

A model of military deployment: Effects of psychological stress and caffeine use
on information processing in male rats

by

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DEDICATIONS

This project is dedicated to the Pettey family.



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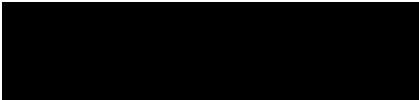
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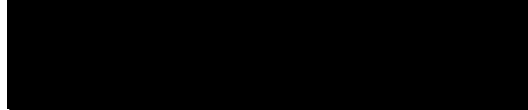
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ABSTRACT

Title of Thesis: A Model of Deployment Stress and Caffeine Use: Acoustic Startle and Environmental Habituation in Male Rats

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Military Service Members consume caffeine in large quantities to maintain performance, particularly in deployed settings under chronic stress and sleep-deprived conditions. Some researchers argue that stimulant drugs consumed in theater worsen Service Members' mental health, potentially contributing to incidence of mental health disorders. However, empirical studies suggest chronic caffeine consumption is associated with psychological and physical health benefits. It is unclear how chronic caffeine consumption affects Service Members' mental health. An animal experiment was used to investigate how caffeine (caf), a model of Warrior Stress (WS), and combined WS and sleep disruption (SDis) conditions impact behavioral measures of information processing in a 2 (no caffeine, caffeine) x 2 (no stress, stress) nested design with repeated measures (baseline, +7 days WS, +14 days WS+ SDis, for stressed groups). Acoustic startle response with and without pre-pulse and locomotor habituation to an open field were used as primary outcome measures because they involve basic non-conscious processes that serve as an important bridge between human and animal research as indices of cognitive function. Seven days of sleep disruption was nested within the Warrior Stress groups. Results indicated that the Time x Caf x WS interaction was significant for Initial Startle Amplitude and approached significance for Startle Amplitude in response to the 110 dB startle/68 dB pp only. Caf increased Initial Startle for non-stressed rats only at

Time 2 (post WS + SDis). In addition, there were main effects for WS and Time on percent inhibition for 110 dB startle/68 dB only, such that WS decreased inhibition over time. There were significant main effects for Caf and WS on body weight (BW), indicating that caffeinated and stressed rats had less increases in BW over time (emerged after start of SDis). Caf and WS did not demonstrate robust effects across measures of information processing. There were no effects of WS or Caf on environmental habituation. There were trends to suggest that caffeine mimics the effects of stress by increasing behavioral reactivity to startle noises, but does not appear to affect already stressed rats. Translated to human conditions, this study did not provide evidence that Caf is either harmful or beneficial to already stressed Service Members with regard to information processing.

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*RMANCOVA= repeated measures analysis of variance

ANCOVA= analysis of covariance

ASR=acoustic startle reflex

CHAPTER 1: Introduction

Warrior Stress, Performance Enhancement, and Mental Health

Military conflicts in Iraq and Afghanistan have placed a heavy burden on United States Service Members for more than a decade. In deployed environments, Service Members manage the stress of continuous operations, sleep disturbance, and constant threat of injury or death (1). Experiencing these stressors often results in negative physical and mental health consequences. In one large scale cohort study, 20.3% of active duty and 42.4% of reserve Soldiers were identified as requiring mental health treatment following deployment (2). Post-traumatic Stress Disorder (PTSD) rates among Service Members range from 5-20% of the current active duty population, with even more Service Members suffering from other mental health disorders often related to stress (3).

Service Members often rely on caffeine and other stimulant drug use to maintain their vigilance and performance under high stake scenarios. Deployed Service Members consume large amounts of caffeine, and caffeine consumption is even recommended by command and placed in many military issued food products (4). For many military occupational specialties, compared to the needs of the general population, unique dietary supplements are required to achieve performance standards (5). While caffeine use might help Service Members perform better, especially while fatigued (6-8), its potential unintended consequences are not well characterized.

In combination with chronic stress, caffeine use itself may influence the trajectory of symptoms. As of 2010, one in six active duty Service Members is prescribed a psychiatric medication (9), often to maintain performance and reduce mental health

symptoms in theater. Some researchers go so far as to argue in opinion pieces that prescription stimulants (e.g., Adderall, Ritalin) cause stressor-related disorders (10; 11). However, studies have not yet investigated stimulant use as a causal factor in the development of mental health disorders related to deployment. These stimulant medications have been called “cogniceuticals” to describe their wide spread use for performance maintenance and enhancement in deployed environments (12).

Military leaders rely heavily on fatigue countermeasures to improve immediate performance, regardless of possible longer-term consequences. However, caffeine appears unique to other stimulants based on its primary mechanism of action and behavioral effects (13). In contrast to identified drugs of abuse, caffeine has been linked to a wide range of mental health benefits, including decreased depressive symptoms, decreased risk of suicide, decreased risk of Parkinson’s and Alzheimer’s, and increased goal-directed activity (14). Therefore, caffeine might be a safer and more effective substitute for other cogniceuticals used to maintain or enhance performance.

Additionally, minimal adverse effects have been identified for caffeine, such as mild to severe withdrawal symptoms (15). However, other clinical researchers argue that caffeine has significant abuse potential, given that chronic users report inability to control their consumption despite experienced adverse effects (16; 17). Regardless of its potential benefits or harm, caffeine consumption in a chronically stressful environment has not been fully investigated. It is important to clarify the impact of caffeine on the mental health of Service Members given the military’s heavy reliance on caffeine as a performance enhancer.

Because stress, sleep disruption, and caffeine consumption often co-occur in a deployed environment, it is important to consider how these factors affect the individual Service Member in ways relevant to mental health. In the current study, a rat model was used to investigate how these conditions impact information processing. Rats were exposed to caffeine, stress, and sleep disruption conditions. Behavioral indices of information processing (i.e., acoustic startle response and habituation) were measured as outcomes relevant to translation to human mental health. In this Introduction, caffeine use, stressors specific to deployed Service Members (i.e., “Warrior Stress”), and sleep disruption are described. Then the effects of caffeine and stress on information processing in humans are discussed. Finally, rationale for animal models, caffeine and stress in animals, and a summary of the Introduction are provided.

Warrior Stress

Stress. Understanding of the physiologic mechanisms and consequences of stress has evolved over time. Stress has been defined as the process by which an organism’s resources are taxed or exceeded by environmental demands (18). Stress can be adaptive in the short-term, but chronic stress is linked to a variety of negative physical and mental health outcomes (19; 20). The Diagnostic Statistical Manual for Mental Disorders, fifth edition includes a new section called “Stressor-Related Disorders” in recognition of the unique consequences of specific stressors and their link to mental health functioning (21). Stressors have been classified in different ways, in terms of their length (i.e., acute versus chronic) and valence (e.g., positive versus negative stress). An organism may encounter direct physical stressors (i.e., heat, physical movement) and anticipatory stressors (i.e., environmental cues of a threat). These stressors produce distinct neurochemical responses

(22). Boonstra (23) argues that stress should not be defined by the length of exposure, but instead by the length of its consequences. For example, a person or animal exposed to a stressful stimulus may develop anticipatory stress that is chronic, as the organism remains vigilant to exposure to that stimulus again. The term allostasis refers to efforts to achieve stability through changing behavior or physiology in response to predictable and unpredictable events (24; 25). Organisms that experience “allostatic load,” or exhaustion of the stability resources, are at greater risk of negative health outcomes (19). There is limited research examining the link between chronic stress and caffeine use.

Warrior Stress. “Warrior Stress” refers to the unique combination of stressors Service Members face when they are deployed. They experience physical stressors (e.g., movements with heavy packs, extreme temperatures, loud noises, and/or sleep disruption) that directly tax physiologic resources, and also experience anticipatory stressors (e.g., high stakes missions, or spots on the road that indicate explosive device presence) that are often cognitively mediated and also produce sympathetic nervous system activation. Isolation from family and familiar surroundings can also present more stress. Warrior Stress encountered during deployments to Iraq and Afghanistan creates an increased risk of negative mental health outcomes, such as anxiety, depression, and PTSD (26; 27) and worsened cognitive function, regardless of whether Service Members experience combat exposure (28). Another longitudinal study indicated that Service Members screened positive for a mental health disorder significantly more often at approximately six months after return home compared to initial screenings (2). This study suggests that Warrior Stress has potential long-term consequences with regard to mental health. The current experiment included a model of Warrior Stress to investigate its impact on the course of

impact on the subject throughout chronic exposure. The Warrior Stress paradigm has been developed over time within the Grunberg laboratory, which is described fully in the Methods section.

Caffeine

Prevalence. Caffeine is the most widely consumed drug in the world, found in coffee, chocolate, tea, and soda, among other foods and drinks (29). Across age groups and sex, 87% of Americans report caffeine consumption in some form, averaging of 193 mg of caffeine per day (the equivalent of approx. 2 cups of coffee) through coffee, soft drinks, and tea (29). Similar to national civilian trends, 82% of active duty US Army Soldiers reported consuming caffeine daily, primarily through coffee, soda, and energy drinks (4). Overall caffeine consumption rates in US Marines have increased since the start of our conflicts in Iraq (2003-2011) and Afghanistan (2001- ongoing) (30). Service Members' caffeine intake is not carefully monitored and there are no clear statistics on how consumption rates in theater compare to rates at home or by civilian counterparts. Caffeine consumption data from the general population and garrison troops, combined with military policy recommendations (e.g., 31) that promotes caffeine use for performance enhancement, suggests that consumption is similar or higher in a deployed environment.

Mechanisms. Caffeine is a methylxanthine drug, distinct from other stimulant drug classes, such as the commonly consumed phenethylamine class that includes amphetamine (32). Caffeine is a stimulant drug that activates the sympathetic nervous system (SNS) and produces physiologic effects similar to stress (33). Caffeine produces tonic, i.e., slow and lasting, behavioral activation (34). Its primary known mechanism of

action is adenosine receptor antagonism (35). In small to moderate doses, caffeine acts on the adenosine receptors to produce tonic motor stimulation and also potentiates dopamine transmission, an effect that is associated with elevated mood (33; 36; 37). Caffeine appears to have many other stimulatory effects, such as decreasing the threshold for neuron activation, increasing spontaneous activity of the sympathetic nervous system, and increasing serotonin (38). Extremely large, toxic doses can induce release of intracellular calcium, block gamma aminobutyric acid (GABA; major inhibitory neurotransmitter) receptors, among other effects, to cause convulsions and possible death (34). Caffeine counteracts the impact of fatigue by acting as an adenosine receptor antagonist. Adenosine is a waste product of adenosine triphosphate (ATP), the body's primary source of energy. As the body uses energy during the day, adenosine increases and acts on adenosine receptors to signal to the body that it needs sleep. Caffeine counters this signal; caffeine molecules act on adenosine receptors and inhibit the activity of these receptors, thereby reducing fatigue (36).

Dose-Response. Caffeine is generally safe and actually has many documented benefits to the user. However, caffeine consumption of more than 400-500 mg at one time is associated with adverse effects, including muscle twitching, tachycardia, and hyperventilation (39). Caffeine also leads to sleep disturbance when consumed in short proximity to bedtime, but people are generally good at regulating their caffeine consumption to minimize adverse effects (40). However, caffeine is relatively safe because reaching a lethal dose is extremely difficult (approximately 10 grams; 41). Whereas the exact mechanisms are still largely unknown, more moderate doses of caffeine demonstrate therapeutic utility, such as to prevent damage caused by hypoxia,

reduce pain, and reduce complications associated with preterm birth (42). Other research indicates that daily consumption of 300-400 mg per day is safe and confers health benefits to the user (43; 44).

Chronic versus acute caffeine consumption appears to produce distinct effects. Chronic is defined here as repeated exposure over time—with regard to caffeine, it is defined as daily use. Chronic caffeine consumption can lead to tolerance. In studies of caffeine, it is commonly speculated that improvements in performance are attributable to reduction of withdrawal symptoms rather than positive effects of caffeine itself. However, other studies have demonstrated that the “Withdrawal Hypothesis” does not fully account for performance effects of caffeine because acute doses appear to produce added benefits even when subjects are not in withdrawal (45; 46). Overall, these studies suggest that the chronic versus acute effects of caffeine consumption are distinct. In the present experiment, caffeine was administered both chronically (daily continuous access) and directly prior to stressors to model the possible range of effects. Caffeine administration and rationale are described in more detail in the Methods section.

Comparison to other stimulants. Arguments for the association between stimulants and stressor-related mental health symptoms are predicated on cognitive enhancement—some researchers argue that stimulant use helps to enhance learning for fear and trauma. Caffeine appears to be a unique stimulant, demonstrating a different effect on anxiety and cognition than other stimulants such as modafinil, or dextroamphetamine (47; 48). Modafinil has been used widely in the military as a “wakefulness promoting” drug, particularly for Service Members who experience frequent shift work (49; 50). Modafinil’s primary effects appear to be mediated by

increases in dopamine levels in the basal ganglia, though the specific mechanisms are largely unclear (51). Other, more “classic,” amphetamine-like drugs increase levels of norepinephrine. Comparisons between caffeine and other stimulants highlight caffeine’s uniquely anxiolytic and neuroprotective properties. Other stimulants (e.g., khat, a chewed amphetamine-like leaf found in Somalia) have been linked with PTSD and specifically paranoia symptoms (52). Whereas some researchers argue that caffeine should be seen as a drug of dependence, neural imaging studies in humans and animals suggest that caffeine has a distinct profile (53). Given the unique aspects of caffeine (i.e., unlike typical drugs of addiction; does not directly stimulate adrenergic responses), caffeine use might be distinct from other stimulants argued to contribute to anxiety and PTSD.

Military Performance. Some studies have demonstrated the utility of caffeine to improve rifle marksmanship performance in military operations under combined sleep deprivation and stress conditions (6; 7). In contrast, one study indicated caffeine had no effect on marksmanship, based on the argument that cold stress in the control group helped to enhance their performance to match the caffeine group (54). The null findings of this study lend support to the hypothesis that caffeine may have a negligible impact on performance if arousal is already high. Another study revealed that caffeine consumption led to overly optimistic subjective performance in pilots, but not objective performance, compared to placebo controls after 37 hours of sleep deprivation (8). Taken together, these military studies suggest that caffeine’s effects depend on baseline arousal and task demands. These studies also did not measure the long-term effects of caffeine and stress together. Whereas the acute effects of caffeine and stress might both be beneficial, their long-term and chronic consequences are not well characterized.

Sleep Disruption

Sleep disruption is a salient obstacle to maintaining performance in a deployed environment. In general, sleep disruption negatively affects cognitive function, mood regulation, and physical performance (55). Acute sleep disruption has subtle, but observable effects on attention and mood. Sleep disruption over time can cause changes in the brain and neuroendocrine systems that increase sensitivity to stress (56; 57). In a survey of Army officers with recent combat experience (N=49), 82.6% reported feeling sleep deprived at some point and reported sleeping an average of 4 hours per night during higher operational tempo (58). More than half of the officers reported that fatigue was a problem in their units. Sleep disruption is the most commonly reported symptom of PTSD and the highest predictor of overall symptom severity in military Service Members (59). Sleep disruption is a unique form of stress that may interact or compound with other forms of stress to adversely affect health and often co-occurs with psychosocial stressors (60; 61).

Adenosine is the body's chemical waste that increases sleepiness and fatigue (62). Caffeine, as an adenosine receptor antagonist, acts on the same receptor as the chemical waste and thereby counters some of the sleepiness-inducing effect. In one study, caffeine and other stimulants restored participants' ability to detect subtle emotional facial expressions after they were sleep deprived (63). A review of studies on sleep disruption and caffeine use indicates that caffeine potentially restores the performance deficits associated with sleep disruption, but may also paradoxically disrupt sleep over time (64). Individuals might have increased arousal during sleep hours after consuming caffeine and thereby experience disrupted sleep. In response to sleep disruption, they may consume

more caffeine. Based on their review of relevant studies, Roehrs *et al.* (64) argue that caffeine simply restores daily performance affected by sleepiness rather than enhance performance past baseline ability.

Information Processing

Information processing is a cognitive term that broadly refers to how an organism consciously and non-consciously attends to and interprets environmental stimuli (e.g., 65). For example, anxious individuals more often attend to threatening stimuli in the environment compared to non-anxious individuals (66; 67). Emerging research in cognitive psychology has identified the prominent role of biases in information processing in the pathogenesis of mood and anxiety disorders (68). High cognitive load and limited cognitive resources might limit a person's ability to intentionally shift attention and regulate negative emotions (69; 70). Working memory training, attentional retraining (71), and other cognitive interventions have demonstrated some efficacy in improving mood and anxiety problems (72; 73). Training working memory through mindfulness helped Service Members preparing for deployment experience less negative emotions (74).

Information processing is a broad concept that is measured in a variety of ways. In the current study, two measures of information processing are highlighted, acoustic startle response (ASR) and habituation, which reflect non-conscious processing of stimuli. In other words, these two constructs measure the way a person automatically attends to the environment. Habituation (reduced sensitivity to a stimulus after repeated exposure) is often included as part of ASR, but is considered distinct from the locomotor habituation measured in the present experiment. Locomotor habituation for rodents is

described in detail in the Methods sections as a measure of gross information processing. ASR and pre-pulse inhibition are described primarily with regard to human studies in the following sections.

Acoustic Startle and Habituation. The current experiment used acoustic startle response (ASR) and habituation (i.e., reduction in activity/exploration) to an environment after repeated exposure as measures of information processing in rodents. In humans, an ASR paradigm is commonly used to understand “motor preparation,” or the initial processes by which a person moves (75). The startle response itself is a musculoskeletal *reaction* to a startling noise. In humans, it is often measured by eyeblink. Many studies have investigated the influence of a startling noise on reaction time in tasks that test speed and accuracy as described above. These studies consistently demonstrated that exposure to a startle sound allows a person to engage in a motor response more quickly than when not exposed to startle noise before the task. Researchers speculate that the startle stimulus initiates the motor response without engaging cortical structures (or conscious decision making), and thereby improves reaction times within other motor tasks (75). It is also widely recognized as a useful bridge between human and animal research because basic, non-cortical brain structures are involved in the reflex.

ASR with and without pre-pulse, and pre-pulse inhibition (PPI) involves both affective and cognitive faculties. The two measures are used in the current study as an index of “sensorimotor-motivational information processing” (76). The “motivational” denotes the influence of emotion, and sensorimotor as a reflexive response, also influenced by cognitive ability. In rodents, ASR continues to be developed and standardized as an important measure of possible genetic and neurobiological

susceptibility to disorders of information processing (77). PPI theoretically reflects how a person filters environmental information and is also sensitive to changes in dopamine levels associated with cognitive disorders in humans (78). PPI shows how a person automatically attends to and uses other environmental information (i.e., a pre-pulse) to anticipate another stimulus and thereby have a reduced startle. In practical terms, when a person cannot effectively attend to and interpret environmental information, he or she might feel flooded, overwhelmed, and have fragmented thoughts. Acoustic startle is also a useful index to better understand the distinct effects of fear versus anxiety (79). Fear is an adaptive, short-term response to a threatening stimulus in the environment. Anxiety is a more chronic response, that manifests in similar physiologic symptoms to fear, but that may lack a specific target and any adaptive utility. Anxiety versus fear activates distinct neural pathways with regard to the acoustic startle reflex (80). Fear, as well as general anxiety, are potential consequences of deployment stress and therefore are of interest in the present experiment.

Measuring both the startle reflex and PPI allows researchers to discriminate between effects of emotion and cognition (pre-attention). In one human study, darkness (i.e., an aversive and potentially threatening environmental condition) led to increases in the startle response (81). Interestingly, increases in startle responses were positively correlated with retrospectively reported childhood fear of darkness. Darkness increased startle but did not increase PPI. This discriminant finding suggests that darkness did not improve attention to auditory stimuli, and increases in startle likely resulted from an affective response, or increased emotional reactivity, rather than a change in pre-attention. Another study showed that startle response increased in subjects exposed to

pictures of angry faces (82). In summary, startle reflex and PPI are independent measures that are influenced more heavily by emotional state and attention respectively (83), though these processes are interrelated, as discussed with regard to the impact of attentional biases on anxiety (e.g., 66).

Caffeine. Caffeine appears to improve reaction times and lower error rates in basic information processing tasks (84). It can also enhance learning and memory encoding, an effect that forms a link between stimulant use and PTSD symptoms (10). PTSD and other stress-induced disorders are considered learned responses to threats, which require memory of the event (85). Increasingly, researchers are testing methods to interfere with memory consolidation and reduce arousal following exposure to trauma or stressful stimuli (86; 87). Therefore, caffeine as a stimulant might serve to enhance the memory of an aversive experience and increase reactivity to stressful stimuli in the future.

Caffeine tends to produce faster reaction times regardless of stimulus characteristics (88). For many tasks, this improved reaction time might be functional. However, this improved reactivity might lead to adverse consequences when individuals are exposed to negative or harmful stimuli. For example, one study showed that healthy subjects had an increased startle response to unpleasant emotional pictures after consuming 300 mg of caffeine (or approximately three 8 oz cups of coffee), compared to placebo controls (89). Attending to pre-pulse stimuli increases PPI in humans and animals. In an investigation of the effect of caffeine and attention on PPI, caffeine increased arousal in general, and at a dose of 4 mg/kg (moderate dose) abolished the effect of controlled attention on PPI (90). Caffeine appears to increase the startle

response, but also improves PPI (91; 92). Enhanced attention and reactivity improve performance, but may not be adaptive for regulating emotions.

