta_p^2 = .94$, and a Physical Stress by Time interaction, $F(5, 50) = 3.22, p = .014, \eta_p^2 = .24$ (see Table 2.2). These effects were characterized by higher heart rate during the loaded than unloaded foot march at minutes 20, 40, 60, 80 and 120 ($ps < .023$; $ds > .89$), but no difference at 0 minutes.

Shoot/don't-shoot performance***Hits***

There were no effects of Physical Stress, Stimulus Ambiguity, nor a Physical Stress by Stimulus Ambiguity interaction, ($ps > .119$; see Table 2.3).

False Alarms

There was a main effect of Stimulus Ambiguity, $F(1, 5) = 7.89, p = .038, \eta_p^2 = .61$, in which false alarms were higher in the high ambiguity than low ambiguity condition (see Table 2.3). There were no effects of Physical Stress nor Physical Stress by Stimulus Ambiguity interactions ($ps > .270$).

Sensitivity (d')

There were no effects of Physical Stress, Stimulus Ambiguity, nor a Physical Stress by Stimulus Ambiguity interaction, ($ps > .404$; see Figure 2.5).

Response Bias (criterion c)

There was a main effect of Stimulus Ambiguity, $F(1, 5) = 7.94$, $p = .037$, $\eta_p^2 = .61$, in which participants were more biased towards responding yes (shooting) in the high ambiguity than low ambiguity condition (see Figure 2.6). There were no effects of Physical Stress nor Physical Stress by Stimulus Ambiguity interactions ($ps > .155$).

Response Time

When examining RT for hits there were no main effects of Physical Stress or Stimulus Ambiguity, nor interactions, ($ps > .16$). When examining RT for false alarms, there were no effects of Physical Stress, Stimulus Ambiguity, nor a Physical Stress by Stimulus Ambiguity interaction, ($ps > .81$; see Table 2.3).

Study 2 Discussion

The present study evaluated whether physical stress induced by load carriage influenced shoot/don't-shoot decisions and ambiguous shoot/don't-shoot decisions. Participants made friend/foe discriminations using camouflage patterns presented on avatars in a virtual environment at rest and during a 120-minute loaded and unloaded treadmill foot march. In addition, we collected heart rate and measures of perceived exertion, affective valence and arousal during both conditions.

First, sample size was a limiting factor, as our sample size of 13 is admittedly less than ideal. Constraints of sampling from a specific population of interest was indeed a challenge of this study. It is difficult to get a large number of Soldiers to take time out of their regular duty schedule to participate in studies (as evidenced by 16 out of the request 70 Soldiers who volunteered to travel to participate in this study). Furthermore, technical error led to as few as 10

complete data sets across conditions for dependent measures of shoot/don't-shoot performance. Using G*Power 3.1 (Faul et al., 2007) to plot power ($1 - \beta$) by total sample size ($n = 10$) yielded 75% power to detect a main effect of stress and even less to detect an interaction between stress and ambiguity. Low power reduces the chance of detecting a true effect, and less intuitively, low power also reduces the likelihood that a statistically significant result reflects a true effect (Button et al., 2013). Therefore, it is to be noted that preliminary conclusions drawn here are limited and should be interpreted with caution.

How does load carriage influence physiological and emotional responses?

Two hours of load carriage led to increased heart rate and perceived exertion. Load carriage also reduced affective valence, leading to less positive, more negative feelings relative to the unloaded condition. These results align with previous studies and support affect exertion models such as the Dual Mode Theory, whereby physical exertion becomes less pleasant with increased exercise intensity (Ekkekakis et al., 2011) during applied activities such as load carriage (Giles et al., 2019). During the loaded condition, participants' percent HR_{max} and VO_{2peak} fell within the upper end of the moderate intensity range (Garber et al., 2011). Interestingly, extant literature has demonstrated that during unloaded exercise at low or moderate intensities, below the ventilatory threshold (VT), affective responses are mostly positive (pleasant). Affective responses become variable near VT, or the point at which ventilation becomes disproportionately high with respect to oxygen consumption. Moreover, intensity, rather than duration or total work, drives these changes in affective responses (Daley & Welch, 2004; Kilpatrick et al., 2007). The present findings suggest that during load carriage Soldiers experienced negative emotion at lower intensities relative to the VT than the Dual Mode Theory would predict (Ekkekakis & Brand, 2019). Typically, during unloaded exercise, affective

responses do not become uniformly negative until exercise intensities are high and surpass the VT (Ekkekakis et al., 2004, 2008). However, during load carriage, physical exertion can be compounded by pain, such as in the lower back (Qu et al., 2020), which likely exacerbates negative emotion beyond what might be expected in unloaded conditions. Nonetheless, changes in heart rate, perceived exertion and emotional valence indicate that load carriage elicited a reliable physical stress response.

How does load carriage influence shoot/don't-shoot decisions?

