Controlling for Acute Caffeine Intake in Cardiovascular Reactivity Research

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ABSTRACT (Academic)

Caffeine substantially affects cardiovascular functioning, yet wide variability exists in caffeine control procedures in cardiovascular reactivity (CVR) research. This study was conducted in order to identify a minimal abstention duration in habitual coffee consumers whereby CVR is unconfounded by caffeine; Six hours was hypothesized (average half-life). Thirty nine subjects (mean age: 20.9; 20 Women) completed a repeated measures study involving hand cold pressor (CP) and memory tasks. Caffeinated and decaffeinated coffee were administered. The following CV indices were acquired during baseline, task, and recovery epochs prior to coffee intake, 30 minutes-, and six hours post-intake: Heart rate (HR), high frequency heart rate variability (hfHRV), root mean squared successive differences (RMSSD), systolic and diastolic blood pressures (SBP, DBP), mean arterial pressure (MAP), pre-ejection period (PEP), left ventricular ejection time (LVET), and total peripheral resistance (TPR). Results support the adequacy of a six-hour abstention in controlling for caffeine-elicited CVR changes. The current study contributes to methodological endeavors in psychophysiology. Further investigations are crucial in establishing ideal caffeine controls, which would promote increasingly valid and reliable cross-study results.

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List of Abbreviations

Abbreviation	Definition
CVR	Cardiovascular reactivity
HR	Heart rate
IBI	Interbeat interval
hfHRV	High frequency heart rate variability
RMSSD	Root mean squared successive differences of R-R intervals
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
MAP	Mean arterial pressure
RR	Respiration rate
ICG	Impedance cardiography
PEP	Pre-ejection period
LVET	Left ventricular ejection time
TPR	Total peripheral resistance
CP	Cold pressor
DASS	Depression Anxiety and Stress Scale
HHQ	Health History Questionnaire
BMIS	Brief Mood Introspection Scale
BPQ	Body Perception Questionnaire

Controlling for Acute Caffeine Intake in Cardiovascular Reactivity Research

1. Introduction

The widely used pharmacological substance, caffeine, modulates myriad cognitive, mood, and motor variables as well as several variables associated with nervous, cardiac, and vascular functioning, which decades of research have strongly indicated. These aforementioned factors are critically important to consider in the practice of psychophysiological research, particularly in the conducting of research involving cardiovascular reactivity (CVR), since caffeine exerts several CV effects.

Despite this, there is a severe lack of consistent methodological control for caffeine. Studies of CVR reveal wide variation in methodological protocols for the numerous cardiovascular effects of this drug. Particularly, no standardized guidelines exist for caffeine abstinence length prior to studies of CVR. This leads to potentially confounded study results and possible difficulty in cross-comparisons of research findings.

The aim of the present study was to address this issue by identifying an ideal duration for participant abstention whereby the drug minimally affects CVR. The hypothesized duration (six hours) is based on the average half-life of the drug. Using a repeated measures design, comparison of CVR to two tasks (hand cold pressor (CP) and working memory) was made across three phases: baseline, 30 minutes- and six hours-following consumption of caffeinated coffee.

2. Literature Review

2.1. Variability in Pre-experimental Abstention

Despite several decades of research examining caffeine's myriad effects, existing literature reveals widely variable results, particularly concerning effects of caffeine on CV

functioning and reactivity. It is probable that this results from factors contributing to interindividual variability in CVR.

Disparate findings are also caused by methodological dissimilarities among investigations. It is likely that variability across studies in pre-experiment caffeine abstention times presents a major confound in CVR research in general. Due to the prevalent consumption of caffeine and its time-dependent effects on cardiovascular functioning, this highlights a considerable, yet largely understudied issue. Both within and across studies, wide between-participant variability with respect to levels of acute versus deprivation effects during experimental sessions likely presents significant methodological confounds. This concern extends beyond caffeine-related research, and to physiological research, more broadly.

2.2. Survey of CVR Journals

In order to explore the general status of commonly used control procedures for caffeine abstention, a review of recently published peer-reviewed studies in the area of CVR was performed (Grant, McGruder, & Friedman, 2016). This survey involved an active search through five prominent journals with studies examining CVR using any of the following measures and key words: *heart rate, heart rate variability, cardiac, blood pressure, impedance cardiography*. The following cited journals, commonly studying CVR, were used: *Psychosomatic Medicine*, *Biological Psychology*, *Journal of Psychosomatic Research*, *Psychophysiology*, and *International Journal of Psychophysiology*. Reviews, meta-analyses, prospective, and retrospective studies were excluded from the survey.

Published CVR studies in 2012, 2013, and 2014 revealed widely discrepant practices in caffeine control (Appendix A). Of the 361 identified studies using the named CV indices, only 82 studies (23%) explicitly mentioned pre-experiment caffeine abstention.

Therefore, it is unclear how or whether possible effects of caffeine were accounted for among the remaining 77% of investigations. Abstention durations for each year of published issues ranged from 1 to 24 hours (SD=7.12), indicating considerable variation across studies concerning how the potential confound of caffeine was controlled for.

The impetus for the current study was rooted in this evidence supporting this severe methodological deficiency. The extreme aforementioned variability in caffeine controls reflects the lack of standardization of caffeine controls in CVR research. As such, there exists a clear need for systematic study of the optimal abstention duration that balances the reduction of acute caffeine effects on CVR with the need to minimize withdrawal effects of caffeine abstention. This need is especially salient in view of the widespread use of caffeine, which is likely to characterize the subsample of the population studied in CVR research, which often included college students. Moreover, the need for such studies is highlighted by the possible inclusion of individuals with caffeine-related disorders and/or anxious symptomatology, as caffeine has anxiogenic potential (Lara, 2010).

Seemingly similar studies often report highly divergent results due in large part to lack of consideration for particular methodological characteristics, as many have theorized (Schneiderman & McCabe, 1989). Mixed results in the literature concerning health benefits and risks associated with caffeine are in large part due to this gap in this field of study. Further examination of this issue will lead to increasingly cogent arguments for or against use of caffeine, especially among those with CV risk factors for developing hypertension, such as pre-hypertension. Hypertension plagues the US population, with nearly 67 million American adults (31%) with the illness (CDC, 2012). Additionally, high blood pressure costs the US $47.5 billion each year (Heidenrich et al., 2011). Standard caffeine control procedures will ultimately aid in promoting methodological reliability and validity in research exploring effects of caffeine on those with hypertension and/or other CV illnesses.

Ultimately, research that is aimed at addressing the potential confound of caffeine in CVR research may have broad implications for methodological rigor in CVR research.

The current study was envisioned as the first in a series of systematic investigations to determine the optimal abstention time in regular caffeine users (i.e., that which minimizes both CVR confounds and caffeine withdrawal effects). The duration chosen for this study (6 hours) represents the reported average half-life of caffeine in the body. Six hours was viewed as an initial point in a program of research that carefully and systematically examines the issue of caffeine confounds in CVR research, with the aim of informing the field and promoting standardization across labs. This experiment explored whether regular consumers of caffeine show changes in indices of CVR, cognitive functioning and mood following six hours of abstention, examining both the acute and deprivation effects of the drug on these variables.

Although individual differences most certainly exist in responses to caffeine, the present study focused on a group that is widely studied in physiological reactivity research: healthy young adults. As such, this study was envisioned as the first in a series of investigations ultimately yielding caffeine abstention recommendations for CVR research across various demographic categories. If adopted, these guidelines will increase the validity of such research by enhancing comparability across studies. That is, standardizing control of the confounding effects of caffeine will reduce extraneous variance, allowing for more consistent examination of the various factors of interest that systematically affect CVR. Such research lends to enhanced understanding of variables affecting CVR, which clearly play considerable roles in both cardiovascular and emotional health.

2.3. Caffeine

Caffeine (1,3,7-trimethylxanthine) is a naturally occurring xanthine derivative and purine alkaloid. This metabolic and central nervous system stimulant is the most widely consumed psychoactive substance in the world and the main export to over 36 countries (Echeverri et al, 2010). Approximately 85% of Americans consume caffeine regularly or daily (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014; Barone & Roberts, 1996). Individuals aged 18-24 consume approximately 288 mg of caffeine daily (Mitchell et al., 2014). In the US, approximately 67% of caffeine intake is consumed in the form of coffee (Barone & Roberts, 1996). Coffee contains over a thousand bioactive compounds, caffeine being its major active component. The drug is also found in a wide variety of commonly consumed beverages and foods including tea, soft drinks, energy drinks, and chocolate. Additionally, many frequently used medications such as pain relievers, cold remedies, and fatigue restoratives include caffeine due to its analgesic properties as well as providing increased efficacy of the medication (Lee et al, 2013).

Caffeine is rapidly and completely absorbed from the intestinal tract, making it 100% bioavailable (Echeverri et al., 2010). It is metabolized within 30-45 minutes on average, with maximum plasmatic concentration at 30–120 minutes following intake (Mort & Kruse, 2008). The elimination half-life of caffeine ranges from 3 (Rizzo et al., 1988) to 10 hours (Robertson et al., 1981), with an average of six hours. Diverse factors affect this half-life including diet, nicotine, antidepressants, oral contraceptives, and alcohol (Kroon, 2007; Parsons & Neims, 1978; Abernethy & Todd, 1985). Caffeine metabolism varies among individuals, as the drug is metabolized by CYP1A2, a hepatic cytochrome enzyme with a genetic polymorphism, partly accounting for such variations (Butler et al., 1992). Furthermore, factors such as sex, weight, and the presence of hepatic diseases are responsible for large interindividual variations in metabolism of caffeine.

2.4. Primary Mechanisms of Action

Multiple mechanisms of action of caffeine have been proposed, including mobilization of intracellular calcium and inhibition of phosphodiasterases which ultimately leads to epinephrine release. However, the majority of caffeine's biological effects are exerted through antagonism of the A_1 and A_2 subtypes of the adenosine receptor (Higdon & Frei, 2006). Adenosine is an endogenous neuromodulator that plays a key role in regulation of tissue function and blood flow to various organs (Hasko et al., 2008). Pharmacological adenosine is prescribed in order to treat various forms of cardiac arrhythmias. Naturally occurring adenosine is vastly present both within and outside of cells, affecting multiple transmitter systems within both the central and peripheral nervous systems (Fredholm & Dunwoodie, 1988).

Among its multiple functions, adenosine serves as a vasodilator and ultimately potentiates sleep. The prevention of inhibitory effects of adenosine eventually lead to CNS excitability, stimulation of medullary, vagal, vasomotor, and respiratory brain networks as well as release of epinephrine. As would follow, caffeine's effects on autonomic nervous system activity are primarily sympathomimetic. Through a series of events related to adenosine blocking effects, physiological responses to caffeine are widespread and broadly include vasoconstriction, increased blood pressure, increased blood flow to the muscles, decreased cutaneous and inner organ blood flow, bradycardia, and tachycardia. Additionally, caffeine promotes the release of glucose by the liver (Pizzol, et al., 1998). The drug also indirectly increases levels of dopamine in the brain through interactions between adenosine A_{2A} and dopamine D_2 receptors (Nieoullon, 2002). Dopamine, a key neurotransmitter, acts on the regulation of cognitive and attention systems, which likely explains the vast use of this relatively harmless drug.

2.5. Behavioral Effects of Caffeine

Many consumers use caffeine daily due to its positive stimulant effects. Desirable acute effects of caffeine at moderate doses include feelings of enhanced mental performance, vigilance, reaction time, attention, memory, and elevated mood (Lara, 2010; Fine et al., 1994; Haskell et al., 2005). Much of the alerting and mood enhancing effects of caffeine may be related to the drug's action on serotonin neurons (Nehlig, Daval, & Debry, 1992).

In recent years, a growing number of fitness and weight loss industries have incorporated caffeine into products to bolster performance during physical exercise. Individuals commonly purchase such products as fitness aids or ultimately for weight loss purposes. Caffeine broadly generates sympathetic outflow, as has been discussed briefly. Sympathetically released noradrenaline activates thermogenesis, which promotes weight loss (Dulloo et al., 2000). Also, caffeine-mediated increases in skeletal muscular blood flow and muscular neuronal firing lead to potentiated mobilization and less exertion (Walton, Kalmar & Cafarelle, 2002). Notably, rather than offering any direct energy source, the greater part of caffeine's stimulatory effects result from an offsetting of physical fatigue that naturally occurs during physical fitness routines.

In fact, this truth may at least partially extend to cognitive benefits of caffeine as well in regards to habitual consumers. A great deal of research supports the notion that habitual users of caffeine consume the drug primarily to avoid symptoms due to deprivation or withdrawal, and that these individuals do not receive any net cognitive benefit for mental alertness and performance (Rogers et al, 2012). Consumer expectations and the possibility of the influence of placebo effects may also play an important role in caffeine-related performance gains. Regardless of actual causes for feelings of physical or cognitive energy

potentiation, the most consistently found benefit among both habitual and non-habitual consumers is increases in motor speed and motor endurance (Rogers et al, 2012).

Aside from caffeine's popular ameliorative effects, the drug may also produce negative physical, cognitive, and emotional side effects, especially when consumed at high or excessive doses (Richardson, Rogers, Elliman, & O'Dell, 1995). Though relatively harmless, the extent of caffeine's negative effects depends primarily on individual sensitivity to caffeine, health condition, and pattern of consumption. A pattern of regular consumption followed by an abrupt abstention often produces considerable withdrawal symptoms. Aversive consequences of caffeine withdrawal include headache, insomnia, gastrointestinal disturbances, irritability, and anxiety (Juliano & Griffiths, 2004). A systematic review concerning caffeine withdrawal revealed that a great portion of studies indicated reporting of impaired cognitive performance (48%) irritability (35%) and lack of motivation (50%) following cessation of caffeine consumption among regular consumers (Juliano & Griffiths, 2004).

The drug also has the capacity to potentiate feelings of anxiety as a function of dosage and consumption pattern, with caffeine-related anxiety production most commonly reported at higher doses and among non-habitual consumers. Caffeine produces its anxiogenic effects though antagonizing of adenosine A_1 and A_2 receptors and through interactions with other neurotransmitter systems (Childs, Hohoff, Deckert, Xu, Badner, & DeWit, 2008). Research also indicates that genes for adenosine and dopamine receptors play a role in anxiety generation with caffeine consumption, and supports an association between ADORA2A and DRD2 polymorphisms and self-reports of anxiety following ingestion of moderate amounts of caffeine (Childs et al., 2008). Caffeine's anxiogenic effects are amplified in clinically anxious populations, where potentiation of anxiety following caffeine ingestion is especially pronounced in those with social anxiety disorder

and panic disorder (Lara, 2010). Clinically anxious individuals are typically high in anxiety sensitivity (i.e. fear of arousal) and anxiety sensitivity and perceived control have also been revealed to affect response to caffeine challenge. In a study by Telch, Silverman, and Schmidt (1996), researchers administered a caffeine challenge to those high and low in anxiety sensitivity who were given either a perceived control or no perceived control instructional set. Results indicated that subjects high in anxiety sensitivity in the "no perceived control" condition displayed significantly greater emotional response to the caffeine challenge (Telch, Silverman, and Schmidt, 1996).

Issues related to particular patterns of caffeine use can impair social and occupational functioning. Regular caffeine users show overall tolerance to and physical dependence on the drug, characterized by withdrawal symptoms upon cessation or reduction in use (Juliano & Griffiths, 2004). In fact, the DSM-5 recognizes caffeine-related disorders (American Psychiatric Association, 2013). Diagnostic criteria for Caffeine Intoxication include recent consumption of a high dose of caffeine (in excess of 250 mg), restlessness, nervousness, excitement, insomnia, and tachycardia or cardiac arrhythmia. Symptoms of Caffeine Withdrawal can occur in regular users within 24 hours of abrupt cessation or reduction in caffeine use, and include headache, fatigue, dysphoric mood or irritability, difficulty concentrating, and flu-like symptoms.

These effects pose a significant confound in behavioral CVR studies. In essence, if lengthy abstention is required, they can interact with both independent and dependent variables that are often cognitive and affective in nature. Hence, the lack of a standard caffeine abstention time in CVR research presents a clear threat to the validity and reliability of such studies in terms of the physiological and psychological variables under study.

2.6. Caffeine's Cardiovascular and Respiratory Effects

As an ANS stimulant, caffeine ingestion initiates changes in several indices of autonomic functioning including effects on both the cardiovascular and neuroendocrine systems. As a result of caffeine's classification as a sympathomimetic drug, it produces actions that roughly mimic the effect of agonists of the sympathetic nervous system. The xanthine produces various direct and indirect effects on the heart and on vascular tissue.

Despite the wealth of literature concerning the presence of caffeine's pressor effects, the magnitude of these effects is not entirely conclusive, while systematic effects on heart rate, HRV, impedance measures and other CV indices are especially variable and unsettled. Caffeine's effects on CVR are complex and may produce antagonistic effects. Some research supports tolerance to effects of the drug on CVR among regular caffeine consumers. However, this is not a consistent finding. Although very sparing caffeine consumption tends to relate to greater CV responses to the drug than regular consumption, complete tolerance to the drug in terms of CVR is not a consistent finding.

2.6.1. Acute pressor effects. Regarding caffeine's effects, the most frequently explored cardiovascular variables are related to pressor effects. The most consistent findings indicate transient post-consumption elevations in both SBP and DBP, likely primarily through systemic increases in vascular resistance (Pincomb et al., 1985, 1987; Lovallo et al., 1989; Nurminen et al., 1999; Noordzij et al., 2005). A meta-analysis suggests that caffeine has a minute effect on the blood pressure of chronic users when ingested through coffee (Noordzij et al, 2005), whereas other studies reveal substantial pressor effects (Robertson et al., 1978). The meta-analysis yielded an overall SBP effect of 2.04 mmHg and DBP effect of 0.73 mmHg following acute consumption of caffeine.

Despite focus on caffeine's hallmark sympathomimetic effects, blood pressure response to caffeine depends on the balance among multiple CV mechanisms including myocardial stimulation and central vasomotor activity as well as central vagal stimulation and peripheral blood vessel dilation. For example, cardiac sympathetic stimulation upregulates blood pressure whereas parasympathetic responses decrease it (Ritchie, 1975). Additionally, caffeine produces both direct and indirect effects on the vasculature that initiate vasodilation and vasoconstriction. Direct vasodilatory effects are produced by diffusion of nitric oxide in the smooth muscle vasculature and local accumulation of intracellular second messenger cyclic adenosine monophosphate (cAMP), which also encourage increased cardiac contractility (Echeverri et al., 2010). By contrast, caffeine-initiated antagonization of adenosine receptors elicits vasoconstriction.

Importantly, a net increase or decrease in blood pressure involves action of multiple opposing mechanisms. Although multiple mechanisms are responsible for upregulation of blood pressure following acute caffeine consumption, this effect primarily results from competitive antagonism of A_1 and A_{2a} adenosine receptors. Adenosine plays a critical role in myocardial and vascular functioning. In terms of neural actions, it possesses primarily inhibitory functions (e.g. inhibiting the release of nearly all known neurotransmitters) (Biaggioni, 1992). However, adenosine potentiates vasodilation in most vasculature, including the coronary arteries (Hansen & Schnermann, 2005; Sato et al., 2005). Despite this singular effect, the similar molecular structures of caffeine and adenosine cause caffeine to inhabit adenosine receptor sites, thereby hindering modulatory effects of adenosine (James, 2004). As such, caffeine displays generally stimulatory effects, which are evident in blood pressure responses to the drug.

Several studies have found that caffeine increases both SBP and DBP (Jeong & Dimsdale, 1990; Nurminen et al., 1999; Pincomb et al., 1987; Bender et al., 1997; Lovallo

et al., 1989; Lovallo et al., 1991; Nussberger et al., 1990; Sung et al. 1994). Robertson et al. (1978) found 250 mg of caffeine administered in a methylxanthine-free beverage caused mean blood pressure to rise 14/10 mmHg as well as a 20 percent increase in respiratory rate one hour after caffeine consumption. Jee et al. (1999) performed a meta-analysis including eleven studies that examined the blood pressure effects of caffeine in a coffee treatment group, where the median dose of coffee was five cups per day, to a control group. The meta-analysis found that systolic blood pressure increased by an average 2.4 mmHg and diastolic blood pressure increased by an average of 1.2 mmHg in the coffee treatment group, independent of study design characteristics and age of subjects. Pincomb et al. (1985) suggested that progressively increased systemic vascular resistance causes caffeine's pressor effects. It is currently well-established that this action is primarily exerted through vascular adenosine receptor blockade (Smits et al., 1987).

Though most literature does not indicate robust findings of detrimental increases in blood pressure from caffeine use among healthy consumers, effects are potentially harmful for those with CV illnesses. Regular consumption of and/or high levels of caffeine intake may cause harmful increases in blood pressure in individuals who are especially susceptible to, or suffering from, hypertension (Nurminen, Niittyen, Korpela, and Vapaatalo, 1999).

2.6.2. Heart rate. Extensive studies examining HR changes following caffeine ingestion reveal somewhat variable results. Most studies report decreases in HR (Pincomb et al., 1985, Pincomb et al., 1987), while others reveal increases (Lane & Manus, 1989), or fail to show a change (Sondermeijer, 2002). The majority of studies indicate either no change or small reductions in HR with this drug. A well-supported explanation for HR decreases resulting from acute ingestion of moderate amounts of caffeine is an offsetting reaction initiated by baroreflex responses to increases in blood pressure. By contrast, increases in HR are often associated with consumption of higher doses of the drug.

Additionally, HR reactivity is closely associated with mixed vagal and sympathetic responses to the drug (Lane, 1990).

2.6.3. Heart rate variability. Heart rate variability reflects the constant dynamic relationship between sympathetic and parasympathetic activity in regulation of HR (Koenig, et al, 2013). The high-frequency component of HRV, which varies with respiration, is reflective of vagal activity. hfHRV has substantial implications on CV and emotional health, and is positively associated with *autonomic flexibility*, or systemic adaptive variability (Friedman, 2007; Friedman & Thayer, 1998). Despite extensive studies concerning HR effects associated with caffeine, examination of changes in HRV following caffeine intake are by comparison, limited. A recent systematic review revealed unclear associations concerning the impact of acute caffeine consumption on vagally mediated time- and frequency-domain-related HRV changes (Koenig et al., 2013). Results across studies varied, with some showing increases, decreases, and no significant changes in HRV following caffeine ingestion.