Warrior Stress. Declines in cognitive function might make Service Members more susceptible to mental health disorders. One large study (N=961) indicated that US Army Soldiers deployed to Iraq performed worse on measures of sustained attention and memory over time than Soldiers who were not deployed (93). For Service Members, pre-existing and deployment-related cognitive deficiencies might make re-integration and adjustment to life back home more difficult. In a sample of 754 OIF/OEF combat veterans, the top problems following return home included: (a) problems controlling anger; (b) thoughts or concerns about hurting someone; (c) increased substance abuse; (d) dangerous driving noted by others, among other reported problems (94).

ASR is a widely used measure that has helped clinical researchers identify possible mechanisms of the pathogenesis of anxiety and related disorders. Results vary across studies, but in general suggest that PTSD is associated with increased startle and enhanced PPI (95-97). For example, Grillon *et al.* (96) reported that inpatient Vietnam veterans (n=34) with PTSD and combat exposed veterans without PTSD (n=17) had significantly higher increases in startle response during fear-potentiated conditions (i.e., lab stress induction) compared to civilian controls (n=14). This study indicated that combat veterans with and without PTSD may have higher stress sensitivity compared to non-combat exposed civilians, and this increased sensitivity was revealed in their response to startling noises. The PTSD group also had enhanced PPI, which suggests improved pre-attentional processing. The authors explained that this finding was likely related to enhanced “non-specific” attention in a threatening environment. Grillon and

Morgan III (98) replicated the finding of increased stress sensitivity in another study comparing ASR in Gulf War veterans with PTSD (n=13) versus veterans from the same unit without PTSD (n=14). The authors used procedure called “differential conditioning” to teach the participants to pair one light with fear and another light with safety. The authors hypothesized that the PTSD group would show increased generalization of learned fear compared to the non-PTSD groups, and the results supported this hypothesis. In general, the distinction between fear of specific stimuli versus fear of an environmental context (more aptly called anxiety) is important to consider in military populations transitioning back from deployment. A twin study of PTSD and response to startling tones indicated that participants with PTSD responded with a larger heart rate increase than their non-PTSD twins (99). In another study, Vietnam veterans showed startle responses comparable to controls, but had reduced PPI (97).

A prospective study of police officers before and after trauma exposure showed that higher startle response and subjective fear rating after repeated exposure to startle sounds, measured prior to trauma exposure, predicted PTSD symptom severity (100). This study demonstrated the predictive validity of the ASR measure and how higher responses might suggest individuals are more sensitive to the negative effects of stress. In these examples, the utility of ASR is demonstrated in its application to human psychopathology and military combat populations. Whereas the studies described are specific to PTSD, the proposed mechanisms shed light on the role of learning and information processing in the experience of anxiety and stress for Service Members.

Rationale for Animal Models

As discussed, habituation (i.e., decreased responding after repeated exposure) and ASR are widely considered a useful bridge between human and animal research. Therefore, the outcome measures of the chosen study were selected based on their face validity and generalizability to human markers of mental health. In addition, stress and caffeine have been studied in animal studies. The primary benefit of animal models is experimental control, which is often limited in human studies because of ethical and logistical limitations. For example, it would be unethical to chronically stress a person for research purposes; so instead, researchers use acute stress paradigms, or observe individuals' natural behavior over time and then make inferences about how stress might cause observed outcomes. In contrast, animal models allow researchers to directly test cause and effect relationships relevant to mental health conditions. In addition, animal models allow for control of genetics, environment, and social interactions, among other variables, that might otherwise confound interpretation of experimental findings. To highlight contributions by animal models and to inform hypotheses for the current study, the following sections focus on animal models of stress, caffeine, and combined stress and caffeine.

Stress. Animal models have made important contributions to our understanding of the human stress response. McEwen and Gianaros (19) argue that animal models are “critical” to our understanding of the causal links between stress and adverse outcomes. Rats respond similarly to stress and demonstrate behavior associated with human mood states. In response to stressors, rats demonstrate biobehavioral changes that are similar in humans, such as alterations in neuroendocrine, cardiovascular, and gastrointestinal

function (101). Common stress paradigms for rats include restraint stress, social crowding, and predator exposure. These stress paradigms can be used to model traumatic stress as well as chronic mild stress. For example, one study validated the use of animals to model PTSD based on behavioral criteria—exploratory activity and response to a startle stimulus (102). Rats demonstrated similar incidence rates to humans exposed to trauma; 90% of rats showed stress symptoms acutely following the traumatic stressor (predator stress or underwater trauma; +1 day) and only 25% showed stress symptoms chronically (+7 days; +30 days), mirroring the trends in humans. The Warrior Stress Paradigm has been used more recently by Grunberg and Yarnell (103), and includes repeated exposures to unpredictable, mildly distressing stimuli. This stress manipulation is described further in the Methods section. In general, the use of animal models allows for a window into the wide range of factors that influence how organisms respond to different types of stress.

Caffeine. Animal models are commonly used in caffeine research. As discussed, caffeine appears to have dose-dependent effects, similar to stress. The size and chronicity of the dose helps to determine its behavioral effects. Common methods of delivery for caffeine include: *ad libitum* access to drinking water, controlled intraperitoneal (IP) or subcutaneous (SC) injection, or implantation of osmotic pumps. Route of delivery is discussed in more detail in the Methods section. One study demonstrated that rats exposed to high doses of “chronic” or continuous access to caffeinated drinking water did not differ from controls on behavioral measures (104). However, caffeine withdrawal after continuous administration reduces motor activity in adolescent and adult rats compared to rats never exposed to caffeine (105). Other rodent studies have shown that

caffeine produces biphasic responses, with small/moderate doses increasing motor activity and larger doses reducing motor activity (105; 106). This biphasic effect appears to mirror patterns in humans with regard to anxiety. Adolescent rats appear to be more sensitive to behavioral effects of caffeine (105; 106). With regard to cognition in rats, chronic caffeine administration appears to improve decision-making (i.e., rats chose more food with delayed presentation, rather than immediate smaller amount of food), with even greater improvements shown after acute administration (107).

Caffeine and stress. Rodent models more generally have helped researchers better understand how the physiologic changes due to stress might make individuals more susceptible to drug addiction (108). Rodent models in particular have been used frequently to understand the mechanisms of caffeine and stress. These studies have primarily focused on anxiety-like behavioral outcomes, given their relevance to mental health. Although the current experiment is focused on cognitive outcomes, anxiety studies provide useful information, and are related to how rats react to a startle noise, as discussed in the description of the cognitive and emotional components of ASR. Hughes *et al.* (109) investigated the effect of stress from bright light on rats' responses to caffeine. The authors injected rats intraperitoneally with low and moderate doses of caffeine prior to the exposure to an open field and the elevated plus maze (i.e., behavioral measures of anxiety in rodents). Caffeine reduced anxiety-like behavior for both stressed and non-stressed male rats. In female rats, however, caffeine reduced anxiety-like behavior in the stress condition only, and caffeine did not appear to affect non-stressed female rats. Another group investigated the effects of caffeine within a chronic unpredictable stress model that included stressors such as light switched on during dark

phase, foot shocks, social crowding (adding another rat to cages), wet bedding, and restraint stress (110; 111). Results from these two studies showed that caffeine reduced anxiety-like behavior in rats exposed to stress. Overall, rodent studies suggest that caffeine might protect against the negative impact of stress, although these effects differ based on: (a) sex of the animal, (b) chronicity of dose, (c) vehicle of administration, and (d) dose amount.

Summary

Chronic caffeine consumption has little known negative effects, and some identified positive long-term effects. Chronic stress, in contrast, has widely known negative effects. Some researchers argue that stimulant use by deployed Service Members could be worsening their mental health functioning (10; 11). Deployed Service Members demonstrate diminished cognitive functioning when they return home, whether or not they were exposed to combat. Meanwhile, the military continues to recommend caffeine as a “cogniceutical” for Service Members to maintain performance under stress and sleep disruption conditions (12). It is important to better understand how caffeine use impacts the stress response to critically examine whether the military’s policy negatively or positively impacts the mental health of its members. Is caffeine harmful or protective for a person under deployment conditions? The present experiment focused on how Warrior Stress, sleep disruption, and caffeine may affect information processing, as measured by reduction in exploratory activity over time (habituation), response to a startle stimulus (sensori-emotional processing), and inhibition of a response to a startle noise when presented with a brief warning stimulus (pre-attention).

CHAPTER 2: Overview and Specific Aims

Overview and Specific Aims

The present experiment was designed to determine the main and combined effects of caffeine, Warrior Stress (WS), and combined WS and sleep disruption (SDis) on behavioral indices of information processing in male rats. Behavioral data were collected at: Baseline (before manipulation); post seven days of caffeine and WS exposure (Time 1); post an additional seven days of caffeine, WS (cumulative 14 days of WS + caffeine), and seven days of SDis exposure. Data from control rats also were collected at these times. The SDis manipulation was nested within the WS. The design is outlined further in the Methods section, but is briefly described in this section to better explain the Specific Aims and Hypotheses. This experiment focused on three Specific Aims—to examine the separate and combined effects of caffeine, WS, and combined WS/ SDis on: (1) general information processing (i.e., environmental habituation); (2) sensori-emotional processing (i.e., Acoustic Startle Response); (3) pre-attention (i.e., pre-pulse inhibition within ASR). The term “separate” is used for main effect and “combined” refers to possible additive, interactive, or no effect on the outcome variables.

Specific Aim 1: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **general information processing** (as measured by speed of locomotor habituation to an open field).

Hypothesis 1a. *Caffeine will impair general information processing (i.e., decrease speed of habituation).*

Hypothesis 1b. *Warrior Stress will impair general information processing (i.e., decrease speed of habituation).*

Hypothesis 1c. *Combined Warrior Stress and Sleep Disruption will impair general information processing compared to Baseline and Time 1.*

Hypothesis 1d. *Caffeine will exacerbate the negative effect of Warrior Stress on general information processing (i.e., interaction effect at Time 1).*

Hypothesis 1e. *Caffeine will exacerbate the negative effect of combined Warrior Stress and Sleep Disruption (i.e., interaction effect at Time 2).*

Specific Aim 2: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **sensori-emotional processing** (as measured by behavioral reactivity to a startle noise).

Hypothesis 2a. *Caffeine alone will impair (i.e., increase sensitivity) sensori-emotional processing (i.e., increase startle response).*

Hypothesis 2b. *Warrior Stress alone will impair (i.e., increase sensitivity) sensori-emotional processing (i.e., increase startle response).*

Hypothesis 2c. *Combined Warrior Stress and Sleep Disruption will impair (i.e., increase sensitivity) sensori-emotional processing.*

Hypothesis 2d. *Caffeine will exacerbate the negative effect of Warrior Stress on general information processing (i.e., interaction at Time 1).*

Hypothesis 2e. *Caffeine will exacerbate the negative effect of combined Warrior Stress and Sleep Disruption (i.e., interaction effect at Time 2).*

Specific Aim 3: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **pre-attention** (as measured by inhibition to a startle noise when preceded by a pre-pulse, or warning).

Hypothesis 3a. *Caffeine alone will improve pre-attention (i.e., increase inhibition).*

Hypothesis 3b. *Warrior Stress* alone will *impair* pre-attention (i.e., decrease inhibition).

Hypothesis 3c. *Combined Warrior Stress and Sleep Disruption* will *impair* pre-attention.

Hypothesis 3d. *Caffeine* will *exacerbate* the negative effect of *Warrior Stress* on pre-attention (i.e., interaction effect at Time 1).

Hypothesis 3e. *Caffeine* will *attenuate* the negative effect of combined *Warrior Stress* and *Sleep Disruption* on pre-attention (i.e., interaction effect at Time 2).

Rationale: Caffeine acts directly at the skeletal muscle level and stimulates the motor response (112). Caffeine also improves attentional performance (e.g., 113). Therefore, caffeine might have divergent effects on the separate measures of ASR—increase the startle response (mediated by its effect on the skeletal muscles), while also increasing pre-pulse inhibition (mediated by its effect on attention). Caffeine primarily increases arousal by reducing fatigue rather than sympathetic nervous system. Sleep disruption is a unique stressor that might combine with caffeine differently than stress alone in humans and animals. Caffeine has distinct effects on fatigued versus well-rested subjects (114). The effect of caffeine might be more pronounced in sleep-disrupted rats than in rats stressed in others ways.

CHAPTER 3: Methods

To address the above hypotheses, this experiment was conducted as a 2 (caffeine, no caffeine) x 2 (stress, no stress) nested design with repeated measures (time: baseline; post-caffeine/stress, post-caffeine/stress and sleep disruption combined). To determine the additional effect of sleep disruption on animal behavior, the second week of stress manipulation included sleep disruption for stressed rodents. Therefore, a combined stress and sleep disruption group was nested within the Warrior Stress group. This experimental design resulted in four experimental conditions measured across three time-points (see Figure 1 for Experimental Timeline). The nested group also allowed for within-subject comparisons of the effect of stress alone versus combined stress and sleep disruption. In this section, animals, husbandry conditions, independent variables, dependent variables, experimental timeline, data analytic strategy, and power analyses are explained.

Animals and Housing Conditions

The current sample consisted of 32 Sprague-Dawley male rats from Charles River Laboratories (Wilmington, Massachusetts). There were eight subjects in each cell (see Table 1) for a total of 32 subjects. Based on past Grunberg laboratory studies, six to eight subjects per cell was typically sufficient to detect an effect of stress on behavioral measures (103; 115-117). In addition, an *a priori* power analysis using G*Power was conducted, based on a mixed factorial analytic plan. This power analysis revealed that 6-8 animals per cell were needed to detect a large effect size with 80% power ($\beta=.2$).

Sprague-Dawley is an outbred, albino strain of rats, bred initially for general-purpose laboratory experiments. This strain was chosen based on its extensive use in stress and drug research in general (118-121) and specifically in the Grunberg lab (115;

116; 122; 123). Also, Sprague Dawley rats are commonly used in caffeine research (119; 124; 125). Rats in the current sample were approximately 50-55 days old upon arrival, comparable to late adolescent age in humans. It is difficult to identify precise age markers to delineate adolescence and adulthood in humans and other animals (126). For male and female rats, adolescence is likely to begin as early as 20 days and end as late as 60 days, a period during which significant neurobehavioral changes tend to happen (126).

Male, late adolescent rats were chosen to model the average demographic characteristics of the US military service member first entering into active duty service. In 2012, the average active duty service member across branches and ranks was 28.7 years old; 85.4% of personnel were male (127). Also, male Service Members tend to consume more caffeine than female Service Members (4). Over the approximately 30-day data collection period, rats aged from 50-55 days old to 80-85 days old, reflecting the age span of our current active duty military personnel. This sequence and age progression model the impact of repeated exposure to stress for a service member over a military tenure.

Animals were housed individually in polycarbonate shoebox cages (42.5 x 20.5 x 20 cm) with hardwood chip bedding (Pine-dri) and given continuous access to food (Harlan Teklad 4% mouse/rat diet 7001). Access to drinking water was manipulated and will be explained in the section describing methods of caffeine administration. The housing room was maintained at approximately 23° C and 50% relative humidity on a 12-h light: 12-h dark reversed cycle (lights on at 1900). Soiled cages were changed twice per week. Rats were housed individually without environmental enrichment, to model the isolating experience of deployment and separation from family members. Additionally,

social and other environmental variables might variably affect the biological and behavioral stress response (128; 129) and confound interpretation of the impact of stress and caffeine alone.

This experiment was conducted under a protocol approved by the USUHS Institutional Animal Care and Use Committee (MPS-14-898; Behavioral investigations of nicotine and caffeine in rats; see Appendix C) and conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (130).

Independent Variables

Caffeine. Caffeine was administered using two methods: (a) *ad libitum* 24-h access to drinking water; and (b) controlled oral syringe prior to stressors. A combined oral self-administration and acute parental administration method has been used in other studies of caffeine (131). This method of administration was chosen based on the aims to: (a) use a minimally invasive/stressful manipulation; and (b) create a face-valid model of caffeine consumption in humans. This study combined a commonly used method for continuous self-administration (i.e., drinking water) with a novel approach of acute administration using voluntary drinking from a syringe. Rationale for these methods and specific procedures are described in this section.

Pilot studies. Prior to collecting primary study data, two pilot studies using 24 rats each (12 female, 12 female) were conducted to determine: (a) the most feasible method of caffeine administration based on the study aims; and (b) a dosing level that produces measurable behavioral responses. Female rats were used in the pilot to inform extensions of the study that include sex comparisons, although the current experiment focused on male rats. The first pilot study compared Jell-O versus drinking water vehicles for

caffeine administration. Jell-O preparation was based on one previous study using Jell-O to administer buprenorphine (132). Results revealed rats may be averse to a Jell-O vehicle; rats tended to attack the 2 mL of caffeinated cherry-flavored Jell-O placed in their home-cages. The second pilot study revealed that rats were receptive to drinking caffeine dissolved in 2 mL of 7% sucrose solution from an adapted syringe (i.e., with the needle removed) after 2-3 training days (i.e., to acclimate rats to the syringe), following methods from another study using oral syringe to administer haloperidol (133). Pilot studies revealed non-significant trends indicating high caffeine doses (80 mg/kg) decreased behavioral activity compared to moderate caffeine doses (40 mg/kg). Overall, the pilot studies indicated that: (a) rats tended to drink more sucrose solution than caffeinated sucrose or tap water; (b) rats were averse to Jell-O vehicle; (c) voluntary drinking from an oral syringe is a feasible method to administer caffeine; (d) there were qualitative (i.e., not statistically significant because of the small sample size) dose-dependent behavioral responses to 40 mg/kg and 80 mg/kg daily caffeine. The administration techniques described below were developed based on these pilot findings.

Oral self-administration. Caffeine was administered chronically to rats in their drinking water over the course of approximately 20 days. Rats in the caffeine condition were exposed to caffeinated drinking water as their only water source for the majority of the day (i.e., 22 hours each day); they were provided with an alternative tap water bottle for two hours each day to prevent dehydration. Tap water consumption during the two-bottle choice period was measured throughout the study. Caffeinated sucrose solution was weighed and replaced with new solution approximately every three days to avoid sucrose decay.

Caffeine was concentrated at 1 gram per liter (1 g/L) in a vehicle of 7% sucrose solution. This caffeine concentration was chosen to reflect a moderate dose, based on prior caffeine studies using drinking water administration (131). Control animals received tap water. Tap water was chosen as a valid control based on its use in previous studies (131). Also, during pilot studies, rats drank significantly more pure sucrose solution than caffeinated sucrose solution. Investigators decided against sucrose solution as a control to avoid the possibility of much higher sugar consumption among control animals compared to caffeine condition animals. Pairing sucrose with caffeine also reflects how humans typically consume caffeine (e.g., energy drinks and other caffeinated beverages high in sugar content). Chronic caffeinated beverage drinkers may consume more sugar than individuals who do not consume caffeine. Water consumption for all groups was measured when bottles were switched out (i.e., every approx. 2-3 days). Bottles were weighed on scale and weights were recorded on paper.

Parental administration. Humans tend to consume more caffeine when under stress. Caffeine was administered to rats approximately 30-60 minutes prior to each stress manipulation. Caffeine was administered at a concentration of 60 mg/kg in 2 mL of a sucrose solution via a syringe without a needle. Gavage attachments (metal needles with an opening at the top; normally used to insert directly into the stomach cavity) were used to cleanly funnel the solution into the rats' mouths (See Figure 2). Rats were trained to drink from the syringes prior to receiving caffeine doses.

Preparation. Caffeine was dissolved into sucrose solution for both self-administration and parental dosing. Pure anhydrous caffeine (99% by HPLC) was ordered through Sigma-Aldrich Co., LLC. All solutions were made using a Corning P-220

stirrer/hotplate (Corning, Inc., Corning, New York). Sucrose was dissolved into tap water to create a 7% sucrose solution. Caffeine was then weighed and added to the 7% sucrose solution to create a 1 g/L concentration. The caffeinated solution was then poured into bottles. Bottles were prepared one day before they were placed into the cages and were stored at room temperature. For the acute dose, rats were weighed prior to syringe preparation. The average weight for the caffeine condition rats was used to calculate correct amount of caffeine for 60 mg/kg mass. All rats were consistently +/- 15% compared to the average weight, so the same solution was used for all rats each week. Syringes were prepared twice per week and stored in a refrigerator.

Acclimation. Oral syringe training took place over three days to acclimate rats to this administration method. Training was conducted after baseline data were collected. During training days, only sucrose solution was used so the rats did not learn initially to associate the syringe with the aversive bitter taste of the caffeine. On Day One, rats received 1-2 droplets of the sucrose solution, so they were able to taste the solution. On Day Two, rats received 1 mL of the sucrose solution. On Day Three, rats received the full 2 mL of the solution—the same volume they received prior to each stress manipulation.

Warrior Stress. Animals were stressed for 14 days under The Warrior Stress Paradigm (WSP). The WSP is a stress manipulation intended to model the experience of the deployed service member using anticipatory predatory stress (i.e., fox urine) and unpredictable environmental stimuli (103). Another common model of predatory stress includes exposing rodents to a live cat; the animals experience stress at the sight of the cat, though they are not in physical contact with it (102; 134). The WSP was initially developed as an isolated psychological stressor that does not physically stress the animal

(135; 136). Predator odors are theorized to produce anticipation of threat in rodents and have been shown to elicit a physiologic stress response (137). This anticipatory predator stress models Service Members' experience while deployed to a combat zone. The WSP has been used in various military-relevant translational studies in the Grunberg laboratory as a model of psychological stress (117; 138; 139).