Shoot/don't-shoot performance did not differ as a function of load. This is contrary to similar work finding decrements in Soldiers' go/no-go performance, such as higher proportion of false alarms and lower response sensitivity, under loaded compared to unloaded conditions (Eddy et al., 2015; Giles et al., 2019). Importantly, previous load carriage work has not included control conditions, making it hard to tell the magnitude of changes in performance, relative to rest. In the present study, even though physical demands of the march increased with load and duration, exercise intensity remained within the moderate range in the loaded condition (due to the need to reduce the load mass for several participants) and may not have reached a level at which cognitive decrements would be realized. Yet, results align with the reticular-activating hypofrontality (RAH) model of acute exercise and empirical work demonstrating that, under unloaded conditions, low to moderate intensities (or levels of physical stress) do not influence cognitive control (Cantelon & Giles, 2021; Davranche et al., 2009; John et al., 2009). In contrast, we did not observe a reliable change in response times, which has been repeatedly demonstrated during unloaded moderate intensity exercise, compared to lower intensity or rest (McMorris & Hale, 2012, 2015). Yet, a lack of difference in response times has previously been observed between carrying loads ranging from 8kg to 43kg (Armstrong et al., 2022; Giles et al., 2019).

How does load carriage influence ambiguous shoot/don't-shoot decisions?

As expected, shoot/don't-shoot performance varied by stimulus ambiguity. Participants were less accurate and more likely to classify friends as foes under conditions of high compared to low ambiguity, as demonstrated by greater rates of false alarm and shifts in response bias. These results are similar to those reported in Study 1 and consistent with extant literature on decision making under uncertainty, or ambiguity (Brunyé & Gardony, 2017; Scriber, 2002). Overall, it appears our manipulation of stimulus ambiguity was effective in altering shoot/don't-shoot performance across levels of varying stimulus clarity.

Despite this, we did not find differences in response to ambiguity under physical stress. It was difficult to fully test this interaction due to technical error causing incomplete data sets, as well as limited sample size. Further, very limited work has explored responses to ambiguity within the domain of physical stress. However, previous empirical work in adjacent fields may help guide interpretation of findings. Previous research examining differences in interpretation of ambiguous social cues during 50 minutes of exercise found no differences in response to ambiguity, or tendency to rate ambiguous facial expressions (i.e. surprise) as positive or negative, under varying exercise intensities (Cantelon et al., 2019). This study examined response to ambiguity under moderate compared to high exercise intensities. Here, we did not find any differences in tendency or ability to identify friends as foes when stimulus ambiguity was high during light compared to moderate intensities, as well as rest. It is possible that realization of differences in performance in response to ambiguity and physical stress may require greater differences in the relative intensity of stress conditions (i.e. low vs. high exercise intensity). Although we did not find differences in response to ambiguity between varying loads, research in this area is very limited with much to be explored. Previous study of load carriage has

aimed to increase ecological validity by testing performance outside laboratory settings (i.e. in the field), or employing operationally relevant load configurations within the lab. Future work should also aim to incorporate factors inherent in high-stakes contexts (i.e. ambiguity) into research examining cognitive performance under load.

Study 2 Limitations and Future Directions

We measured shoot/don't-shoot performance at a single time point, late in the exercise bout when fatigue was likely to be high. This decision was driven by evidence suggesting that decrements due to absolute loads ranging from 40-50kg may only become apparent after 45 minutes of physical activity (Giles et. al. 2019; Eddy et al., 2015). However, more recent work has demonstrated no effects of medium (26kg), high (33 kg), or very high (43 kg) loads, compared to low load (21 kg), on a similar auditory go/no-go task after 105 and 120 minutes of exercise (Armstrong et al., 2022). Manipulation of march duration was not a primary independent variable in this study, but single sampling of cognitive performance limited our ability to determine temporal dynamics of shoot/don't-shoot performance over the exercise bout. For example, during a prolonged bout of load carriage, performance may increase or decrease at varying time points as top-down (cognitive and physical efforts) and bottom-up processes (bodily sensations) may act in parallel of arousing mechanisms to dynamically influence cognitive performance across time (Schmit & Brisswalter, 2018). Changes in cognition during longer durations are essential to understand for military personnel and emergency responders, who must remain cognitively intact in prolonged, physically demanding situations.

Although we sampled from the military population, the recruited participants (comprising a representative sample of combat engineer and cavalry Military Occupation Specialties (MOS)) had variable load carriage experience and aerobic fitness levels (see Table 2.1). The load in this