Frequency-domain measures revealed more clarity than time-domain measures. Despite caffeine's primarily sympathomimetic effects, overall, studies revealed caffeine-induced increases in hfHRV. No confirmed mechanism of these changes has been discussed in the literature, however, some have suggested that caffeine plays a role in the modulation of parasympathetic nerve activity (Hibino, Moritani, Kawada, & Fushiki, 1997). Given sympathetic influences of caffeine, vagal increases due to caffeine could conceivably be part of a co-activation of the sympathetic and parasympathetic nervous systems, perhaps as part of a parasympathetic compensatory response. However, effects on HRV still remain unclear and results are difficult to integrate. The authors cite that this lack of clarity results from wide variations in study designs and methodology; more specifically, populations studied (healthy versus clinical), experimental conditions, time of measurement, and the

dose-response relation (Koenig et al., 2013). The effect of caffeine on HRV in healthy habitual consumers of moderate amounts of caffeine has yet to be clarified, likely due to such considerations.

2.6.4. Respiratory effects. Previous research examining caffeine's effects in non-human primates and rodents have established that xanthines such as caffeine affect inhalation and exhalation by affecting central mechanisms controlling respiration (Howell et al., 1990; Wessberg et al., 1985). Caffeine generally elicits increases in respiratory rate, likely through the sensitization of medullary centers to carbon dioxide (Martinet & Debry, 1992; Benowitz, 1990).

2.6.5. Cardiac contractility effects. It is currently somewhat equivocal whether systemic increases in SBP and DBP following acute caffeine ingestion are predominantly potentiated by changes in either cardiac output or systemic vascular resistance. Robust findings previously suggested that such pressor increases were due to elevations in systemic vascular resistance coupled with a lack of change in cardiac output (Pincomb, et al., 1993). Notably, these studies were conducted using male samples. The mechanisms by which blood pressor increases due to caffeine consumption may likely differ as a function of sex.

In support of this, research has indicated that despite similar acute pressor responses to caffeine, women appear to experience blood pressure elevations due to cardiac output and stroke volume increases, whereas men indicate such pressor changes due to increases in systemic vascular resistance (Hartley, Lovallo, & Whitsett, 2004).

Few investigations examine caffeine's acute effects on systolic time intervals such as pre-ejection period (PEP) and left-ventricular-ejection time (LVET). Of studies examining these variables in relation to caffeine, most reveal no drug-induced changes (Conrad, Blanchard, & Trang, 1982). A study using a female sample found acute decreases

in PEP and LVET (Hasenfratz & Battig, 1992). These effects are certainly conceivable, due to systematic sympathetic effects of caffeine; however, further research is necessary to clarify the effects of caffeine on contractility indices.

2.7. Caffeine's Effects on Neuroendocrine Reactivity

Neuroendocrine effects are also evident in CVR to caffeine. A number of studies have indicated caffeine-induced increases in catecholamines such as epinephrine and norepinephrine among both regular- and non-consumers of caffeine. Endocrine effects tend to attenuate to some extent with regular consumption of caffeine, although not completely (Robertson et al., 1981).

A dose-dependent effect of caffeine on catecholamines has been found, such that greater amounts of caffeine cause greater increases in secretion of these hormones (Papadelis, et al., 2003). Both consumption of caffeine and acute psychological stress potentiate elevations in plasma and urinary epinephrine and norepinephrine. Studies reveal additive effects of stress and caffeine consumption on secretion of these hormones. In a study by Lane et al (2002), examining caffeine effects on neuroendocrine activation at work and home among healthy habitual consumers of coffee indicated that caffeine significantly increased levels of free epinephrine by 32% during the workday and evening. These results were accompanied by significant caffeine-elicited increases in ambulatory blood pressure (4/3 mm Hg) and reductions in heart rate (2 bpm).

These and similar results suggest that caffeine causes increases in stress-related hormones and elevated sympathetic-medullary reactivity to acute stress. Because caffeine activates the stress axis, it also exerts glucocorticoid effects. Caffeine increases both (adrenocorticotropin) ACTH and cortisol in humans (Lovallo et al, 1996).

2.8. Caffeine and Stress Reactivity

At rest, caffeine causes cardiovascular reactivity patterns as discussed. Importantly, caffeine consumption is often associated with stressful moments in individuals' daily lives (Conway, Vickers, Ward, 1979). Therefore, it is fundamental to examine caffeine's effects during mental and/or physical stress as well as during rest. Ample evidence supports the notion of combined cardiovascular effects of caffeine and stress. Research examining hemodynamic responses to both caffeine and stress have indicated an additive effect, and suggest a synergetic interaction between stress and caffeine. For example, greater blood pressure reactivity during stress typically occurs following acute consumption of caffeine (Lane & Williams, 1987).

Importantly, research in this area has primarily studied such effects in male samples. Women and men indicate varied cardiovascular responses to mechanisms underlying mental stress reactivity. Men tend to reveal vascular reactivity to a variety of mental stress tasks, whereas women tend to be myocardial reactors to mental stress (Girdler, Turner, Sherwood, & Light, 1990; Hartley, et al., 2004). It remains to be seen whether women reveal differing hemodynamic responses to combined effects of caffeine and stress, as very minimal research has focused on sex differences in this domain. Hemodynamic differences in CVR to combined caffeine and stress are suggested based on previous research, but not confirmed. For example, one study by Farag et al. (2006) revealed similar enhancement in sympathetic CV responses to a laboratory stressor following caffeine ingestion among both male and female subjects. More specifically, pre-task caffeine consumption generated an increase in cardiac index and a decrease in peripheral resistance index.

Finally, it is essential to consider that cardiovascular responses to caffeine are a partial product of individual differences in stress reactivity at multiple levels (Lovallo,

2016). Specifically, Level I involves central nervous system responses such as those grounded in frontal-limbic processes (e.g. appraisals, coping responses, working and declarative memory). Level II concerns brainstem and hypothalamic influences on autonomic outputs and affective bias. Level III influences involve peripheral organ and tissue function (e.g. adrenoreceptor sensitivity). Research using caffeine challenges have suggested that individual differences in CV responses to caffeine (e.g. vascular-related pressor increases) are strongly peripheral in nature (Level III) (Lovallo, 2016; al'Absi, Bongard, & Lovallo, 2000; Hartley et al., 2000).

2.9. Cardiovascular Withdrawal Effects of Caffeine

Individuals who regularly consume caffeine prior to an abrupt cessation of intake commonly experience deprivation or withdrawal symptoms. Research has not typically highlighted symptoms associated with abstinence from caffeine that are directly CV in nature. A critical review of caffeine withdrawal includes studies suggesting that caffeine abstinence may be associated with increases in cerebral blood velocity and decreased blood pressure (Juliano & Griffiths, 2004).

Increases in cerebral blood flow have been associated with caffeine deprivation (Jones et al., 2000). This has been examined using study designs in which acute caffeine abstention is compared to that during a preceding baseline condition and caffeine administration condition (Jones et al., 2000). Accordingly, consumption of caffeine is associated with acute decreases in cerebral blood flow (Dodd, Kennedy, Riby, & Haskell-Ramsay, 2015). Theories posit that headaches related to caffeine withdrawal are associated with vascular mechanisms that underlie relative increases in blood flow following deprivation from the drug. While this appears plausible, further research is required to confirm these effects as signs of withdrawal from caffeine.

Caffeine withdrawal is presumed to be associated with decreases in blood pressure (Phillips-Bute & Lane, 1998; Lane, 1997). Findings indicate mean arterial pressure decreases of approximately 5-6 mmHg. This conclusion regarding decreases in blood pressure is based on studies using comparisons similar to the aforestated methodology linking caffeine withdrawal to increases in cerebral blood flow. Given the far-reaching cardiac, vascular, and broad autonomic effects of caffeine, it is likely that withdrawal from caffeine causes changes in blood pressure. However, further research on this topic is necessary in order to more precisely determine the magnitude of and mechanisms associated with these effects.

2.10. Caffeine Tolerance and Peripheral Physiological Changes

It has long been proposed that regular dietary use of caffeine leads to tolerance, or more specially, that habitual consumers of caffeine are hemodynamically unresponsive to caffeine, particularly regarding pressor responses. However, this notion has been refuted based on a multitude of findings.

Research strongly supports the notion of incomplete tolerance to caffeine's effects with repeated intake. It is suggested that tolerance to caffeine-provoked pressor effects occurs only in approximately half of regular consumers in acute laboratory tests. In fact, persistent elevations in blood pressure may occur among some habitual consumers of caffeine, indicating possible clinical significance (Farag et al., 2005). A critical review of the relationship between dietary caffeine and blood pressure emphasizes the relative dearth of studies demonstrating complete tolerance among habitual consumers, as compared to those indicating only partial tolerance (James, 2004). Additionally, no systematic difference in reactivity between high- and low- habitual consumers exists.

This conflicts with the tolerance hypothesis in that hemodynamic reactivity should be a function of amounts of caffeine consumed; a relationship not in fact seen to be evident. In fact, in some instances, caffeine's pressor effect has even been shown to be proportional to systemic levels of caffeine, regardless of current history (James, 2004). In short, consistent tolerance effects do not appear to span Central Nervous System (CNS) and Peripheral Nervous System (PNS) effects, but rather, they appear to exist primarily and most reliably in CNS domains of cognitive functioning rather than PNS measures of CVR including blood pressure responses.

2.11. Additional Considerations

In addition to methodological considerations for subject abstention, the continuation of the enduring debate regarding coffee and caffeine's effects on CVR is due in large part to a lack of specification of some important factors influencing the drug's effects on the autonomic nervous system. Caffeine produces various effects on cardiovascular (CV) activity that fluctuate with myriad factors including dosage and method of administration (e.g. via coffee or caffeine pills). Caffeine content in coffee is also directly associated with method of preparation (e.g. grinding method or filtering methods) (Bell, Wetzel, & Grand, 1996). Additionally, effects of caffeine on CVR reveal important individual differences related to variables including consumption pattern, age, and sex. For example, older women have shown greater systolic (SBP) and diastolic blood pressure (DBP) increases than younger women following acute ingestion of caffeine (Arciero & Orsmbee, 1998). Though caffeine may not pose a serious health threat in most users, evidence of its exact CV effects are somewhat inconclusive.

2.11.1. Expectancy effects. Phenomena related to placebo effects are the subject of a long history of control-comparison condition studies of drug effects (Kirrsch, 1999).

Caffeine expectancies may indirectly influence caffeine's effects on CV, affective, and cognitive indices. Placebo effects are proposed to underlie these effects such that even in the absence of the drug, individuals may show increased performance on cognitive tasks as well as mood or physiological changes resulting from the expectation of having consumed the drug (Fillmore et al., 1994; Fillmore & Vogel-Sprott, 1992). For example, one study revealed that individuals who incorrectly believed that they received caffeinated coffee exhibited significantly smaller decreases in blood pressure than control subjects who received caffeinated coffee (Walach, Schmidt, Dirhold, Nosch, 2002). These and several additional considerations must be made when drawing conclusions about CV effects of coffee.

3. Present Study

The aim of the present study was to suggest guidelines for participant abstention from caffeine whereby the drug minimally affects CVR. Specifically, the goal was to identify a duration that allows for maximal avoidance of both acute effects (e.g. pressor increases) and deprivation effects of caffeine (e.g. mood changes, headaches). This was performed by assessing an abstention period of six hours in a healthy young adult sample of regular consumers of moderate amounts of coffee. This duration is equivalent to the average half-life of the drug. The repeated measures study design included comparison of CVR to two tasks across three phases: baseline, 30 minutes- and six hours-following consumption of caffeinated coffee on one day, and decaffeinated coffee on another day

Given the additive effects of caffeine on stress reactivity, the examination of CVR to stress inductions was of particular interest. Two prototypical laboratory tasks assessing CVR were selected. A hand CP task was used as a *passive* stressor whereas a working memory task was selected in order to index *active* stress (Obrist, 1981). The primary

distinction between *passive* and *active* coping tasks is based on degree of psychological engagement as well as opportunity to influence the outcome of the task (Vella & Friedman, 2007). Furthermore, differing cardiovascular mechanisms underlying stress responses are associated with these task types.

The CP is a laboratory stress induction task commonly used in stress and pain research involving immersion of either a hand or foot in cold water (Lovallo, 2016). Water temperatures are typically in the range between 0 and 5° Celcius, although responses can be reliably expected with temperatures in the 4 to 10° Celcius range (Porcelli, 2014; Silverthorn & Michael, 2013). The water temperature for the current study ranged from 6 to 8° Celsius. Among its wide array of experimental uses, this task is used primarily as a method of pain elicitation or as a manipulation to examine vascular responses to acute stress.

The task was included in order to assess alpha-adrenergic reactivity, which causes significant vasoconstriction, and is associated with increases in DBP. Due to its robust pressor effects, CP responses have also been commonly used as an indicator of existing cardiovascular illness or risk for the development of hypertension (Wood, 1984). The CP is a reliable elicitor of sympathetic vascular activation and has been used extensively in our laboratory for this purpose (McGinley & Friedman, 2014; Knepp & Friedman, 2008; Vella & Friedman, 2007; Friedman & Santucci, 2003). The task causes transient increases in both SBP and DBP. A three-minute long hand CP task was employed in the current study as a systemic active coping task to examine interactive and/or additive effects of physiological responses and time-based effects of caffeine.

The original Sternberg memory task was developed in an effort to study components of and working memory processes related to reaction time (Sternberg, 1966). A modified

version of this task was used in the present study for the purposes of including a laboratory stressor that indexes beta-adrenergic reactivity. Such tasks primarily affect sympathetic myocardial performance. This working memory task is moderately difficult, but motivating for a monetary reward (Richter, Friedrich, & Gendolla, 2008). The inclusion of this task was designed to assess reactivity of the physiological and cognitive variables of interest to this type of acute stressor combined with effects of caffeine.

3.1. Aims and Hypotheses

The ultimate goal for the present study was to promote a standard practice for caffeine abstention durations in CVR research, which will potentially enhance comparability of results across studies and increase reliability of findings. Time-varying physiological effects of caffeine intake on task reactivity of the following measures were examined: systolic and diastolic blood pressure (SBP, DBP), mean arterial pressure (MAP), heart rate (HR), high-frequency heart rate variability (hfHRV), root mean squares successive differences (RMSSD), and the following impedance cardiogram (ICG) indices: (total peripheral resistance (TPR), pre-ejection period (PEP), and left-ventricular ejection time (LVET)). Respiration rate (RR) was acquired for potential use in controlling for possible effects of respiration on HRV indices. State mood was also assessed at each phase on both days in order to examine possible changes in general mood associated with time-varying effects of caffeine.

Impedance Cardiography (ICG) was employed in order to derive hemodynamic variables and to differentiate cardiac versus vascular mechanisms underlying blood pressure responses to acute caffeine intake as well as six hours-post consumption. TPR, PEP, and LVET were derived from the ICG output to indicate inotropic and chronotropic sympathetic activity, respectively. PEP is inversely related to myocardial contractility and is inferred to

index beta-adrenergic influences on the heart (Newlin & Levenson, 1979; Obrist, Light, James, & Strogatz, 1987; Cacioppo, et al., 1994). In general, lower values of PEP reflect heightened sympathetic activation (Sherwood et al., 1990; Thayer & Uijtdehaage, 1990).

Specific aims for the study were as follows:

1. The primary aim was to examine a hypothesized duration for the minimal abstention duration for healthy regular consumers of moderate amounts of caffeine whereby cardiovascular (CV) reactivity is unconfounded by caffeine, by first testing six hours of waking abstention, based on the average half-life of the drug. It was hypothesized that a lack of statistically significant differences would occur between CVR during stress-inducing laboratory tasks (CP and working memory task) prior to caffeine consumption and CVR six hours following caffeine intake.

2. The secondary aim was generally to examine acute and deprivation effects of caffeine consumption on CV variables. It was hypothesized that following acute intake, subjects would reveal mean increases in SBP, DBP, MAP, TPR, hfHRV, and RMSSD. Acute decreases in HR, PEP and LVET were anticipated. To test these hypotheses, comparisons of baseline measures of CV variables to task CVR following acute caffeine intake were made.

3. To examine psychological variables to view possible negative mood changes or cognitive performance decrements on a working memory task following six hours of caffeine abstention. General self-reported awareness of bodily sensations was also explored for possible associations with CVR.

A rationale for the use of coffee. Coffee was selected over other caffeine-containing substances (e.g. caffeine pills) in order to examine the effects of caffeine in its most commonly used form. In a largescale study of 18-24 year old students, individuals

obtained most of their caffeine intake from coffee and espresso rather than from energy drinks (Norton, Lazev, & Sullivan, 2011). Moreover, caffeine pills contain higher amounts of caffeine than that which is normally consumed by moderate caffeine consumers.

4. Method

4.1. Subjects

Approval from the Virginia Tech Institutional Review Board was obtained (View Appendix B for risks and deception). Based on G*Power software's analysis, ability to attain a medium effect size (.3) for the repeated measures analyses with a .95 power value at α=0.05 requires 40 subjects. Forty three subjects were recruited for participation in the study. Thirty nine subjects had useable data. Participant age ranged from 18 to 24 years (M=20.9 years, SD=1.9). The sample consisted of 20 women (19 men) from Virginia Tech and the New River Valley community (Refer to Table 1 for participant characteristics). Individuals were recruited using multiple methods: Virginia Tech Psychology Department's SONA online research system, University student listervs, flyers posted in local coffee shops and academic buildings at local colleges and universities.

Subjects were healthy regular consumers of moderate amounts of caffeine (average two to three cups of coffee daily; at 120-200 mg caffeine/day; Arciero et al., 1998). Other inclusion criteria included right-handed individuals. Exclusion criteria included history of CV disease, metabolic disease, neurological, respiratory, or psychiatric disorder, history of traumatic brain injury or recent history of brain injury, smoking within the past year, those currently taking medications with known cardiovascular effects, and prescription medications taken less than one month prior to the study. Subjects completed a total of four sessions: two morning sessions of 1 hour and 45 minutes in duration; two afternoon sessions of 30 minutes in duration each. Subjects were monetarily compensated at the end of each afternoon for participation in each of the sessions.

Prior to participation in the experiment, subjects were provided specific instructions for study preparation. These included instruction and reminders to abstain from consumption of alcohol within 24 hours, from caffeine for 12 hours, and to sleep for approximately eight hours overnight prior to each morning session. Instruction also included refraining from eating or vigorous exercise within one hour prior to each morning and afternoon session. Subjects completed a detailed journal chronicling their daily intake of all caffeine containing beverages and foods for one week leading up to the first session, as well as on the days in between experimental days (Appendix C). (Refer to Appendix D for participant caffeine consumption details).

4.2. Self-Report Measures

4.2.1. Caffeine consumption journal. Subjects were asked to begin completing a journal documenting their caffeine consumption exactly one week prior to the first session of the study and during the days in between the first and second days of the study. This log included ingestion of caffeine from all sources (e.g. coffee, tea, chocolate, medications). This questionnaire was incorporated into the study in order to collect information regarding subjects' average caffeine consumption prior to the study, as this may play a role in between-subject and between-day differences in results.

4.2.2. Screening questionnaires. All subjects were screened prior to study participation, using the following battery of self-report questionnaires:

4.2.2.1. Mind-Body Laboratory health history questionnaire (HHQ). Following expressed interest in the study, potential subjects completed the HHQ via the Virginia Tech Qualtrics survey website. These questionnaires were designed to screen subjects based on exclusion and inclusion criteria pertaining to patterns of coffee and caffeine consumption as

well as health conditions that may likely interfere with the variables of interest. Eligible individuals were recruited for the study (Appendix E).

4.2.2.2. Recent health history questionnaire (R-HHQ). This questionnaire is a standard instrument administered to subjects in the Mind-Body Lab prior to the initiation of study sessions, and includes questions about subjects' recent health behaviors. In the present study, the purpose of this questionnaire was to ensure that individuals adhered to abstention requirements concerning caffeine, alcohol, food, and exercise. Responses from the R-HHQ also provide information about the timing of the menstrual cycle phase (for women), and duration of sleep acquired during the preceding night, as these factors may systematically relate to variables of interest (Appendix F).

4.2.3. Questionnaires administered during the study

4.2.3.1. *Depression Anxiety and Stress Scale (DASS).* The primary purpose for inclusion of the DASS in the present study was to explore broadly whether depressive, anxious, and stress-related emotions were associated with physiological responses to caffeine. The DASS is a 42-item self-report instrument designed to index the negative emotional states characteristic of depression, anxiety and stress/tension (Lovibond & Lovibond, 1995b). Each of the three scales contains 14 items, subsisting of two to five items with comparable content. Items on the Depression scale assess dysphoria, devaluation of life, hopelessness, self-deprecation, anhedonia, lack of interest or involvement in daily activities, and inertia. The Anxiety scale indexes autonomic arousal, skeletal muscle effects, situational anxiety, and the subjective experience of anxious affect. Items on the Stress scale assess levels of chronic non-specific arousal; essentially nervous arousal, difficulty relaxing, and irritability or impatience. Respondents were instructed to use four-point scales to rate the frequency and extent to which they had experienced each emotional state over the past

week. Scoring is such that greater scores for each dimension are associated with greater-self-ratings.

The psychometric properties of the DASS have been evaluated in a normal sample of N=717 (DASS; Lovibond & Lovibond, 1995a). Exploratory and confirmatory factor analyses both revealed satisfactory internal consistency among all scales. The DASS has utility in discriminating among the three constructs (Appendix G).

4.2.3.2. ***Brief Mood Introspection Scale (BMIS).*** Inclusion of this self-report measure allowed for brief general pre-caffeinated, 30 minute- and six hour-post-caffeinated measures of current mood states. Subjects were instructed to respond to 16 items, on a four-point Likert scale, to indicate how well each adjective described the individual's present mood. The scale yields measures on the following dimensions of mood: Pleasant-Unpleasant, Arousal-Calm, Positive-Tired, and Negative-Calm. The BMIS is scored such that greater numerical responses for each dimension are associated with greater scores (e.g. Larger scores for "Pleasant" or "Arousal" correspond with greater self-rated pleasantness or arousal). The BMIS was found to have good factor validity and Cronbach's alpha reliabilities ranging from 0.76 to 0.83, which are quite satisfactory (Mayer & Gaschke, 1988) (Appendix H).

4.2.3.3. ***Body Perception Questionnaire (BPQ; Awareness Subscale).***The BPQ is comprised of five subscales. The *Awareness* subscale of the BPQ was employed to provide an index of self-reported awareness of bodily sensations, and whether this awareness is associated with CVR to tasks or caffeine consumption (Porges, 1993). The *Awareness* subscale is designed as an index of awareness of bodily processes. The addition of this instrument provided a general indirect trait index of self-assessed interoceptive processes. Subjects were instructed to indicate on a five-point Likert scale (from (a) through (e)) their

level of awareness during most situations of bodily processes. For example, items include: "how fast I am breathing", and "muscle tension in my back and neck". The instrument is scored such that greater numbers on the BPQ correspond with greater bodily awareness (Appendix I).