Rats in the stress condition were transported to a separate room and placed in smaller, polycarbonate mouse cages (19 x 18 x 12 cm). Cotton balls were saturated in synthetic fox urine (15 mL per cotton ball; Buck Stop, Stanton, MI) and placed in the rats' smaller cages for 20 minutes on the first day and left for 10 minutes for all days after the first one. Once the cotton balls were removed, the rats were exposed to unpredictable stressful stimuli, including: whistle blasts, shaking coin noise, flickering lights, and cage shakes. Figure 3 shows the order and timing of the unpredictable stressful stimuli for each day of the stress manipulation.

Combined Warrior Stress and Sleep Disruption. The WSP consisted of two seven-day periods (see Figure 3). As part of the nested design, sleep disruption was added as an additional stressor to the WSP for the second 7-day period (see Figure 1). Animals in the stress condition were transported to a separate room during their inactive cycle, or their sleep period, for the sleep disruption manipulation. Exposure to the sleep disruption stimuli lasted for ten hours (2100-0700) each night. Sleep disruption stimuli were created using high and low frequency environmental sounds (banging, bells, voices, shattering glass, etc.) at random intervals (135). Sleep disturbance sounds were recorded on a compact disc (CD) and played on a CD player, emitting sounds ranging between 65 and 90 decibels. Other studies have demonstrated that environmental noises are a valid

method of disrupting sleep in rats, as evidenced by weight loss, cognitive deficits, and other behavioral indicators in exposed rats (140-142).

Dependent Variables

Environmental habituation. Activity in an open field (Open Field Activity; OFA) is considered an animal's unconditioned response to its environment (143) and has been used in other drug and stress research (117; 136; 144). OFA is commonly used to understand gross motor function, general health, and depression-like and anxiety-like responses (145). Horizontal Activity (HA) is the rat's movement around the floor of the chamber and is used as a proxy of general health and also exploratory activity (145). Using changes in HA over time, within sessions and between sessions, is a common method of assessing habituation to an environment in rodents (146). Habituation may parallel cognitive mechanisms associated with the process of re-integration following deployment, as the Service Member re-acclimates to his or her home environment.

Rats were placed individually in a clear polycarbonate chamber (40 cm x 40 cm x 30 cm) with a ventilated Plexiglas lid. Once rats were placed in the chamber, the investigator turned the lights and computer monitor off. Rats were then monitored continuously for 60 minutes, each time during their active cycle. Rats' movement was measured using Accuscan Superflex Sensor Version 2.2 infrared photocell system (Accuscan Instruments Inc., Columbus, OH). Infrared photocells (located side-by-side along the walls of the chamber) measured movement within the chamber. Sixteen pairs of Superflex Sensors measured HA at 2.5 cm above the chamber floor; and 16 pairs measured VA at 10.5 cm above the floor. Data were transmitted automatically and continuously to a computer via a universal serial bus (USB) connector. Chambers were

cleaned using Clidox solution (Pharmaca, Nuagatuck, CT) between trials. These OFA methods and materials have been used previously in the Grunberg laboratory (e.g., 139).

Habituation to the chamber was measured based on HA counts over 12 five-minute periods. Rats were exposed to the chambers four times throughout this experiment: (1) acclimation (i.e., no measurements were recorded); (2) baseline; (3) post-seven days Warrior Stress; (4) post-combined Warrior Stress (cum. 14 days) and sleep disruption (seven days).

Acoustic startle response. The acoustic startle response (ASR) is the whole body reaction to a startling noise (78-80; 147). ASR with and without a pre-pulse is considered an index of information processing (147) and possibly attention. Rats are exposed to 20 millisecond (ms) startle sounds at 110 or 120 decibels (dB) sometimes preceded by a longer, lower sound, called a pre-pulse (100 ms at 82 or 68 dB, 1kHz). Rats with intact information processing are expected to reflexively lurch in response to the startle sound. When startle sounds have a pre-pulse, rats are expected to react less than when there is not a pre-pulse. The diminishing effect on the response of the pre-pulse is called pre-pulse inhibition (PPI) and signifies that the rat processed the pre-pulse effectively as a pre-cursor to the startle noise. The Initial Startle response was analyzed in this study. This data point reflects the rat's unconditioned response to the acoustic stimulus without opportunity to habituate.

For this measure, rats are placed in a ventilated holding cage on a weight-sensitive platform. The size of the holding cage restricts extensive movement, but allows for smaller movements. Each 15-minute assessment period consisted of 48 randomly ordered trials. Trial types included: (1) 120 dB stimulus; (2) 110 dB stimulus; (3) 120 dB

stimulus preceded by a 82 dB pre-pulse; (4) 120 dB stimulus preceded by a 68 dB pre-pulse; (5) 110 dB stimulus preceded by an 82 dB pre-pulse; and (6) 110 dB stimulus preceded by a 68 dB pre-pulse. Each trial type was presented eight times and yielded a metric called “amplitude,” a calculation based on the release of weight from the platform when the rats were startled. Higher amplitudes reflect increased startle responses. Methods and materials for ASR were used in previous Grunberg laboratory studies (115; 122; 123). Rats were acclimated to the startle chambers during two acclimation periods prior to baseline testing. Acclimation is necessary because the environment can influence the startle amplitudes when rats are not acclimated to it (123).

CHAPTER 4: Data Analytic Strategy

First, repeated-measures analyses of variances (RMANOVAs) were conducted to investigate trends in body weight and water consumption. Estimated amount of caffeine consumed was then calculated based on water consumption analyses. Repeated measures two-way analyses of covariance (RMANCOVA; covarying for baseline scores) were conducted using: (1) Stress and (2) Caffeine, as independent variables. A separate RMANCOVA was conducted with each of the dependent variables: (1) Area Under of the Curve (AUC) of Horizontal Activity over Time (148); (2) Initial Startle Amplitude (ASR); (3-8) Startle Amplitudes with and without Pre-Pulse (ASR); (9-12) Pre-Pulse Inhibition Percentages (%PPI; ASR). Each RMANCOVA used the respective baseline measure as the covariate. This method was selected to conserve power (relative to RMANOVA across three time-points). In addition, because groups were randomized, systematic differences at baseline were not hypothesized to exist and therefore were not included in the primary analyses, but used as covariates instead to account for individual differences across rats. There were not baseline differences between groups identified. When omnibus RMANCOVAs indicated significant effects or trends, follow-up analyses were conducted for individual time-points. Data were checked for normality prior to analyses, which indicated one or two outliers per group across percent PPI measures within ASR. To conserve power, these outliers were retained in the analyses because the data were widely dispersed in general and no outlier exceeded three standard deviations from the mean. Also, ANCOVA is considered a robust analysis that is not sensitive to normality assumption violations (149).

The OFA data were extracted and plotted based on horizontal activity (HA) across five-minute increments. The area under the curve (AUC) for each time-point was calculated based on the trapezoidal rule, a technique used to approximate the definite integral of a curve. For example, the HA count for one five-minute increment was 1,000; the AUC during that five-minute increment is 5,000. The areas for the 12 five-minute increments were summed for each animal to yield an overall AUC at each time-point.

ASR submeasures included: (1) Initial Startle Amplitude; (2) Startle Amplitudes with and without pre-pulse (six trial types); (3) pre-pulse inhibition (PPI) percentages (four trial types), as used in previous Grunberg laboratory studies (e.g., 115; 103). The initial startle response was the amplitude of the rat's response to the 120 dB noise without a pre-pulse, as the first startle noise it received when it was in the chamber. The PPI percentage was calculated as the amount of inhibition (amplitude of response without pre-pulse – amplitude with pre-pulse) divided by the rat's response to startle noises without a pre-pulse [(amount of inhibition/amplitude of response without pre-pulse) x 100] as calculated in, Acri *et al.* (115). The PPI percentage, rather than simply PPI, is less sensitive to the strength of the startle itself and therefore more easily compared against other scores. The ASR data produced a PPI percentage for each of the four trial types [listed as, startle noise (pre-pulse)]: (1) 110 dB (68 dB); (2) 110 dB (82 dB); (3) 120 dB (68 dB); (4) 120 dB (82 dB). Bivariate correlation analyses revealed percentages of PPI across trial types were not significantly correlated with each other ($p > .05$; $r < \pm .20$). Therefore, a separate analysis was conducted for each trial type, as the lack of correlation suggests that each trial type measures a unique behavior.

CHAPTER 5: Results

Body Weight

Descriptives (unadjusted means and Standard Deviations, SDs) of body weight by time and group are presented in Table 3. Body weight data were collected at eight time points, approximately every 3-4 days. As Table 3 indicates, caffeine administration began at the time of third body weight data-point, the Warrior Stress Paradigm (WSP) began at the time of the fourth data-point, and Sleep Disruption (SDis) was added to WSP at the time of the sixth data-point. Body weight over time is presented graphically by condition in Figure 7. An RMANCOVA was conducted using the seven body weight data-points as outcome measures and baseline body weight as a covariate. For each RMANCOVA, the Standard Error (SE) and adjusted means (adjusted based on baseline covariate) are presented. The body weight data violated the sphericity assumption. Therefore, results are presented using the Huynh-Feldt correction (Table 3). The omnibus test indicated significant Time x Caffeine ($F(3,162)=9.08, p<.01$ partial $\eta^2=.25$) and Time x Stress ($F(3,162)=3.32, p<.05$, partial $\eta^2=.11$) interactions, showing that rats exposed to either caffeine ($M(\text{adj.})=358.61$ g, $SE=6.91$, for Caffeine Only; $M(\text{adj.})=340.32$ g, $SE=6.78$, for Caffeine + Stress) or stress ($M(\text{adj.})=360$ g, $SE=6.77$) weighed less over time than control animals ($M(\text{adj.})=383.19$, $SE=6.84$).

Internal analyses. As Figure 7 indicates, possible differences are visually identifiable at +12 days, +16 days, and +19 days, following the start of SDis. Therefore, follow-up analyses were conducted to identify differences at specific time-points. For each of the three time-points, an ANCOVA was conducted using baseline body weight as a covariate. At +22 days, there was a main effect of caffeine on body weight, $F(1,27)=11.50, p<.01$, partial $\eta^2=.30$. Caffeine Only ($M(\text{adj.})=353.69$ g, $SE=6.54$),

Caffeine + Stress (M(adj.)=346.92 g, SE=6.42) weighed significantly less than the Stress Only (M(adj.)=358.86 g, SE=6.41) and Control group (M(adj.)=372.53 g, SE=6.54). At +26 days, again, there was a main effect of caffeine on body weight ($F(1,27)=5.62$, $p<.05$, partial $\eta^2=.17$). At +29 days, there was a main effect of caffeine ($F(1,27)=10.42$, $p<.01$, partial $\eta^2=.28$) and stress ($F(1,27)=9.33$, $p<.01$, partial $\eta^2=.26$) on body weight. Caffeine Only (M(adj.)=358.61 g, SE=6.91), Stress Only (M(adj.)=360.02 g, SE=6.77), and Caffeine + Stress (M(adj.)=340.32 g, SE=6.78) weighed significantly less than the Control group (M(adj.)=383.19 g, SE=6.84). However, a Caffeine x Stress interaction was non-significant.

Daily Water Consumption

Water bottles were weighed approximately every three to four days. Daily averages were calculated for comparison. Descriptives of daily water consumption are presented in Table 7. Figure 8 shows daily water consumption over time by group. An overall RMANOVA was conducted (see Table 8) for water consumption over time. The analysis was not co-varied for baseline scores because there was no true baseline measure of water consumption; rats were exposed to a titrated amount of caffeine (1/2 g per L) from the start of water measurement. These data violated the sphericity assumption and therefore results are presented using the Huynh-Feldt correction. The RMANOVA results showed a main effect of caffeine, $F(4.7,28)=15.17$, $p<.01$, partial $\eta^2=.35$, indicating that rats in the caffeine condition consumed significantly less water than Stress Only or Control rats. Also, results indicate water consumption changed significantly over time, $F(4.7,131.6)=9.36$, $p<.01$, partial $\eta^2=.25$. It should be noted that Time x Caffeine, Time x Stress, or Time x Caffeine x Stress effects were non-significant.

Caffeine consumption. Caffeine consumption estimates (i.e., for Caffeine Only and Caffeine + Stress groups) were calculated based on water consumption, caffeine concentration, and natural leakage estimates. “Dummy” water bottles were placed in empty cages and measured for 24 hours suggested. Results from this procedure suggested that approximately 15 mL naturally leaks per day. Table 9 shows estimated daily caffeine consumption in mg/kg and reflects calculations adjusted for natural leakage. Caffeine consumption did not differ for stressed versus non-stressed animals—there were non-significant findings for the main effect of Stress, $F(1,14)=0.90$, $p=.36$, partial $\eta^2=.06$., and effect of Time x Stress, $F(3.12, 43.7)=0.50$, $p=.69$, partial $\eta^2=.04$ (Huynh-Feldt corrected). When controlling for baseline caffeine consumption (i.e., prior to stress manipulation), caffeine consumption was not significantly different between stressed and non-stressed animals, $F(1,13)=0.00$, $p=.98$.

Open Field Habituation, Area under the Curve

Habituation was represented as area under the Curve (AUC) which reflected the reduction in exploratory behavior (Horizontal Activity) over time. Descriptives for AUC by time and group are presented in Table 10. Baseline AUC was used as a covariate for a comparison of Initial Startle at Time One (T1; post-stress) and Time Two (T2; post-combined stress/sleep disturbance) across caffeine and stress groups. Results for an overall RMANCOVA are presented in Table 11. Results demonstrated a main effect of Time, $F(1,27)=4.43$, $p=.05$, partial $\eta^2=.14$, indicating that rats habituated to the open field faster after repeated exposures to the chamber, regardless of experimental condition. However, there was no significant effect of experimental manipulation (i.e., for stress or caffeine).

Acoustic Startle Response

Initial Startle. For each data collection point, 3 minutes after rats were placed in the acoustic startle chamber, they were exposed to a 120 dB startle noise without a pre-pulse. The physical response to this first startle noise is called “Initial Startle” in these analyses. Raw descriptives of the startle amplitudes, including Initial Startle, are presented in Table 12. Baseline Initial Startle was used as a covariate for a comparison of Initial Startle at Time One (T1; post-stress) and Time Two (T2; post-combined stress/sleep disturbance) across caffeine and stress groups. Overall RMANCOVA results are presented in Table 13. Results revealed that baseline scores had a significant effect on amplitudes at T1 and T2 when controlling for caffeine and stress, $F(1,27)=4.07$, $p=.05$, partial $\eta^2=.13$. Results also demonstrated a significant three-way, Time x Caffeine x Stress interaction, $F(1,27)=4.31$, $p=.05$, partial $\eta^2=.07$. A Time x Caffeine interaction also trended toward significance, $F=3.39(1, 27)$, $p=.08$, partial $\eta^2=.11$. Figure 10 graphically shows the Initial Startle response across each time point and provides more information on the nature of the three-way interaction. As indicated by Figure 10, for Initial Startle Amplitude, the control group decreased over time, while rats in three experimental groups (Caffeine only, Stress only, and Caffeine + Stress) had an equivalent or increased Initial Startle Amplitude over time. Follow-up analyses were conducted to investigate this pattern further.

Internal analyses. A two-way ANCOVA was conducted for Initial Startle at Time One (i.e., after 7 days of WSP and caffeine administration), using baseline Initial Startle as a covariate. Results revealed no significant effects. Another ANCOVA was

conducted for Initial Startle at Time Two. Results indicated a significant Caffeine x Stress interaction at Time Two (i.e., after the second 7 days of WSP combined with 7 days of SDis), $F(1,27)=6.03$, $p=.02$, partial $\eta^2=.18$. Caffeine increased Initial Startle for non-stressed rats ($M(\text{adj.})=19.12$, $SE=1.32$, for Caffeine Only) compared to non-stressed rats not exposed to caffeine ($M(\text{adj.})=14.06$, $SE=1.32$). However, the Caffeine Only rats appeared to not differ from Stress Only ($M(\text{adj.})=19.25$, $SE=1.30$) or Caffeine + Stress ($M(\text{adj.})=18.05$, $SE=1.34$). The second ANCOVA was conducted using Time One Initial Startle as a covariate to isolate the possible unique effects of combined WSP and SDis. Results again indicated a significant interaction effect of Caffeine x Stress, $F(1,27)=5.80$, $p=.02$, partial $\eta^2=.18$. At Time Two, An ANCOVA within stressed rats revealed no significant differences between caffeinated and non-caffeinated rats. An ANCOVA within the non-stressed rats revealed that caffeinated rats had significantly higher (Caffeine x Stress, $F(1,27)=6.03$, $p=.02$, partial $\eta^2=.18$) amplitudes for Initial Startle ($M=19.09$; $SD=1.69$) than non-caffeinated rats ($M=14.03$; $SD=5.82$) at Time Two.

Startle Amplitudes (with and without pre-pulse). Raw descriptive statistics are presented for the startle amplitudes across ASR trial types by condition and time (see Table 12). An RMANCOVA was conducted for each of the six ASR trial types: (1) 110 dB startle/no pre-pulse (Table 16); (2) 120 dB startle/no pre-pulse (Table 17); (3) 110 dB startle/68 dB pre-pulse (Table 18); (4) 110 dB startle/82 dB pre-pulse (Table 19); (5) 120 dB startle/68 dB pre-pulse (Table 20); (6) 120 dB/82 dB pre-pulse (Table 21). Figure 11 graphically shows ASR amplitudes over time for each trial type. Results demonstrated non-significant effects for Time, Caffeine, Stress, and interactions across trials. As the results indicate, the variance in startle amplitudes was largely predicted by baseline

individual differences for trials with a pre-pulse, $F(1, 27) > 30$, $p < .001$, partial $\eta^2 > .30$, and for trials without pre-pulse, $F(1, 27) > 12$, $p < .001$, partial $\eta^2 > .30$. Across trials with pre-pulse (but not for trials without a pre-pulse), there were trends towards significance for three-way Time x Caffeine x Stress interactions. For the 110 dB startle/ 68 dB pre-pulse trials, the Time x Caffeine x Stress approached significance, $F(1, 27) = 3.83$, $p = .06$, partial $\eta^2 = .12$. Follow-up analyses were conducted to interpret this trend.

Internal Analyses. Two-way ANCOVAs of startle amplitudes were conducted at each time-point for the 110 dB startle/68 dB trial type. At Time One (see Table 22), results from the ANCOVA indicated non-significant effects of Caffeine ($p = .25$), Stress ($p = .48$), and Caffeine x Stress ($p = .94$) interaction. At Time Two (see Table 23), results from the ANCOVA again indicated non-significant effects of Caffeine ($p = .83$), Stress ($p = .09$), and Caffeine x Stress ($p = .08$) interaction.

Percent Pre-Pulse Inhibition (PPI). An RMANCOVA was conducted for each of the four pre-pulse inhibition (PPI) percentage trial types. Raw descriptives of ASR %PPI across trial types by condition and time are presented in Table 24. Of note, control rats did not demonstrate significant PPI across trials and individual percent PPI varied widely [BL, $M = 3.15$ ($SD = 25.11$); T1, $M = -3.97$ ($SD = 20.61$); T2, $M = 4.69$ ($SD = 19.87$)]. Other studies (e.g., 123) have consistently shown much larger percent PPI (approximately 40-80%). The RMANCOVA results are shown for the four trial types: 110 dB startle/68 dB pre-pulse (Table 25); 110 dB startle/82 dB pre-pulse (Table 26); 120 dB startle/68 dB pre-pulse (Table 27); 120 dB startle/82 dB pre-pulse (Table 28). Figure 12 graphically shows %PPI over time for each trial type. Results revealed non-significant results for all trial types, except for the 110 dB startle noise with a 68 dB pre-pulse. Results for

RMANCOVA for 110 dB startle/68 dB pre-pulse trials revealed significant main effects of Stress, $F(1, 27)=4.56$, $p=.04$, partial $\eta^2=.14$, and Time, $F(1, 27)=5.51$, $p=.03$, partial $\eta^2=.17$. Overall, for the 110 dB/68 dB trials, rats exposed to WSP showed less pre-pulse inhibition ($M(\text{adj.})=-9.08$; $SE=4.08$, for stressed groups) than rats not exposed to WSP ($M(\text{adj.})=3.58$, $SE=4.08$, for non-stressed rats).

Internal analyses. An ANCOVA at each time-point was conducted to further interpret the main effects of stress and time found in the omnibus test. At Time One (see Table 29), there were no significant effects of Stress, Caffeine, or Stress x Caffeine interaction. At Time Two (see Table 30), again, there were no significant effects of Stress, Caffeine, or Stress x Caffeine interaction.

CHAPTER 6: Evaluation of Hypotheses

Specific Aim 1: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **general information processing** (as measured by speed of locomotor habituation to an open field).

Hypothesis 1a. *Caffeine* will *impair* general information processing (i.e., slow speed habituation). The hypothesis that caffeine would *impair* general information processing as shown by reducing the speed of habituation to an open field apparatus was **not supported**. Results indicated non-significant effects of all experimental manipulations on open field habituation.

Hypothesis 1b. The hypothesis that *Warrior Stress* would *impair* general information processing (i.e. decrease speed of habituation) was **not supported**. Results indicated non-significant effects of all experimental manipulations on open field habituation.

Hypothesis 1c. The hypothesis that *Combined Warrior Stress and Sleep Disruption* would further *impair* general information processing compared to Baseline and Time 1 (i.e., Time x Stress interaction) was **not supported**. Results indicated non-significant effects of all experimental manipulations on open field habituation.

Hypothesis 1d. The hypothesis that *Caffeine* would *exacerbate* the negative effect of *Warrior Stress* on general information processing was **not supported**. Results indicated non-significant effects of all experimental manipulations on open field habituation.