study was on the upper end of what Soldiers are typically required to carry in training (Headquarters, 2022) and beyond what the present sample typically trained, that is the 12-mile foot march carrying 35 lbs. in their rucksack, in addition to the M4/M16/rifle/carbine, in under three hours (Department of the Army Headquarters, 2020). Consequently, participants were allowed to decrease their weight during the foot march in order to safely and successfully complete the study. They did so in 10% increments resulting in final loads of 23.6 ± 12.0 kg (27.5 ± 7.2 excluding two Soldiers who dropped their pack entirely, see Table 2.1). This led to differences in the relative magnitude of the physical stressor between participants at the time of performing the DeMUS task. Interestingly, recent work from Armstrong and colleagues (2022) found no differences between absolute loads of 26 and 43 kg on response inhibition accuracy (hits and correct rejections) during an operationally-relevant visual go/no-go task (where friendly targets were depicted as uniformed Soldiers and enemy targets were depicted as non-uniformed individuals). This suggests that there may be a range whereby effects on cognitive control may be negligible between absolute load masses (26-43 kg). End load (when participants performed the DeMUS task) for 54% of our participants fell within this range, suggesting that for these individuals the difference between starting and ending weights may have been negligible with regards to effects on cognitive control. In contrast, 46% of participants had greater reductions in the load carried and may have completed the DeMUS task with no relative difference between physical stress conditions (loaded and unloaded). Combined with a small sample size, this variability may have reduced generalizability of our findings. Thus future work should aim to understand the dose response of load carriage under Soldier relevant loads (Headquarters, 2022; Orr et al., 2021), both relative to body size and absolute, to better understand impacts on Soldier cognitive performance.

Future research should carefully consider methodological approaches in order to both reduce attrition and increase the quantity and quality of cognitive research on load carriage. In the present study, 4 out of 13 participants were able to complete the entire 120-minute march while loaded to 50% body weight. Exclusion of participants who were unable to complete the experimental conditions as intended (i.e. they incrementally reduced their load) would've yielded an attrition rate of 70%. Unfortunately, when reported, attrition is a common issue among load carriage research (Armstrong et al., 2022; 70% attrition). Still the carrying of heavy loads is an essential aspect of military operations and is often mission essential (Knapnik & Reynolds, 2012). Indeed, loads often remain unchanged regardless of changes in threat (which require varying degrees of personal protection equipment), environmental conditions and mission characteristics (Armstrong, 2017). Furthermore, even at rest, Soldiers typically wear additional load mass, which has been shown to influence cognitive performance (Son et al., 2019). Thus, research methodologies need to strike a balance between optimizing participant comfort and safety versus ethically assessing how factors inherent to load carriage impact cognitive performance. For instance, engaging in shoot/don't-shoot decisions during load carriage involves an inherent dual-task environment and heavy equipment induces physical strain. Future work could explore how factors such as relative vs. absolute load, equipment type and configuration (i.e. plate carrier vs. tactical vest) and recovery time between sessions may impact attrition as well as cognitive performance.

Study 2 Conclusions

To date, there is a paucity of research exploring the effect of load on cognition, with even less work examining physical and cognitive demands concurrently. Previous work suggests that load carriage influences cognitive processes across several load configurations, exertion

intensities, and duration's. However, further work with larger samples is required to determine the dose response relationship for the effect of these factors, as well as ambiguity, on shoot/don't-shoot performance. It is imperative for future work to characterize cognition under operationally relevant conditions that apply to a range of physically fatiguing careers, including military personnel and first responders.

STUDY 2 TABLES**Table 2.1** Sample Characteristics (n = 12 men, 1 woman).

	Average	SD	Minimum	Maximum
Age (years)	23.1	3.9	19	34
Years in Military	3.2	2.2	0	6
BMI (kg/m ²)	25.2	3.6	21.4	31.5
VO ₂ peak (ml/kg/minute)	43.1	5.0	34.7	50.3
HR max at VO ₂ peak (bpm)	196.2	4.4	186.0	202.0
Load at Foot March Start (kg)	45	6.95	21.6	50.5
Load at Foot March End (kg)	23.6	12.0	0.0	38.7
Load at Foot March End (% body weight)	30.1	19.4	0.0	50.0