4.2.3.4. Task Ratings. Following completion of tasks, subjects provided task Likert scale ratings based on perceived difficulty of each memory task as well as pain level experienced during each CP task (National Institute of Clinical Studies, 2011). Ratings were completed at each experimental phase following recovery periods. Memory task difficulty ratings ranged from 1 (very easy) to 9 (very difficult). Ratings for the CP test indexed perceived pain during the task where responses ranged from 0 (no pain) to 10 (worst possible pain) (McCaffrey, 1999) (Appendix J).

4.3. Physiological Measures

Physiological signals were collected using BIOPAC Systems, Inc. software and MP150 hardware (BIOPAC Systems Inc, Goleta, CA). Signals were amplified and then integrated as analog data before reaching a PC computer in an adjacent laboratory room. The ECG signal was amplified through the ECG 100C system. EBI 100C system amplified the ICG signal. These signals were then routed to the MP150 interface to be digitally sampled at 1000 Hz, then transmitted to the computer which recorded physiological data using Biopac AcqKnowledge 4.3 (BIOPAC Systems Inc, Goleta, CA). This software was employed in an effort to detect and remove movement artifact. Both ECG and ICG were collected with ConMed Suretrace conductive ag/ag chloride adhesive pre-gelled electrodes (#1800) (Refer to Appendix K for diagram of electrode placement). All physiological measures were collected in real time, then separately averaged across baseline, task, and recovery epochs prior to statistical analyses.

4.3.1. Electrocardiogram (ECG). ECG was measured using a standard two-lead electrode configuration involving placement of electrodes on the thorax: One below the right collar bone, and the second below the chest on the left ribcage. Beat-to-beat distances between the peaks of the QRS (R-spikes) were computed using this detector system in order to calculate interbeat interval (IBI) values. IBI's (distance between two consecutive R-spikes) were derived from the ECG signal. IBI's were then imported into Kubios software (Version 2.2, 2004, University of Eastern Finland) for both heart rate and heart rate variability (HRV) measures. Values were obtained using spectral analysis of this signal and yielded variability in the high frequency spectrum corresponding to respiration (0.12-0.40 Hz). Specifically, a Fast-Fourier Transform function was performed for the IBI time series in order to derive power (ms^2) in this frequency band. Calculations were then converted with a natural logarithm transformation. High frequency heart rate variability (hfHRV) was regarded as an index of vagal activity. RMSSD was used as a time-based proxy for hfHRV. RMSSD values were also natural-log transformed prior to performing of statistical analyses. RMSSD and HR were additionally calculated using Kubios software.

4.3.2. Respiration. Respiration rate values were obtained using a Biopac respiratory effort transducer TP-TSD-201 respiration belt. The respiration belt was wrapped around the subject at the sternum and fastened close but comfortably (Etzel et al., 2006). The respiration waveform was resampled at 62.5 samples per second and transformed using a digital band pass filter with low- and high-frequency cutoffs fixed at .05 and 1.0 Hz, respectively. The waveform was also fixed at 5000 coefficients, based on guidelines suggesting number of coefficients be fixed at 4x(Waveform Sampling Rate/Lowest Frequency Cutoff for Filter) (Biopac Systems Inc., AcqKnowledge Software Guide).

4.3.3. Blood pressure. Blood pressure measurements were collected using the Fusion Noninvasive Semi-continuous Blood Pressure Measurement System Unit

(Medwave, Inc., Danvers, MA). The monitor includes a sensor contained in a Velcro wrist strap, which was placed on the subject's left wrist atop the radial artery. The device sampled both SBP and DBP values four times per minute. The signal was amplified by the NIBP100B system (BIOPAC Systems Inc, Goleta, CA). Mean Arterial Pressure (MAP) values were obtained using the following formula: MAP= (2x DBP) + SBP / 3. SBP and DBP were separately averaged across each epoch prior to the performing of the MAP calculations. Epochs containing greater than 45 consecutive seconds (approximately 3 samples) of an absent signal were treated as missing data.

4.3.4. Impedance cardiography. Noninvasive thoracic impedance measurements were taken using electrode placement and the EBI100C system (Biopac Systems Inc., Goleta, CA). A standard tetrapolar electrode configuration was employed involving placement of electrodes on the spine of each subject. A distance of 25-35 centimeters was between the second and third electrodes from the uppermost electrode.

The impedance waveform (delta Z) was derived using Acqknowledge 4.4 adaptive template matching. The software extracted the dZ/dt waveform, providing the following: C-point location using adaptive template matching, B-point location using the minimum derivative in the C-QRS interval, and X-point location using the minimum dZ/dt 150 -275 ms after the C point. Each dZ/dt waveform was visually inspected and manually edited where necessary, for accuracy. Subjects' height and weight as well as distance between electrodes were entered into the analysis routine.

4.4. Experimental Tasks

Two tasks (hand CP and Sternberg working memory tasks) were counterbalanced across subjects and sessions to control for potential order effects. The memory computer task was created specifically for use in the present study using E-Prime 2.0 stimulus presentation software (Psychology Software Tools, Inc., Sharpsburg, PA). The CP task was

designed using a standard water cooler filled with water 6 to 8° Celcius. A water circulator attached to an inside wall of the cooler provided movement of the water to promote stable water temperature surrounding the hand.

4.5. Baseline Videos

"Vanilla" baseline periods were employed for the collection of resting physiological measures. This commonly used assessment technique has indicated equality or superiority (i.e. stability and generalizability) to traditional "resting" baseline measures as well as lengthier baseline periods (Jennings et al., 1992). In the present study, subjects watched engaging but non-stimulating videos, which were muted to prevent potential confounding reactivity effects stemming from responses to music. Videos displaying marine life were viewed by subjects during all three- minute baseline epochs (Coral Sea Dreaming; Plankton Productions and MLJ). The study employed six different selections of segments from this video for the baselines. Another video was played for participant viewing during completion of questionnaires following consumption of coffee. This video consisted of scenes portraying everyday life in various cultures (Powaqaatsi: Life in Transformation, 1988). All videos were counterbalanced across subjects and sessions in order to avert potential order effects.

4.6. Procedure

Subjects entered the laboratory at either 8:00 a.m. or 10:00 a.m. following a 24- hour fast from alcohol and a 12-hour fast from caffeine, as confirmed through self-report. Subjects were also instructed to abstain from vigorous exercise, eating, or drinking within one hour of the morning sessions. Upon entering the lab, researchers collected the completed consumption logs. Subjects read and signed a consent form approved by the Virginia Tech Institutional Review Board. Physiological recording equipment for ICG,

ECG, and respiration was attached to subjects. The following segment of the study involved completion of two questionnaires: a brief health screening questionnaire and the Brief Mood Inventory Scale (BMIS; Mayer & Gaschke, 1988). Blood pressure measures were then acquired following attachment of the wrist cuff. Recording of all physiological measures continued simultaneously throughout the course of the sessions, while the individual remained in a seated position. Following approximately one to two minutes of additional resting to allow further acclimation to physiological equipment, subjects then began a series of two successive vanilla baseline, task, and recovery epochs, each of three minutes in duration. Resting "vanilla" baseline measures were recorded prior to completion of one of two stated tasks, which were counterbalanced across subjects and sessions.

For the CP task, subjects immersed the right hand in cold water (6 to 8° C; McGinley & Friedman, 2015) for the duration of the epoch while keeping the hand relatively still. For the memory task, each trial involves presentation of four slides: On the first slide, a white fixation cross appeared against a black background on a laptop computer screen for 750 ms. This preceded a series of four white capital nonsense letters presented for 550 ms. On the following slide, the letter series appeared with a single blue capital letter of the same size above the masked series for 550 ms. On the final slide of the trial, subjects were shown a single blue letter in isolation in the center of the screen for a maximum of 2550 ms. At this point, subjects pressed a key indicating whether or not the blue letter was presented in the previously shown series of letters (the number 1 indicating "yes' or 2 indicating 'no'. On certain trials (masking trials) this letter was the masking letter. On other trials, this letter was indeed present in the letter string. Trials were presented randomly (Refer to Appendix L). During the first session only, subjects completed a series of 14 practice trials prior to completing 44 trials of the task. Prior to initiation of each memory task, individuals were instructed to perform optimally well (90% or greater across sessions)

in order to receive a monetary reward at the end of the fourth session. Each task period was followed by a three-minute recovery period in which the subject sat quietly. Immediately subsequent to the recovery period, subjects provided ratings via pen and paper on the tasks in order to generally assess subjective ratings of perceived task difficulty and pain intensity.

Subjects then consumed 8 ounces of either hot caffeinated coffee (light roast; 230 mg caffeine) or an identical amount of hot decaffeinated coffee (medium roast; 5 mg caffeine) depending on the condition assigned. Coffee was brewed via a standard Keurig cup coffee maker. Coffee conditions were counterbalanced across morning sessions. Subjects were offered the opportunity to have a maximum of two small packets of creamer and sugar (approximately 8 grams of carbohydrates) or artificial sweetener added to the beverage, as the experience of consuming coffee without cream or sweetener may have produced an aversive reaction among those who normally do not consume coffee this way. Additionally, no consistent evidence reveals systematic acute effects of substances in creamer or sugar of the named amounts on the variables of interest in this study, although cardiovascular effects are evident for much greater amounts of carbohydrates (e.g. 1 gram glucose per kg body weight) (Jern, 1991; Synowski et al., 2013).

Subjects were allowed a maximum of five minutes to consume the beverage in its entirety. Once consumed, researchers allowed exactly 30 minutes following consumption for the caffeine to take effect. During this period, individuals completed the Depression Anxiety Stress Scale (DASS; Lovibond & Lovibond, 1995) prior to watching another non-stimulating video (Powaqqatsi: Life in Transformation; Vella & Friedman, 2007) for the remainder of the 30 minute period. Subjects then briefly completed the BMIS once again to assess possible mood changes associated with acute reactivity to caffeine. The end of this period initiated completion of another baseline, task, and recovery sequence, involving both tasks as outlined, in order to assess acute physiological reactivity to caffeine. At the end of

the session, researchers obtained height and weight measures in order to derive body mass index (BMI). Subjects were instructed to return to the lab six hours post-coffee consumption. Researchers requested a brief, general, hour-by-hour account of activities engaged in during the six-hour period (Appendix M). Subjects were instructed to abstain from consuming caffeine from any source during this period, and were also urged not to consume food or beverages within one hour of the afternoon session. Additionally, vigorous exercise was prohibited within one hour of the following session.

Subjects returned to the lab in the afternoon (six hours post-coffee consumption). During the afternoon sessions, study procedure was repeated, excluding beverage consumption. Following completion of the Recent Health History Questionnaire, identical physiological measures were collected during completion of only one baseline, task, and recovery sequence that included both tasks. At the end of the afternoon session, subjects were instructed to resume his or her regular consumption of caffeinated beverages until the abstention period prior to the next morning session, as well as to continue completing the caffeine consumption log. Subjects received monetary compensation for completion of the first two sessions.

On a separate day during the following week, subjects returned to the lab to complete an experimental procedure nearly identical to that of the first day, in the opposing coffee condition. Additionally, the memory task was not preceded by practice trials on the second day. Researchers debriefed subjects at the last session and subjects received monetary compensation for the third and fourth sessions (Refer to Figure 1 for schematic of study design).

4.7. Data Analyses

4.7.1. Physiological measures. Prior to performing analyses, measures from each epoch were averaged separately, with data expressed as mean values for each physiological variable. Multiple statistical tests were performed to specifically address the research questions.

Four-factor repeated measures Multivariate Analysis of Variance (MANOVA) tests using mean values were performed separately for each physiological measure to examine the presence of significant within-subject differences of means based on four factors of interest. Factors included: *Condition* (Caffeine, Decaffeinated), *Experimental Phase* (1, 2, 3), *Task* (memory, CP), and *Epoch* (baseline, task, recovery). Phases are coded as follows: (1) measures taken prior to consumption of coffee, (2) acute measures collected 30 minutes following intake of coffee, and (3) measures collected six hours following consumption of coffee.

Repeated measures MANOVA's using within-subject physiological change scores from baseline to task were also performed in order to index physiological reactivity. Reactivity scores were calculated by subtracting mean baseline from mean task values. In order to index physiological recovery, recovery epoch values were also taken into account by subtracting recovery scores from baseline scores in order to assess the extent to which subjects' physiological measures returned or "rebounded" to baseline values within the allotted three-minute recovery period.

BMI was analyzed as a covariate, computing analyses adjusted for BMI. Use of oral contraceptives (OC) was also controlled for, as these medications are associated with sustained increases in blood pressure indices during consistent use (Nichols et al., 1993). Use of OC was treated as a coded variable (1: OC use; 0: no OC use), and analyzed as a

covariate. A Bonferonni confidence interval adjustment was used for all MANOVA and pairwise comparison analyses.

4.7.2. Self-Report measures. Analyses of DASS and BPQ data were performed using a univariate repeated measures ANOVA, with Condition (Caffeine, Decaffeinated) as a factor. This analysis was performed to examine any possible effects of condition on self-reported perceptions of depression, anxiety, stress, and self-felt bodily awareness variables. DASS and BPQ data were also analyzed using correlational analyses to examine possible associations with HR and HRV measures on each day. Analysis of BMIS responses was performed using a two-factor repeated measures MANOVA, with Phase (1,2,3) and Condition as factors. Self-reported difficulty and pain ratings for tasks were analyzed using an identical analysis.

BMIS responses were scored on the following dimensions: Pleasant-Unpleasant and Arousal-Calm. Items included in the former dimension were: Active, Calm, Caring, Content, Happy, Lively, Loving, Peppy, Drowsy, Fed up, Gloomy, Grouchy, Jittery, Nervous, Sad, and Tired. Items included in the latter dimension were: Active, Caring, Fed up, Gloomy, Jittery, Lively, Loving, Nervous, Peppy, Sad, Calm, and Tired. Unpleasant and Calm items were reverse scored.

Finally, to examine the possibility of gender-related influences, multivariate ANOVAs were performed to assess the presence of gender effects on mean, reactivity, and recovery values for each physiological measure. Gender differences were also assessed for mood via ANOVA tests for self-report responses as well on the caffeinated day. Again, BMI and birth control were accounted for in the models as covariates.

Note: Data from some subjects of the total sample of 39 were not included in certain analyses due to equipment malfunctioning or confounding effects that appeared during the

study. Specifically, three individuals were excluded from HR and HRV analyses and data from two subjects were not included in the blood pressure analyses. Other missing data was imputed using a multiple imputation technique (MI). A maximum of 4% of data for each dependent measure was absent prior to imputation. Seven iterations were used based on accepted recommendations (Graham et al., 2007).

Note: Results derived from impedance cardiography measures (PEP, TPR, LVET) will be analyzed in the near future.

5. Results

For specific mean values by task, task reactivity, and recovery "rebound" for physiological variables, refer to Tables 2 through 4.

5.1. Heart Rate (HR)

A pairwise comparison test revealed significant differences between Phases 1 and 2 (p<.000), and 2 and 3 (p<.000), but no significant difference between Phases 1 and 3 (p=.88). A repeated measures MANOVA revealed a marginal main effect for mean values of HR for Phase [Wilks λ= .83, F(2,32)=3.25, p=.052]. Lowest HR was at phase 2, whereas higher HR values were at phases 1 and 3. Several significant interactions appeared for mean HR values. A Condition x Phase interaction revealed greatest reduction in HR at phase 2 in the Caffeine condition [Wilks λ=.76, F(2,32)=5.03, p=.013] (Figure 2). A significant Condition x Task interaction was found, where the memory task showed slightly lower HR values than the CP task, but only in the caffeinated condition [Wilks λ=.88, F(2,32)=4.35, p=.045]. A marginal Phase x Task interaction was found, where lowest values appeared at Phase 2, but lower values were found for the memory task versus the CP [Wilks λ=.83, F(2,32)=3.22, p=.053]. A significant Condition x Epoch interaction revealed acute increases in mean HR during the tasks, with overall lower mean values for the caffeinated condition

across epochs [Wilks λ=.72, $F(2,32)=6.34$, $p=.005$]. A marginal Phase x Epoch interaction appeared: [Wilks λ=.75, $F(4,30)=2.50$, $p=.063$]. Finally, a significant three-way interaction for Condition x Phase x Task revealed a similar pattern of mean HR values for Condition and Phase, but greater mean HR values during the CP task as compared to the memory task [Wilks λ= .78, $F(2,32)=4.56$, $p=.018$].

A repeated measures MANOVA test revealed a significant main effect for HR reactivity, each for Condition and Phase [Wilks λ=.74, $F(1,33)=11.36$, $p=.002$;Wilks λ=.80, $F(2,32)=4.13$, $p=.025$, respectively] (Figures 3 through 5). Greatest reactivity was found for the caffeine condition, which indicated a mean increase in HR of 3.6 beats per minute (BPM), versus the decaffeinated condition (mean increase of 3.3 BPM). Overall, HR reactivity was greatest at Phase 1. Collapsed across conditions, a significant Phase x Task interaction was found, where lowest reactivity was revealed at Phase 2 for the memory task (+3.06 BPM), and greatest reactivity was at Phase 2 for the CP task (+4.79 BPM) [Wilks λ=.75, $F(2,32)=5.31$, $p=.010$] (Figures 6 and 7). Among all time points, HR reactivity was greatest during the acute caffeinated period, though this difference was not significant. A significant three-way interaction (Condition x Phase x Task) occurred for recovery "rebound" [Wilks λ= .83, $F(2,32)=3.37$, $p=.047$].

5.2. High Frequency Heart Rate Variability (Ln hfHRV)

A repeated measures MANOVA test of mean values did not reveal significant effects for mean hfHRV values for Phase. However, significant interactions for Condition x Task, Condition x Epoch, and Task x Epoch existed [Wilks λ=.78, $F(1,33)=9.71$, $p=.004$; Wilks λ=.74, $F(2,32)$, $p=.008$; Wilks λ=.79, $F(2,32)=4.26$, $p=.023$, respectively]. Greater mean HRV was found for the CP task than the memory task, but only on the caffeinated day. Mean HRV values were greatest during tasks, and the caffeine condition was

associated with greater mean values overall, compared to the decaffeinated condition. For mean values, a Condition x Phase x Task interaction was found, where values were overall greatest during Phase 2, but especially on the caffeinated day [Wilks λ=.79, $F(2,32)=4.28$, $p=.023$].

A significant main effect of Condition was found for hfHRV reactivity, where greatest acute task-elicited increases were revealed on the caffeinated day [Wilks λ=.76, $F(1,33)=10.56$, $p=.003$]. Additionally, Phase was an important factor, with a marginal main effect [Wilks λ=.84, $F(2,32)=2.95$, $p=.067$]. In other words, collapsed across conditions, the test revealed significant chronological increases in positive hfHRV reactivity across time points (Figures 8 through 10).

A significant interaction for Phase x Task was found for the difference between mean recovery and baseline values [Wilks λ=.79, $F(2,32)=4.19$, $p=.024$]. Largest discrepancy between baseline and recovery hfHRV values were revealed during acute phase following decaffeinated coffee.

5.3. Root Mean Squared Successive Differences of R-R Intervals (Ln RMSSD)

The repeated measures MANOVA test for mean values revealed multiple significant two-way interactions: Condition x Phase [Wilks λ=.83, $F(2,32)=3.37$, $p=.047$] (Figure 11), Condition x Epoch [Wilks λ=.70, $F(2,32)=6.79$, $p=.003$], Phase x Task [Wilks λ=.82, $F(2,32)=3.59$, $p=.039$], and Phase x Epoch [Wilks λ=.72, $F(4,30)=2.88$, $p=.040$]. Greatest mean values were found in the caffeinated condition during the acute caffeinated experimental phase. Significant three way interactions were found for Condition x Phase x Task [Wilks λ=.64, $F(2,32)=9.03$, $p=.001$] and Phase x Task x Epoch [Wilks λ=.73, $F(4,30)=2.80$, $p=.044$]. Greatest mean values were found during the recovery epochs.

For reactivity values, two separate significant main effects were found for Condition (Figure 12) and Phase (Figure 13) [Wilks λ=.70, $F(1,33)= 13.92$, $p=.001$; Wilks λ=.82, $F(2,32)=3.62$, $p=.038$], respectively. A negligible increase in reactivity was found during the acute caffeinated phase, whereas a small decrease appeared for the acute decaffeinated phase. For recovery "rebound" values, a main effect for Phase was found [Wilks λ=.73, $F(2,32)=3.62$, $p=.038$]. A significant Condition x Task interaction was found [Wilks λ=.89, $F(1,33)=4.28$, $p=.047$]. A marginal trend was found for Phase x Task [Wilks λ =.84, $F(2,32)=2.96$, $p=.066$]. Finally, a significant Condition x Phase x Task interaction was found [Wilks λ =.79, $F(2,32)= 4.33$, $p=.022$]. In both conditions, overall positive differences between recovery and baseline were found at Phase 2. The memory task was associated with an increase during the acute caffeinated period, whereas the opposite was true for the CP.

5.4. Diastolic Blood Pressure (DBP)

Greatest DBP reactivity to tasks appeared at Phase 1, though no significant results were found for either mean, reactivity, or recovery values of DBP (Figure 14).

5.5. Systolic Blood Pressure (SBP)

MANOVA tests revealed no significant effects for mean values of SBP. However, pairwise comparisons of mean SBP values revealed significant differences between Phases 1 and 2 ($p<.00$), as well as 2 and 3($p<.00$), but no significant differences between Phases 1 and 3 ($p=.30$).

No significant results were shown for SBP reactivity to tasks, though SBP increased in response to tasks at all time points during the caffeinated and decaffeinated days. Greatest SBP reactivity values appeared at Phase 2 following consumption of caffeinated

coffee, whereas least SBP reactivity occurred during the acute phase following intake of decaffeinated coffee (Figure 15).

For the difference between baseline and recovery values for SBP, a marginal Condition x Task interaction was found [Wilks λ=.89, $F(1,34)=4.06$, $p=.052$], whereby recovery values were closer to baseline for the memory task versus the CP. This occurred to a greater extent on the caffeinated versus the decaffeinated day.

5.6. Mean Arterial Pressure (MAP)

Regarding mean arterial pressure (MAP), no significant results were found from a MANOVA test for either mean, reactivity, or recovery values. However, a pairwise comparison test revealed significant differences in mean MAP values between Phases 1 and 2 ($p<.000$), and 2 and 3 ($p<.000$), but no significant differences between Phases 1 and 3 ($p=.052$), with greatest values at Phase 2 (Figure 16).