Hypothesis 1e. The hypothesis that *Caffeine* will *exacerbate* the negative effect of *combined Warrior Stress and Sleep Disruption* was **not supported**. Results

indicated non-significant effects of all experimental manipulations on open field habituation.

Specific Aim 2: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **sensori-emotional processing** (as measured by behavioral reactivity to a startle noise).

Hypothesis 2a. The hypothesis that *Caffeine* alone would *impair* (i.e., increase sensitivity) sensori-emotional processing (i.e., increase startle response) was **partially supported**. Results demonstrated a significant caffeine x stress interaction at Time 2, and there were trends in the other omnibus tests for ASR trials of Caffeine x Stress x Time interactions approaching significance. Overall, this pattern suggests that caffeine might increase the startle response for control rats, but not for rats already under stress. Because there may have been a ceiling effect for how much the rats could demonstrate a startle response, there are indications that caffeine might have had a main effect or the same effect of stress. Therefore, this hypothesis was not fully supported, but *partially* supported, and is worthy of further investigation.

Hypothesis 2b. The hypothesis that *Warrior Stress* alone would *impair* (i.e., increase sensitivity) sensori-emotional processing (i.e., increase startle response) was **not supported**. Results indicated non-significant effects at Time 1 for all stressed groups.

Hypothesis 2c. The hypothesis that *Combined Warrior Stress and Sleep Disruption* would further *impair* (i.e., increase sensitivity) sensori-emotional processing compared to Baseline and Time 1 was **partially supported**. Results indicated a significant caffeine x stress interaction at Time 2, and there were trends in the other

omnibus tests for ASR trials of Caffeine x Stress x Time interactions approaching significance. Overall, this pattern suggests that the WS + SDis, WS + SDis + Caffeine, and Caffeine Only differed significantly from the control group. While this explanation does not suggest a main effect of WS + SDis, it does suggest that the WS + SDis increased the startle response relative to the control group.

Hypothesis 2d. The hypothesis that *caffeine* would *exacerbate* the negative effect of Warrior Stress on sensor-emotional processing was **not supported**. Results indicated that stressed rats without caffeine did not significantly differ from stressed rats with caffeine at Time 1, without the added stress of SDis.

Hypothesis 2e. The hypothesis that *caffeine* would *exacerbate* the negative effect of combined Warrior Stress and Sleep Disruption was **not supported**. However, results for Time 2 analyses suggested there was an opposite moderation effect than hypothesized. There was a medium-sized interaction effect of caffeine and combined WS/SDis on Initial Startle at Time 2. Caffeine increased behavioral reactivity to the initial 120 dB noise (i.e., Initial Startle) for non-stressed rats, but not for stressed rats.

Specific Aim 3: To examine the effects of caffeine, Warrior Stress, and combined Warrior Stress and Sleep Disruption on **attention** (as measured by inhibition to a startle noise when preceded by a pre-pulse, or warning).

Hypothesis 3a. The hypothesis that *Caffeine* alone would *improve* attention (i.e., increase inhibition) was **not supported**. The effects of caffeine were non-significant across trials for %PPI.

Hypothesis 3b. The hypothesis that *Warrior Stress* alone would *impair* attention (i.e., decrease inhibition) was **not supported**. The effect of WS was non-significant across %PPI trials at Time 1. It appears the WS manipulation alone did not produce a significant effect on attention.

Hypothesis 3c. The hypothesis that *Combined Warrior Stress and Sleep Disruption* would *impair* attention was **partially supported**. There was a significant Time x Stress effect for one trial type (110 dB startle/68 dB pre-pulse) that indicated the Stress group showed increased startle at Time 2 (i.e., after SDis).

Hypothesis 3d. The hypothesis that *caffeine* would *exacerbate* the negative effect of Warrior Stress on attention (i.e., interaction effect at Time 1) was **not supported**. The effects of caffeine and WS alone were non-significant across %PPI trials.

Hypothesis 3e. The hypothesis that *caffeine* would *attenuate* the negative effect of combined Warrior Stress and Sleep Disruption (i.e., interaction effect at Time 2) was **not supported**. The effect of caffeine was non-significant across trials for %PPI, and the effect of WS + SDis was partial or limited.

CHAPTER 7: Discussion

This experiment investigated the unique and combined effects of stress, caffeine, and sleep disruption on behavioral measures of information processing in male rats. A Warrior Stress Paradigm (WSP) was used to induce chronic mild stress over 14 days. Sleep disruption using auditory stimuli was added to the WSP for the second seven days of the manipulation. Sleep disruption was measured in a nested, non-counterbalanced group and therefore its effects could not be isolated. Caffeine was administered using a combined self (continuous access in drinking water) and parental (directly prior to WSP) administration paradigm. Outcome variables included: (1) decrease in horizontal activity over time (environmental habituation); (2) response to startle noises (sensori-emotional processing); (3) inhibition to startle noises presented with a pre-pulse (pre-attention). In this section, specific results are summarized and discussed in the context of their translation to deployment conditions for Service Members. Next, limitations and future directions are summarized. Finally, a summary and conclusion are provided.

Commentary on Outcome Measures

Body Weight. Rats exposed to either caffeine, stress, or both weighed significantly less than the control group at the end of the experiment. Internal analyses revealed that caffeine impacted weight over time and stress did not demonstrate a main effect on body weight until the last data-point (+19 days) after SDis. It is possible that SDis combined with WSP impacted weight more than WSP alone, or that the cumulative effect of WSP did not appear until later in the stress manipulation. This finding was interesting, given that previous studies have indicated that 40 days of restraint stress and chronic caffeine administration (1 g/L in drinking water) did not affect body weight

(150). Body weight was also a useful manipulation index of caffeine and stress, given that there were no other bioassays to determine caffeine consumption.

Water and Caffeine Consumption. Daily water consumption rates were significantly lower in rats drinking caffeinated water versus tap water. These differences were not present in previous studies that used tap water control conditions (110; 111). Other studies also indicated high caffeine consumption rates for similar caffeine concentrations (151). However, previous studies did not include a two-bottle choice period for the caffeinated rats, providing them access to tap water for two hours per day. It is unclear whether this discrepancy was caused by differences in actual consumption rates, or differences in measurement techniques across studies.

Open Field Habituation (AUC). There were no significant effects of experimental manipulation on open field habituation. There was a significant effect for Time, which supports the validity of the OFA horizontal activity reduction as a measure of habituation, as rats decreased exploratory activity after repeated exposure to the chamber.

Initial Startle Amplitude. Initial startle was used a measure of anxious and aroused state. Consider a service member who has returned home and is sitting in traffic. He hears a honk from behind him and is jolted in his seat—maybe he is more reactive to this startle noise than other drivers who have not recently returned from deployment. For the rats in ASR, this initial, spontaneous startle sound 3 minutes after they have been placed in the chamber is similar to a spontaneous startle noise for returning Service Members. Service Members might be less reactive to the honking after they have been sitting in traffic for longer and have heard more honks—therefore, this initial response is

a unique phenomenon and not impacted by habituation to the noise. This response may be considered a common reaction to other startling, novel stimuli prior to habituation and repeated exposure.

The results of this study indicated that the rats exposed to caffeine, stress, or both, demonstrated a stronger response to the startle noise than the control group. However, this effect was small. Though there were trends for other interactions, there were no statistically significant differences within the three experimental groups at each time point. These results suggest that caffeine alone can mimic the effects of stress and that caffeine does not significantly attenuate or exacerbate the stress response with regard to physical reactivity to an initial startle noise.

ASR: Startle Amplitudes. Results indicated that rats exposed to caffeine had stronger responses to the startle noise than controls at 110 dB with a 68 dB pre-pulse after exposure to Warrior Stress and sleep disruption combined. There were also similar, non-significant trends for the other trials with pre-pulses, but no indication of this effect with trials without a pre-pulse. The effect of the caffeine alone appeared similar to the effect of WSP/SDis, and WSP/SDis + Caffeine. In other words, there were no differences between experimental groups; caffeine did not appear to attenuate or potentiate the stress response with regard to startle response, though it did appear to mimic the effects of WSP/SDis. Another possible interpretation is that caffeine disrupted sleep for rats. Chronic caffeine consumption has been shown to disrupt sleep in human and rodents (152).

ASR: Percent PPI. Results of PPI percentages demonstrated that stressed rats (with and without caffeine) had less pre-pulse inhibition than non-stressed rats for one trial type only (110 dB/68 dB) cumulatively over both time-points, but not at either time-

point individually. However, results should be interpreted with caution given that no PPI was found for control rats. Possible explanations include apparatus or software malfunction. Results for other trial types indicated non-significant effects for stress, caffeine, and time. Notably, this trial type reflects the lower startle noise (110 dB versus 120 dB) and the lower pre-pulse sound (68 dB versus 82 dB). It is possible that exposure to WSP sensitized animals to lower dB startle noises.

Limitations

Internal validity. This study was intended to be a face-valid animal model of stress exposure and caffeine consumption in a combat deployment environment. To enhance face validity, a self-administration paradigm was used and therefore, caffeine exposure was not tightly controlled. Stress may have influenced the amount of caffeine rats consumed, resulting in differences in caffeine exposure between stressed and non-stressed groups. While the omnibus results did not indicate significant differences between stressed versus non-stressed groups for caffeine consumption, examination of means and standard deviations at specific time-points suggest there may have been variability among groups. Rats that were exposed to caffeine were also exposed to 7% sucrose solution, while control rats were exposed to regular tap water. Therefore, sucrose may have contributed to small differences found in caffeine versus control rats. Because of these two design limitations – uncontrolled self-administration of caffeine, and increased sucrose exposure in the caffeine group, one cannot confidently make causal interpretations about the effects of caffeine alone or a specific dose.

Animal to human translation. There is not a direct translation from animal models to human problems. Consistent with the limitations of most animal models, this

study could not directly replicate a deployed environment. In the current study, the primary translational issues were: (a) pharmacokinetic/pharmacodynamic non-equivalence; and (b) behavior based methods of data collection combined with the motor stimulation effects of caffeine. In terms of the pharmacologic effects of caffeine, moderate doses in rats do not necessarily have a human equivalent. Given the importance of dosing and timing, tolerance to caffeine is an important consideration when using proxy, behavior-based methods to assess cognitive and mood-related phenomena. For animals, tolerance to caffeine's effects is primarily measured by observed changes in locomotor activity. Caffeine increases locomotor activity in rats, and diminished activity, or return to baseline, suggests tolerance. Tolerance in rats happens at different rates depending on the method of administration. For example, Lau and Falk (153) found that rats returned to baseline locomotor activity after four days of IP injection and after only one day of self-administered caffeinated drinking water. In the current study, rats were exposed to caffeine more than seven days prior to behavioral testing. The influence of motor stimulation effects of caffeine are expected to be minimal, but still might have played a role.

Pre-Attention Outcomes. PPI was not found in control rats during pre-pulse trials of ASR. This unexpected finding casts doubt on the validity of the PPI measure and therefore, PPI findings as an index of pre-attention should be interpreted with caution.

Caffeine. Limitations with regard to caffeine included: (a) estimated consumption rates did not match with previous studies (appeared to be lower); (b) self-administration method allowed for wide individual variability in individual consumption rates; (c) oral syringe method also introduced some variability among caffeine condition rats as some

rats were more averse to the method than others; (d) there were no biological assays to estimate actual caffeine consumption. Because caffeine was self-administered, consumption was not tightly controlled. In addition, only estimates of caffeine consumption could be made based on water bottle weights and natural leakage. Blood or urine assays to detect the concentration of caffeine are preferable for calculating caffeine consumption. The acute, parental dosing method also introduced some limitations. Individual differences among rats and also investigator delivery technique (i.e., for the acute doses) created variance in how much rats consumed out of the total syringe volumes. Some rats drank the caffeine solution quickly and completely, while other rats appeared more averse to the syringe method, taking longer to drink the volume and spitting some out. Again, actual caffeine consumed was not measured and exact caffeine solution spillage could not be measured.

Warrior Stress. It is not clear if the WSP was a robust and effective experimental manipulation, given that there were not physiologic (e.g., cortisol or adrenergic activity) or behavioral manipulation checks. However, its effect on body weight is consistent with a stress manipulation. Also, WSP demonstrated effects on open field activity in previous Grunberg laboratory research (103).

Sleep disruption. The nested design without a crossover-counterbalanced manipulation prevented isolation of the effects of sleep disruption specifically. Significant results at the second time point cannot be interpreted without considering the accumulation of chronic stress exposure in combination with sleep disruption. In addition, there was no manipulation check for sleep disruption. It was not confirmed that rats experienced disrupted sleep compared to rats in the control condition.

Summary

Design. The present experiment was conducted to understand how caffeine combines with stress to influence information processing. Caffeine was administered chronically (continuous access, self-administration) and acutely (directly prior to stressor, parental administration via oral syringe drinking). The Warrior Stress Paradigm (WSP) included daily exposure to synthetic fox urine for 10 minutes at a time (20 minutes for the first day only) and 10 minutes of a schedule of unpredictable stressful stimuli (e.g., coin shake). Sleep disruption (SDis) consisted of exposing rats to a variable schedule of loud sounds during their typical sleep cycle. Rats in the stress condition were exposed to WSP for 14 days, and SDis for the second seven days of WSP. Rats' behavior was measured at baseline, after seven days WSP, and after seven additional days WSP combined with SDis.

Hypotheses and rationale. Caffeine and Warrior Stress separately were expected to impair locomotor habituation to an environment, increase the startle response, and increase pre-pulse inhibition. The combined effect of caffeine and Warrior Stress was expected to be additive (i.e., caffeine was expected to worsen the negative effect of stress) across all information processing measures. Also, caffeine was expected to impact information processing differently when SDis was presented, given that caffeine reduces fatigue and appears to have stronger effect for sleep deprived humans and rodents.

Results. Caffeine, Stress Only, and Caffeine + Stress groups had significantly higher startle amplitudes for the initial startle sound (the first startle presented at each trial) than controls. However, there were no differences among experimental groups. Consistent with this interpretation, follow-up analyses revealed a significant Caffeine x

Stress interaction at Time Two. Caffeine increased the amplitude under non-stressed conditions, but had no effect under the stressed conditions.

Though results were not significant for other trials, similar trends were found in trials that included pre-pulse, but not with trials without a pre-pulse. The strongest trend was found for the 110 dB startle/68 dB pre-pulse trial. Results were non-significant for the percent pre-pulse inhibition (% PPI) trials, except for a main effect found for stress in the 110 dB startle/ 68 dB pre-pulse trial in the omnibus test, but not at individual time-points.

Interpretation. The effects of SDis versus cumulative stress cannot be distinguished, but the data suggest that SDis may have created added stress as demonstrated by later effects on body weight, and increased startle amplitude at Time 2. An interaction effect of caffeine and stress on Initial Startle was revealed at Time 2, which might indicate that caffeine produces stress-like impairment over time. Caffeine may have disrupted sleep in rats and created similar conditions to the stress group. However, this interpretation is made with caution, as increased sucrose consumption in the caffeine groups may have also influenced these outcomes.

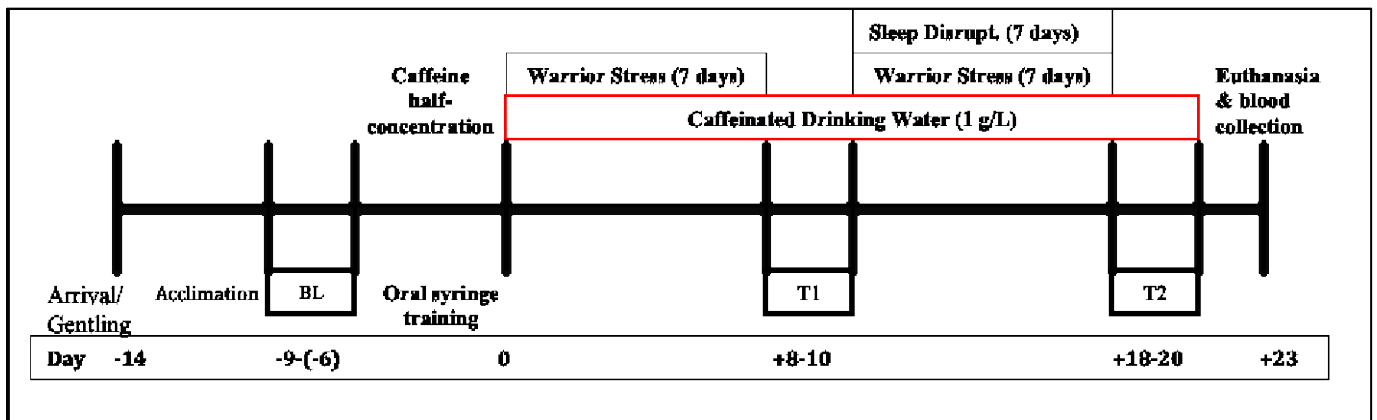
CHAPTER 8: Conclusions

Based on the current experiment, a moderate caffeine dose does not appear to adversely or positively impact rats under already stressed conditions with regard to startle response, pre-attention, and behavioral habituation. It is possible that the true effect size for caffeine is smaller than anticipated, and therefore the experiment was not sufficiently powered. Some unanticipated findings included the additive effects of caffeine and stress on body weight, whereby caffeine and stress decreased the rate of weight gain, but the effect of stress emerged later in the study. Whereas there were significant effects of caffeine and stress on body weight in this model, the information processing effects are much more subtle if any exist. Interactive effects of caffeine emerged only after the introduction of sleep disruption and the accumulation of chronic mild stress for the lower startle sound, with the lower pre-pulse. However, caffeine appears to mimic the effects of stress when administered alone, specifically for acoustic startle responses. If this trend translates to the human response to caffeine and stress, then this study suggests that caffeine alone might predispose individuals to poorer mental health outcomes, but when added to stress, may not add any vulnerability. As discussed above, increased sucrose exposure may have also influenced outcomes. Future studies should explore individual differences (e.g., comparing genetic strains of rats), group differences, specifically with regard to sex, and dose-response effects of stress and/or caffeine. It is possible that other dosing sizes of caffeine and stress will combine differently to affect information processing in rats. In addition, adding biomarkers of stress and caffeine metabolism increase an understanding of the mechanisms of these potential effects. Also, the literature review of this study exposed a gap in observed data regarding how much and

when Service Members are consuming caffeine. Also, human studies also have focused on the immediate or short-term effects of caffeine on performance, but not on emotional functioning. Continued human research on the effects of caffeine and stress is needed to justify current command policies advocating for caffeine use in theater.

APPENDIX A: Figures

Figure 1: Experimental Timeline



* Day 0 indicates start of experimental manipulations

Please note: "Warrior Stress (7 days)" included daily parental caffeine administration (60 mg).