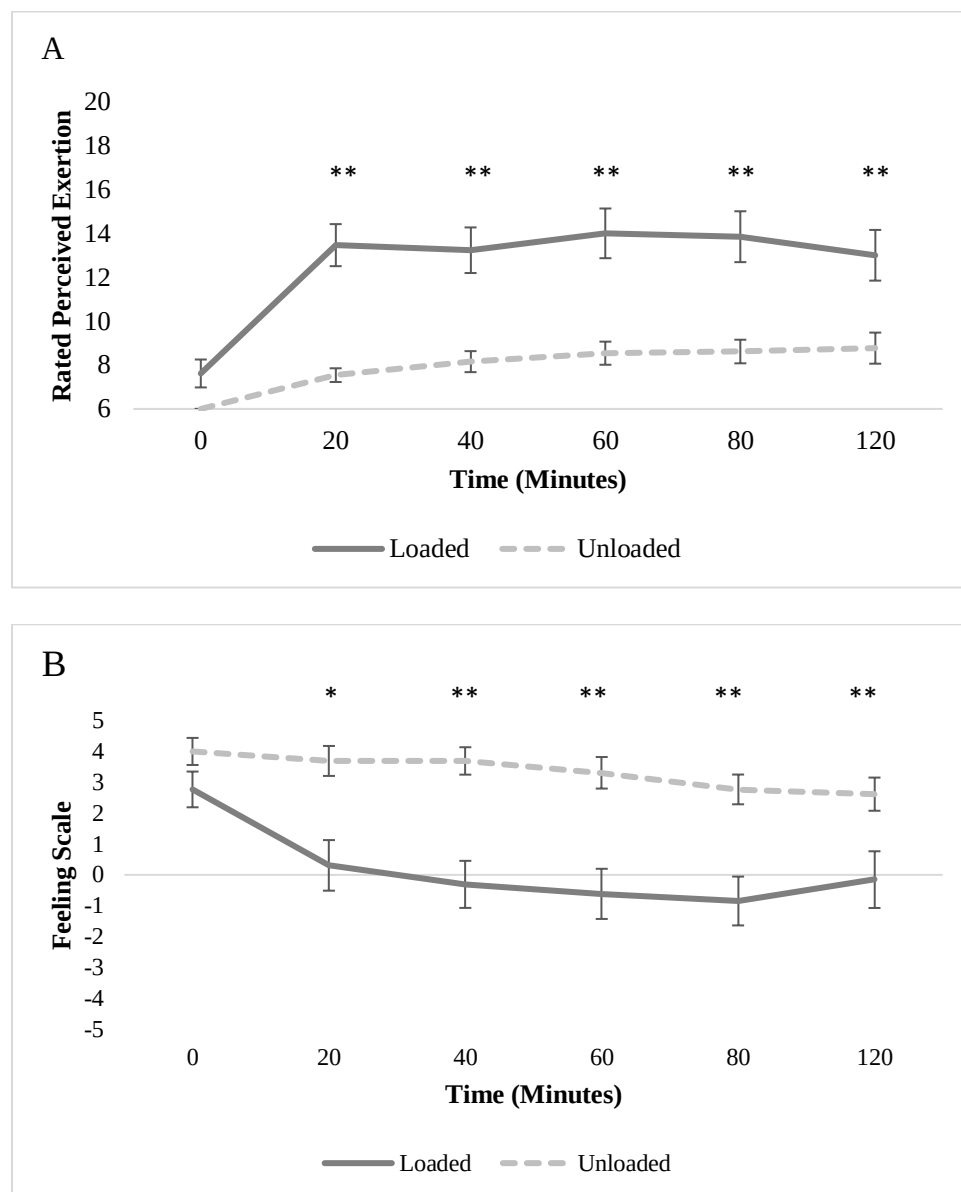
Table 2.2 HR, percent HR_{max} and VO₂peak means $\pm$ SD during a two-hour treadmill foot march (n = 13).

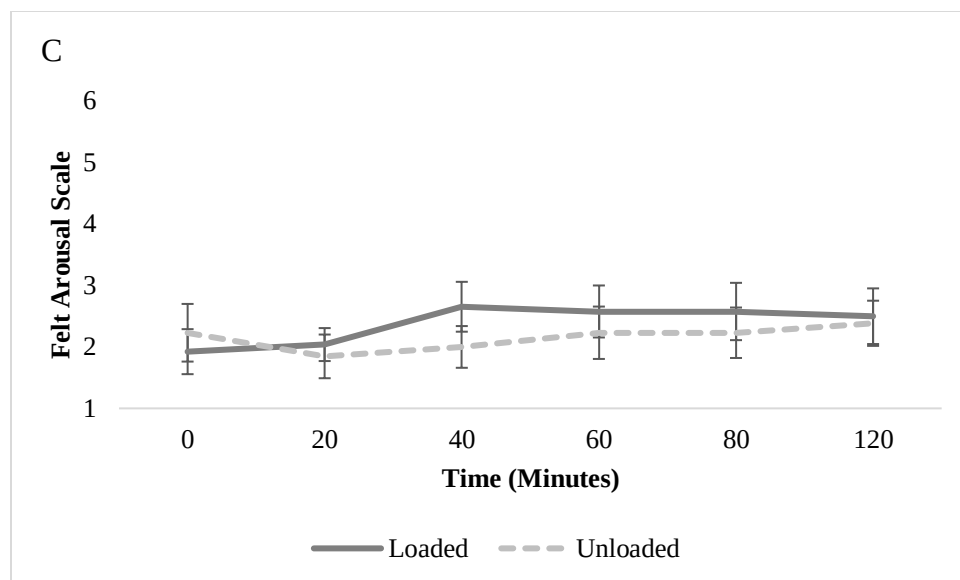
		Minute 0	Minute 20	Minute 40	Minute 60	Minute 80	Minute 120
Average HR	Loaded	94.7 $\pm$ 17.7	150.1 $\pm$ 14.2	151.1 $\pm$ 14.2	151.7 $\pm$ 18.7	151.5 $\pm$ 21.5	143.4 $\pm$ 14.8
	Unloaded	82.2 $\pm$ 10.3	118.7 $\pm$ 9.5	123.7 $\pm$ 7.6	126.4 $\pm$ 6.7	125.9 $\pm$ 6.0	121.1 $\pm$ 6.3
Percent HR _{max}	Loaded	47.8 $\pm$ 4.8	-	76.6 $\pm$ 5.2	-	76.0 $\pm$ 5.8	71.9 $\pm$ 4.6
	Unloaded	41.3 $\pm$ 2.8	-	62.7 $\pm$ 2.1	-	63.7 $\pm$ 1.8	60.9 $\pm$ 2.2
Percent VO ₂ peak	Loaded	16.2 $\pm$ 1.0	-	53.9 $\pm$ 2.1	-	52.3 $\pm$ 2.0	46.4 $\pm$ 2.0
	Unloaded	13.4 $\pm$ 0.9	-	43.6 $\pm$ 1.7	-	44.0 $\pm$ 1.6	37.3 $\pm$ 1.6