5.7. Memory Task Performance

For mean reaction time (RT) across memory task trials, results of a repeated measures MANOVA showed a significant main effect of Phase [Wilks λ= .56, $F(2,34)=13.27$, $p=.000$]. RT's decreased sequentially from Phase 1 to Phase 3. A significant main effect for accuracy was also found for Phase [Wilks λ=.58, $F(2,34)=12.25$, $p= .000$]. Accuracy increased sequentially from Phase 1 to Phase 3. No significant reaction time or accuracy effects were found for Condition. (Refer to Table 5 for mean memory task performance values; Figures 18 and 19 for significant effects).

5.8. Questionnaires

5.8.1. BMIS. On the Pleasant-Unpleasant dimension, a two-factor repeated measures MANOVA (Condition, Phase) revealed a significant main effect of Phase [Wilks λ=.73, $F(2,37)=6.87$, $p=.003$]. Specifically, similar self-rated pleasantness occurred at Phase

1 on both days, but overall greater pleasant mood in the caffeine condition during Phases 2 and 3 as compared to the decaffeinated condition, wherein subjects reported overall decreases from Phase 1 to Phase 2 prior to increases at Phase 3. While no significant main effect of Condition was found, indices of pleasantness were highest during the afternoon session on the caffeinated day (Refer to Table 6 and Figure 20). On the Arousal-Calm dimension, no significant main effects or interactions were found.

5.8.2. DASS. As hypothesized, univariate repeated measures ANOVA revealed no significant main effect of Condition. Pearson correlations showed a significant positive relationship between the DASS Anxiety subscale and HRV reactivity to the memory task at Phase 2 only on the caffeinated day (r=.38, p=.02).

5.8.3. BPQ (Awareness Subscale). A univariate ANOVA revealed no significant main effect of Condition on BPQ Awareness responses. On the caffeinated day, a marginal trend revealed a positive correlation between responses on the BPQ Awareness subscale and HRV reactivity to the CP at Phase 2 (r=.32, p=.06).

5.9. Gender Effects

MANOVA tests revealed no consistent significant results for gender differences on mean, reactivity, or recovery "rebound" values. Multivariate tests revealed no significant gender differences in responses on either dimension of the BMIS, nor on DASS, or BPQ subscales.

5.10. Task Ratings

Results from a two-factor repeated measures MANOVA revealed a significant main effect of Phase for difficulty ratings for the memory task [Wilks λ=.72, F(2,35)=6.83, p=.003]. Greatest perceptions of difficulty occurred at Phase 1 on both the caffeinated and

decaffeinated days. On both days, average ratings of difficulty decreased at Phase 2, and again at Phase 3 (Table 7 and Figure 21).

Pain perception ratings for the CP task also revealed a significant main effect for Phase, whereby on average, greatest pain ratings occurred at Phase 1 and decreased over the measurement phases following [Wilks λ=.82, $F(2,34)=3.75$, p=.034]. Additionally, a significant interaction between Condition and Phase was found, with lowest ratings occurring at Phase 2 in the caffeine condition, but greatest ratings given at Phase 2 in the decaffeinated condition [Wilks λ=.78, $F(2,34)=4.81$, p=.014]. (Table 8 and Figure 22).

5.11. Consumption Journals

Subjects consumed a daily average of 309 mg of caffeine in the week preceding the study. Prior to the second day of the study, subjects consumed an average of 286 mg of caffeine daily. These averages include estimated amounts of caffeine from all sources (Appendix D).

5.12. Debrief

At debrief, some subjects were asked to provide a best guess about whether they had consumed caffeine on the first or second day of the study. Approximately half of subjects guessed correctly. They explained that their guesses were based on self-perceptions of comparative energy levels, memory task performance, taste of the coffee, and headaches on the decaffeinated day.

6. Discussion

The primary aim of the present study was to examine the efficacy of a six-hour abstention period in minimizing the confounding acute and negative abstention effects of caffeine on CVR (SBP, DBP, MAP, HR, hfHRV, RMSSD, and TPR, PEP, LVET), overall mood, and working memory performance in habitual consumers of moderate amounts of the

drug. A repeated measures design was employed for systematic examination of physiological reactivity to an alpha- and a beta-adrenergic laboratory stressor prior to, 30 minutes- and six hours-following consumption of coffee containing 230 mg of caffeine on one day and 5 mg on another day. In line with overall hypotheses, there was no evidence that CVR was elevated by caffeine six hours following consumption of coffee as indicated by results from pairwise comparisons between experimental phases. Similarly, no evidence suggested significant changes in mood or working memory ability following six hours of deprivation.

The most frequently studied cardiovascular responses to caffeine intake are blood pressure and HR. Well-established findings reveal acute increases in blood pressure following consumption of caffeine. Literature concerning HR responses is slightly variable but most studies support the existence of HR decreases that accompany an upregulated pressor response. In this scenario, baroreceptor reflexes mediate HR activity in response to caffeine-induced blood pressure increases. In support of this, results of the current study revealed overall acute decreases in mean HR following caffeine consumption. This was indicated in marginal trends toward decreases in average HR from morning measures obtained prior to caffeinated coffee consumption to measures obtained 30 minutes following caffeine consumption. HR values collected in the afternoon appeared to approximate values obtained during the first morning session.

While no systematic main effects or interactions appeared for blood pressure means or reactivity as a function of caffeine, mean SBP and MAP values indicated the hypothesized pattern: relatively low values during the first phase, and greatest values during the acute caffeinated phase, followed by decreases returning to values approximating the pre-caffeinated phase. This overall pattern of pressor responses is consistent with hypothesized time-dependent cardiovascular responses to caffeine. As predicted, these

results would suggest that six hours appears to adequately control for acute and deprivation effects of caffeine on HR and blood pressure. Despite lack of significant effects of Condition on pressor reactivity, mean values suggest the existence of additive effects of actions of caffeine and stress response systems, which much research has established (Lane et al., 2002; Lane & Williams, 1987).

Concerning blood pressure reactivity to tasks across phases on the caffeinated day, mean blood pressure values decreased across time points. Regardless of Condition, greatest overall pressor reactivity occurred during the morning sessions prior to consumption of coffee. It is well-established that the physiology of the cardiovascular system shows rhythmic 24-hour patterns due to circadian effects (Portaluppi et al., 2012). Circadian effects cause a tendency for highest blood pressure in the early morning hours, followed by a gradual decrease by the evening hours, which is associated with decreased sympathetic nerve traffic (Giles, 2006; Mancia, DiRienzo, & Parati, 1993).

While this is true, no consistent circadian patterns appeared across measures in the present study. For example, although reactivity values tended to peak during the first phase, mean pressor values were greatest during the acute caffeinated phase. Additionally, the existence of significant circadian HRV effects would reveal a tendency for high frequency HRV indices to peak during early morning and evening hours; a pattern not evident in results of the present study (Vandewalle et al., 2007; Molgaard, Sorensen, & Bjerregaard, 1991). Moreover, the inclusion of an identical day in which subjects consumed decaffeinated coffee provided a reasonably sufficient methodological control for circadian effects on CV indices as they relate to effects of caffeine consumption. Greatest blood pressure reactivity during the first experimental phase may be due to novelty effects (Van den Buuse, Acker, Fluttert, de Kloet, 2001).

As predicted, consistently positive reactivity values for blood pressure and HR across all task periods indicate the efficacy of the tasks in inducing sympathetic stress responses. The magnitude of responses to the CP exceeded that of the stressful but motivating memory task. Observed task-dependent variation in reactivity magnitudes is likely due in part to differential CV activation during stress that is driven by either primarily alpha-adrenergic (CP) or beta-adrenergic (memory task) systems (Montoya et al., 1997). For example, the CP elicited significantly greater DBP reactivity than the memory task, which is expected due to alpha- adrenergic mechanisms.

No significant main effects of task appeared for HR reactivity, but significant interactions between phase and task revealed increased HR reactivity for the CP following caffeine consumption. By contrast, HR reactivity to the memory task decreased during the acute caffeine consumption phase. This suggests that beyond alpha- versus beta- adrenergic categorization of tasks, other factors prove important. For example, the CP task is associated with HR increases in addition to vasoconstriction (Saab et al., 1993). Also, perceived stress plays in important role in these task differences in CVR. For example, memory task-elicited CV response magnitudes depend heavily on task difficulty, or the level of cognitive processing required. Results suggest that the task was not highly difficult.

In addition to reactivity effects, which were of primary interest in the current study, it was also deemed important to examine recovery values. The extent to and time by which values of cardiovascular indices return to baseline is informative in the study of cardiovascular dynamics. In terms of recovery, on average, individuals' mean SBP values following the CP task failed to return to baseline within the three minutes given, again reflecting task differences. Pressor responses to the CP task may also differ from the memory task in terms of temporal dynamics. The task causes an upregulation in vascular

sympathetic responses and sustained increases in blood pressure magnitude (Cui et al., 2002; Sendowski et al., 2000).

In an effort to address the relative lack of research examining effects of caffeine on indices of vagal activity, particularly concerning time-varying effects of the drug, the current study included measurement of heart rate variability in the high frequency domain (hfHRV) and a time-domain measure of HRV associated with vagally mediated cardiac control (RMSSD). Regarding caffeine's effects overall, expected patterns emerged, where significantly greater hfHRV and RMSSD values appeared on the caffeinated day. For hfHRV, this occurred specifically during the acute caffeinated phase, though this particular result did not reach significance.

RMSSD reactivity revealed small but significant decreases 30 minutes following caffeine intake, whereas the opposite was true for hfHRV. Notably, significant results pertaining to RMSSD reactivity in the present study are minimal in terms of magnitude of values, which presents difficulty in forming conclusions for implications. Significant findings concerning acute increases in vagal indices of HRV in response to both stressors likely reflects effective stress regulation mechanisms. Positive reactivity values occurred in response to both tasks. Positive reactivity to the cold pressor task was expected due to thermoregulating mechanisms. In fact, high frequency power has been shown to increase, as has sympathetic nervous activity, during cold stress but not heat stress, and this is due to subcutaneous thermoregulation (Huang et al., 2010). However, positive hfHRV reactivity to the memory task was not expected. Perhaps this response could be reflective of adaptive emotion regulation strategies during the task (Park & Thayer, 2014). Additionally, vagal fibers have been suggested to be part of a negative feedback loop that adjusts sympathoadrenal activity (Mravec, 2011).

Caffeine may also play a role in this possible occurrence given possible associations with parasympathetic activity. hfHRV reactivity associated with caffeine may also depend upon type of stressor. In the caffeinated condition, acute caffeinated task reactivity showed hfHRV increases for the memory task, but decreases for the CP. Subjects revealed systematic increases in hfHRV in response to tasks on both the caffeinated and placebo decaffeinated days but tended to show greater reactivity increases 30 minutes following ingestion of caffeine. For both conditions, greatest hfHRV reactivity occurred during the afternoon sessions. For the memory task, caffeinated day reveal hypothesized patterns (greatest reactivity during the second experimental phase). Importantly, following effects of caffeine on HRV reactivity at Phase 2, there did not appear to be any enduring effects of the drug six hours following consumption.

In alignment with findings of increased vagal activity in response to caffeine, results may generally suggest that hfHRV was positively associated with caffeine ingestion. Acute increases in HRV following consumption of caffeinated beverages have been demonstrated in recent research, which suggests a vagally-mediated response to the drug (Zimmermann-Viehoff et al., 2015; Monda et al., 2009). Though caffeine's more reliable effects appear to influence primarily sympathetic activity, research has indicated that moderate doses of caffeine (e.g. 240 mg) enhance modulation of parasympathetic activity (Hibino et al., 1997). Further research is required to specify vagally-mediated CV responses to caffeine. Nevertheless, results from the current study highlight the complexity of vagal and sympathetic activation related to caffeine use as well as acute reactivity to stress, despite caffeine's primarily sympathomimetic effects.

Additional considerations should be made in drawing conclusions regarding caffeine's CV effects. For instance, results imply that expectancy effects may be substantial enough to cause systematic effects on CVR, as suggested by certain unexpected patterns of

CVR to decaffeinated coffee (e.g. increased pressor reactivity following consumption of decaffeinated coffee in certain instances). Caffeine-associated stimuli (e.g. decaffeinated coffee) have been shown to increase both subjective and physiological indices of arousal such as skin conductance level and startle reflex magnitude, and decrease HR (Flaten & Blumenthal, 1999). Within a pharmacological classical conditioning framework, this effect has been postulated to result from the pairing of caffeine-related stimuli (i.e. coffee) with a conditioned response to caffeine. Also, caffeine expectancy effects have been shown to modulate respiratory responses to caffeine in low-vs. high anxiety sensitive individuals (Pané-Farré et al, 2014). For the purposes of the present study however, inclusion of the decaffeinated condition served as a placebo control.

Notably, results from the current study are intended only to generalize to populations with comparable sample characteristics (healthy college-aged individuals who are regular consumers of moderate amounts of caffeine) and may not generalize to those with CV illnesses, younger or older populations, or those with vastly different caffeine consumption behavior. Despite the inherent inability to generalize to differing populations, the sample selection in the current study allows for increased comparability across a large number of studies conducted in University laboratories that examine CVR, being that college students are commonly recruited in this type of research. Furthermore, caffeine consumption has been shown to be widespread among college students, where 89% of participants in a largescale study reported past-30-day caffeine use and respondents were most likely to consume caffeine on a daily basis (Norton, 2011).

Additional important considerations include sex differences. Based on prior research, sex differences were not predicted for mean or reactivity magnitudes. However sex differences in ICG indices were predicted. Primarily, caffeine-induced increases in blood pressure among female individuals are expected to be due to greater cardiac output,

whereas male counterparts are hypothesized to reveal greater increases in systemic vascular resistance (Hartley et al., 2004).

6.1. Questionnaire Responses

Responses from the Brief Mood Introspection Scale (BMIS) indicate that general indices of mood were significantly associated with study phases. Specifically, afternoon measures of self-rated pleasant mood were greatest compared to ratings collected at both morning time points. Despite lacking significant mean differences of Pleasant-Unpleasant mood based on Condition, BMIS responses suggest that subjects may have experienced more positive mood following caffeine consumption, an occurrence long supported in the literature (Ruxton, 2008). This was indicated by greater scores on the caffeinated day than on the decaffeinated day, at Phases 2 and 3. Importantly, subjects did not report negative mood changes following the six-hour deprivation period, supporting hypotheses.

A significant positive relationship between the DASS Anxiety subscale and hfHRV reactivity to the memory task at Phase 2 on the caffeinated day was unexpected, as state anxiety is generally associated with increased vagal withdrawal (Friedman, 2007). This unexpected finding may suggest an offsetting of anxiety-induced vagal withdrawal by acute caffeine consumption, which has been shown in the present study to potentiate momentary increases in hfHRV. However, this presumption must come with caution, as subjects were informed to report their experiences of anxiety over the past week. Therefore, in this instance, state anxiety is not necessarily specific to present emotions. Additionally, this correlational result prevents causational explanations. Further research must investigate how caffeine may modulate the relationship between stress, anxiety, and hfHRV effects.

The Awareness subscale of the BPQ indexes self-reported overall awareness of bodily processes including cardiac, gastrointestinal, muscular, and respiratory reactions.

Those who reported a tendency to notice these physiological changes when they occur showed marginal trends toward greater hfHRV reactivity to the CP following consumption of caffeinated and not decaffeinated coffee. This finding indicates differential responding on both days of the study, which is interesting, considering that the scale is designed to assess trait measures of bodily awareness. Perhaps caffeine is able to influence self-perceptions of bodily awareness to a certain extent.

Regarding subjects' perceptions of the tasks, both ratings and physiological reactivity suggest that the CP task was generally more aversive than the memory task, although ratings were on different scales (pain versus difficulty) and task aversion was not directly assessed. Aside from differences in physiological reactivity to tasks resulting from differing adrenergic sources, each of the two tasks involve highly complex physiological, cognitive, and emotional responses that markedly diverge.

The incidence of greatest pain ratings during the first experimental phase was predicted, given that individuals tend to report greater pain upon initial pain experiences due to pain habituation mechanisms (Rennefeld, Wiech, Schoell, Lorenz, & Bingel, 2010). While pain severity was rated as moderate at all experimental phases, some degree of nociceptive tolerance appears to have occurred over time. Responses also suggest the presence of known analgesic effects of caffeine, as the CP task was rated as least aversive during the acute caffeinated phase.

6.2. Memory Task Performance

Performance on the modified Sternberg memory task was expected to increase as a result of acute caffeine consumption, however, individuals indicated progressive increases in task performance measures across both days. This did not vary as a function of Condition. Sequential reaction time decreases and accuracy increases likely reflect the

presence of practice effects, and are not likely to reflect momentary improvements in working memory ability or ease of focus following caffeine ingestion.

7. Conclusions

Caffeine has known effects on CVR, yet there exists no standardization nor guidelines for caffeine abstention duration for subjects prior to study participation. Hence, methodological rigor and the replicability of findings across studies is compromised by lack of consistent control for acute caffeine intake. The current experiment addresses this understudied issue that has significant implications for research in psychophysiology and behavioral medicine. The primary aim of the study was to elucidate the optimal caffeine abstention duration prior to CV studies that minimizes both the confounding effects of caffeine and aversive effects regular caffeine users may experience from extended abstention. Abstention effects are manifested both physiologically and psychologically, and pose as much of a threat to the validity of a study as caffeine itself.

Given the exceedingly vast use of caffeine, the need to adequately and consistently control for the drug in CVR research is clearly evident. Potential confounds associated with caffeine are likely to partially underlie widely variable results in research examining caffeine's effects on psychophysiological indices. The current investigation and studies similar to this provide information that is crucial to the understanding of the behavior of cardiovascular systems in response to caffeine. Implications for health are significant, as such investigations inform caffeine-relevant as well as CVR research, more broadly.

In evaluating the overall cardiovascular effects of caffeine at each time point, results support the adequacy of the six-hour abstention duration for CVR study participation among habitual consumers of moderate amounts of the drug. This duration appeared adequate in reducing acute effects of caffeine on CVR and avoiding deprivation effects. This is

concluded based on the absence of confounding effects of caffeine on CVR during the afternoon session. As expected, magnitude of results indicate somewhat divergent results depending on which physiological measure is examined. Certain CV indices may be more sensitive to effects of caffeine. Additionally, patterns of cardiovascular reactivity to caffeine partly depend upon factors such as the particular type of stress induction (i.e. perhaps alpha- versus beta-adrenergic). Indices derived from impedance cardiography (PEP, LVET, and TPR) will provide much useful information, when completed. Systolic time intervals and volumetric indices will elucidate caffeine's temporal effects on sympathetic activation and CVR at the time points of interest.

Potential limitations of the study include: self-reported caffeine abstention (vs. biologically verified (e.g. by saliva assay), practice effects on the memory stressor, and individual differences due to genetics and caffeine metabolism rates. Controlling for these inherent limitations was unfeasible in this type of study design. Finally, given that the purpose of the study was to examine the adequacy of a six-hour abstention from caffeine in a particular population (regular consumers of moderate amounts of caffeine (via coffee), ages 18-24), generalizability is inherently and somewhat intentionally limited.

This is the first known published study to systematically examine CVR to caffeine in a repeated measures study in a specific attempt to identify the ideal abstention duration that lends to unconfounded CVR affects. The repeated measures design allowed for examination of within-subject changes across multiple time points. The use of multiple factors in the current study allowed for specific examination of variables that systematically affect CVR to caffeine in different situations. Inclusion of several CV indices and manipulated variables allowed for a more definitive picture of the effects of caffeine on CVR over time.

7.1. Future Directions

The current study is envisioned as the first in a series of investigations aiming to ascertain an ideal abstention duration for CVR to caffeine. Replication of this investigation will be crucial in identification of ideal methodological standards for caffeine controls. A six-hour abstention duration was used based on the average half-life of the drug. Future research may examine effects using a shorter period of time, which would be practically ideal. Furthermore, results from the present study may provide methodological guidelines for experimental sessions run in the afternoon, but may not apply to running of morning experimental sessions. For that reason, it is necessary to examine these relationships at varying times of the day.

Investigations examining CVR effects of withdrawal and deprivation are virtually nonexistent. Psychological and general health effects of caffeine deprivation such as headaches and fatigue are well-documented. However, there is an essential lack of evidence indicating the extent to which deprivation from caffeine among regular consumers affects CV indices. Moreover, it is unclear which autonomic indices are most affected by withdrawal. This lack of information demands investigations in this domain. Additional physiological and self-reported indices pertaining specifically to deprivation effects should be included in future research.

Research interests regarding the influence of caffeine have been guided by a primary focus in caffeine's sympathomimetic effects. Historically, research concerning CVR to caffeine has almost exclusively focused on the role of sympathetic activation. A great need exists to investigate the potential role of caffeine in acutely or more persistently altering HRV. Since caffeine affects both sympathetic and parasympathetic nervous system activity and HR is influenced by innervation from both systems, there is great value in elucidating the actions of caffeine on the interplay of these systems. Furthermore, it is currently

uncertain how caffeine may affect moment-to-moment variations in blood pressure. More investigations that include measures of baroreceptor sensitivity may provide a more lucid understanding of how caffeine may differentially affect individuals' CVR across short durations of time.

Finally, replicating this study by examining various demographic groups (e.g. older populations) is also necessary in order to gain a superior understanding of ideal caffeine control procedures in research using various subpopulations. The current study and future studies in this line of research ultimately have significant implications for physical and emotional health, as maximally valid psychophysiological research hinges upon adequate control for the most commonly consumed psychoactive substance worldwide.