Figure 2: Caffeine Administration Equipment



Photo by M.C. Baisley, 2015

Figure 3: Warrior Stress Paradigm: Schedule of Stressors

Timeline of Stress Days		
Stress Day	Predator Stress	Unpredictable Event
1	Fox Urine (20 min)	None
2	Fox Urine (10 min)	Whistle at 12, 15 & 19 min
3	Fox Urine (10 min)	Coin Shake at 11, 14, & 17 min
4	Fox Urine (10 min)	Flashing Lights at 13, 16, & 19 min
5	Fox Urine (10 min)	Cage Shake at 12, 15, & 18 min
6	Fox Urine (10 min)	Flashing Lights at 12, 16, & 19 min
7	Fox Urine (10 min)	Whistle at 11, 13, 16 & 18 min
8	Fox Urine (10 min)	Coin Shake at 12, 16, & 19 min
9	Fox Urine (10 min)	Flashing Lights at 11, 15, 19 min
10	Fox Urine (10 min)	Cage Shake at 11, 14, & 17 min
11	Fox Urine (10 min)	Coin Shake at 13, 16, & 19 min
12	Fox Urine (10 min)	Whistle at 12, 14, 17 min
13	Fox Urine (10 min)	Flashing Lights at 11, 14, 18 min
14	Fox Urine (10 min)	Cage Shake at 12, 15, & 18 min

Figure 4: Warrior Stress Paradigm Equipment



Photo by A.M. Yarnell, 2012

Figure 5: Open Field Activity Equipment

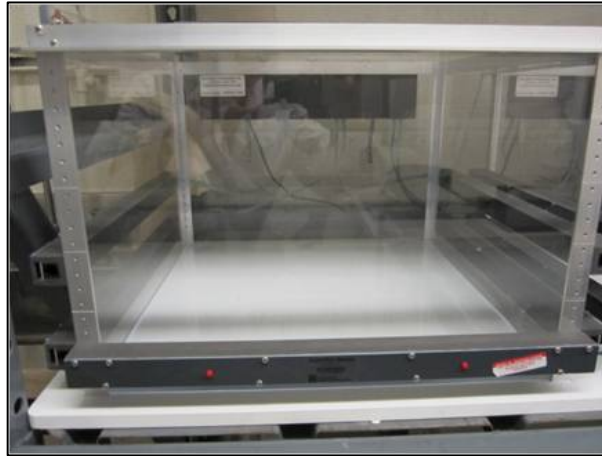


Photo by A.M. Yarnell, 2012

Figure 6: Acoustic Startle Response (ASR) Equipment



Photo by A.M. Yarnell, 2013

Figure 7: Body Weight (grams) over Time

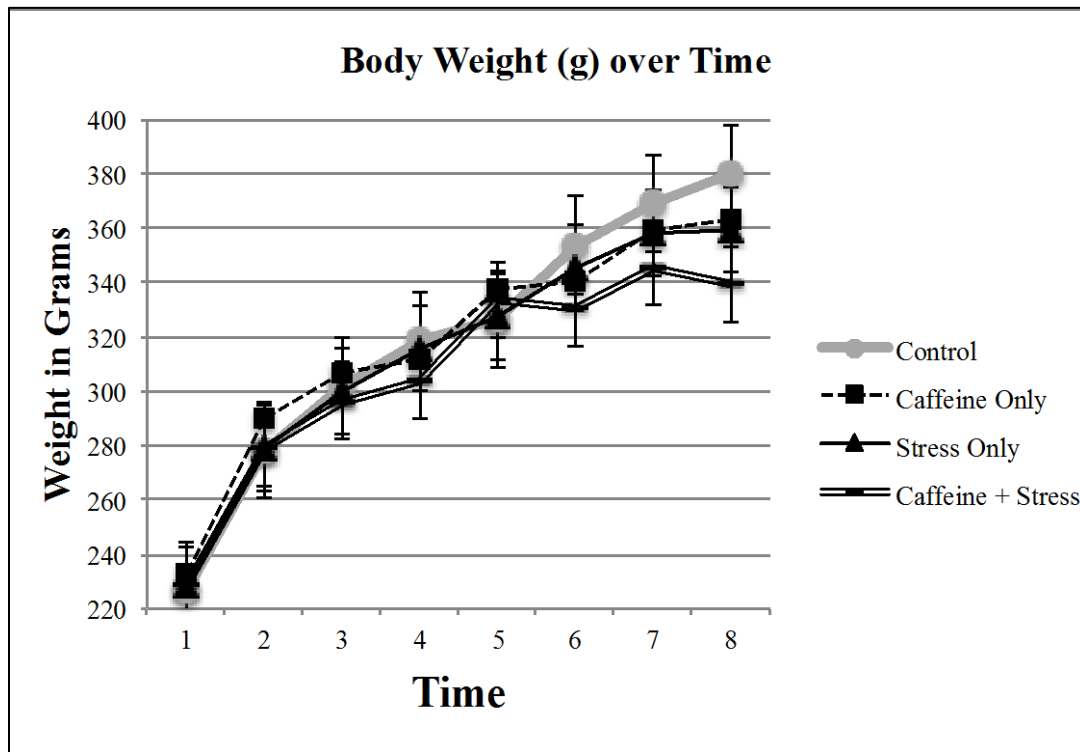


Figure 8: Daily Drinking Water Rates (mL/day) across Groups

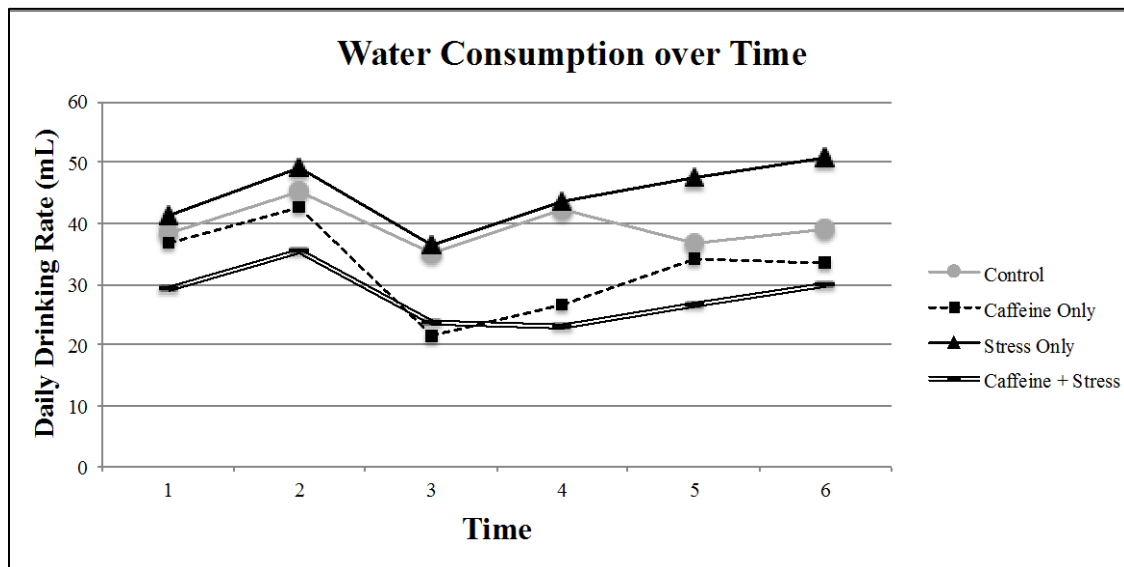


Figure 9: Habituation Curves at Each Time-Point

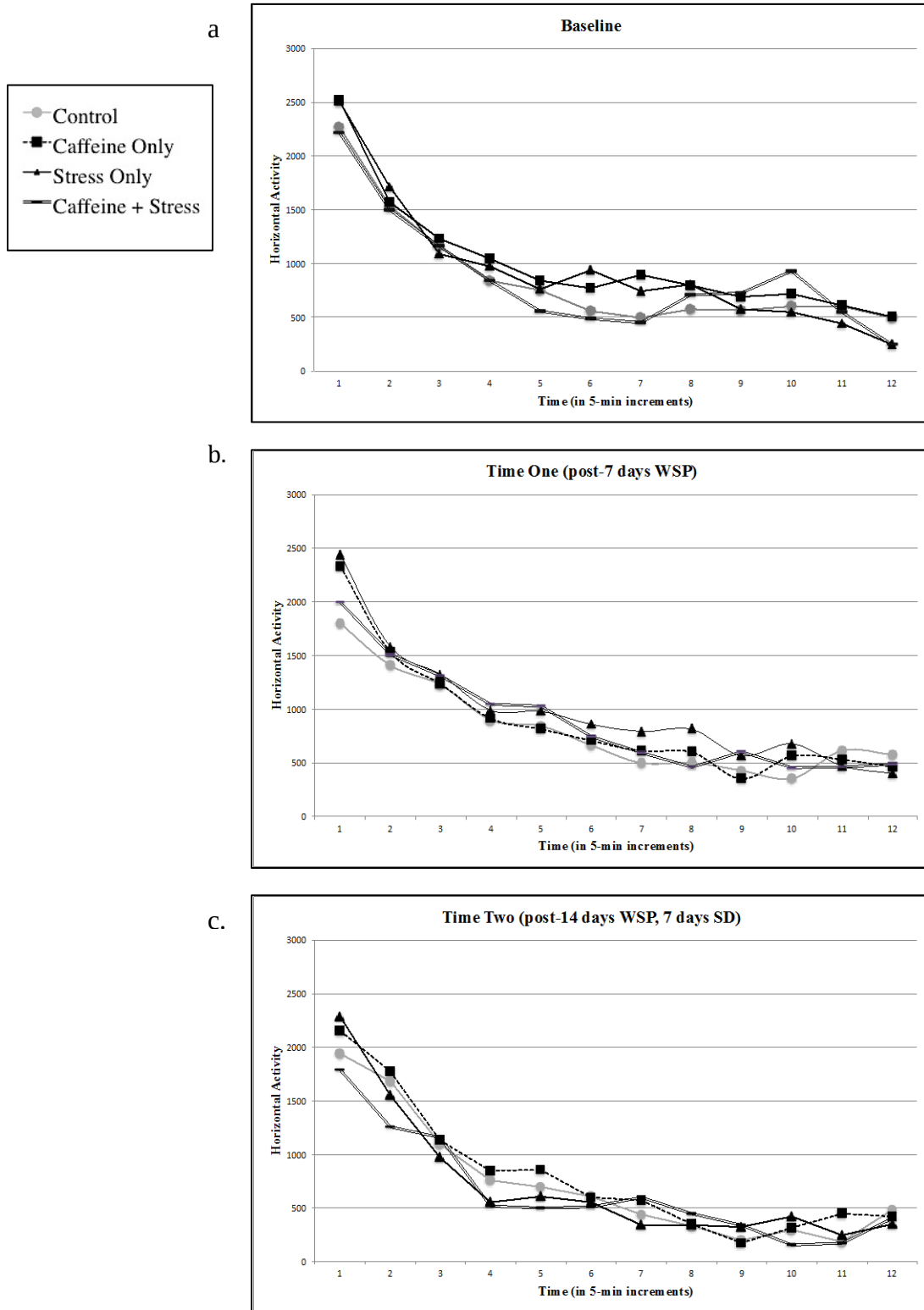


Figure 10: Initial Startle –Time x Group

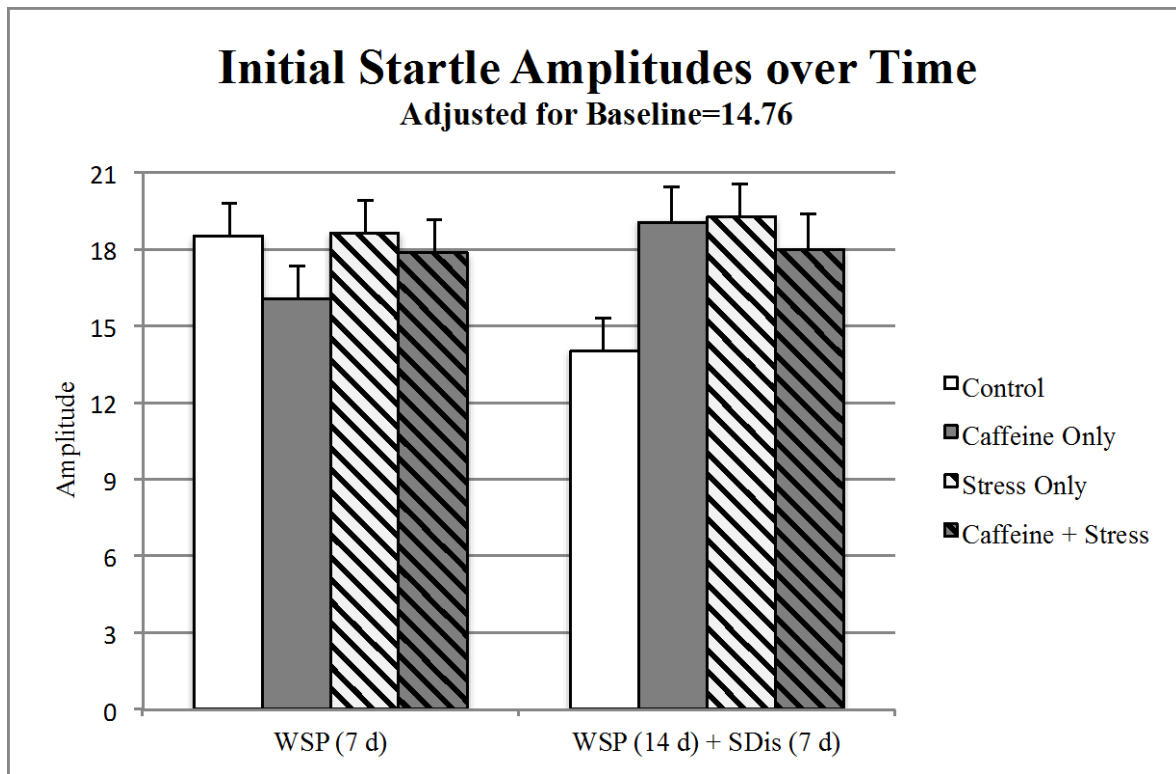
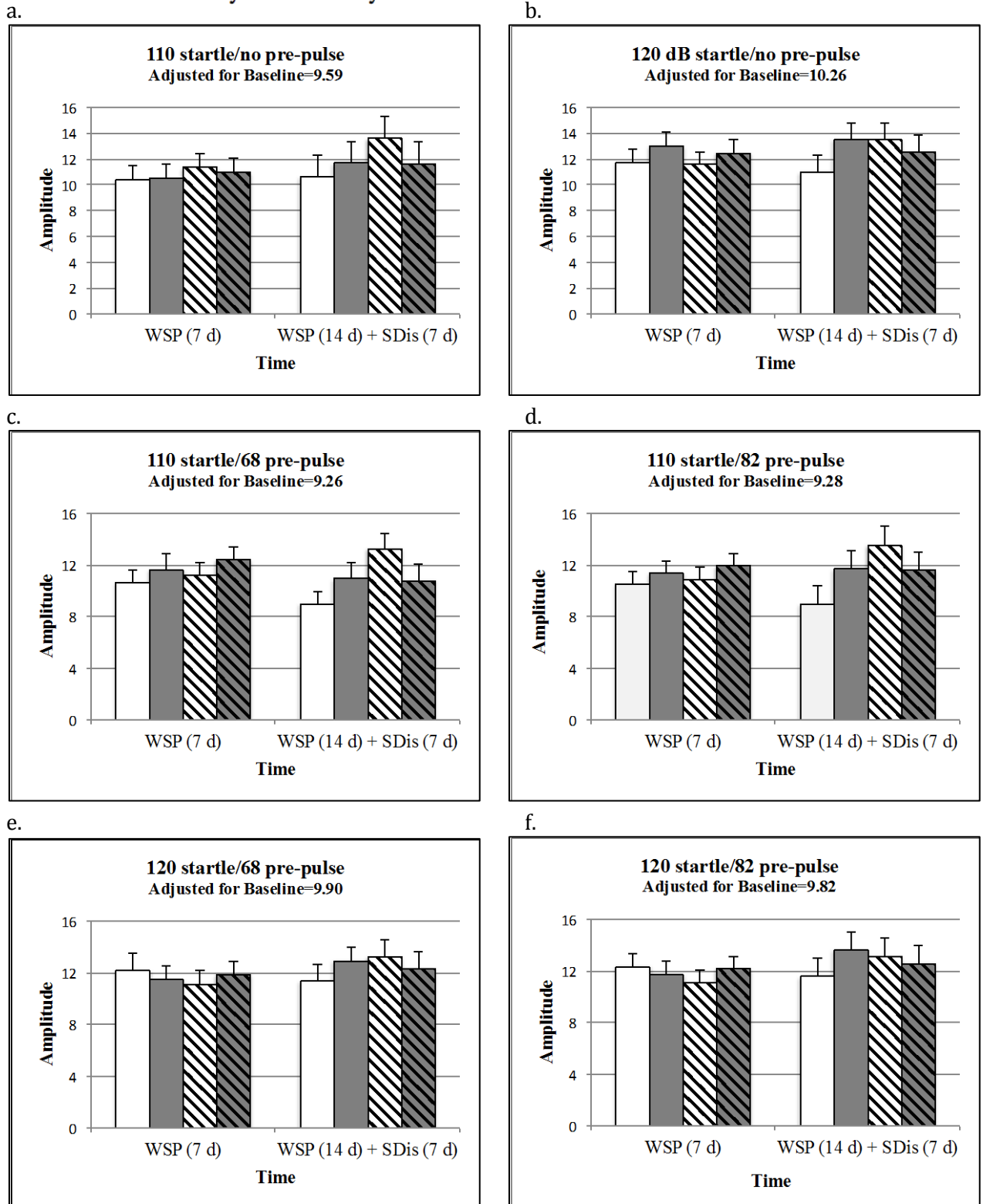


Figure 11: Acoustic Startle Trials – Time x Group

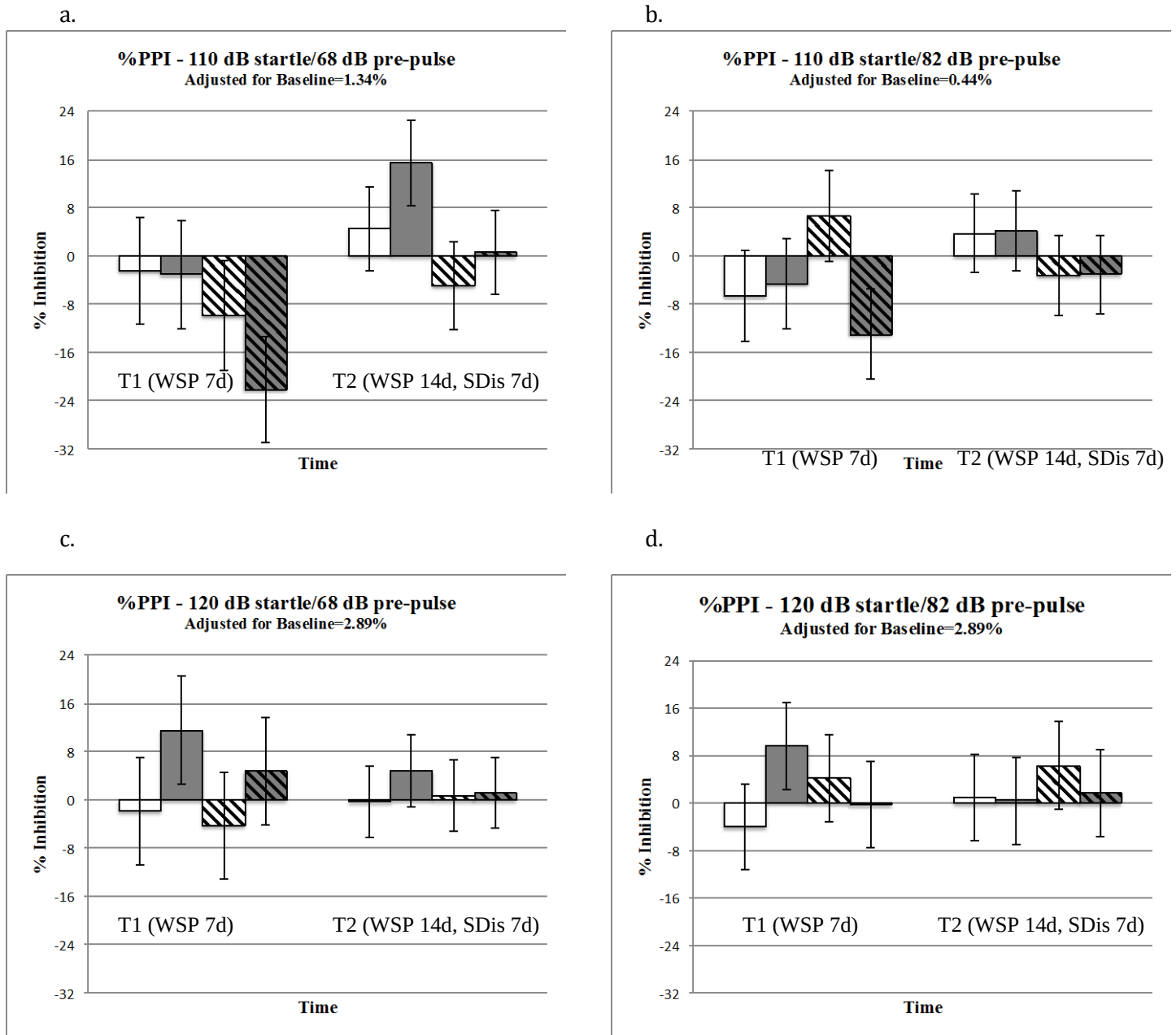
□ Control ■ Caffeine Only ▨ Stress Only ▩ Caffeine + Stress



*For Time (horizontal axes): “WSP (7 d)”= Warrior Stress Paradigm for 7 days for stress groups (i.e., T1); “WSP (14 d) + SDis (7 d)”= Warrior Stress Paradigm for cum. 14 days combined with sleep disruption for 7 days for stress groups (i.e., T2).

Figure 12: Percent Pre-Pulse Inhibition (% PPI) Trials – Time x Group

□ Control ■ Caffeine Only ▨ Stress Only ▩ Caffeine + Stress



*For Time (horizontal axes): “T1 (WSP 7d)”= Warrior Stress Paradigm for 7 days for stress groups; “T1 (WSP 14d, SDis 7d)”= Warrior Stress Paradigm for cum. 14 days combined with sleep disruption for 7 days for stress groups (i.e., T2).