Table 2.3 Shoot/don't-shoot hit rate, false alarm rate, and response time means $\pm$ SD by Physical Stress and Stimulus Ambiguity.

Stress Condition	Stimulus Ambiguity	Hit Rate			False Alarm Rate		Response Time (Sec)	
		N	M	SD	M	SD	M	SD
Rest	Low	11	0.65	0.37	0.33	0.31	21.32	2.71
	High	11	0.65	0.38	0.35	0.41	23.00	2.81
Unloaded	Low	10	0.47	0.43	0.19	0.34	20.37	7.19
	High	10	0.86	0.16	0.50	0.27	23.06	2.87
Loaded	Low	11	0.46	0.39	0.35	0.39	22.38	3.63
	High	11	0.58	0.26	0.51	0.32	22.27	3.11

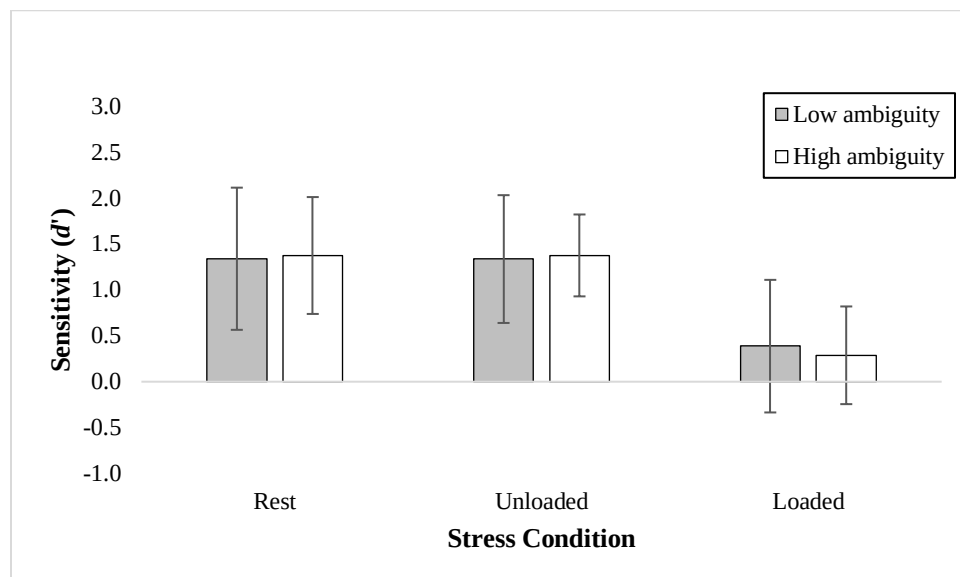
STUDY 2 FIGURES

Figure 2.4 Emotional Response means (SEM) by Physical Stress and Time.



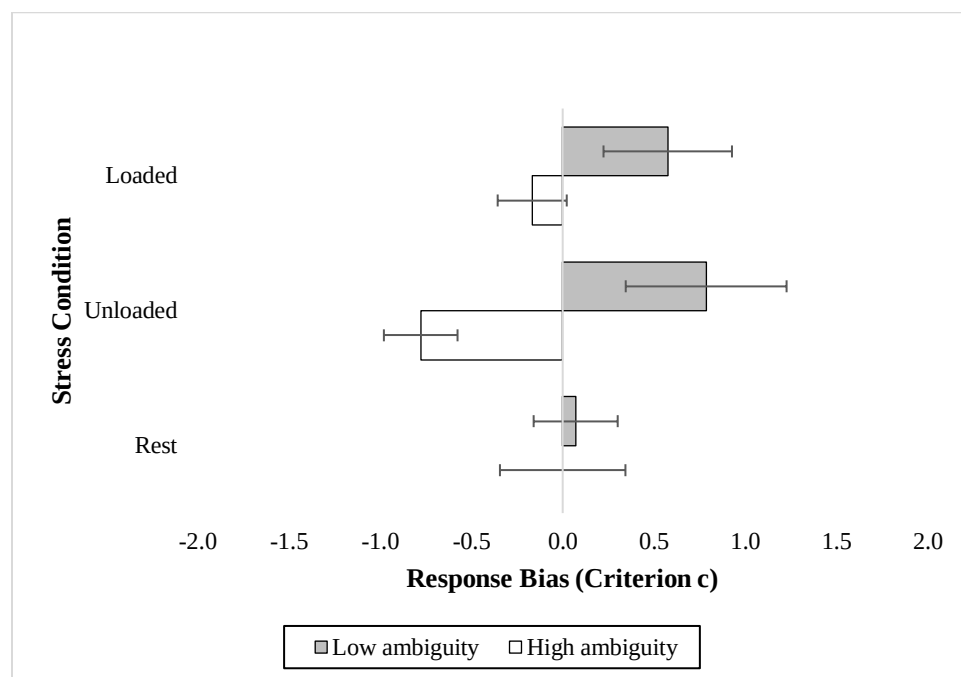
Rated perceived exertion (A), emotional valence (B) and emotional arousal (C) during two-hour treadmill foot march under loaded (to 50% body weight) and unloaded conditions. * $p < .05$, ** $p < .01$.