Table 1. *Participant characteristics*

Demographic Measures	**Mean ± S.D. or N (%)**
Age (years)	20.90 ± 1.92
Race	
Caucasian	26 (60.4%)
African American / Black	1 (2.3%)
Asian	11 (25.5%)
Latino / Hispanic	1 (2.3%)
BMI (kg/m^2)	
Male	25.9 ± 3.43
Female	24.0 ± 3.52
Baseline Physiological Measures	**Mean**
HR (bpm)	72.42
RR (bpm)	17.14
Ln hfHRV (ms^2)	6.63
Ln RMSSD (ms)	3.81
SBP (mm Hg)	120.42
DBP (mm Hg)	69.50
MAP (mm Hg)	86.76

Table 2. *Mean physiological values by task*

CV Measure	Mean Memory	Mean Cold Pressor
	Caffeinated day	
HR (bpm)	74.58	76.04
RR (bpm)	19.04	16.71
Ln hfHRV (ms^2)	6.99	6.92
Ln RMSSD (ms)	3.87	3.89
SBP (mmHg)	124.94	132.53
DBP (mmHg)	73.11	79.35
MAP (mmHg)	90.84	97.52
	Decaffeinated day	
HR (bpm)	75.27	77.49
RR (bpm)	18.89	17.05
Ln hfHRV (ms^2)	6.87	6.65
Ln RMSSD (ms)	3.81	3.80
SBP (mmHg)	124.71	134.50
DBP (mmHg)	72.03	79.49
MAP (mmHg)	**89.69**	**97.06**

Table 3. *Mean reactivity on caffeinated day (Task – Baseline)*

CV Measure	Experimental Phase	Task	Mean Reactivity	SD
Heart Rate (HR) (Beats per minute (bpm))	1	Memory	3.88	5.80
		Cold Pressor	3.54	5.72
	2	Memory	3.06	7.24
		Cold Pressor	4.79	6.74
	3	Memory	3.77	5.73
		Cold Pressor	2.58	0.77
High Frequency Heart Rate Variability (Ln hfHRV (ms^2))	1	Memory	0.14	0.90
		Cold Pressor	0.29	0.76
	2	Memory	0.43	1.01
		Cold Pressor	0.03	0.73
	3	Memory	0.26	0.75
		Cold Pressor	0.27	0.77
Root Mean Squared Successive Differences (LnRMSSD (ms))	1	Memory	0.05	0.32
		Cold Pressor	0.06	0.36
	2	Memory	0.11	0.39
		Cold Pressor	-0.06	0.28
	3	Memory	0.09	0.36
		Cold Pressor	0.06	0.30
Systolic Blood Pressure (SBP) (mmHg)	1	Memory	5.69	6.98
		Cold Pressor	13.98	8.22
	2	Memory	3.14	6.11
		Cold Pressor	11.97	10.29
	3	Memory	3.73	5.64
		Cold Pressor	11.84	7.42
Diastolic Blood Pressure (DBP) (mmHg)	1	Memory	3.41	5.20
		Cold Pressor	11.34	6.30
	2	Memory	2.39	5.62
		Cold Pressor	10.41	7.66
	3	Memory	2.88	4.20
		Cold Pressor	7.69	6.21
Mean Arterial Pressure (MAP) (mmHg)	1	Memory	4.29	5.57
		Cold Pressor	12.76	7.62
	2	Memory	2.84	5.34
		Cold Pressor	10.24	11.35

	3	Memory	3.41	4.51
		Cold Pressor	9.98	7.42

Table 4. *Mean recovery "rebound" values on caffeinated day (Recovery – Baseline)*

CV Measure	Experimental Phase	Task	Recovery Rebound	SD
Heart Rate (HR) (Beats per minute (bpm))	1	Memory	1.19	3.03
		Cold Pressor	-1.88	3.42
	2	Memory	2.29	3.97
		Cold Pressor	-0.85	4.70
	3	Memory	0.25	4.05
		Cold Pressor	-2.52	4.84
High Frequency Heart Rate Variability (Ln hfHRV (ms^2))	1	Memory	-0.11	0.64
		Cold Pressor	0.41	0.69
	2	Memory	0.17	0.53
		Cold Pressor	0.06	0.62
	3	Memory	0.11	0.56
		Cold Pressor	0.18	0.79
Root Mean Squared Successive Differences (LnRMSSD) (ms)	1	Memory	-0.01	0.27
		Cold Pressor	0.17	0.25
	2	Memory	0.02	0.24
		Cold Pressor	0.03	0.25
	3	Memory	0.08	0.27
		Cold Pressor	0.15	0.26
Systolic Blood Pressure (SBP) (mmHg)	1	Memory	-0.12	4.52
		Cold Pressor	2.92	6.95
	2	Memory	-0.57	7.84
		Cold Pressor	3.52	7.22
	3	Memory	0.90	5.26
		Cold Pressor	3.82	5.09
Diastolic Blood Pressure (DBP) (mmHg)	1	Memory	-0.70	3.87
		Cold Pressor	2.01	5.05
	2	Memory	-0.91	4.98
		Cold Pressor	2.72	4.14
	3	Memory	0.29	4.10
		Cold Pressor	1.36	4.91
Mean Arterial Pressure (MAP (mmHg)	1	Memory	-0.75	4.14
		Cold Pressor	3.28	4.05
		Memory	-0.35	5.71

	2			
		Cold Pressor	2.96	4.62
	3	Memory	0.69	4.31
		Cold Pressor	2.49	4.54

Table 5. *Memory task reaction time (RT) and accuracy*

Reaction Time

Condition	Phase	Mean (ms)	Std. Error
Caffeinated	1	661.391	24.217
	2	639.428	22.352
	3	605.457	21.391
Decaffeinated	1	665.563	31.026
	2	647.783	31.215
	3	605.227	22.326

Accuracy

Condition	Phase	Mean (% Correct)	Std. Error
Caffeinated	1	92	.013
	2	94	.015
	3	96	.013
Decaffeinated	1	93	.020
	2	95	.016
	3	95	.014

Table 6. *Self-reported pleasantness, Brief Mood Introspection Scale (BMIS)*

	Mean	SD
Caffeinated, Phase 1	47.03	5.470
Caffeinated, Phase 2	47.56	5.046
Caffeinated, Phase 3	49.44	4.576
Decaffeinated, Phase 1	47.13	5.492
Decaffeinated, Phase 2	45.77	6.106
Decaffeinated, Phase 3	47.97	6.434

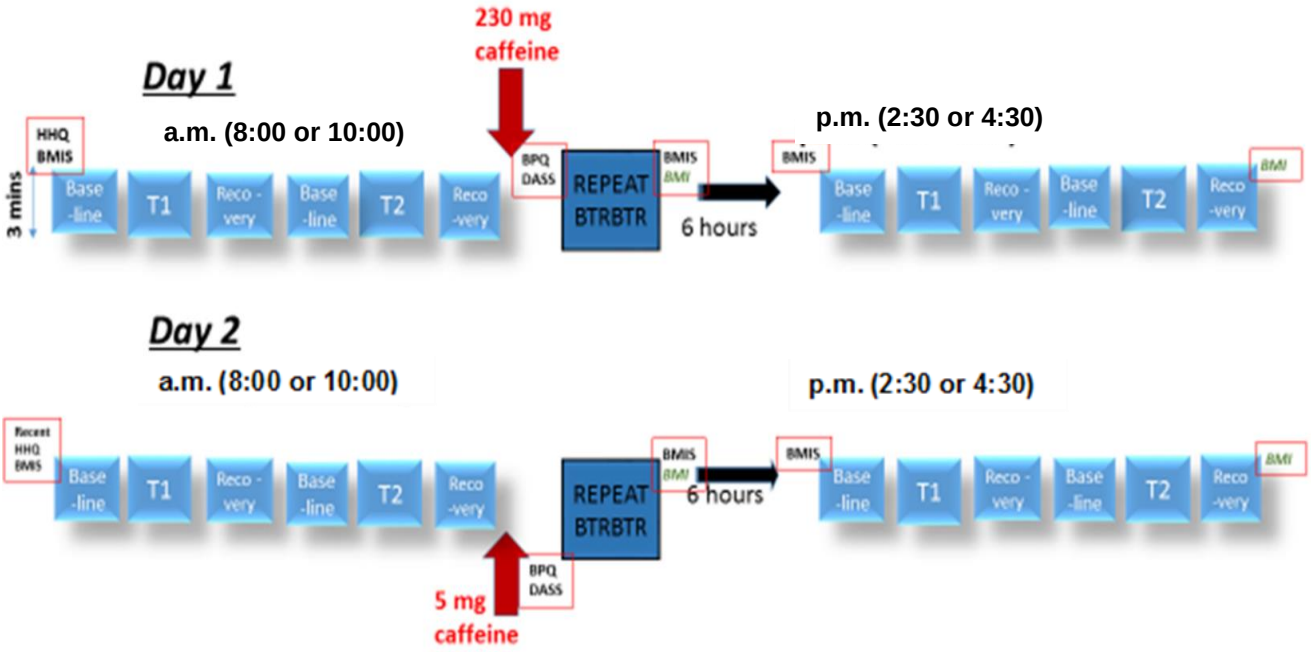
Table 7. *Self-reported difficulty following memory task*

Condition	Phase	Mean	Std. Error
Caffeinated	1	3.973	.371
	2	3.324	.340
	3	3.243	.362
Decaffeinated	1	3.595	.364
	2	3.297	.340
	3	3.108	.319

Table 8. *Self-reported pain for cold pressor task*

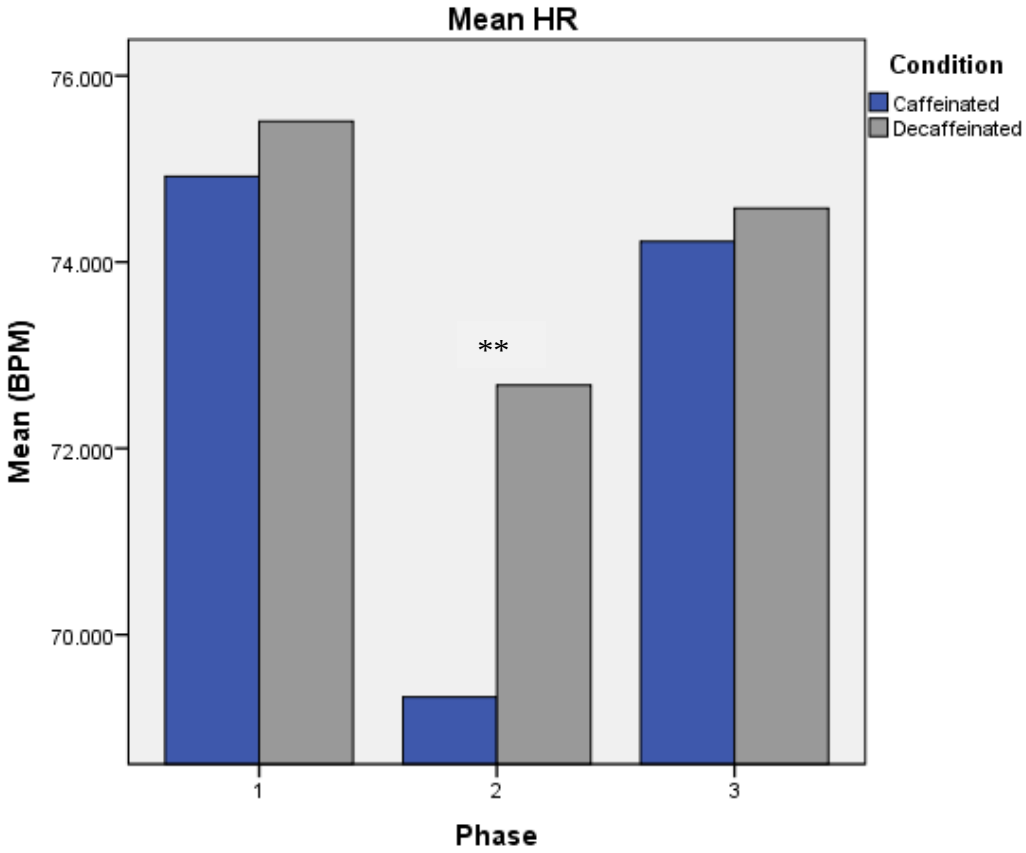
Condition	Phase	Mean	Std. Error
Caffeinated	1	5.833	.307
	2	5.417	.296
	3	5.472	.307
Decaffeinated	1	5.806	.267
	2	5.861	.282
	3	5.306	.281

Figure 1. *Study Design*



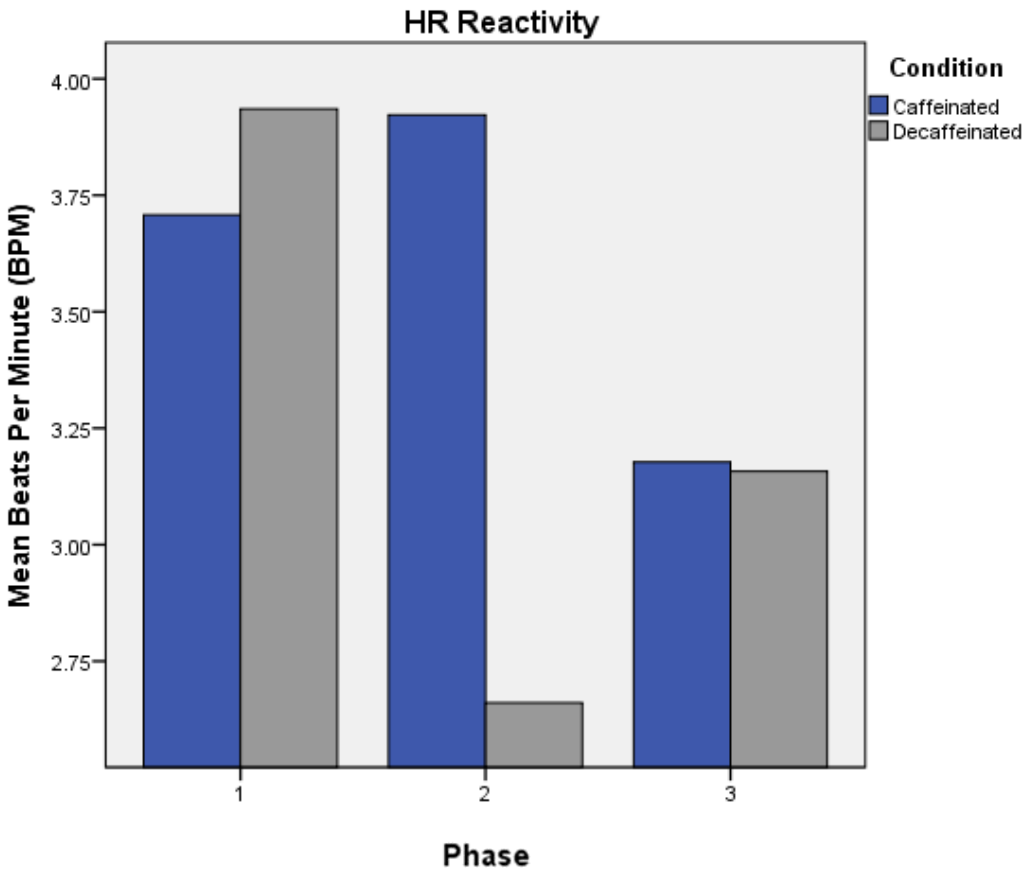
Notes. "T"= Task; "Repeat BTRBTR"= Repetition of sequence involving baseline, task, and recovery epochs for both tasks; Conditions, tasks and baseline videos were counterbalanced across subjects and sessions.

Figure 2. *Mean heart rate (HR), Condition x Phase*



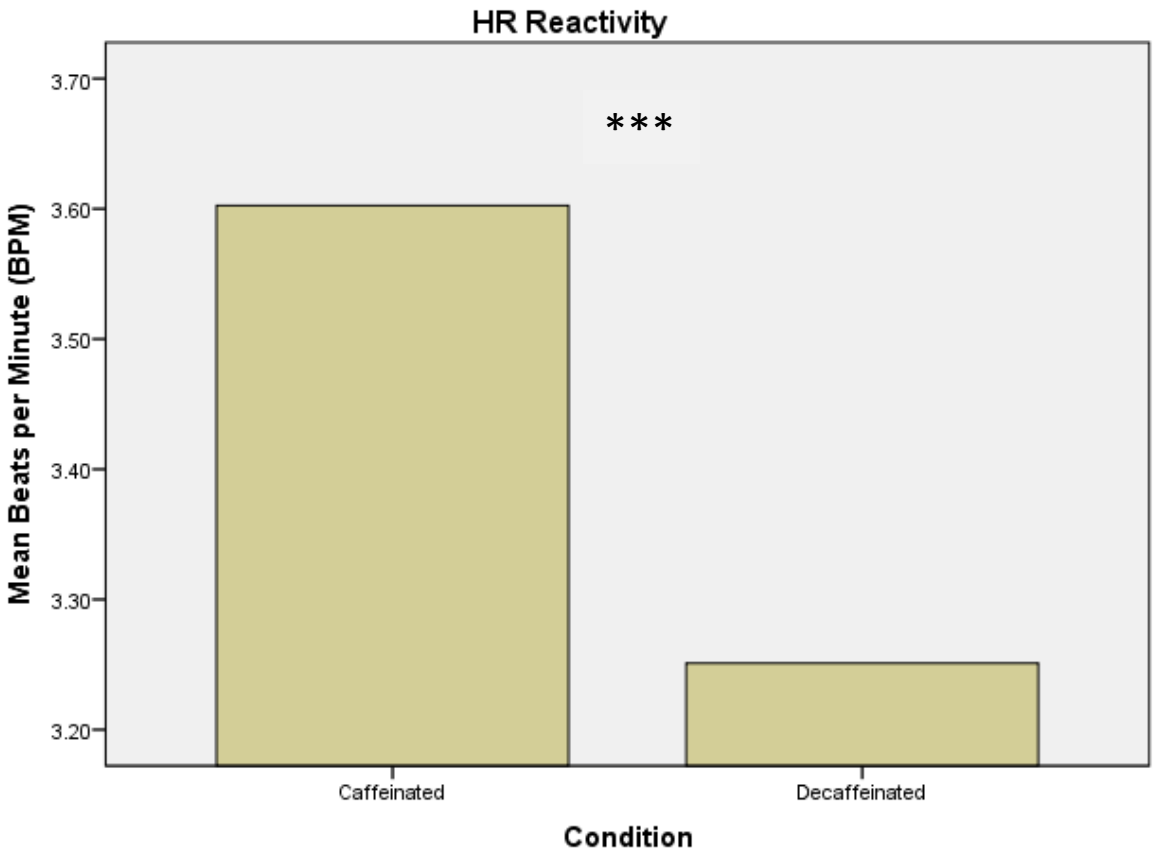
**Significant at p<.05

Figure 3. *Mean HR reactivity, Condition and Phase*



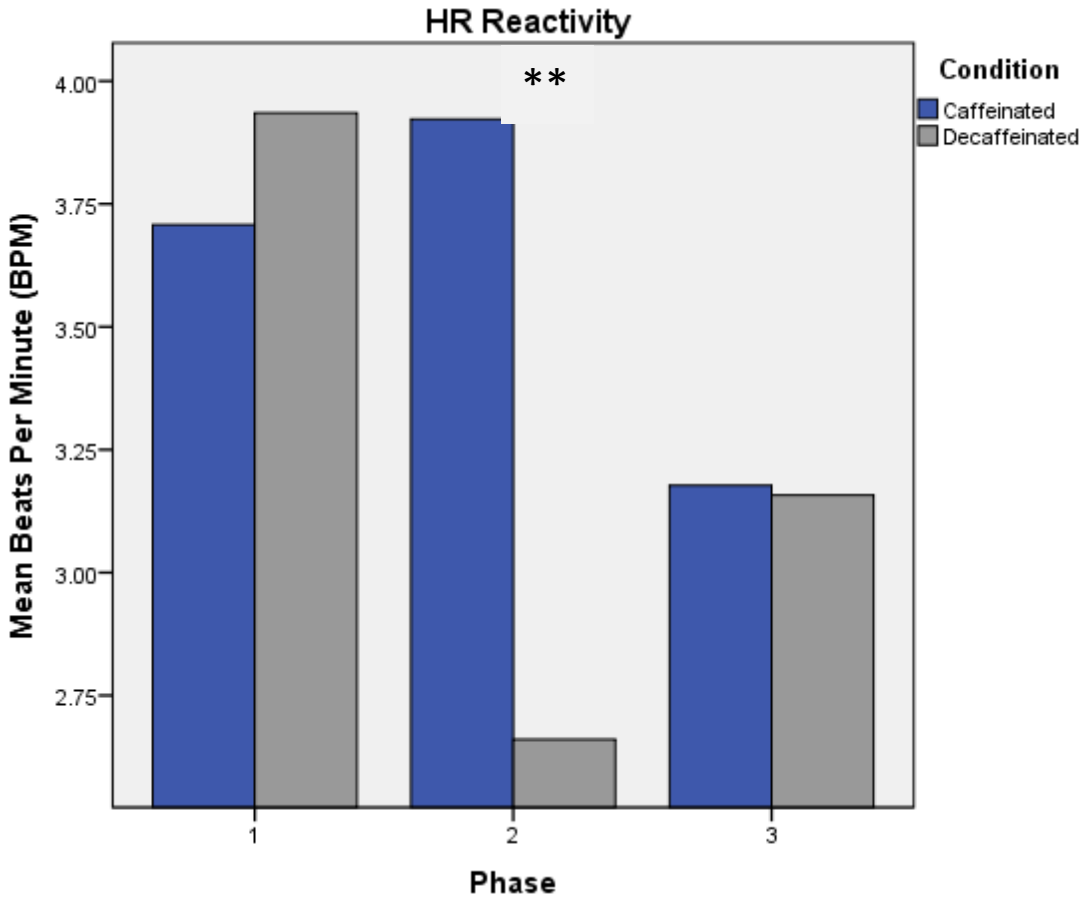
Note. Two separate main effects for Condition and Phase were significant at $p<.05$