APPENDIX B: Tables

Table 1: Treatment Cell Breakdown

Condition	No WSP ^a	WSP (+nested SDis ^b at T2)
Water/sucrose	n=8	n=8
Caffeine	n=8	n=8

^aWSP=Warrior Stress Paradigm; ^bSDis=Sleep Disruption

Table 2: Body Weight Descriptives

Descriptives [Means (SDs)] of Body Weight (in grams) – Time x Group								
	-13 Days	-7 Days	*-3 Days	*+6 Days	+9 Days	*+12 Days	+16 Days	+19 Days
Control	226.74 (10.54)	278.56 (13.30)	301.77 (13.49)	318.26 (15.26)	326.31 (19.97)	353.78 (19.00)	368.89 (21.42)	380.05 (17.27)
Caffeine Only	232.97 (7.43)	290.49 (16.73)	306.57 (19.57)	312.11 (22.97)	337.92 (25.82)	340.11 (25.49)	359.00 (28.11)	363.19 (28.88)
Stress Only	228.9 (6.71)	279.25 (8.76)	299.95 (10.60)	316.05 (12.18)	327.26 (21.00)	345.11 (15.40)	358.33 (16.61)	359.56 (20.97)
Caffeine + Stress	228.49 (12.77)	279.08 (15.39)	296.37 (18.26)	303.58 (18.80)	333.34 (16.27)	330.44 (21.38)	345.78 (22.89)	339.33 (20.58)

*Experimental manipulations: ½ caffeine concentration at -3 days; WSP starting at +6 days; combined WSP/SD starting at +12 days

Table 3: Omnibus RMANCOVA of Body Weight

Overall RMANCOVA of Body Weight over Time (Huynh-Feldt corrected)							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	477.56	1	477.56	0.48	.49	.02	.10
Baseline BW ^a	29297.55	1	29297.55	29.51	.00*	.52	1.00
Caffeine	4485.64	1	4485.64	4.52	.04*	.14	.54
Stress	3020.97	1	3020.97	3.04	.09	.10	.39
Caffeine x Stress	103.22	1	103.22	0.10	.75	.00	.06
Time	1024.76	3	341.89	1.63	.19	.06	.41
Time x Baseline BW	911.97	3	304.26	1.45	.23	.05	.37
Time x Caffeine	5692.29	3	1899.09	9.08	.00*	.25	1.00
Time x Stress	2080.48	3	694.10	3.32	.02*	.11	.74
Time x Caffeine x Stress	238.71	3	79.64	0.38	.77	.01	.12
Error (time)	16935.00	162	104.54				
Error (measure)	26809.94	27	992.96				

^aBW=Body Weight
*Indicates p≤.05

Table 4: ANCOVA of Body Weight at +12 Days

ANCOVA of Body Weight at +12 Days							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	7991.50	4	1997.87	8.67	.00*	.56	1.00
Intercept	0.75	1	0.75	0.00	.96	.00	.05
Baseline Body Weight	5710.84	1	5710.84	24.77	.00*	.48	1.00
Caffeine	2650.42	1	2650.42	11.50	.00*	.30	.91
Stress	442.69	1	442.69	1.92	.18	.07	.27
Caffeine x Stress	149.62	1	149.62	0.65	.43	.02	.12
Error	6224.39	27	230.53				
Total	3765002.49	32					
Corrected Total	14215.88	31					
*Indicates $p \leq .05$							

Table 5: ANCOVA of Body Weight at +16 Days

ANCOVA of Body Weight at +16 Days							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	7614.98	4	1903.75	5.79	.00*	.46	.96
Intercept	36.26	1	36.26	0.11	.74	.00	.06
Baseline Body Weight	5461.10	1	5461.10	16.60	.00*	.38	.98
Caffeine	1847.98	1	1847.98	5.62	.03*	.17	.63
Stress	832.18	1	832.18	2.53	.12	.09	.34
Caffeine x Stress	92.54	1	92.54	0.28	.60	.01	.08
Error	8882.60	27	328.99				
Total	4117738.42	32					
Corrected Total	16497.58	31					
*Indicates $p \leq .05$							

Table 6: ANCOVA of Body Weight at +19 Days

ANCOVA of Body Weight at +19 Days							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	10773.68	4	2693.42	7.34	.00*	.52	.99
Intercept	278.89	1	278.89	0.76	.39	.03	.13
Baseline Body Weight	4067.70	1	4067.70	11.09	.00*	.29	.89
Caffeine	3823.69	1	3823.69	10.42	.00*	.28	.88
Stress	3422.40	1	3422.40	9.33	.01*	.26	.84
Caffeine x Stress	46.20	1	46.20	0.13	.73	.01	.06
Error	9904.74	27	366.84				
Total	4180163.50	32					
Corrected Total	20678.42	31					
*Indicates $p \leq .05$							

Table 7: Water Consumption Descriptives

Descriptives [Means (SDs)] of Daily Water Consumption (in mL) – Time x Group						
	^a Days -6(-)1	^b Days +0-2	Days +3-6	^c Days +7-9	Days +10-13	Days +14-17
Control	38.23 (7.50)	45.07 (7.01)	35.05 (9.48)	42.40 (12.73)	36.72 (10.02)	39.17 (9.50)
Caffeine Only	36.73 (10.20)	42.64 (25.50)	21.59 (5.92)	26.72 (13.31)	34.22 (15.77)	33.44 (11.60)
Stress Only	41.27 (9.45)	49.06 (13.05)	36.42 (6.26)	43.55 (15.88)	47.48 (11.96)	50.61 (10.82)
Caffeine + Stress	29.29 (6.47)	35.57 (15.84)	23.66 (6.45)	22.93 (8.87)	26.52 (8.08)	30.04 (5.76)
^a No Shading indicates half-concentration (1/2 g/L) was being administered when data were collected						
^b Lighter shading indicates ongoing WSP at the time of data collection						
^c Darker shading indicates ongoing WSP + SDIs at the time of data collection						

Table 8: Omnibus RMANOVA of Water Consumption

Overall RMANOVA of Water Consumption over Time (Huynh-Feldt corrected)							
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	251356.39	1	251356.39	570.08	.00*	.95	1.00
Caffeine	6690.26	1	6690.26	15.17	.00*	.35	.96
Stress	6.56	1	6.56	0.02	.90	.00	.05
Caffeine x Stress	1163.78	1	1163.78	2.64	.12	.09	.35
Time	3408.01	4.7	725.23	9.36	.00*	.25	1.00
Time x Caffeine	673.43	4.7	143.31	1.85	.11	.06	.60
Time x Stress	236.97	4.7	50.43	0.65	.65	.02	.22
Time x Caffeine x Stress	472.66	4.7	100.58	1.30	.27	.04	.43
Error (time)	10196.66	131.6	77.50				
Error (measure)	12345.65	28	440.92				

Table 9: Caffeine Consumption Descriptives

Descriptives [Means (SDs)] of Estimated Daily* Caffeine Consumption over Time (mg/kg)						
	^a Days -6(-)1	^b Days +0-2	Days +3-6	^c Days +7-9	Days +10-13	Days +14-17
Control	0	0	0	0	0	0
Caffeine Only	35.27 (16.24)	86.25 (73.99)	19.12 (17.54)	35.97 (35.54)	53.76 (44.29)	51.27 (31.69)
Stress Only	0	0	0	0	0	0
Caffeine + Stress	23.89 (9.95)	66.02 (46.76)	25.84 (19.05)	24.27 (24.60)	33.01 (21.78)	44.10 (15.91)
*Daily caffeine consumption does not include acute doses						
^a No Shading indicates half-concentration (1/2 g/L) was being administered when data were collected						
^b Lighter shading indicates ongoing WSP at the time of data collection						
^c Darker shading indicates ongoing WSP + SDIs at the time of data collection						

Table 10: Open Field Habituation Descriptives

Descriptives of Area Under The Curve (Open Field)			
	BL Mean (SD)	T1 Mean (SD)	T2 Mean (SD)
Control	52261.88 (13642.16)	48985.63 (14276.37)	43696.88 (5491.28)
Caffeine Only	53723.13 (17932.25)	53176.88 (22153.57)	42753.13 (21773.32)
Stress Only	63899.38 (23007.15)	59371.88 (11214.67)	48219.38(12073.76)
Stress + Caffeine	51838.13 (10451.61)	53465.63 (34743.68)	39286.25 (29474.05)

Table 11: Omnibus RMANCOVA of Open Field Habituation

Overall RMANCOVA of Open Field Area under The Curve							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	2112271432	1	2112271432	3.80	.06	.12	.47
Baseline AUC	4005444015	1	4005444015	7.21	.01	.21	.74
Caffeine	806115.21	1	806115.21	0.00	.97	.00	.05
Stress	4209560.69	1	4209560.69	0.01	.93	.00	.05
Caffeine x Stress	20814935.68	1	20814935.68	0.04	.85	.00	.05
Time	893273469.90	1	893273469.90	4.43	.05*	.14	.53
Time x Baseline AUC	373745384.30	1	373745384.30	1.85	.19	.06	.26
Time x Caffeine	22754623.34	1	22754623.34	0.11	.74	.00	.06
Time x Stress	152779378	1	152779378	0.76	.39	.03	.13
Time x Caffeine x Stress	36120719.01	1	36120719.01	0.18	.68	.01	.07
Error (Time)	5445641514	27	201690426.40				
Error (Measure)	14998666427	27	555506164				

Table 12: ASR Descriptives

Descriptives of ASR – Initial Startle and Trials (with and without pre-pulse) – Time x Group				
	Trial Type	BL Mean (SD)	T1 Mean (SD)	T2 Mean (SD)
Control	Initial Startle (120 dB)	17.20 (4.53)	19.42 (1.09)	14.03 (5.82)
	110 dB (no pre-pulse)	10.75 (4.34)	11.04 (2.49)	11.31 (6.60)
	120 dB (no pre-pulse)	10.86 (3.72)	12.19 (3.27)	11.48 (5.39)
	110 dB (68 dB)	9.90 (4.08)	11.06 (3.09)	9.59 (5.03)
	110 dB (82 dB)	10.37 (3.34)	11.46 (2.70)	9.84 (5.71)
	120 dB (68 dB)	10.05 (3.14)	12.25 (2.69)	11.48 (5.56)
	120 dB (82 dB)	9.95 (3.57)	12.46 (2.91)	11.69 (5.96)
Caffeine Only	Initial Startle (120 dB)	17.14 (3.97)	17.00 (4.43)	19.09 (1.69)
	110 dB (no pre-pulse)	10.01 (3.65)	10.73 (3.32)	11.96 (3.74)
	120 dB (no pre-pulse)	10.00 (3.01)	12.84 (2.57)	13.31 (2.64)
	110 dB (68 dB)	8.61 (3.10)	11.18 (3.59)	10.34 (3.73)
	110 dB (82 dB)	9.16 (2.80)	11.25 (4.14)	11.57 (3.77)
	120 dB (68 dB)	9.73 (2.66)	11.31 (3.27)	12.77 (3.62)
	120 dB (82 dB)	9.43 (3.18)	11.43 (3.25)	13.26 (3.67)
Stress Only	Initial Startle (120 dB)	12.89 (5.10)	17.94 (3.50)	19.27 (2.04)
	110 dB (no pre-pulse)	8.37 (3.83)	10.65 (5.02)	12.84 (4.80)
	120 dB (no pre-pulse)	10.60 (4.40)	11.81 (5.71)	13.79 (4.90)
	110 dB (68 dB)	9.15 (4.24)	11.13 (5.22)	13.17 (5.52)
	110 dB (82 dB)	8.43 (3.80)	10.11 (5.45)	12.85 (4.85)
	120 dB (68 dB)	10.26 (4.34)	11.32 (5.11)	13.50 (4.62)
	120 dB (82 dB)	10.26 (4.29)	11.43 (5.79)	13.55 (6.24)
Stress + Caffeine	Initial Startle (120 dB)	11.79 (5.66)	16.77 (5.17)	18.08 (3.02)
	110 dB (no pre-pulse)	9.25 (4.12)	10.79 (3.55)	11.41 (5.64)
	120 dB (no pre-pulse)	9.59 (3.65)	11.98 (3.26)	12.03 (4.92)
	110 dB (68 dB)	9.39 (4.32)	12.49 (2.92)	10.93 (5.06)
	110 dB (82 dB)	9.16 (4.36)	11.87 (3.43)	11.50 (5.43)
	120 dB (68 dB)	9.58 (4.71)	11.66 (4.05)	12.05 (5.50)
	120 dB (82 dB)	9.63 (4.46)	12.04 (4.11)	12.38 (6.03)

Table 13: Omnibus RMANCOVA of Initial Startle

Overall RMANCOVA of Initial Startle (ASR)							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	1251.27	1	1251.27	1251.80	.00*	.81	1.00
Baseline Initial Startle	43.35	1	43.35	4.07	.05*	.13	.49
Caffeine	.482	1	.482	0.05	.83	.00	.06
Stress	28.23	1	28.23	2.65	.12	.09	.35
Time	42.13	1	42.13	2.92	.10	.10	.38
Caffeine x Stress	21.39	1	28.23	2.00	.17	.07	.28
Time x Caffeine	48.96	1	48.96	3.39	.08	.11	.43
Time x Stress	3.85	1	3.85	0.27	.61	.01	.08
Time x Caffeine x Stress	62.16	1	62.16	4.31	.05*	.07	.40
Error (time)	829.45	27	14.81				

Table 14: ANCOVA of Initial Startle at Time One

ANCOVA of Initial Startle (ASR) at Time One							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	417.11	1	417.11	34.45	.00	0.56	1.00
Baseline Initial Startle	92.15	1	92.15	7.61	.01*	0.22	.76
Caffeine	19.87	1	19.87	1.64	.21	0.06	.24
Stress	5.61	1	5.61	0.46	.50	0.02	.10
Caffeine x Stress	5.31	1	5.31	0.44	.51	0.02	.10
Error	326.93	27	12.11				
Total	10573.06	32					
*Indicates $p \leq .05$							

Table 15: ANCOVA of Initial Startle at Time Two

ANCOVA of Initial Startle (ASR) at Time Two							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	876.29	1	876.29	67.55	.00	0.71	1.00
Baseline Initial Startle	0.08	1	0.08	0.01	.94	0.00	.05
Caffeine	29.58	1	29.58	2.28	.14	0.08	.31
Stress	26.47	1	26.47	2.04	.17	0.07	.28
Caffeine x Stress	78.24	1	78.24	6.03	.02*	0.18	.66
Error	350.24	27	12.97				
Total	10429.41	32					
*Indicates $p \leq .05$							

Table 16: Omnibus RMANCOVA of ASR Trial 110 dB Startle (no pre-pulse)

Overall RMANCOVA of ASR - Trial 110 dB startle/ no pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	289.61	1	289.61	11.94	.00	.31	.92
Baseline Startle	291.26	1	291.26	12.01	.00*	.31	.92
Time x Baseline Startle	0.83	1	0.83	0.10	.75	.00	.06
Caffeine	1.27	1	1.27	0.05	.82	.00	.06
Stress	17.17	1	17.17	0.71	.41	.03	.13
Time	0.50	1	0.50	0.06	.81	.00	.06
Caffeine x Stress	2.18	1	2.18	0.26	.61	.01	.08
Time x Caffeine	0.36	1	0.36	0.04	.84	.00	.06
Time x Stress	12.01	1	12.01	0.50	.49	.02	.10
Time x Caffeine x Stress	6.82	1	6.82	0.82	.37	.03	.14
Error (Measure)	654.85	27	24.25				
Error (Time)	225.05	27	8.34				
*Indicates $p \leq .05$							

Table 17: Omnibus RMANCOVA of ASR Trial 120 dB Startle (no pre-pulse)

Overall RMANCOVA of ASR - Trial 120 dB startle/no pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	149.97	1	149.97	10.33	.00*	.28	0.87
Baseline Startle	435.11	1	435.11	29.97	.00*	.53	1.00
Time x Baseline Startle	0.75	1	0.75	0.11	.74	.00	0.11
Caffeine	13.46	1	13.46	0.93	.34	.03	0.15
Stress	0.60	1	0.60	0.04	.84	.00	0.05
Time	0.06	1	0.06	0.01	.93	.00	0.01
Caffeine x Stress	14.74	1	14.74	1.02	.32	.04	0.16
Time x Caffeine	0.39	1	0.39	0.06	.81	.00	0.06
Time x Stress	5.28	1	5.28	0.77	.39	.03	0.77
Time x Caffeine x Stress	9.62	1	9.62	1.40	.25	.05	1.40
Error (Measure)	392.06	27	14.52				
Error (Time)	185.25	27	6.86				
*Indicates $p \leq .05$							

Table 18: Omnibus RMANCOVA of ASR Trial 110 dB Startle (68 dB pre-pulse)

Overall RMANCOVA of ASR - Trial 110 dB startle/ 68 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	128.44	1	128.44	9.76	.00*	.27	.85
Baseline Startle	566.62	1	566.62	43.04	.00*	.62	1.00
Time x Baseline Startle	9.22	1	9.22	1.73	.20	.06	.25
Caffeine	2.86	1	2.86	0.22	.65	.01	.07
Stress	30.26	1	30.26	2.30	.14	.08	.31
Time	12.25	1	12.25	2.30	.14	.10	.31
Caffeine x Stress	17.67	1	17.67	1.34	.26	.07	.28
Time x Caffeine	7.52	1	7.52	1.41	.25	.05	.21
Time x Stress	7.73	1	7.73	1.45	.24	.05	.21
Time x Caffeine x Stress	20.34	1	20.34	3.83	.06	.12	.47
Error (Measure)	355.46	27	13.17				
Error (Time)	143.61	27	5.32				
*Indicates $p \leq .05$							

Table 19: Omnibus RMANCOVA of ASR Trial 110 dB Startle (82 dB pre-pulse)

Overall RMANCOVA of ASR - Trial 110 dB startle/ 82 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	92.46	1	92.46	6.01	.00*	.18	.66
Baseline Startle	518.48	1	518.48	33.69	.00*	.56	1.00
Time x Baseline Startle	0.45	1	0.45	0.05	.82	.00	.06
Caffeine	7.48	1	7.48	0.49	.49	.02	.10
Stress	29.21	1	29.21	1.90	.18	.07	.26
Time	0.98	1	0.98	0.12	.73	.00	.06
Caffeine x Stress	18.76	1	18.76	1.219	.28	.04	.19
Time x Caffeine	1.40	1	1.40	0.17	.69	.01	.07
Time x Stress	12.51	1	12.51	1.50	.23	.05	.22
Time x Caffeine x Stress	23.97	1	23.97	2.86	.10	.10	.37
Error (Measure)	415.51	27	15.39				
Error (Time)	225.96	27	8.37				

*Indicates $p \leq .05$

Table 20: Omnibus RMANCOVA of ASR Trial 120 dB Startle (68 dB pre-pulse)

Overall RMANCOVA of ASR - Trial 120 dB startle/ 68 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	148.38	1	148.38	9.51	.00*	.26	.84
Baseline Startle	470.44	1	470.44	30.15	.00*	.53	1.00
Time x Baseline Startle	6.44	1	6.44	0.90	.64	.01	.08
Caffeine	0.58	1	0.58	0.04	.85	.00	.05
Stress	0.39	1	0.39	0.03	.88	.00	.05
Time	1.65	1	1.65	0.23	.64	.01	.08
Caffeine x Stress	17.67	1	17.67	1.34	.26	.07	.28
Time x Caffeine	0.37	1	0.37	0.05	.82	.00	.06
Time x Stress	3.51	1	3.51	0.49	.49	.02	.10
Time x Caffeine x Stress	15.66	1	15.66	2.19	.15	.08	.30
Error (Measure)	421.27	27	15.60				
Error (Time)	193.58	27	7.17				

*Indicates $p \leq .05$

Table 21: Omnibus RMANCOVA of ASR Trial 120 dB Startle (82 dB pre-pulse)

Overall RMANCOVA of ASR - Trial 120 dB startle/ 82 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	91.29	1	91.29	5.33	.03	.17	.61
Baseline Startle	698.39	1	698.39	40.81	.00*	.60	1.00
Time x Baseline Startle	7.89	1	7.89	1.14	.30	.04	.18
Caffeine	4.15	1	4.15	0.24	.63	.01	.08
Stress	0.13	1	0.13	0.01	.93	.00	.05
Time	1.97	1	1.97	0.28	.60	.01	.08
Caffeine x Stress	0.83	1	0.83	0.05	.83	.00	.06
Time x Caffeine	1.12	1	1.12	0.16	.69	.01	.07
Time x Stress	1.69	1	1.69	0.24	.63	.01	.08
Time x Caffeine x Stress	19.04	1	19.04	2.74	.11	.09	.36
Error (Measure)	462.11	27	17.12				
Error (Time)	187.69	27	6.95				

*Indicates $p \leq .05$

Table 22: Time One ANCOVA of ASR Trial 110 dB Startle (68 dB pre-pulse)

ANCOVA of ASR - Trial 110 dB/68 dB at Time One							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	110.02	1	110.02	15.48	.00	0.36	.97
Baseline Initial Startle	215.65	1	215.65	30.35	.00*	0.53	1.00
Caffeine	9.82	1	9.82	1.38	.25	0.05	.21
Stress	3.70	1	3.70	0.52	.48	0.02	.11
Caffeine x Stress	0.05	1	0.05	0.01	.94	0.00	.05
Error	191.88	27	7.11				
Total	4625.22	32					

*Indicates $p \leq .05$

Table 23: Time Two ANCOVA of ASR Trial 110 dB Startle (68 dB pre-pulse)

ANCOVA of ASR - Trial 110 dB/68 dB at Time Two							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	30.68	1	30.68	2.70	.11	0.09	0.35
Baseline Startle	360.19	1	360.19	31.66	.00*	0.54	1.00
Caffeine	0.55	1	0.55	0.05	.83	0.00	.06
Stress	34.29	1	34.29	3.01	.09	0.10	.39
Caffeine x Stress	37.97	1	37.97	3.34	.08	0.11	.42
Error	307.20	27	11.38				
Total	4601.12	32					

*Indicates $p \leq .05$

Table 24: Pre-Pulse Inhibition Percentage Descriptives

Descriptives of %PPI (ASR) – Time x Group				
	Trial Type	BL Mean (SD)	T1 Mean (SD)	T2 Mean (SD)
Control	110 dB (68 dB)	6.46 (23.66)	-4.20 (32.62)	3.31 (30.10)
	110 dB (82 dB)	-0.24 (16.82)	-6.63 (19.72)	3.92 (30.85)
	120 dB (68 dB)	3.79 (24.46)	-0.97 (12.67)	-0.18 (12.55)
	120 dB (82 dB)	2.59 (35.48)	-4.08 (17.44)	11.69 (5.96)
Caffeine Only	110 dB (68 dB)	10.82 (21.61)	-6.20 (22.19)	13.19 (15.76)
	110 dB (82 dB)	5.62 (17.61)	-4.79 (17.16)	2.47 (12.63)
	120 dB (68 dB)	0.48 (23.75)	11.82 (16.55)	4.45 (15.99)
	120 dB (82 dB)	5.82 (12.95)	10.02 (23.32)	13.26 (3.67)
Stress Only	110 dB (68 dB)	-10.21 (24.14)	-6.15 (21.76)	-2.22 (11.91)
	110 dB (82 dB)	-4.69 (27.61)	6.85 (19.72)	-1.66 (16.73)
	120 dB (68 dB)	4.76 (11.20)	-4.47 (42.67)	0.85 (12.39)
	120 dB (82 dB)	2.39 (20.23)	4.04 (21.05)	13.55 (6.24)
Stress + Caffeine	110 dB (68 dB)	-1.70 (19.03)	-21.11 (23.46)	1.36 (17.95)
	110 dB (82 dB)	1.08 (24.50)	-12.98 (21.61)	-3.30 (10.92)
	120 dB (68 dB)	2.54 (15.39)	4.80 (14.16)	1.14 (23.44)
	120 dB (82 dB)	0.77 (16.09)	-0.62 (20.09)	12.38 (6.03)