Figure 2.5 Shoot/don't-shoot Sensitivity means (SEM) by Physical Stress and Stimulus Ambiguity.



Sensitivity did not differ for low and high ambiguity stimuli between rest, unloaded and loaded conditions ($ps > .404$).

Figure 2.6 Shoot/don't-shoot Response Bias means (SEM) by Physical Stress and Stimulus Ambiguity.



Response bias did not differ between rest, unloaded and loaded conditions ($p > .155$), but participants had a more liberal response bias and were more likely identify targets as an enemy than friendly in the high than low ambiguity condition ($p = .037$). Note, the mean for high stimulus ambiguity rest condition is zero.

GENERAL DISCUSSION

The goal of the present studies was to determine how operationally-relevant emotional and physical stress impacted shoot/don't-shoot performance under varying conditions of ambiguity. To do this, we used a Soldier-relevant cognitive task in an ecologically valid virtual environment. An additional strength of these studies included measurement of behavior during stress manipulation, as well as validation of subjective and objective stress response. Despite these strengths, we found mixed evidence of successful stress manipulation across studies and limited evidence that threat of shock or load carriage influenced shoot/don't-shoot decisions, nor did ambiguity interact with stress to influence decisions.

It has been argued that in order to increase the translation of stress and cognition research to military-specific contexts, performance should be examined using operationally relevant tasks. In the present studies, we used a novel shoot/don't-shoot task designed to represent the demands of operational scenarios in terms of the cognitive tasks required by the Soldier, as well as incorporation of realistic sources of uncertainty. For instance, Soldiers are often tasked with identifying allied vs. enemy forces based on camouflage patterns worn. As such, the demands of our DeMUS task represented ecologically valid demands faced in high stakes contexts.

One way to emulate military-specific contexts is through virtual environments (VE's). In the present studies we used semi-immersive (Study 1) and non-immersive (Study 2) VE's to present high dimensional, interactive, and ecologically valid stimuli while maintaining a high degree of internal validity (Bohil et al., 2011; Schilbach, 2015). Previous research has demonstrated that when interactive VE's are paired with stress induction it can lead to a greater sense of presence (i.e., the subjective experience of "being there") (Beeli et al., 2008), which may produce greater emotional and physiological reactions (Asbee & Parsons, 2021; Parsons &

Rizzo, 2008). This was not the case in Study 1, which used a semi-immersive environment and found little evidence of changes in subjective emotional or physiological response. Study 2 demonstrated significant changes in physiological and emotional responses, yet this was more likely due to the physical stress manipulation than the non-immersive task environment. However, to date, there have been few studies leveraging controlled VE's to study cognitive performance in general, or under conditions of stress or ambiguity. Although VE's can increase realism, precisely mimicking real-world scenarios is often a challenge of military-cognition research. Military operations are often dangerous in nature, which complicates the possibility of creating realistic content and context for transfer. Despite practical limitations, when it comes to shoot/don't-shoot decision making, questions remain as to whether simulation is a comparable substitute to live fire (Blacker et al., 2019). Future research could further explore the impacts of environmental realism by examining the extent to which stress and ambiguity influence shoot/don't-shoot decisions in fully immersive VE's, or during simulated compared to live-fire contexts.