Figure 4. *Mean HR reactivity by Condition*



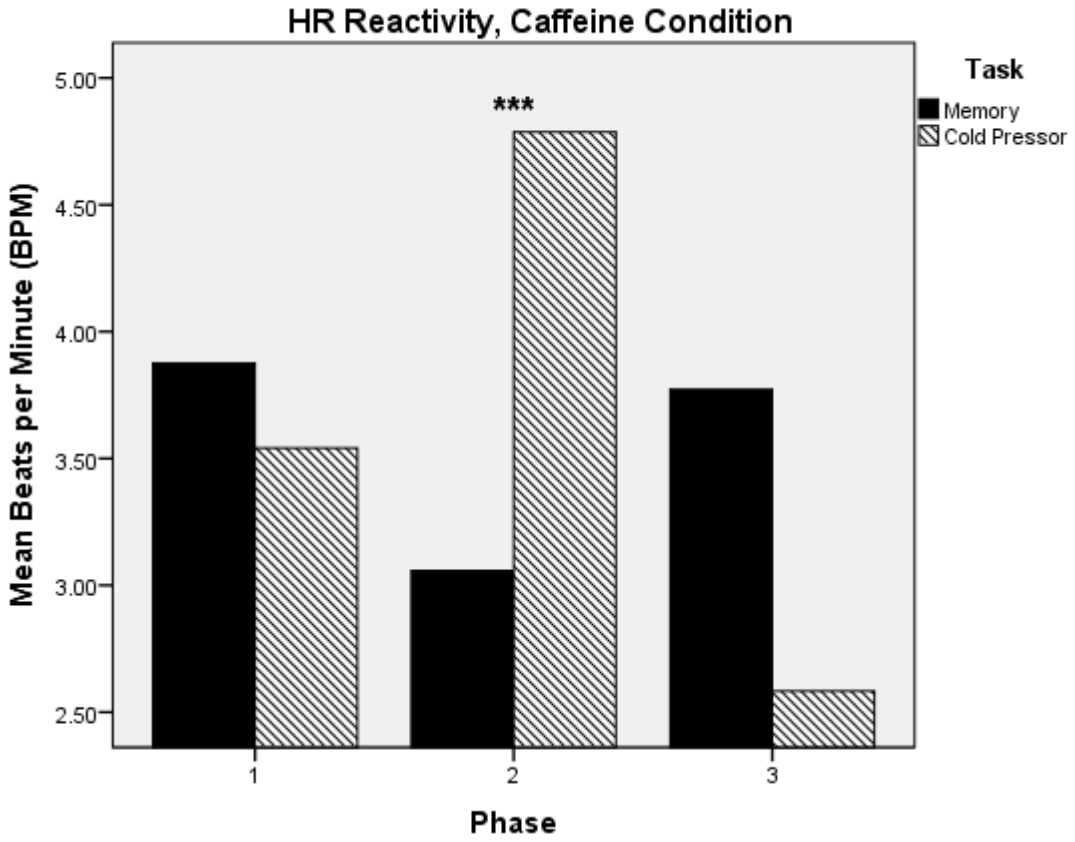
*** Significant at p<.01

Figure 5. *Mean HR reactivity by Phase*



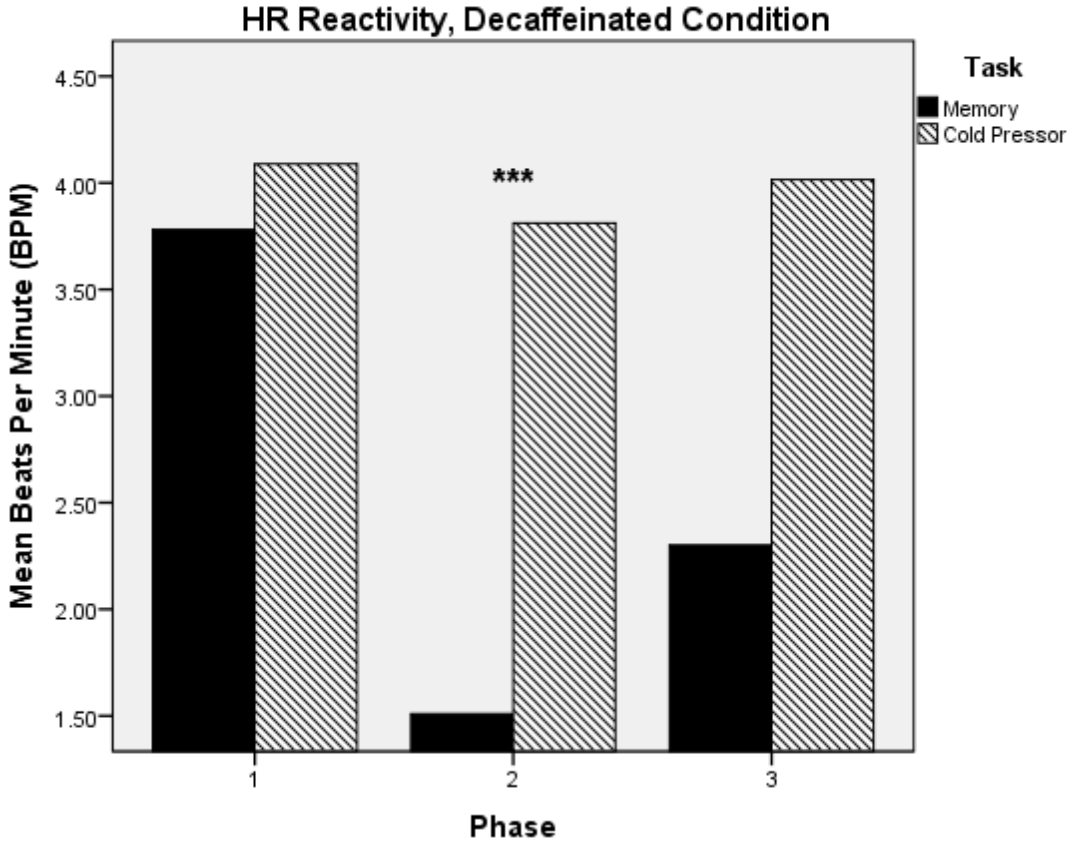
** Significant main effect of Phase at p<.05

Figure 6. *Mean HR reactivity, Phase x Task (Caffeine)*



*** Phase x Task Interaction significant at p=.01

Figure 7. *Mean HR reactivity, Phase x Task (Decaffeinated)*



*** Phase x Task interaction significant at p=.01

Figure 8. *Mean natural log transformed hfHRV reactivity, Phase by Condition*

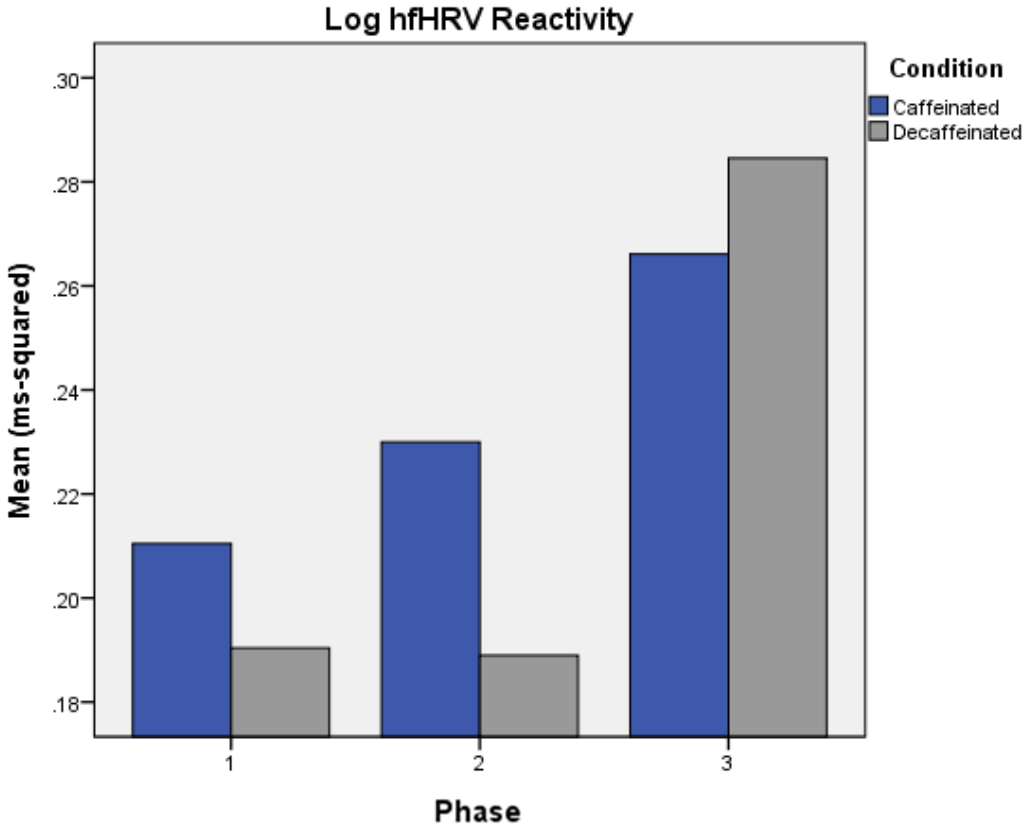
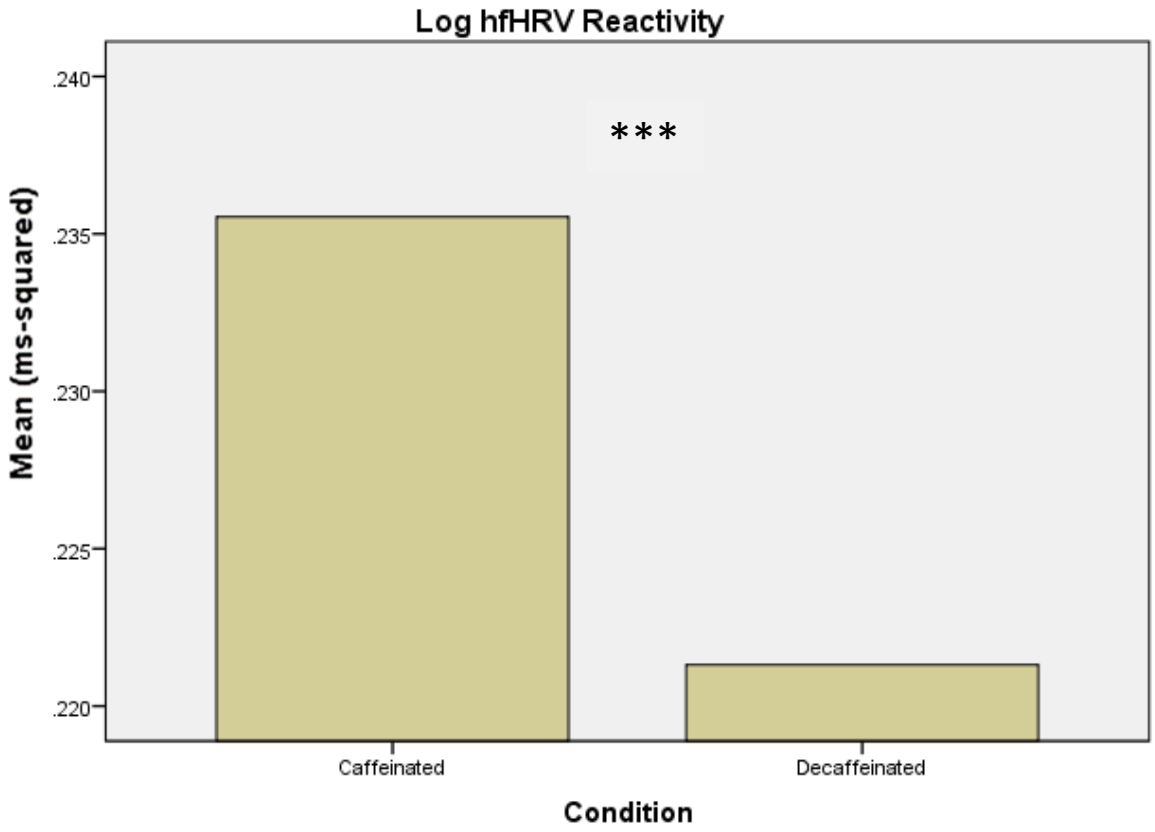
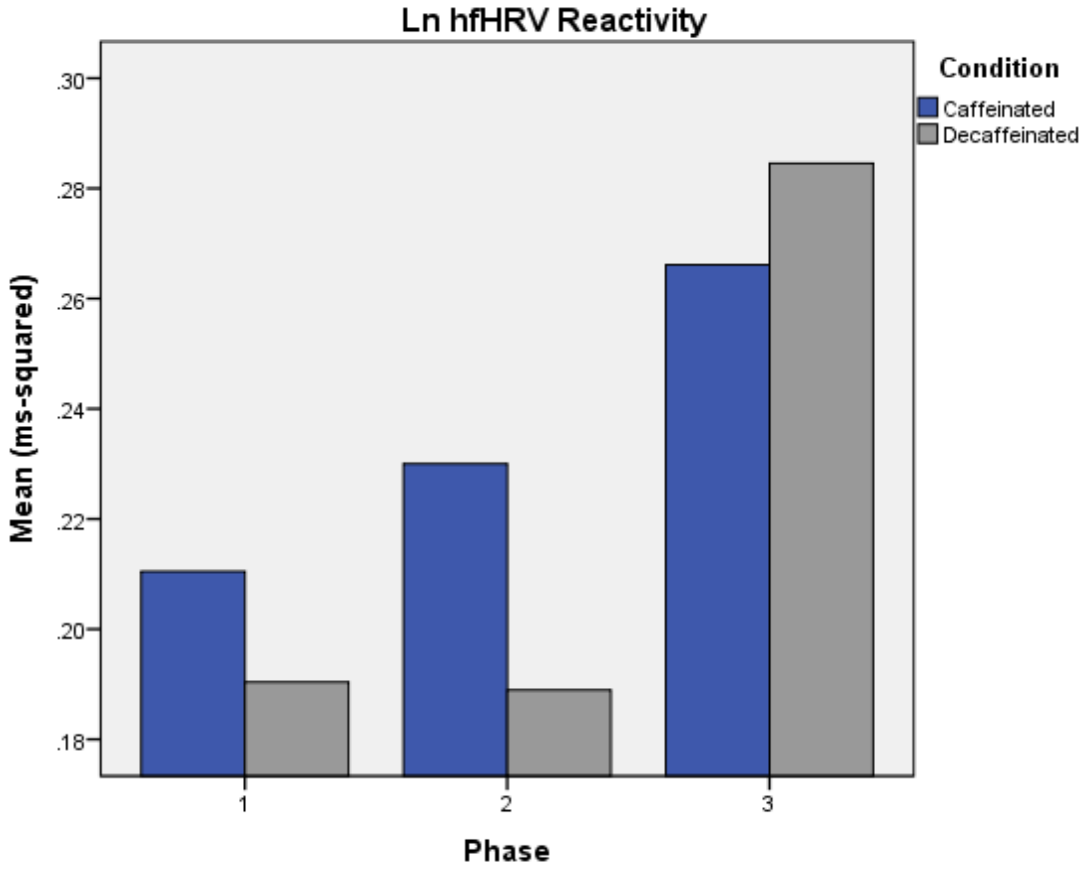


Figure 9. *Mean natural logarithm transformed hfHRV Reactivity by Condition*



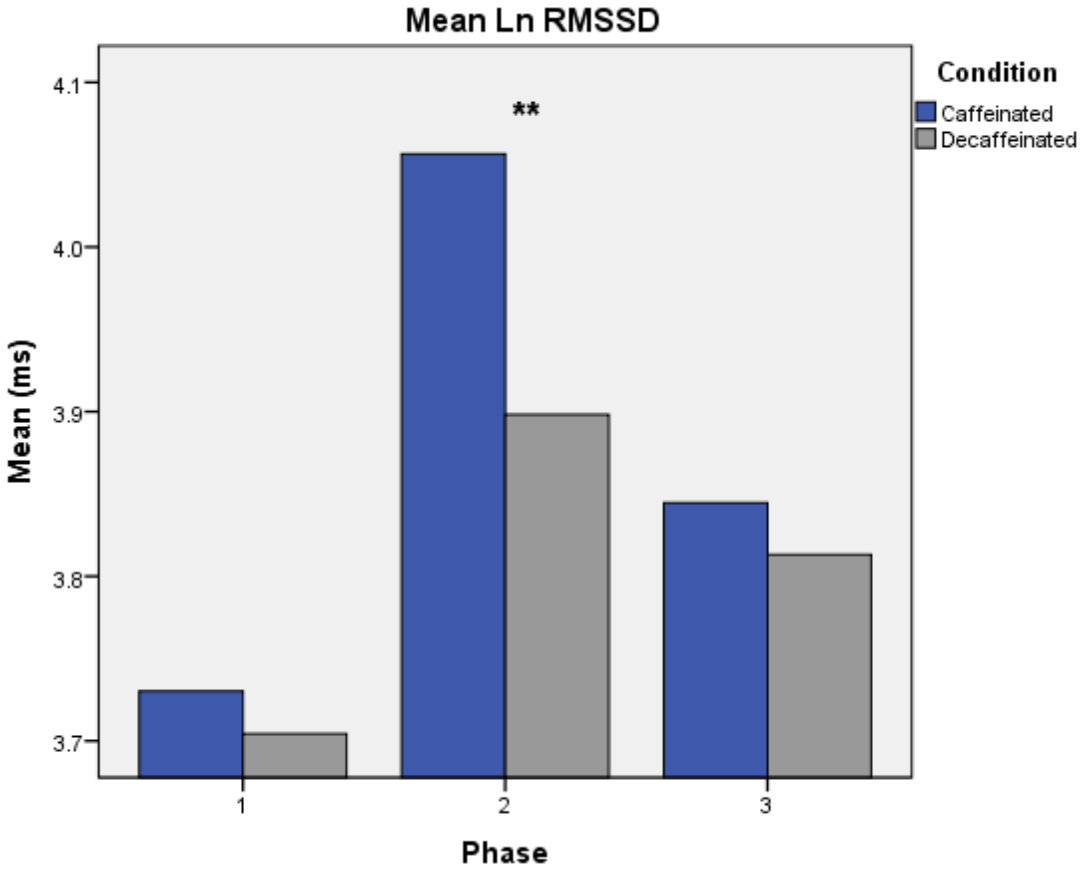
*** Significant at p<.01**.**

Figure 10. *Mean natural logarithm transformed hfHRV Reactivity by Phase*



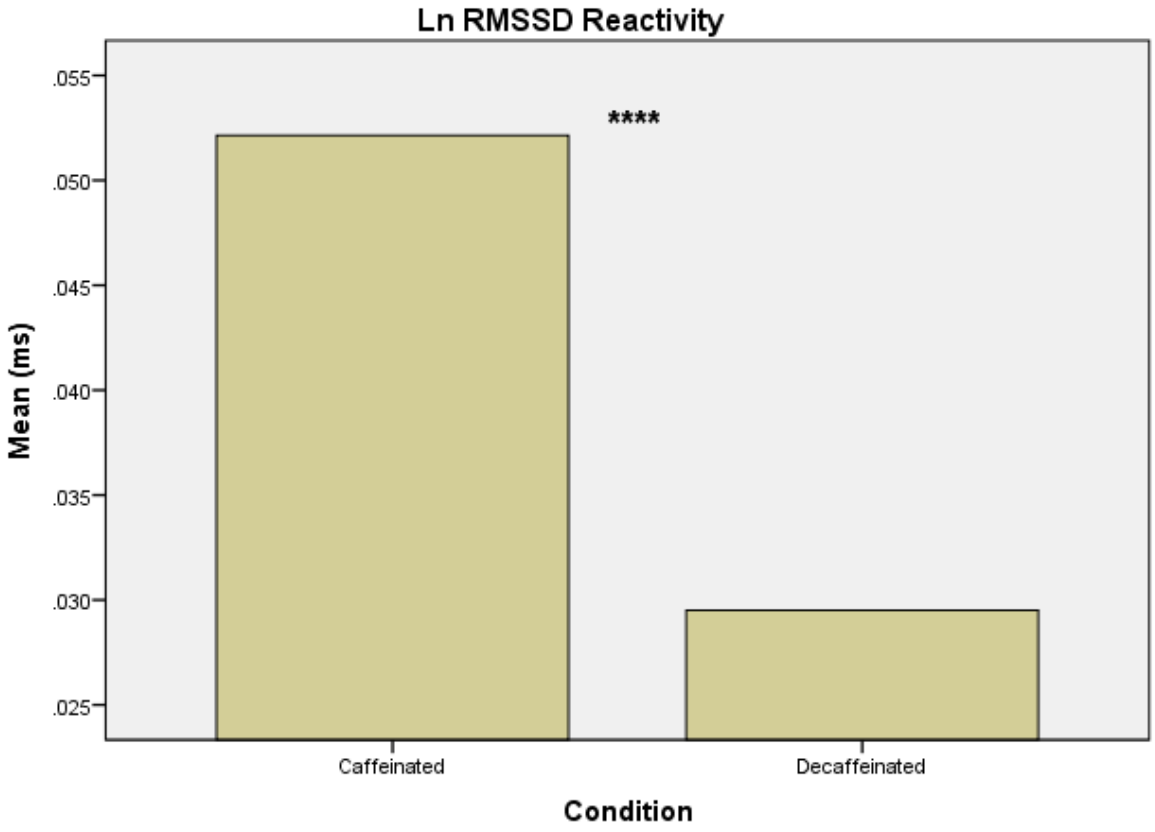
Note: Marginal trend for Phase at p=.067

Figure 11. *Mean natural logarithm transformed RMSSD, Condition x Phase*



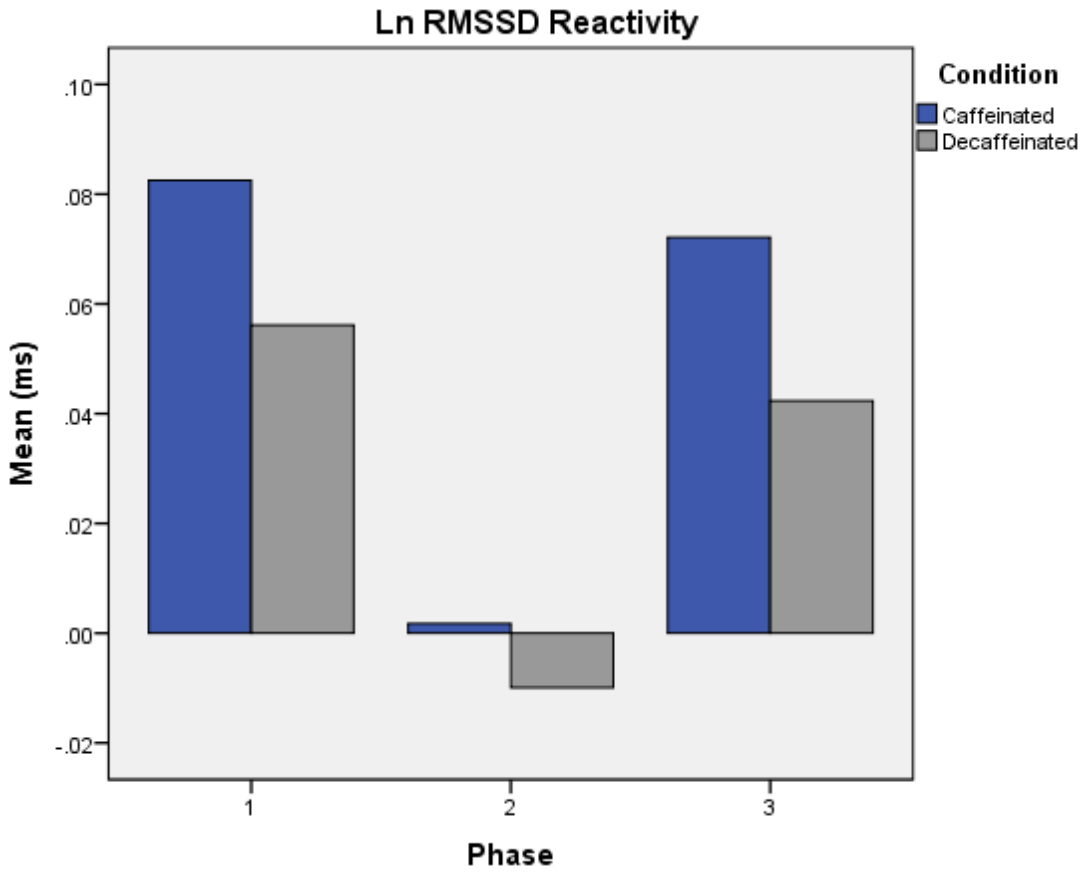
** Condition x Phase interaction significant at $p<.05$.