Table 25: Omnibus RMANCOVA of %PPI (110 dB startle, 68 dB pre-pulse)

Overall RMANCOVA of %PPI (ASR) - Trial 110 dB startle/ 68 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	359.35	1	359.35	0.72	.40	0.03	0.13
Baseline %PPI	2184.19	1	2184.19	4.36	.05*	0.14	0.52
Caffeine	13.67	1	13.67	0.03	.87	0.00	0.05
Stress	2283.36	1	2283.36	4.56	.04*	0.14	0.54
Caffeine x Stress	286.05	1	286.05	0.57	.46	0.02	0.11
Time	2780.53	1	2780.53	5.51	.03*	0.17	0.62
Time x Baseline %PPI	52.26	1	52.26	0.10	.75	0.00	0.06
Time x Caffeine	838.69	1	838.69	1.66	.21	0.06	0.24
Time x Stress	3.74	1	3.74	0.01	.93	0.00	0.05
Time x Caffeine x Stress	39.66	1	39.66	0.08	.78	0.00	0.06
Error (Measure)	13525.45	27	500.94				
Error (Time)	13629.00	27	504.78				
*Indicates $p \leq .05$							

Table 26: Omnibus RMANCOVA of %PPI (110 dB startle, 82 dB pre-pulse)

Overall RMANCOVA of %PPI (ASR) - Trial 110 dB startle/ 82 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	240.30	1	240.30	0.62	.44	.02	.12
Baseline %PPI	821.14	1	821.14	2.13	.16	.07	.29
Caffeine	285.43	1	285.43	0.74	.40	.03	.13
Stress	82.99	1	82.99	0.22	.65	.01	.07
Time	379.29	1	379.29	0.92	.35	.03	.15
Caffeine x Stress	17.67	1	17.67	1.34	.26	.07	.28
Time x Caffeine	320.25	1	320.25	0.78	.39	.03	.14
Time x Stress	360.92	1	360.92	0.88	.36	.03	.15
Time x Caffeine x Stress	461.12	1	461.12	1.12	.30	.04	.18
Error (Measure)	11102.78	27	411.21				
Error (Time)	193.58	27	7.17				

Table 27: Omnibus RMANCOVA of %PPI (120 dB startle, 68 dB pre-pulse)

Overall RMANCOVA of %PPI (ASR) - Trial 120 dB startle/ 68 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	261.33	1	261.33	0.51	.48	.02	.11
Baseline %PPI	0.28	1	0.28	0.00	.98	.00	.05
Caffeine	780.89	1	780.89	1.52	.23	.05	.22
Stress	139.78	1	139.78	0.27	.61	.01	.08
Time	43.05	1	43.05	0.11	.75	.00	.06
Caffeine x Stress	17.67	1	17.67	1.34	.26	.07	.28
Time x Caffeine	281.58	1	281.58	0.70	.41	.03	.13
Time x Stress	42.75	1	42.75	0.11	.75	.00	.06
Time x Caffeine x Stress	0.01	1	0.01	0.00	.99	.00	.05
Error (Measure)	13858.02	27	513.26				
Error (Time)	10847.91	27	401.77				

Table 28: Omnibus RMANCOVA of %PPI (120 dB startle, 82 dB pre-pulse)

Overall RMANCOVA of %PPI (ASR) – Trial 120 dB startle/ 82 dB pre-pulse							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	237.71	1	237.71	0.54	.47	.02	.11
Baseline %PPI	495.53	1	495.53	1.13	.30	.04	.18
Caffeine	16.38	1	16.38	0.04	.85	.00	.05
Stress	23.57	1	23.57	0.05	.82	.00	.06
Time	0.04	1	0.04	0.00	.99	.00	.05
Caffeine x Stress	489.08	1	489.08	1.11	.30	.04	.05
Time x Caffeine	206.06	1	206.06	0.49	.49	.02	.10
Time x Stress	69.66	1	69.66	0.17	.69	.01	.07
Time x Caffeine x Stress	193.64	1	193.64	0.46	.50	.02	.10
Error (Measure)	11854.69	27	439.06				
Error (Time)	11276.73	27	417.66				

Table 29: Time One ANCOVA of %PPI (110 dB startle, 68 dB pre-pulse)

ANCOVA of %PPI (ASR) – Trial 110 dB/68 dB at Time One							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	2569.53	1	2569.53	4.18	.05	0.13	.50
Baseline %PPI	1456.09	1	1456.09	2.37	.14	0.08	.32
Caffeine	319.11	1	319.11	0.52	.48	0.02	.11
Stress	1235.90	1	1235.90	2.01	.17	0.07	.28
Caffeine x Stress	269.37	1	269.37	0.44	.51	0.02	.10
Error	16606.33	27	615.05				
Total	22379.75	32					

Table 30: Time Two ANCOVA of %PPI (110 dB startle, 68 dB pre-pulse)

ANCOVA of %PPI (ASR) – Trial 110 dB/68 dB at Time Two							
Source	Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	570.35	1	570.35	1.46	.24	0.05	.21
Baseline %PPI	780.37	1	780.37	2.00	.17	0.07	.28
Caffeine	533.25	1	533.25	1.37	.25	0.05	.20
Stress	1051.20	1	1051.20	2.69	.11	0.09	.35
Caffeine x Stress	56.34	1	56.34	0.14	.71	0.01	.07
Error	10548.12	27	390.67				
Total	12862.71	32					

APPENDIX C: Administrative Documents



UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
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April 2, 2014

MEMORANDUM FOR DR. NEIL GRUNBERG, DEPARTMENT OF MEDICAL AND
CLINICAL PSYCHOLOGY

SUBJECT: IACUC Approval of Protocol – Initial Review

The following application was reviewed and approved by the Uniformed Services University of the Health Sciences (USUHS) Institutional Animal Care and Use Committee (IACUC) via Designated Member Review on April 2, 2014:

Title of Application: “Behavioral investigations of nicotine and caffeine in rats (*Rattus norvegicus*)”

USUHS Protocol Number: MPS-14-898

Expiration Date: April 1, 2017

Supporting Grant(s) Number: E072194414

Name of Principal Investigator: Dr. Neil Grunberg

The USUHS has an Animal Welfare Assurance on file with the Office for Laboratory Animal Welfare (OLAW), National Institutes of Health (NIH). The Assurance Number is A3448-01. The IACUC approved the above referenced application as submitted.

An annual review is required for each of the three years of this protocol. This review must be completed by the anniversary date of the protocol. If work is to be continued past the expiration date, a triennial review must be completed prior to the expiration date in order for work to be uninterrupted. Protocol expiration dates may not be extended, and no animal work may be done without an approved protocol. Although the IACUC may send reminders, it is the investigator's responsibility to submit an annual review form (Form 3206A) at least 30 days in advance, or a new Form 3206 for triennial review at least 60 days in advance of expiration.

Prior to placing your first animal order, please contact MAJ. Amanda Christy to schedule a pre-protocol planning meeting (295-3708). This meeting must occur to ensure animal numbers are loaded in the CART system and LAM resources are available to meet your needs.


Brian M. Cox, Ph.D.
Chair, Institutional Animal
Care and Use Committee

cc: Office of Research

USUHS FORM 3206B
ANIMAL STUDY PROTOCOL
(modification/addendum)

Animal Protocol Number: MPS-14-898
Grant Number: E072194414
Principal Investigator: Neil E. Grunberg, Ph.D.
Department: MPS
Phone: 295-9673

Animal Protocol Title: Behavioral investigations of nicotine and caffeine in rats (*Rattus norvegicus*)
(If animal species not identified in title, list parenthetically after title, i.e., rat, mice, etc.)

Grant Title (if different from above): Behavioral effects of stress, nicotine, and caffeine in male and female rats

Description of the proposed modification:

Significant changes to an Animal Study Proposal must be submitted to the USUHS IACUC for review and subsequent coordination for approval. Significant changes include, but are not limited to, a change in one or more of the overall aims or objectives of the study which would affect animal use; an increase in animal pain, distress or discomfort; a change of principal investigator; a change or addition of personnel having a role in the care and handling of animals as approved in the original proposal; a significant change in the animal number; a major change in procedures, surgery, or treatment; addition of the use of hazardous or radioactive materials; a change in species.

Explain the proposed modification(s) to the study. Include a brief description of the reasons for the change(s). Use additional sheets if necessary.

We request the following changes to our protocol:

1. Use of 24 pilot animals to assess preferable route of administration.
2. Change in vehicle of administration for caffeine from drinking water to Jell-O.
3. Addition of the elevated plus maze (EPM) as another behavioral measure of anxiety-related behaviors.
4. Separate housing for male and female animals.

(1, 2) Based on an additional literature review, we would like to pilot drinking water versus Jell-O as potential routes of administration for caffeine. The purpose of this investigation is to enhance experimental control. Based on the lack of precedence of Jell-O administered caffeine, we request the pilot animals to determine appropriate dosage levels, tolerance effects, and confirm whether this method of administration is preferable to drinking water. Two mL of sugar-free Blackcurrent Jell-O will be mixed with the assigned amount of caffeine. The Jell-O will be placed in a small bowl or cup in the rat's cage. These methods are consistent with Leach and colleagues' (2010) study involving Jell-O administration. We would also like to add an acute caffeine administration via oral syringe. Animals are trained to drink out of the syringe. This method was identified as an effective substitute to intrusive methods of involuntary oral dosing (e.g., Schleimer et al., 2005; Atcha et al., 2010). To better model the warrior stress experience, we want to include both chronic caffeine administration as well as acute doses prior to stressors.

To serve these aims, a 2 (female versus male) x 2 (Jell-O versus drinking water) x 2 (control

versus caffeine) factorial design will be used, with 3 animals per cell. Table 1 describes the planned dosing methods that will be used to compare the routes of administration and assess tolerance effects. The pilot animals will be housed for two weeks. Data from the elevated plus maze and open field activity will be collected at multiple time-points to observe behavioral effects of different caffeine administrations. These data will be used to make a final decision on which route of administration to use for the study. Time animals will be tested will not exceed 1 hour/day (only 1 behavior/day).

Table 1: Caffeine dosage plan for pilot animals

Route	Jell-O	Drinking water	Caffeine dosage
Acute dose	20 mg/kg Jell-O 30 min before behaviors	20 mg/kg syringe (oral) 30 min before behaviors	20 mg/kg in a 2 mL 5% aqueous sucrose solution
Chronic dose	2 x 40 mg/kg Jell-O per day	Ad libitum (1 g/L; avg 100 mg/kg/day)	80-100 mg/kg

(3) We would like to add the elevated plus maze (EPM; Pellow et al., 1985) as an additional behavioral measure. EPM is an established measure of anxiety-related behavior in rodents. The addition of this measure is consistent with the initial study aim to investigate the impact of caffeine on the stress response as demonstrated by various proxies of anxiety in rats. We would like additional measures of anxiety-related behavior to enhance validity of our study. EPM requires that the animals are recorded in the apparatus for five-minutes at each data-point. The EPM apparatus consists of two intersecting wooden bars, which create four arms. Two arms are closed in by walls and the other two arms are open. Anxiety-related behavior in EPM is demonstrated by the longer time spent in the enclosed spaces and less entries into the open arms. After the animals are recorded for five minutes in the apparatus, they are returned to their home cages. For the current study, we plan to collect EPM data across four time-points (two baseline measures & two post-stressor measures). The additional impact on the animals in terms of time and pain is negligible. This measure has been used previously in our lab for over ten years (e.g., Elliott et al., 2004; Elliott et al., 2005).

(4) We would like separate housing due to rats' highly sensitive olfactory senses and the influence opposite-sex pheromones may have on the behavior of both male and female rats (e.g., Zufall & Leinders-Zufall, 2007). We have concerns that this influence may confound our results.

References

- Atcha, Z., Rourke, C., Neo, A. H., Goh, C. W., Lim, J. S., Aw, C. C., ... & Pemberton, D. J. (2010). Alternative method of oral dosing for rats. *Journal of the American Association for Laboratory Animal Science: JAALAS*, 49(3), 335.
- Elliott, B. M., Faraday, M. M., Phillips, J. M., & Grunberg, N. E. (2004). Effects of nicotine on elevated plus maze and locomotor activity in male and female adolescent and adult rats. *Pharmacology Biochemistry and Behavior*, 77(1), 21-28.
- Elliott, B. M., Faraday, M. M., Phillips, J. M., & Grunberg, N. E. (2005). Adolescent and adult female rats differ in sensitivity to nicotine's activity effects. *Pharmacology Biochemistry and Behavior*, 80(4), 567-575.
- Leach, M. C., Forrester, A. R., & Flecknell, P. A. (2010). Influence of preferred foodstuffs on the antinociceptive effects of orally administered buprenorphine in laboratory rats. *Laboratory Animals*, 44(1), 54-58.
- Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, 14(3), 149-167.
- Schleimer, S. B., Johnston, G. A., & Henderson, J. M. (2005). Novel oral drug administration in an animal model of neuroleptic therapy. *Journal of neuroscience methods*, 146(2), 159-164.

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REFERENCES

1. Vasterling JJ, Proctor SP, Friedman MJ, Hoge CW, Heeren T, *et al.*. 2010. PTSD symptom increases in Iraq deployed soldiers: Comparison with nondeployed soldiers and associations with baseline symptoms, deployment experiences, and postdeployment stress. *Journal of Traumatic Stress* 23:41-51
2. Milliken CS, Auchterlonie JL, Hoge CW. 2007. Longitudinal assessment of mental health problems among active and reserve component soldiers returning from the Iraq war. *Jama* 298:2141-8
3. Ramchand R, Schell TL, Karney BR, Osilla KC, Burns RM, Caldarone LB. 2010. Disparate prevalence estimates of PTSD among service members who served in Iraq and Afghanistan: possible explanations. *Journal of Traumatic Stress* 23:59-68
4. Lieberman HR, Stavinoha T, McGraw S, White A, Hadden L, Marriott BP. 2012. Caffeine use among active duty US Army soldiers. *Journal of the Academy of Nutrition and Dietetics* 112:902-12, 12 e1-4
5. Oria M, Greenwood M. 2008. *Use of dietary supplements by military personnel*. National Academies Press: Washington, DC
6. McLellan TM, Kamimori GH, Bell DG, Smith IF, Johnson D, Belenky G. 2005. Caffeine maintains vigilance and marksmanship in simulated urban operations with sleep deprivation *Aviation, Space, and Environmental Medicine* 76:39-45
7. Tharion WJ, Shukitt-Hale B, Lieberman HR. 2003. Caffeine Effects on Marksmanship During High-Stress Military Training with 72 Hour Sleep Deprivation. *Aviation, Space, and Environmental Medicine* 74:309-14
8. Lohi JJ, Huttunen KH, Lahtinen T, Kilpeläinen AA, Muhli AA, Leino TK. 2007. Effect of caffeine on simulator flight performance in sleep-deprived military pilot students. *Military Medicine* 172:982-7
9. Tilghman A, McGarry B. March 17, 2010. Medicating the military. *Army Times* <http://archive.armytimes.com/article/20100317/NEWS/3170315/Medicating-military>
10. Correll D. 2013. *Terrifying Our Soldiers: Stimulant-Induced PTSD in the Armed Forces*. Trinity College Digital Repository: Hartford, CT <http://digitalrepository.trincoll.edu/cgi/viewcontent.cgi?article=1018&context=trinitypapers>
11. Friedman RA. April, 2012. Why are we drugging our soldiers. *The New York Times* http://www.nytimes.com/2012/04/22/opinion/sunday/why-are-we-drugging-our-soldiers.html?_r=0
12. Russo MB. 2007. Recommendations for the ethical use of pharmacologic fatigue countermeasures in the US military. *Aviation, Space and Environmental Medicine* 78:B119-B27
13. Bizarro L, Patel S, Murtagh C, Stolerman I. 2004. Differential effects of psychomotor stimulants on attentional performance in rats: nicotine, amphetamine, caffeine and methylphenidate. *Behavioural Pharmacology* 15:195-206

14. Lara DR. 2010. Caffeine, mental health, and psychiatric disorders. *Journal of Alzheimer's Disease* 20:239-48
15. Juliano LM, Griffiths RR. 2004. A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology* 176:1-29
16. Ogawa N, Ueki H. 2007. Clinical importance of caffeine dependence and abuse. *Psychiatry and Clinical Neurosciences* 61:263-8
17. Juliano LM, Evatt DP, Richards BD, Griffiths RR. 2012. Characterization of individuals seeking treatment for caffeine dependence. *Psychology of Addictive Behaviors* 26:948-954
18. Baum A, Gatchel RJ, Krantz DS. 1997. *An Introduction to Health Psychology*. McGraw-Hill: New York, NY
19. McEwen BS, Gianaros PJ. 2011. Stress-and allostasis-induced brain plasticity. *Annual Review of Medicine* 62:431-445
20. Juster R-P, McEwen BS, Lupien SJ. 2010. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience & Biobehavioral Reviews* 35:2-16
21. American Psychiatric Association (APA). 2013. *The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition: DSM 5*. APA: Washington, DC
22. Riebe CJ, Wotjak CT. 2011. Endocannabinoids and stress. *Stress* 14:384-97
23. Boonstra R. 2013. Reality as the leading cause of stress: rethinking the impact of chronic stress in nature. *Functional Ecology* 27:11-23
24. McEwen BS, Wingfield JC. 2003. The concept of allostasis in biology and biomedicine. *Hormones and Behavior* 43:2-15
25. McEwen BS, Stellar E. 1993. Stress and the individual: mechanisms leading to disease. *Archives of Internal Medicine* 153:2093-101
26. Hoge CW, Auchterlonie JL, Milliken CS. 2006. Mental health problems, use of mental health services, and attrition from military service after returning from deployment to Iraq or Afghanistan. *Jama* 295:1023-32
27. Seal KH, Bertenthal D, Miner CR, Sen S, Marmar C. 2007. Bringing the war back home: Mental health disorders among US veterans returning from Iraq and Afghanistan seen at Department of Veterans Affairs Facilities. *Archives of Internal Medicine* 167:476-82
28. Vasterling JJ, Proctor SP. 2011. Understanding the neuropsychological consequences of deployment stress: a public health framework. *Journal of the International Neuropsychological Society* 17:1-6
29. Frary CD, Johnson RK, Wang MQ. 2005. Food sources and intakes of caffeine in the diets of persons in the United States. *Journal of the American Dietetic Association* 105:110-3
30. Horton JL, Phillips CJ, White MR, LeardMann CA, Crum-Cianflone NF. 2014. Trends in New US Marine Corps Accessions During the Recent Conflicts in Iraq and Afghanistan. *Military medicine* 179:62-70
31. Insitute of Medicine (IOM) Committee on Military Nutrition Research. 2001. *Caffeine for the sustainment of mental task performance: Formulations for military operations*. National Academies Press

32. Fredholm BB. 1980. Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends in Pharmacological Sciences* 1:129-32
33. Nehlig A, Daval J-L, Debry G. 1992. Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Research Reviews* 17:139-70
34. Nehlig A. 2004. *Coffee, tea, chocolate, and the brain* ed. Prasad, C. CRC Press: Boca Raton, FL
35. Daly JW, Bruns RF, Snyder S. 1981. Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. *Life Sciences* 28:2083-97
36. Fisone G, Borgkvist A, Usiello A. 2004. Caffeine as a psychomotor stimulant: mechanism of action. *Cellular and Molecular Life Sciences CMLS* 61:857-72
37. Ferré S. 2008. An update on the mechanisms of the psychostimulant effects of caffeine. *Journal of neurochemistry* 105:1067-79
38. Walton C, Kalmar JM, Cafarelli E. 2002. Effect of caffeine on self- sustained firing in human motor units. *The Journal of Physiology* 545:671-9
39. Winston AP, Hardwick E, Jaber N. 2005. Neuropsychiatric effects of caffeine. *Advances in Psychiatric treatment* 11:432-9
40. Smith A. 2002. Effects of caffeine on human behavior. *Food and Chemical Toxicology* 40:1243-55
41. Walton K, Dorne J, Renwick A. 2001. Uncertainty factors for chemical risk assessment: interspecies differences in the in vivo pharmacokinetics and metabolism of human CYP1A2 substrates. *Food and Chemical Toxicology* 39:667-80
42. Jacobson KA, Gao Z-G. 2006. Adenosine receptors as therapeutic targets. *Nature Reviews Drug Discovery* 5:247-64
43. Butt MS, Sultan MT. 2011. Coffee and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition* 51:363-73
44. Floegel A, Pischon T, Bergmann MM, Teucher B, Kaaks R, Boeing H. 2012. Coffee consumption and risk of chronic disease in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Germany study. *The American Journal of Clinical Nutrition* 95:901-8
45. Addicott MA, Laurienti PJ. 2009. A comparison of the effects of caffeine following abstinence and normal caffeine use. *Psychopharmacology* 207:423-31
46. Einöther SJ, Giesbrecht T. 2013. Caffeine as an attention enhancer: reviewing existing assumptions. *Psychopharmacology* 225:251-74
47. Warot D, Corruble E, Payan C, Weil J. 1993. Subjective effects of modafinil, a new central adrenergic stimulant in healthy volunteers: A comparison with amphetamine, caffeine and placebo. *European Psychiatry* 8:201-208
48. Killgore WD, Kahn-Greene ET, Grugle NL, Killgore DB, Balkin TJ. 2009. Sustaining executive functions during sleep deprivation: a comparison of caffeine, dextroamphetamine, and modafinil. *Sleep* 32:205-216
49. Buguet A, Moroz DE, Radomski MW. 2003. Modafinil—medical considerations for use in sustained operations. *Aviation, Space, and Environmental Medicine* 74:659-63