Using a realistic task allowed for high external validity but this also came with limitations. For example, it is more likely that a Soldier will make a few critical friend/foe discriminations than hundreds, back to back. Thus, the present task employed a relatively low number of trials to mirror real-world decisions. Low number of repeated trials limits both statistical power and ability to measure a stable estimate of behavioral response due to stress or ambiguity (Baker et al., 2020). While increasing the number of trials can help to overcome this limitation, there are often restrictions on the number of trials an individual can complete, e.g., due to fatigue. Furthermore, using a predictable performance-contingent emotional stress manipulation, like shock, can also limit the number of trials that are feasible. This is especially

true when using a one-to-one, error-to-feedback delivery schedule, where Soldiers' receive a shock upon every shoot/don't-shoot error. To account for these limitations, we tried to increase participant motivation and compliance through our aversive feedback (i.e., shock) in Study 1. Additionally, across both studies we required participants to meet 0.8 accuracy criterion for identification of enemy and friendly patterns and to perform several practice trials prior to beginning the DeMUS task. Nonetheless, future research should expand upon these findings by examining impacts of stress and ambiguity on a greater number of friend/foe discriminations. This will help determine reliability of effects of independent variables of interest, as well as emulating "target rich" environments of modern urban warfare (Blacker et al., 2019).

In addition to number of trials, the ratio of friends-to-foes can also impact shoot/don't-shoot errors. Indeed, a higher proportion of foe relative to friendly targets will increase the likelihood of commission errors, or false alarms, in shoot/don't-shoot scenarios, which parallels work using basic go/no-go paradigms (Wessel, 2018; Wilson et al., 2014, 2016). However, this effect appears to occur in an exponential rather than linear fashion (Wilson et al., 2015). As such, it is possible that a threshold may exist whereby strength of prepotent motor activity predisposes shooting errors. This is thought to occur somewhere between 50% to 89% probability of foes to friends. However, the extent to which this threshold varies by stress or ambiguity remains unknown. In the present studies we presented an even number of enemy and friendly trials, with 3 additional filler trials to throw off the 50/50 odds (not included in analysis). This ratio is similar to previous work demonstrating effects of threat of shock on shoot/don't-shoot decision accuracy (Gamble et al., 2018; Patton, 2014). These studies used 50/50 friend-friend/friend-foe target pair proportions. However, these ratios are on the lower end of the alleged friend/foe error threshold. Future work should more closely examine the functional relationship between friend–

foe probabilities and shoot/don't-shoot errors, as well as how this may interact with stress and stimulus clarity.

The decision to shoot or withhold fire is critical. In many cases, threats versus non-threats can be easily disambiguated and have been the focus of much of the prior work on shoot/don't-shoot decision errors (Biggs et al., 2015; Biggs & Pettijohn, 2022; Hamilton et al., 2019; Scribner et al., 2005; Vickers & Lewinski, 2012; Wilson et al., 2014). For example, previous work has explored impacts of time pressure, target saliency, cognitive load, and sleep deprivation, to name a few (Burke, 2007; Head et al. 2017; Scribner, 2002; Scriber et al., 2005; Smith et al., 2019; van Peer et al., 2019). Here we sought to expand upon previous work by examining the impacts of ambiguity under stress. To do this, we compared sensitivity, or the ability to discriminate, and response bias, or the tendency to identify, targets as friends and foes under conditions of low and high ambiguity. As such, stimuli included camouflage patterns that elicited low uncertainty (100% enemy or friendly) and moderate to high levels of uncertainty (e.g., friendly if the pattern is 63% friendly and 37% enemy). We found significant effects of stimulus clarity, or ambiguity, on shoot/don't-shoot classifications, yet no impacts of stress (either emotional or physical) nor interactions between the two variables. During stress, or heightened states of arousal, ambiguity may simply increase time to decision (i.e., response times), but may not alter sensitivity or response bias, as demonstrated here. However, similar to the friend/foe error threshold described above, there may be a certain level of uncertainty that must be met and/or surpassed in order to impact performance. Comparison of two discrete levels of ambiguity may have limited ability to detect stress-induced changes.

Also, responses to ambiguity can be exacerbated by individual differences. Indeed, research in related fields has demonstrated that individuals vary in their tendency to perceive

ambiguous postures or facial expressions as either threatening, or negative, as compared to non-threatening, or positive (Biggs et al., 2021; Kim et al., 2003; Neta et al., 2009, 2017).

Furthermore, interpretations of ambiguity may be dependent upon stress reactivity (Brown et al., 2017; Ito et al., 2017; Park et al., 2016). Research points to the inherent uncertainty surrounding a stressor as a seminal driver of these neurophysiological responses (de Berker, 2016; Dickerson & Kemeny, 2004; Starck & Brand, 2016). The tightly coupled relationship between stress and uncertainty raises the important question of how stress exposure affects the way individuals behave when encountering ambiguity in their environment. However, interactions between ambiguity and stress have received little attention, limiting conclusions that be drawn from current findings. Additional research is needed to acquire a more comprehensive understanding of stress and ambiguous decision making.