Figure 12. *Mean natural logarithm transformed RMSSD Reactivity by Condition*



** Main effect of Condition significant at p=.001.

Figure 13. *Mean natural logarithm transformed RMSSD Reactivity, Phase by Condition*



Note: Main effect of Phase significant at p<.05

Figure 14. *Mean DBP reactivity by Phase by Condition*

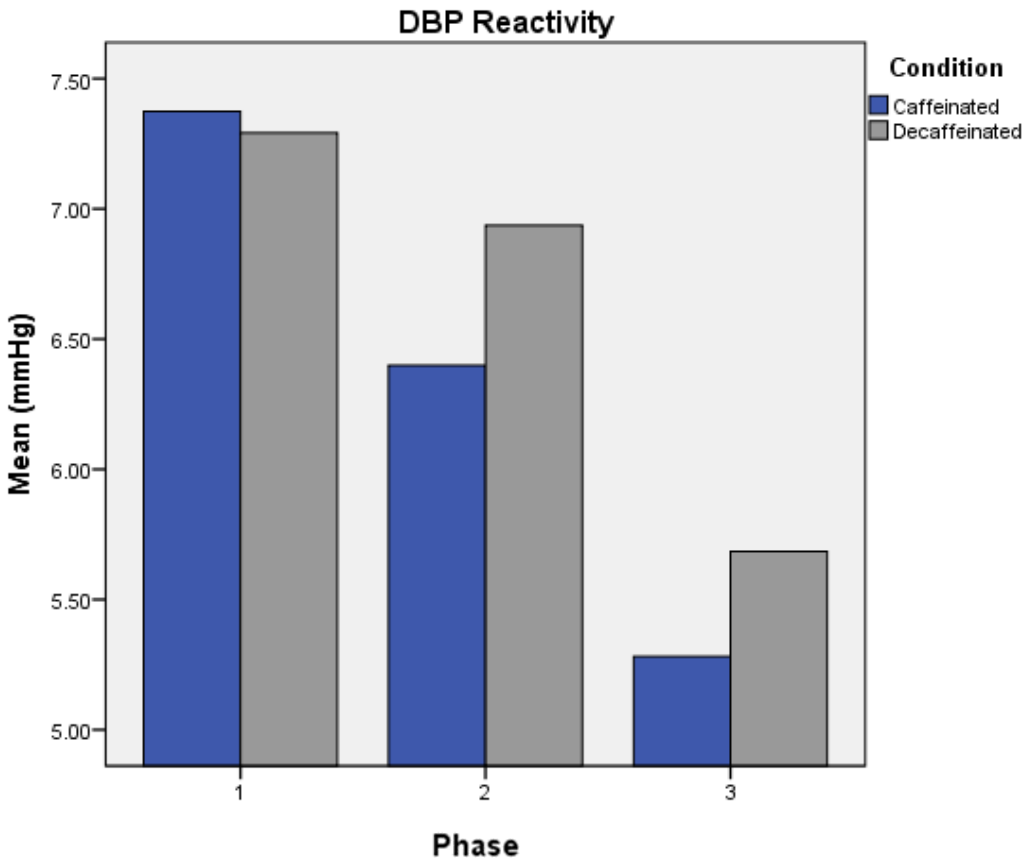


Figure 15. *Mean SBP reactivity, Phase by Condition*

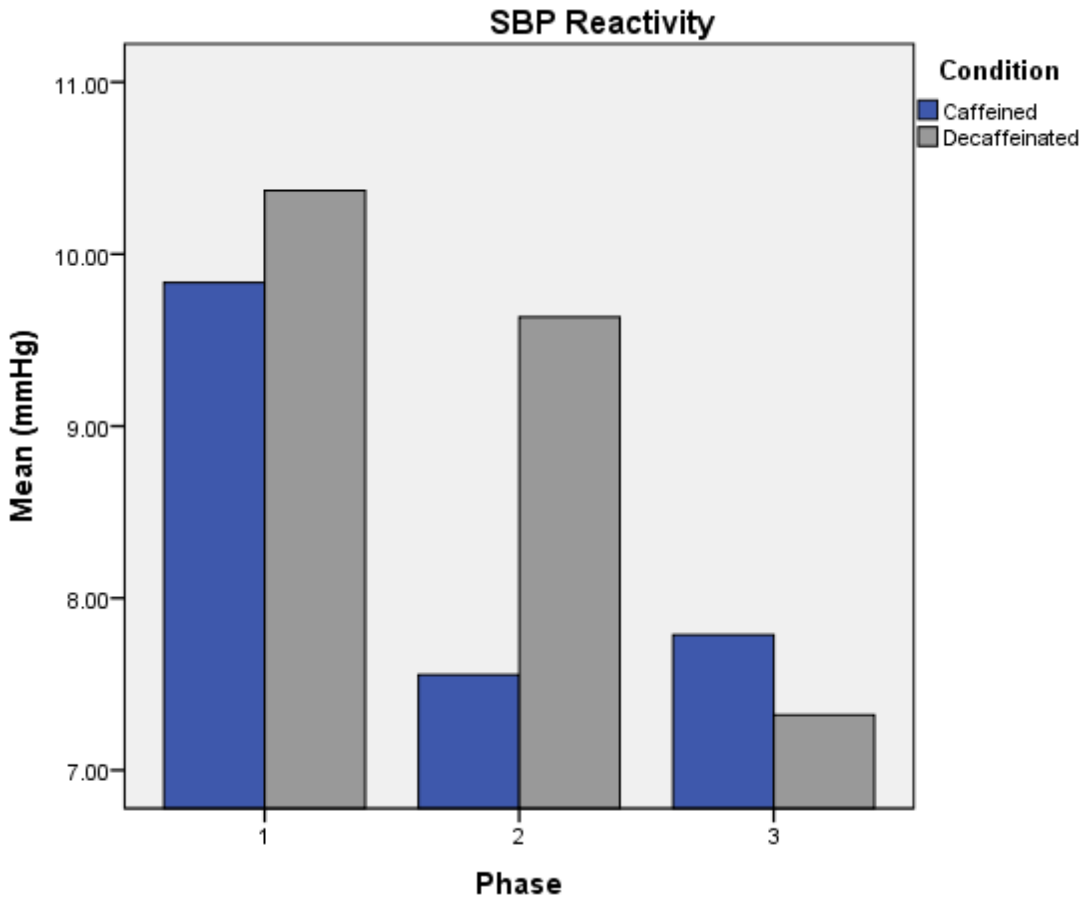
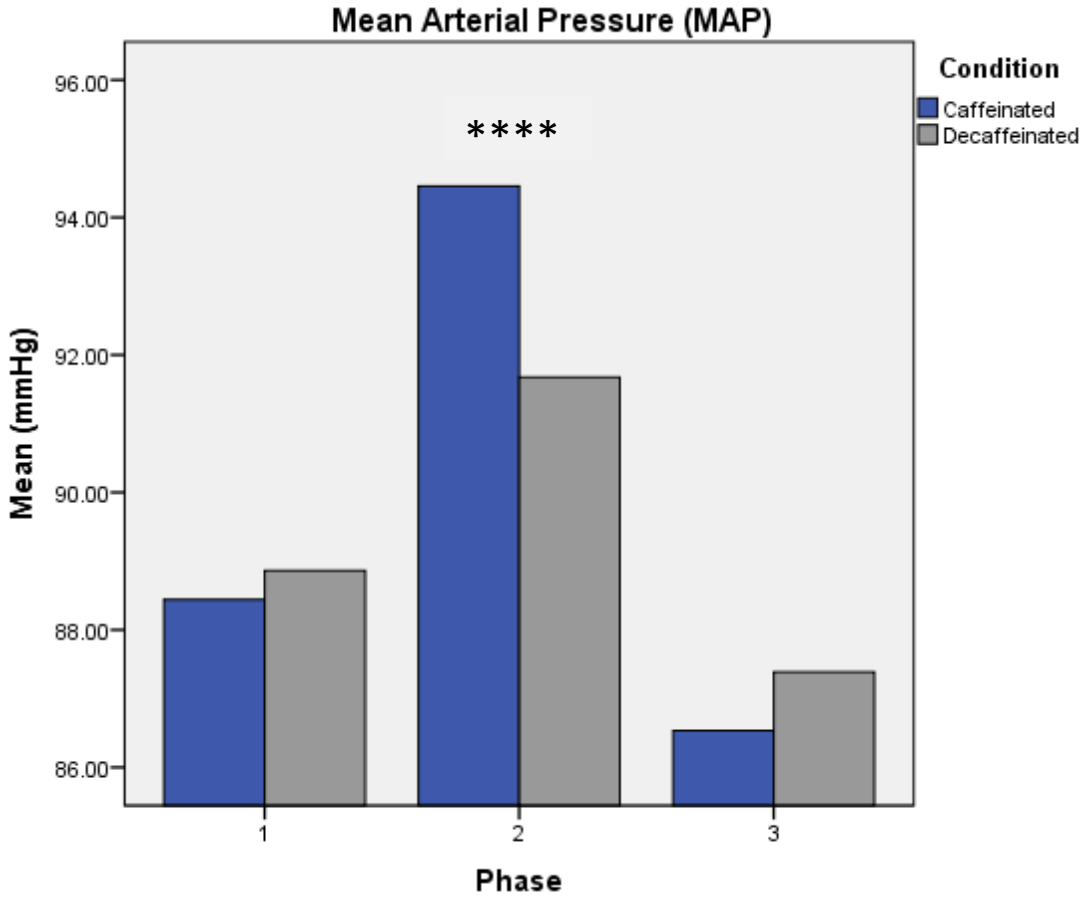


Figure 16. *Pairwise comparisons for mean MAP values*



Note: Significant differences between Phases 1 and 2 (p<.000), and 2 and 3 (p<.000), but no significant differences between Phases 1 and 3 (p=.052).

**** Main effect of Phase significant at p=.000

Figure 17. *Mean MAP reactivity, Phase x Condition*

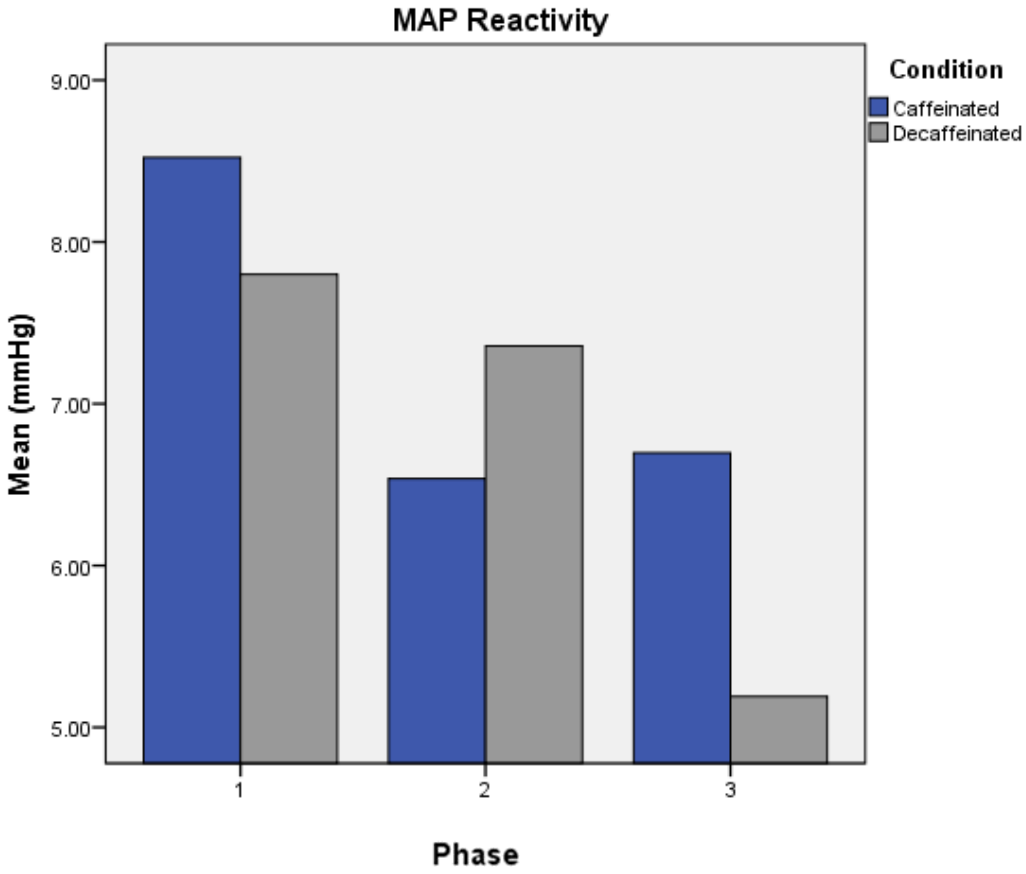
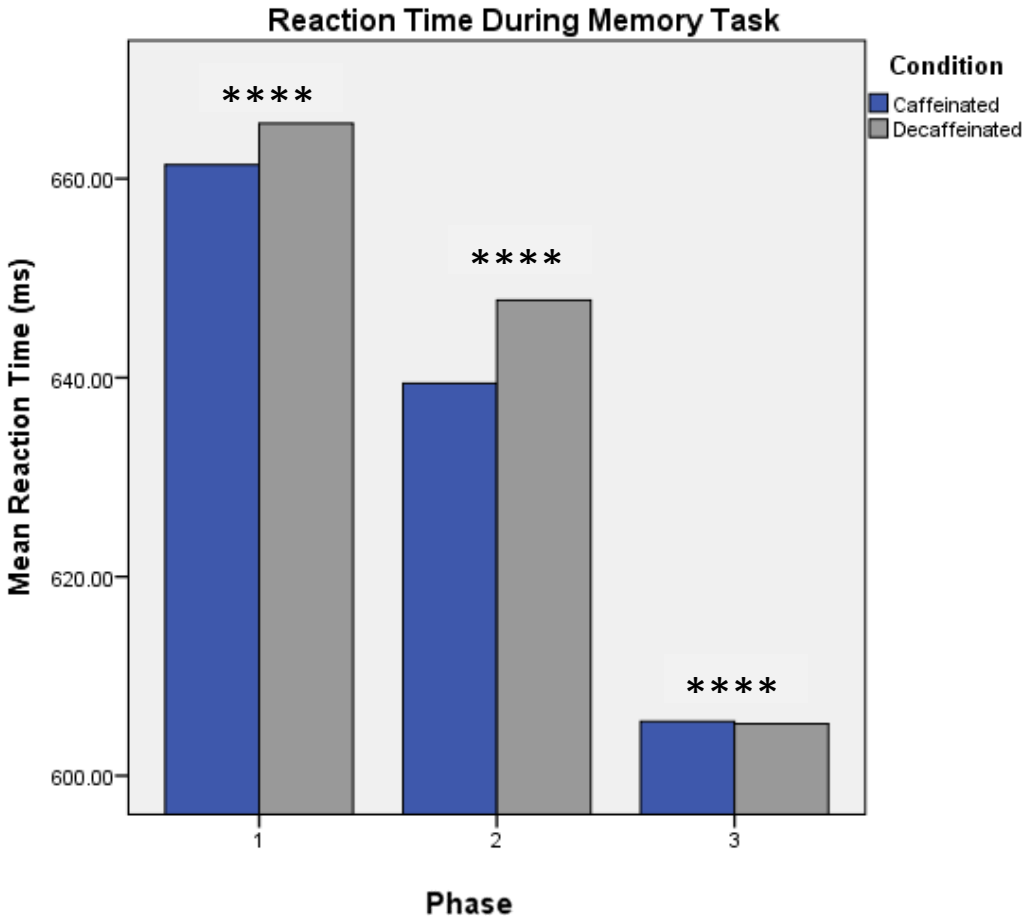


Figure 18. *Mean reaction time (RT) during memory tasks*



**** "Phase" significant at p=.000

Figure 19. *Mean percentage of trials correct for memory task*

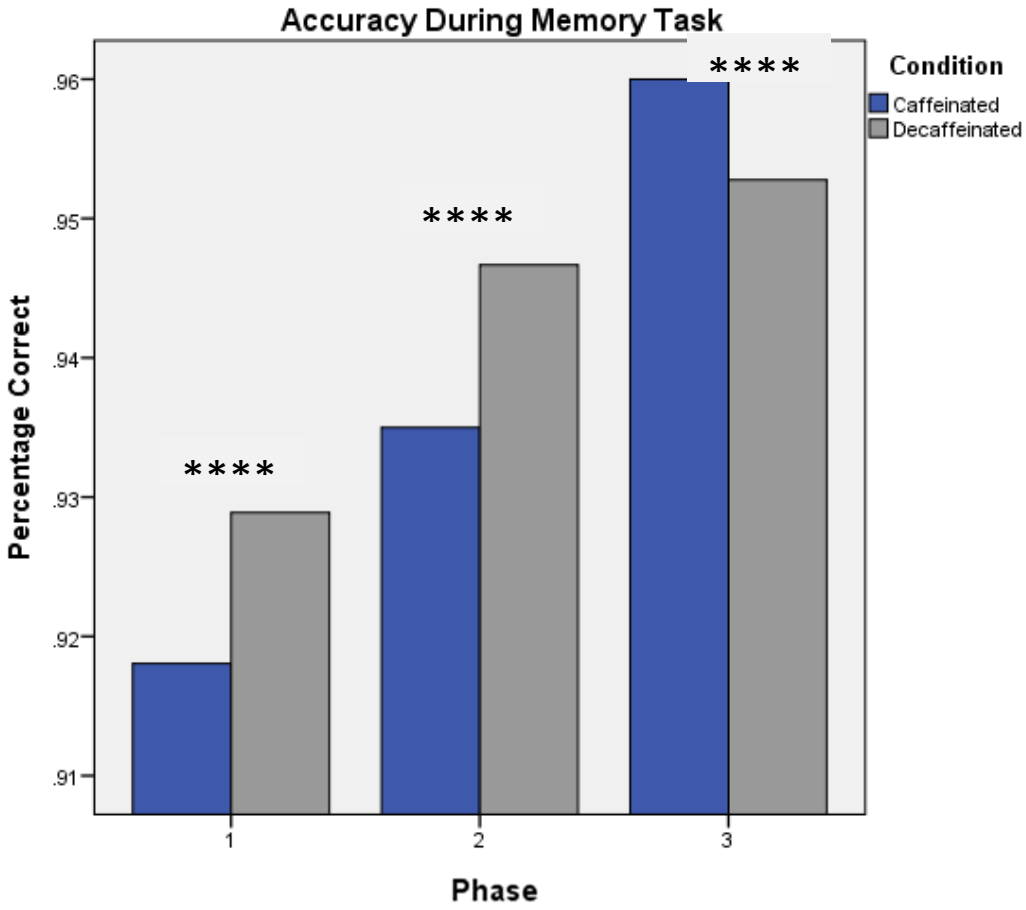
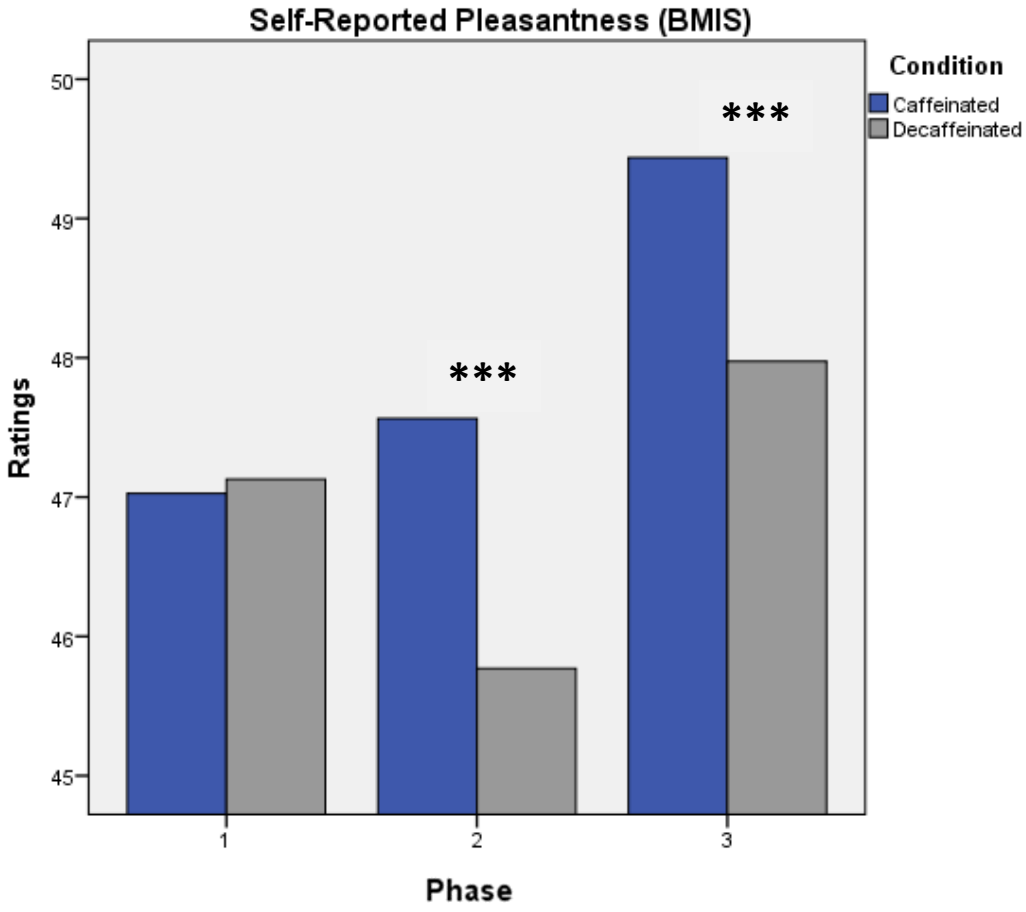
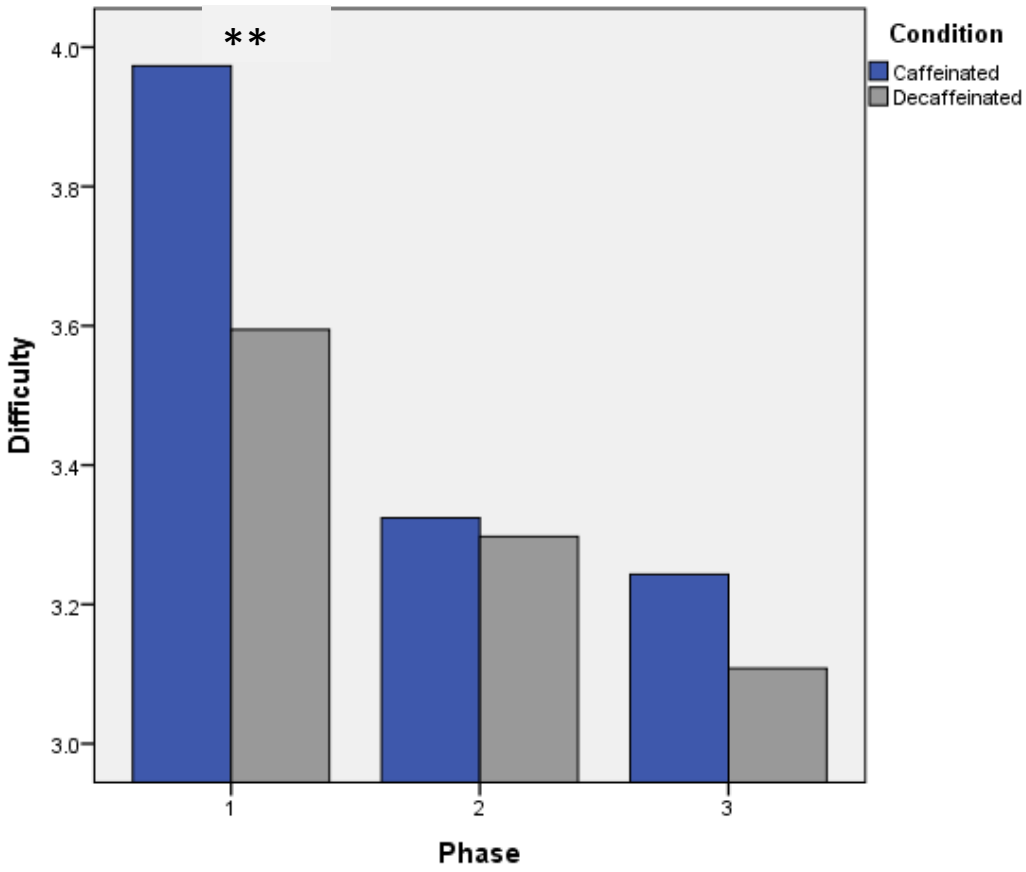