50. Estrada A, Kelley AM, Webb CM, Athy JR, Crowley JS. 2012. Modafinil as a replacement for dextroamphetamine for sustaining alertness in military helicopter pilots. *Aviation, space, and environmental medicine* 83:556-67
51. Zolkowska D, Jain R, Rothman RB, Partilla JS, Roth BL, *et al.*. 2009. Evidence for the involvement of dopamine transporters in behavioral stimulant effects of modafinil. *Journal of Pharmacology and Experimental Therapeutics* 329:738-46
52. Odenwald M, Hinkel H, Schauer E, Schauer M, Elbert T, *et al.*. 2009. Use of khat and posttraumatic stress disorder as risk factors for psychotic symptoms: a study of Somali combatants. *Social Science & Medicine* 69:1040-8
53. Nehlig A. 1999. Are we dependent upon coffee and caffeine? A review on human and animal data. *Neuroscience & Biobehavioral Reviews* 23:563-76
54. Gillingham R, Keefe AA, Keillor J, Tikuisis P. 2003. Effect of caffeine on target detection and rifle marksmanship. *Ergonomics* 46:1513-30
55. Reynolds AC, Banks S. 2010. Total sleep deprivation, chronic sleep restriction and sleep disruption. *Progress in Brain Research* 185:91-103
56. Meerlo P, Sgoifo A, Suchecki D. 2008. Restricted and disrupted sleep: effects on autonomic function, neuroendocrine stress systems and stress responsivity. *Sleep Medicine Reviews* 12:197-210
57. Meerlo P, Mistlberger RE, Jacobs BL, Heller HC, McGinty D. 2009. New neurons in the adult brain: the role of sleep and consequences of sleep loss. *Sleep Medicine Reviews* 13:187-94
58. Miller NL, Shattuck LG, Matsangas P. 2011. Sleep and fatigue issues in continuous operations: a survey of US Army officers. *Behavioral Sleep Medicine* 9:53-65
59. McLay RN, Klam WP, Volkert SL. 2010. Insomnia is the most commonly reported symptom and predicts other symptoms of post-traumatic stress disorder in US service members returning from military deployments. *Military Medicine* 175:759-62
60. Roman V, Hagewoud R, Luiten PG, Meerlo P. 2006. Differential effects of chronic partial sleep deprivation and stress on serotonin 1A and muscarinic acetylcholine receptor sensitivity. *Journal of Sleep Research* 15:386-94
61. Åkerstedt T. 2006. Psychosocial stress and impaired sleep. *Scandinavian Journal of Work, Environment & Health* 6:493-501
62. Porkka-Heiskanen T, Alanko L, Kalinchuk A, Stenberg D. 2002. Adenosine and sleep. *Sleep Medicine Reviews* 6:321-32
63. Huck NO, McBride SA, Kendall AP, Grugle NL, Killgore WD. 2008. The effects of modafinil, caffeine, and dextroamphetamine on judgments of simple versus complex emotional expressions following sleep deprivation. *International Journal of Neuroscience* 118:487-502
64. Roehrs T, Roth T. 2008. Caffeine: sleep and daytime sleepiness. *Sleep medicine reviews* 12:153-62
65. Shiffrin RM, Schneider W. 1977. Controlled and automatic human information processing: II. Perceptual learning, automatic attending and a general theory. *Psychological Review* 84:127-138

66. Bar-Haim Y, Lamy D, Pergamin L, Bakermans-Kranenburg MJ, Van Ijzendoorn MH. 2007. Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychological Bulletin* 133:1-24
67. Cisler JM, Koster EH. 2010. Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. *Clinical Psychology Review* 30:203-16
68. Taylor CT, Bomyea J, Amir N. 2011. Malleability of attentional bias for positive emotional information and anxiety vulnerability. *Emotion* 11:127-138
69. Ochsner KN, Gross JJ. 2005. The cognitive control of emotion. *Trends in Cognitive Sciences* 9:242-9
70. Mitchell DG, Nakic M, Fridberg D, Kamel N, Pine D, Blair R. 2007. The impact of processing load on emotion. *Neuroimage* 34:1299-309
71. Amir N, Beard C, Taylor CT, Klumpp H, Elias J, *et al.*. 2009. Attention training in individuals with generalized social phobia: A randomized controlled trial. *Journal of Consulting and Clinical Psychology* 77:961-973
72. Bomyea J, Amir N. 2011. The effect of an executive functioning training program on working memory capacity and intrusive thoughts. *Cognitive Therapy and Research* 35:529-35
73. Eysenck MW, Derakshan N, Santos R, Calvo MG. 2007. Anxiety and cognitive performance: attentional control theory. *Emotion* 7:336-353
74. Jha AP, Stanley EA, Kiyonaga A, Wong L, Gelfand L. 2010. Examining the protective effects of mindfulness training on working memory capacity and affective experience. *Emotion* 10:54-64
75. Carlsen AN, Maslovat D, Lam MY, Chua R, Franks IM. 2011. Considerations for the use of a startling acoustic stimulus in studies of motor preparation in humans. *Neuroscience & Biobehavioral Reviews* 35:366-76
76. Koch M, Schnitzler H-U. 1997. The acoustic startle response in rats—circuits mediating evocation, inhibition and potentiation. *Behavioural Brain Research* 89:35-49
77. Swerdlow NR, Sprock J, Light GA, Cadenhead K, Calkins ME, *et al.*. 2007. Multi-site studies of acoustic startle and prepulse inhibition in humans: initial experience and methodological considerations based on studies by the Consortium on the Genetics of Schizophrenia. *Schizophrenia Research* 92:237-51
78. Swerdlow NR, Braff DL, Taaid N, Geyer MA. 1994. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Archives of General Psychiatry* 51:139-54
79. Davis M, Walker DL, Lee Y. 1997. Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex. *Annals of the New York Academy of Sciences* 821:305-31
80. Davis M. 2006. Neural systems involved in fear and anxiety measured with fear-potentiated startle. *American Psychologist* 61:741-756
81. Grillon C, Pellowski M, Merikangas KR, Davis M. 1997. Darkness facilitates the acoustic startle reflex in humans. *Biological Psychiatry* 42:453-60
82. Dunning JP, Auriemmo A, Castille C, Hajcak G. 2010. In the face of anger: Startle modulation to graded facial expressions. *Psychophysiology* 47:874-8
83. Hawk LW, Cook EW. 2000. Independence of valence modulation and prepulse inhibition of startle. *Psychophysiology* 37:5-12

84. Dixit A, Goyal A, Thawani R, Vaney N. 2012. Effect of caffeine on information processing: evidence from stroop task. *Indian Journal of Psychological Medicine* 34:218-22
85. Yehuda R, LeDoux J. 2007. Response variation following trauma: a translational neuroscience approach to understanding PTSD. *Neuron* 56:19-32
86. Vaiva G, Ducrocq F, Jezequel K, Averland B, Lestavel P, *et al.*. 2003. Immediate treatment with propranolol decreases posttraumatic stress disorder two months after trauma. *Biological Psychiatry* 54:947-9
87. Henry M, Fishman JR, Youngner SJ. 2007. Propranolol and the prevention of post-traumatic stress disorder: Is it wrong to erase the “sting” of bad memories? *The American Journal of Bioethics* 7:12-20
88. Lorist MM, Tops M. 2003. Caffeine, fatigue, and cognition. *Brain and Cognition* 53:82-94
89. Domschke K, Gajewska A, Winter B, Herrmann MJ, Warrings B, *et al.*. 2012. ADORA2A Gene variation, caffeine, and emotional processing: a multi-level interaction on startle reflex. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 37:759-69
90. Flaten MA, Elden Å. 1999. Caffeine and prepulse inhibition of the acoustic startle reflex. *Psychopharmacology* 147:322-30
91. Andrews SE, Blumenthal TD, Flaten MA. 1998. Effects of caffeine and caffeine-associated stimuli on the human startle eyeblink reflex. *Pharmacology Biochemistry and Behavior* 59:39-44
92. Schicatano EJ, Blumenthal TD. 1998. The effects of caffeine and directed attention on acoustic startle habituation. *Pharmacology Biochemistry and Behavior* 59:145-50
93. Vasterling JJ, Proctor SP, Amoroso P, Kane R, Heeren T, White RF. 2006. Neuropsychological outcomes of army personnel following deployment to the Iraq war. *Jama* 296:519-29
94. Sayer N, Noorbaloochi S, Frazier P, Carlson K, Gravely A, Murdoch M. 2010. Reintegration problems and treatment interests among Iraq and Afghanistan combat veterans receiving VA medical care. *Psychiatric Services* 61:589-97
95. Grillon C, Baas J. 2003. A review of the modulation of the startle reflex by affective states and its application in psychiatry. *Clinical Neurophysiology* 114:1557-79
96. Grillon C, Morgan CA, Davis M, Southwick SM. 1998. Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. *Biological Psychiatry* 44:1027-36
97. Grillon C, Morgan CA, Southwick SM, Davis M, Charney DS. 1996. Baseline startle amplitude and prepulse inhibition in Vietnam veterans with posttraumatic stress disorder. *Psychiatry Research* 64:169-78
98. Grillon C, Morgan CA. 1999. Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *Journal of Abnormal Psychology* 108:134-142
99. Orr SP, Metzger LJ, Lasko NB, Macklin ML, Hu FB, *et al.*. 2003. Physiologic responses to sudden, loud tones in monozygotic twins discordant for combat

- exposure: association with posttraumatic stress disorder. *Archives of General Psychiatry* 60:283-8
100. Pole N, Neylan TC, Otte C, Henn-Hasse C, Metzler TJ, Marmar CR. 2009. Prospective prediction of posttraumatic stress disorder symptoms using fear potentiated auditory startle responses. *Biological Psychiatry* 65:235-40
 101. Stam R. 2007. PTSD and stress sensitisation: a tale of brain and body Part 2: animal models. *Neuroscience and Biobehavioral Reviews* 31:558-84
 102. Cohen H, Zohar J, Matar MA, Zeev K, Loewenthal U, Richter-Levin G. 2004. Setting apart the affected: the use of behavioral criteria in animal models of post traumatic stress disorder. *Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology* 29:1962-70
 103. Yarnell AM. 2013. *Characterization of psychological and biological factors in an animal model of Warrior Stress*. Doctoral Dissertation. Uniformed Services University of the Health Sciences, Bethesda, MD
 104. File SE, Baldwin HA, Johnston AL, Wilks LJ. 1988. Behavioral effects of acute and chronic administration of caffeine in the rat. *Pharmacology Biochemistry and Behavior* 30:809-15
 105. Rhoads DE, Huggler AL, Rhoads LJ. 2011. Acute and adaptive motor responses to caffeine in adolescent and adult rats. *Pharmacology Biochemistry and Behavior* 99:81-6
 106. Marin MT, Zancheta R, Paro AH, Possi AP, Cruz FC, Planeta CS. 2011. Comparison of caffeine-induced locomotor activity between adolescent and adult rats. *European Journal of Pharmacology* 660:363-7
 107. Diller JW, Saunders BT, Anderson KG. 2008. Effects of acute and repeated administration of caffeine on temporal discounting in rats. *Pharmacology Biochemistry and Behavior* 89:546-55
 108. Kosten TA, Ambrosio E. 2002. HPA axis function and drug addictive behaviors: insights from studies with Lewis and Fischer 344 inbred rats. *Psychoneuroendocrinology* 27:35-69
 109. Hughes RN, Hancock NJ, Henwood GA, Rapley SA. 2014. Evidence for anxiolytic effects of acute caffeine on anxiety-related behavior in male and female rats tested with and without bright light. *Behavioural Brain Research* 271:7-15
 110. Pechlivanova D, Tchekalarova J, Nikolov R, Yakimova K. 2010. Dose-dependent effects of caffeine on behavior and thermoregulation in a chronic unpredictable stress model of depression in rats. *Behavioural Brain Research* 209:205-11
 111. Pechlivanova DM, Tchekalarova JD, Alovera LH, Petkov VV, Nikolov RP, Yakimova KS. 2012. Effect of long-term caffeine administration on depressive-like behavior in rats exposed to chronic unpredictable stress. *Behavioural Pharmacology* 23:339-47
 112. Tarnopolsky M, Cupido C. 2000. Caffeine potentiates low frequency skeletal muscle force in habitual and nonhabitual caffeine consumers. *Journal of Applied Physiology* 89:1719-24
 113. Brunyé TT, Mahoney CR, Lieberman HR, Taylor HA. 2010. Caffeine modulates attention network function. *Brain and Cognition* 72:181-8
 114. Lorist MM, Snel J, Kok A, Mulder G. 1994. Influence of caffeine on selective attention in well-rested and fatigued subjects. *Psychophysiology* 31:525-34

115. Acri JB, Brown KJ, Saah MI, Grunberg NE. 1995. Strain and age differences in acoustic startle responses and effects of nicotine in rats. *Pharmacology Biochemistry and Behavior* 50:191-8
116. Faraday MM, O'Donoghue VA, Grunberg NE. 2003. Effects of nicotine and stress on locomotion in Sprague–Dawley and Long–Evans male and female rats. *Pharmacology Biochemistry and Behavior* 74:325-33
117. Yarnell AM. 2012. *A Neurobehavioral Phenotype of Blast Traumatic Blast Injury and Psychological Stress in Male and Female Rats*. Master's Thesis. Uniformed Services University of the Health Sciences, Bethesda, MD
118. Braun AA, Skelton MR, Vorhees CV, Williams MT. 2011. Comparison of the elevated plus and elevated zero mazes in treated and untreated male Sprague–Dawley rats: effects of anxiolytic and anxiogenic agents. *Pharmacology Biochemistry and Behavior* 97:406-15
119. Lim B-V, Jang M-H, Shin M-C, Kim H-B, Kim Y-J, *et al.*. 2001. Caffeine inhibits exercise-induced increase in tryptophan hydroxylase expression in dorsal and median raphe of Sprague–Dawley rats. *Neuroscience Letters* 308:25-8
120. Rosario LA, Abercrombie ED. 1999. Individual differences in behavioral reactivity: correlation with stress-induced norepinephrine efflux in the hippocampus of Sprague-Dawley rats. *Brain Research Bulletin* 48:595-602
121. Teague CR, Dhabhar FS, Barton RH, Beckwith-Hall B, Powell J, *et al.*. 2007. Metabonomic studies on the physiological effects of acute and chronic psychological stress in Sprague-Dawley rats. *Journal of Proteome Research* 6:2080-93
122. Faraday MM, Blakeman KH, Grunberg NE. 2005. Strain and sex alter effects of stress and nicotine on feeding, body weight, and HPA axis hormones. *Pharmacology Biochemistry and Behavior* 80:577-89
123. Faraday MM, Grunberg NE. 2000. The importance of acclimation in acoustic startle amplitude and pre-pulse inhibition testing of male and female rats. *Pharmacology Biochemistry and Behavior* 66:375-81
124. Lazenka MF, Moeller FG, Negus SS. 2015. Effects of caffeine and its metabolite paraxanthine on intracranial self-stimulation in male rats. *Experimental and Clinical Psychopharmacology* 23:71-80
125. Johansson S. 1981. Cardiovascular lesions in Sprague-Dawley rats induced by long-term treatment with caffeine. *Acta Pathologica Microbiologica Scandinavica Section A Pathology* 89:185-91
126. Spear LP. 2000. Neurobehavioral changes in adolescence. *Current Directions in Psychological Science* 9:111-4
127. Military One Source. 2012. 2011 Demographics: Profile of The Military Community, from militaryonesource.mil
128. Elliott BM, Grunberg NE. 2005. Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. *Behavioural Brain Research* 165:187-96
129. Prager EM, Bergstrom HC, Grunberg NE, Johnson LR. 2011. The importance of reporting housing and husbandry in rat research. *Frontiers in Behavioral Neuroscience* 5: 1-4

130. Sciencies TNRCoTNAo. 2010. Guide for the Care and Use of Laboratory Animals: Eight Edition. The National Academies Press: Washington, DC
131. Rhoads DE, Huggler AL, Rhoads LJ. 2011. Acute and adaptive motor responses to caffeine in adolescent and adult rats. *Pharmacology Biochemistry, and Behavior* 99:81-6
132. Leach MC, Forrester AR, Flecknell PA. 2010. Influence of preferred foodstuffs on the antinociceptive effects of orally administered buprenorphine in laboratory rats. *Laboratory Animals* 44:54-8
133. Schleimer SB, Johnston GA, Henderson JM. 2005. Novel oral drug administration in an animal model of neuroleptic therapy. *Journal of Neuroscience Methods* 146:159-64
134. Berardi A, Trezza V, Campolongo C. 2012. Modeling specific phobias and posttraumatic stress disorder in rodents: the challenge to convey both cognitive and emotional features. *Reviews in the Neurosciences* 23:645-57
135. Perry ME. 2009. *Effects of acute and recurrent stress during adolescence on subsequent indices of adult behavioral health*. Doctoral Dissertation. Uniformed Services University of the Health Sciences, Bethesda, MD
136. Hamilton KR. 2010. *Impulsive action, psychological stress, and behavioral sensitization to nicotine in a rat model of impulsivity*. Doctoral Dissertation. Uniformed Services University of the Health Sciences, Bethesda, MD
137. Masini C, Sauer S, Campeau S. 2005. Ferret odor as a processive stress model in rats: neurochemical, behavioral, and endocrine evidence. *Behavioral Neuroscience* 119:280-292
138. Yarnell AM, Chwa A, Hamilton KR, Grunberg NE. 2011. *Warrior Stress paradigm for rats*. USUHS Research Week, Bethesda, MD
139. Moosey MJ. 2014. *Effects of Nicotine and Stress on Anxiety-related and Depression-related Behavior in Rats*. Master's Thesis. Uniformed Services University of the Health Sciences Bethesda, MD
140. Rabat A, Bouyer J, Aran J, Courtiere A, Mayo W, Le Moal M. 2004. Deleterious effects of an environmental noise on sleep and contribution of its physical components in a rat model. *Brain Research* 1009:88-97
141. Rabat A, Bouyer J, Aran J, Le Moal M, Mayo W. 2005. Chronic exposure to an environmental noise permanently disturbs sleep in rats: inter-individual vulnerability. *Brain Research* 1059:72-82
142. Rabat A, Bouyer J, George O, Le Moal M, Mayo W. 2006. Chronic exposure of rats to noise: relationship between long-term memory deficits and slow wave sleep disturbances. *Behavioural Brain Research* 171:303-12
143. Denenberg VH. 1969. Open-Field Behavior in the Rat: What Does It Mean?. *Annals of the New York Academy of Sciences* 159:852-9
144. Katz R, Roth K, Carroll B. 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. *Neuroscience & Biobehavioral Reviews* 5:247-51
145. Whimbey AE, Denenberg VH. 1967. Two independent behavioral dimensions in open-field performance. *Journal of Comparative and Physiological Psychology* 63:500-504

146. Leussis MP, Bolivar VJ. 2006. Habituation in rodents: a review of behavior, neurobiology, and genetics. *Neuroscience & Biobehavioral Reviews* 30:1045-64
147. Swerdlow N, Caine S, Braff D, Geyer M. 1992. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. *Journal of Psychopharmacology* 6:176-90
148. Hartley S, Barbot F, Machou M, Lejaille M, Moreau B, *et al.*. 2013. Combined caffeine and bright light reduces dangerous driving in sleep-deprived healthy volunteers: A Pilot Cross-Over Randomised Controlled Trial. *Neurophysiologie Clinique/Clinical Neurophysiology* 43:161-9
149. Schmider E, Ziegler M, Danay E, Beyer L, Bühner M. 2010. Is it really robust? Reinvestigating the robustness of ANOVA against violations of the normal distribution assumption. *Methodology: European Journal of Research Methods for the Behavioral and Social Sciences* 6:147-151
150. Pettenuzzo LF, Noschang C, von Pozzer Toigo E, Fachin A, Vendite D, Dalmaz C. 2008. Effects of chronic administration of caffeine and stress on feeding behavior of rats. *Physiology & Behavior* 95:295-301
151. Noschang CG, Pettenuzzo LF, von Pozzer Toigo E, Andreazza AC, Krolow R, *et al.*. 2009. Sex-specific differences on caffeine consumption and chronic stress-induced anxiety-like behavior and DNA breaks in the hippocampus. *Pharmacology Biochemistry and Behavior* 94:63-9
152. Paterson L, Wilson S, Nutt D, Hutson P, Ivarsson M. 2009. Characterisation of the effects of caffeine on sleep in the rat: a potential model of sleep disruption. *Journal of Psychopharmacology* 23:475-86
153. Lau CE, Falk JL. 1994. Tolerance to oral and IP caffeine: locomotor activity and pharmacokinetics. *Pharmacology Biochemistry and Behavior* 48:337-44