Yet, stress is both fickle and fascinating. One of the fundamental challenges of stress and cognition research is how to induce a stress response in the laboratory that most closely mimics a response in the real world. Standardized acute emotional stress induction protocols exist because key factors that govern the magnitude of the stress response, such as uncontrollability or socio-evaluation, can be difficult to manipulate in the lab. (Allen et al., 2016; Dickerson & Kemeny, 2004; Kirschbaum et al., 1993; Koolhaas et al., 2011; Roos et al., 2017; Schwabe et al., 2008). Furthermore, in addition to difficulty in defining and measuring stress, stress exerts effects through diverse biological mechanisms, which can be further modulated by factors such as sex, age, and stress appraisals (e.g., viewing a stressor as a challenge rather than a threat) (Denson et al., 2009; Dickerson & Kemeny, 2004; Juster et al., 2012; Lennartsson et al., 2012). Therefore, it is crucial to validate the stress induction using stress-specific biomarkers. To date, the Trier Social Stress Test (TSST) elicits the most robust and reliable stress response through cortisol

increase, the gold standard of stress-specific biomarkers (Allen et al., 2014, 2016; Goodman et al., 2017; Shields et al., 2017). However, despite the effectiveness of this paradigm, the TSST may fail to reflect what Soldiers experience during training and operations.

Additionally, to date, few paradigms exist that allow for concurrent stress induction and cognitive assessment. Thus, one advantage of threat of shock manipulation is that it allows for measurement of behavior during the stressor itself (Robinson et al., 2013b). Yet, the effects of emotional stress on most cognitive processes are, on average, small-to-moderate in magnitude when measured in the lab (Shields et al., 2016, 2017; Starcke and Brand, 2016). Therefore, the use of a threat of shock paradigm here was two-fold: to determine the effectiveness for eliciting stress in military populations and examine impacts on shoot/don't-shoot cognitive performance. However, it appears this paradigm is more effective as an arousal manipulation and does not produce cortisol increases consistent with other stress manipulations of the intended severity (i.e. TSST), even when more severe torso shocks are delivered. It will be a challenge for future work to design representative emotional stress manipulations that mirror the severity of combat.

Relatedly, a growing body of work has investigated the effects of acute, or single bouts of, exercise (or physical stress) on cognitive function. However, this research has largely focused on changes following exercise, with less focus on cognitive changes during exercise. Furthermore, studies examining cognition during exercise have focused on durations of 45 min or less (Cantelon & Giles, 2021). Changes in cognition over relatively short durations are pertinent to the general population aiming to meet the Physical Activity Guidelines (American College of Sports Medicine, 2018), or athletes who perform in discrete playing periods. However, changes in cognition during longer duration exercise are essential to understand for

military personnel and first responders, who must remain cognitively intact in prolonged, physically demanding situations.

Likewise, the influence of military specific exercise on aspects of cognitive function relevant to military operations is poorly understood. Previous findings suggest that load carriage and physical exertion can impair cognitive control, particularly response inhibition required for shoot/don't-shoot decisions (Armstrong et al., 2022; Giles et al., 2019; Eddy et al, 2015). Notably, such degradations occur with greater effect sizes than degradations to cognition under unloaded physical exertion (Chang et al, 2012; Eddy et al., 2105; Giles et al., 2019). However, our understanding of the cognitive impacts of load carriage spanning the matrix of exercise intensities and durations is incomplete. Such examination may provide insights into when and/or how fatigue leads to declines in cognitive performance. With the increasing physical and cognitive loads placed on military personnel (Mahoney et al., 2007), this interaction is fundamental to understanding operational performance (Russo et al., 2005).

Although we did not find significant stress-induced cognitive changes in our laboratory settings, it is still likely that stress plays a fundamental role in Soldier cognitive performance during training and operations. Indeed, both anecdotal evidence and field work point to decrements in performance across several cognitive domains (Vrijkotte et al., 2016) during a variety of operational stressors (i.e. strenuous bouts of load carriage and physical exertion, sleep loss, thermal load, prolonged mental states of isolation, ambiguity, danger and threat, powerlessness, and high workload) (Adam et al., 2008; Bartone, 2006; Parsons et al., 2019; Sullivan-Kwantes et al., 2021; Troxel et al., 2015)). There is currently no objective data-driven means to accurately monitor and predict Soldier cognitive performance on combat tasks in training and operational environments. This is in part due to incomplete understanding of the

specific impacts of varying types of representative combat stress on operationally-relevant cognitive outcomes. It is imperative for future research to expand this knowledge in order to design effective training and mitigation strategies for maintaining peak performance in high stakes environments.

Conclusions

In sum, high levels of stress and uncertainty characterize military operations. To address this gap, we sought to examine the influence of an operationally relevant emotional stressor (threat of shock) and physical stressor (load carriage) on a novel Soldier-relevant cognitive task across two studies. We also examined how ambiguity impacted shoot/don't-shoot decisions. However, stress experienced in military contexts can be difficult to induce in laboratory settings. Although our hypotheses related to stress-induced changes in shoot/don't-shoot performance were not supported, results highlight several important methodological nuances that should be considered when measuring and interpreting responses to ambiguous information in contexts that co-occur with high levels of operationally relevant stress. Importantly, even slight shifts in interpretive biases may determine differences in perceiving information as safe vs. threatening, which could have significant behavioral consequences for military personnel. Thus, future research should continue to characterize decision-making in high-stakes environments.

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