Figure 20. *Responses on Pleasant-Unpleasant scale of BMIS*



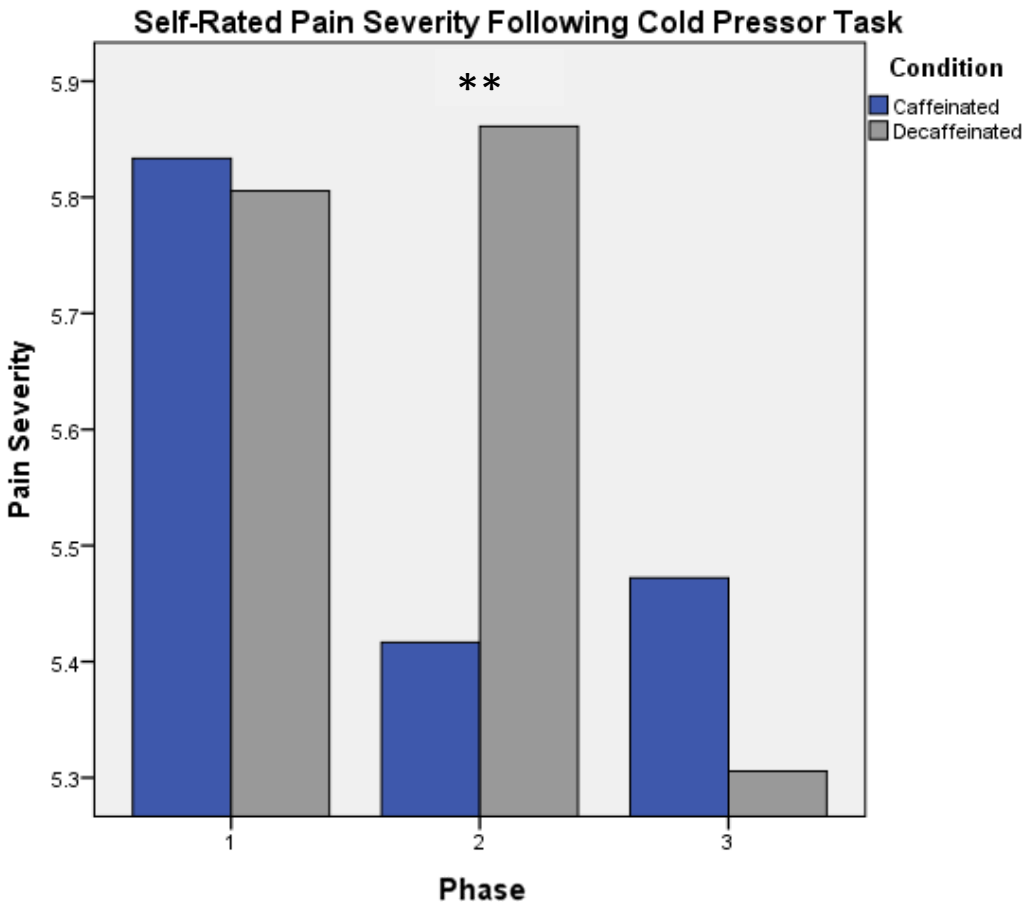
*** "Phase" significant at p<.01.

Figure 21. *Self-reported difficulty for memory task, by Phase*



*** Main effect of Phase significant at p<.01.

Figure 22. *Self-rated pain severity for cold pressor, Phase x Condition*



**Significant at p<.05

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Appendices

APPENDIX A

Journal Survey: 2012, 2013, 2014

Journal	CV studies with Controls*	Abstention Range (hours)	Median Abstention (hours)	Standard Deviation (hours)
Psychosomatic Medicine	7/60 (12%)	1-12 (11)	4.75	6.24
Biological Psychology	20/83 (24%)	2-24 (22)	6.67	6.57
Journal of Psychosomatic Research	6/40 (15%)	12-24 (12)	24	6.93
Psychophysiology	23/77 (30%)	1-24 (23)	6.33	9.35
International Journal of Psychophysiology	26/101 (26%)	1-24 (23)	4	6.52
Mean Overall Totals	**82/361 (23%)**	**1-24 (23)**	**9.15**	**7.12**

Note. Caffeine controls in published CVR studies among five prominent journals in 2012-2014. Reviews, meta-analyses, prospective, and retrospective studies were excluded from the survey.

APPENDIX B

Risks and Ethical Considerations

Potential risks of this study were minimal at most. Subjects may have potentially experienced mild caffeine deprivation symptoms. They may have experienced mild transient increases in blood pressure following acute caffeine consumption. On rare occasion, some individuals reported mild skin reactions (e.g., temporary irritation or discoloration) to the disposable electrodes. There was potential discomfort due to the CP task. This task is commonly used, without incident, for prior research in the Mind-Body Lab and researchers are very experienced in its administration. Subjects were fully informed of all potential risks and informed of their liberty to withdraw from the study at any time.

Deception

Subjects were minimally deceived regarding monetary compensation, as they were told that they would have to satisfactorily complete the memory tasks (scoring an average of 90% or above across all tasks) in order to receive a cash bonus. In actuality, all subjects received the bonus, regardless of task performance. This was at attempt to increase motivation in completing the memory tasks to the best of the subjects' abilities, indirectly increasing subjective stress as compared to completing a task void of any personal consequence as far as performance. Information regarding this deception was revealed during the debriefing.

APPENDIX C

Caffeine Consumption Journal

Please take note of any caffeine-containing beverages or foods that you consume ***in the week (7 days) leading up to the first session*** as well as the days ***in between the second and third sessions of the study***. In other words please bring the completed journal to the first and the second morning sessions. (When taking note of caffeine consumed, please know that this includes consumption of coffee, tea, soft drinks, energy drinks, or chocolate).

Amount (cup size/oz.)	**Specific Type of Beverage (e.g. espresso, light roast coffee)**	**Brand of Beverage (e.g. Starbucks)**
Date:		
_____ _	_____	_____
_____ _	_____	_____
_____ _	_____	_____
_____ _	_____	_____
Date:		
_____	_____	_____
_____	_____	_____
_____ _	_____	_____
_____ _	_____	_____
Date:		
_____ _	_____	_____
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_____ _	_____	_____
Date:		
_____	_____	_____
_____	_____	_____
_____ _	_____	_____

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APPENDIX D

Self-Reported Acute Symptoms Related to Caffeine Use

Symptom	Number of Subjects	Percentage of Subjects
Anxiety	1	8
Increased Heart Rate	9	75
Jitteriness/ General Arousal	5	42
Urge to Urinate	1	8
Nausea	1	8

**31% of subjects reported regularly experiencing symptoms following consumption of caffeine. Data from these 12 of 39 total subjects. Values indicate the number of subjects from the subset of 12 who reported regularly experiencing the above symptoms. Symptoms from which to choose were not presented as mutually exclusive. Data collected prior to sessions.*

Self-Reported Symptoms Related to Deprivation from Caffeine

Symptom	Number of Subjects	Percentage of Subjects
Headache	10	63
Irritability	7	44
Difficulty Concentrating	8	50
Stomach Discomfort	1	6
Other (Fatigue)	2	13

** 41% of subjects reported regularly experiencing symptoms following deprivation from caffeine. Data from these 16 of 39 total subjects. Values indicate the number of subjects from the subset of 16 who reported regularly experiencing the above symptoms. Symptoms from which to choose were not presented as mutually exclusive. Data collected prior to sessions.*

Self-Reported Duration of Time before Appearance of Deprivation Symptoms

Duration	Number of Subjects	Percentage of Subjects
1-3 Hours	4	25
3-24 Hours	7	44
>1 Day	2	12
>2 Days	3	19

**Subjects indicated experiencing symptoms following the stated durations of abstention.*

Self-Reported Most Common Reasons for Consuming Coffee

Reason	Number of Subjects	Percentage of Subjects
Energy/Avoid Fatigue	18	46
Focus/Concentration	17	44
Taste	15	38
Part of Routine/Habit	10	26
Assists during Physical Exercise	1	3
Mood Elevation	4	10
Avoidance of Headaches	1	3

**Total 39 subjects single-sentence responses to open-ended question: "For what reasons do you most commonly consume coffee?" Categories are not mutually exclusive.*

Mean Self-Estimated Caffeine Intake Prior to Sessions

Average Daily Caffeine Intake

Time Period	Mean Caffeine Intake (mg/day)
Pre-Week One	308.78
Pre-Week Two	280.50

**Collected from Caffeine Consumption Journals*

**Pre-Week One averages include estimates from the seven days preceding the first session. Pre-Week Two averages include estimates from the 3-11 days in between the first and second days of the study. Most subjects returned for the second day of sessions seven days following the first day, however inter-day gaps ranged from 3 to 11 days due to subjects' scheduling constraints.*

APPENDIX E

Mind-Body Laboratory Health History Questionnaire

A very brief medical history must be obtained as part of the experimental protocol. It is very important that you be completely honest. This information will be kept strictly confidential.

1. What is your age, height, weight, and gender?

Age: _____ years

Height: _____ feet, _____ inches

Weight: _____ pounds

Sex: ___M ___F

2. Since birth, have you ever been hospitalized or had any major medical problems?

___ Yes ___ No

If Yes, briefly explain:

3. Have you ever experienced a concussion or lost consciousness due to a blow to the head?

___ Yes ___ No

If Yes, briefly explain:

4. Have you ever had problems that required you to see a counselor, psychologist, or psychiatrist?

___ Yes ___ No

If Yes, briefly explain:

5. Do you use tobacco products of any kind?

___ Yes ___ No

If Yes, describe what kind how often/much:

6. Have you ever been diagnosed with a psychological disorder?

___ Yes ___ No

If Yes, briefly explain:

7. Do you currently have or have you ever had any of the following?

___ Yes ___ No Strong reaction to cold weather

___ Yes ___ No Circulatory problems

___ Yes ___ No Tissue disease

___ Yes ___ No Skin disorders (other than facial acne)

___ Yes ___ No Arthritis

___ Yes ___ No Asthma

___ Yes ___ No Lung problems

___ Yes ___ No Cardiovascular disorder/disease

___ Yes ___ No Diabetes

___ Yes ___ No Hypoglycemia

___ Yes ___ No Hypertension (high blood pressure)

___ Yes ___ No Hypotension (low blood pressure)

___ Yes ___ No Hepatitis

___ Yes ___ No Neurological problems

___ Yes ___ No Epilepsy or seizures

___ Yes ___ No Brain disorder

___ Yes ___ No Stroke

If you responded Yes to any of the above conditions, briefly explain:

8. Have you ever been diagnosed as having:

___ Yes ___ No Learning deficiency or disorder

___ Yes ___ No Reading deficiency or disorder

___ Yes ___ No Attention deficit disorder

___ Yes ___ No Attention deficit hyperactivity disorder;

9. Do you have:

___ Yes ___ No Claustrophobia (extreme fear of small closed spaces)

___ Yes ___ No Blood phobia (extreme fear of needles or blood)

___ Yes ___ No Phobia of any type (if Yes, briefly explain:)

___ Yes ___ No Generalized anxiety disorder

___ Yes ___ No Anxiety disorder of any type (if Yes, briefly explain:)

If you responded Yes, briefly explain here:

10. List any over-the-counter or prescription medications you are currently taking:

11. List the symptoms that these drugs are treating

12. List any other medical conditions that you have or have had in the past:

13. What is your average daily caffeine consumption (approximate number of cups/glasses of coffee, tea, or caffeinated soda)?

14. What is your average weekly alcohol consumption (approximate number of alcoholic beverages)?

15. How many hours of sleep do you average per night?

APPENDIX F

Mind-Body Laboratory Recent Health Behaviors Questionnaire

A very brief medical history must be obtained as part of the experimental protocol. It is very important that you be completely honest. This information will be kept strictly confidential.

1. When was the last time that you have had any alcohol before the study began?
2. When was the last time you have had a caffeinated beverage before the study began?
3. When was the last time that you ate before the study began?
4. What phase of the menstrual cycle are you currently in (beginning, middle, end, or N/A)?
5. How many hours of sleep did you get last night?
6. Did you engage in vigorous exercise within the last 2 hours?

APPENDIX G

Australian Centre for Posttraumatic Mental Health

Depression Anxiety and Stress Scale (DASS)

The DASS is a 42-item questionnaire which includes three self-report scales designed to measure the negative emotional states of depression, anxiety and stress. Each of the three scales contains 14 items, divided into subscales of 2-5 items with similar content. The Depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia. The Anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The Stress scale (items) is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient. Respondents are asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week.

Scoring:

Scores of Depression, Anxiety and Stress are calculated by summing the scores for the relevant items. The depression scale items are 3, 5, 10, 13, 16, 17, 21, 24, 26, 31, 34, 37, 38, 42. The anxiety scale items are 2, 4, 7, 9, 15, 19, 20, 23, 25, 28, 30, 36, 40, 41. The stress scale items are 1, 6, 8, 11, 12, 14, 18, 22, 27, 29, 32, 33, 35, 39. To use the Scoring Template (below) print on to a plastic overhead. The score for each of the respondents over each of the sub-scales, are then evaluated as per the severity-rating index below.

	Depression	Anxiety	Stress
Normal	0 – 9	0 - 7	0 – 14
Mild	10 – 13	8 – 9	15 – 18
Moderate	14 – 20	10 – 14	19 – 25
Severe	21 – 27	15 – 19	26 – 33
Extremely Severe	28+	20+	34 +

Norms: Normative data are available on a number of Australian samples. From a sample of 2914 adults the means (and standard deviations) were 6.34 (6.97), 4.7 (4.91), and 10.11 (7.91) for the depression, anxiety, and stress scales, respectively. A clincical sample reported means (and standard deviations) of 10.65 (9.3), 10.90 (8.12), and 21.1 (11.15) for the three measures.

Source: www.psy.unsw.edu.au/groups

Reference : Lovibond, S.H. & Lovibond, P.f. (1995). Manual for the Depression anxiety Stress Scales. (2nd Ed) Sydney: Psychology Foundation.

A centre of excellence supported by the Australian Government

DASS

Name: *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, lifts, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Reminder of rating scale:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

APPENDIX H

Brief Mood Introspection Scale (BMIS)

by John D. Mayer

INSTRUCTIONS: Circle the response on the scale below that indicates how well each adjective or phrase describes your present mood.

(definitely do not feel) (do not feel) (slightly feel) (definitely feel)

XX X V VV

Lively	XX X V VV	Drowsy	XX X V VV
Happy	XX X V VV	Grouchy	XX X V VV
Sad	XX X V VV	Peppy	XX X V VV
Tired	XX X V VV	Nervous	XX X V VV
Caring	XX X V VV	Calm	XX X V VV
Content	XX X V VV	Loving	XX X V VV
Gloomy	XX X V VV	Fed up	XX X V VV
Jittery	XX X V VV	Active	XX X V VV

Overall, my mood is:

Very Unpleasant — Very Pleasant

-10 –9 –8 –7 –6 –5 –4 –3 –2 –1 0 1 2 3 4 5 6 7 8 9 10

Please Note: The "Overall, my mood is" section is usually omitted, although some people use it and fold it into the overall score.

Original Citation: Mayer, J. D., & Gaschke, Y. N. (1988). The experience and meta-experience of mood. Journal of Personality and Social Psychology, 55, 102-111. [Scoring instructions are described there]

Some Other Articles that Have Used the Scale:*

- Examination of the paths between personality, current mood, its evaluation, and emotion regulation. Kokkonen, Marja; Pulkkinen, Lea; European Journal of Personality, Vol 15(2), Mar-Apr 2001. pp. 83-104.
- Resolution of lexical ambiguity by emotional state. Halberstadt, Jamin B.; Niedenthal, Paula M.; Kushner, Julia; Psychological Science, Vol 6(5), Sep 1995. pp. 278-282.
- Intrusive thoughts as determinants of distress in parents of children with cancer. Hall, Martica; Baum, Andrew; Journal of Applied Social Psychology, Vol 25(14), Jul 1995. Special issue: Rumination and intrusive thoughts. pp. 1215-1230.
- Mood inductions for four specific moods: A procedure employing guided imagery vignettes with music. Mayer, John D.; Allen, Joshua P.; Beauregard, Keith; Journal of Mental Imagery, Vol 19(1-2), Spr-Sum 1995. pp. 151-159.
- Mood-congruent judgment over time. Mayer, John D.; Hanson, Ellen; Personality & Social Psychology Bulletin, Vol 21(3), Mar 1995. pp. 237-244.

*The scale has been used in many other articles; I do not have a comprehensive list at this time. If you know of other uses, I would be delighted to hear of them.

APPENDIX I

BODY PERCEPTION QUESTIONNAIRE (BPQ)

Stephen W. Porges, Ph.D.

The BODY PERCEPTION QUESTIONNARIE has five sub-tests: 1) Awareness, 2) Stress Response, 3) Autonomic Nervous System Reactivity, 4) Stress Style, and 5) Health History Inventory. Each of the 122 items in the BODY PERCEPTION QUESTIONNARE are to be answered on the 5-point scoring scale described in the beginning of each sub-test. Read the instructions for each sub-test and designate your answers for each of the 122 items on the provided answer sheet.

I: AWARENESS

Image how aware you are of your body processes. Select the answer that most accurately describes you. Rate your awareness on each of the characteristics described below using the following 5-point scale:

a) Never b) Occasionally c) Sometimes d) Usually e) Always

During most situations I am aware of:

1. Swallowing frequently
2. A ringing in my ears
3. An urge to cough to clear my throat
4. My body swaying when I am standing
5. My mouth being dry
6. How fast I am breathing
7. Watering or tearing of my eyes
8. My skin itching
9. Noises associated with my digestion
10. Eye fatigue or pain
11. Muscle tension in my back and neck
12. A swelling of my body or parts of my body
13. An urge to urinate
14. Tremor in my hands
15. An urge to defecate
16. Muscle tension in my arms and legs
17. A bloated feeling because of water retention
18. Muscle tension in my face
19. Goose bumps

20. Facial twitches
21. Being exhausted
22. Stomach and gut pains
23. Rolling or fluttering my eyes
24. Stomach distension or bloatedness
25. Palms sweating
26. Sweat on my forehead
27. Clumsiness or bumping into people
28. Tremor in my lips
29. Sweat in my armpits
30. Sensations of prickling, tingling, or numbness in my body
31. The temperature of my face (especially my ears)
32. Grinding my teeth
33. General jitteriness
34. Muscle pain
35. Joint pain
36. Fullness of my bladder
37. My eye movements
38. Back pain
39. My nose itching
40. The hair on the back of my neck "standing up"
41. Needing to rest
42. Difficulty in focusing
43. An urge to swallow
44. How hard my heart is beating
45. Feeling constipated

APPENDIX J

Subject ID#:_____ Day: _____ Session: _____

Memory Task _____

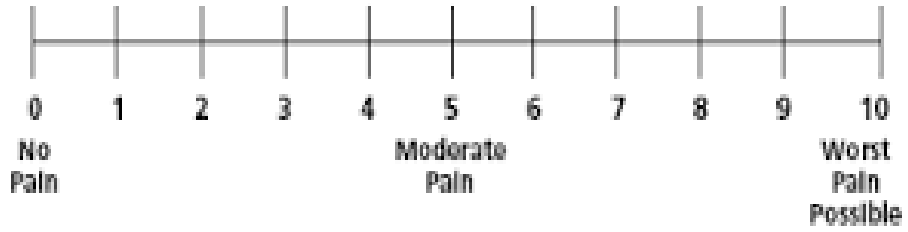
Rate Task Difficulty:

1 =(very easy); 9=(very difficult)

1 2 3 4 5 6 7 8 9

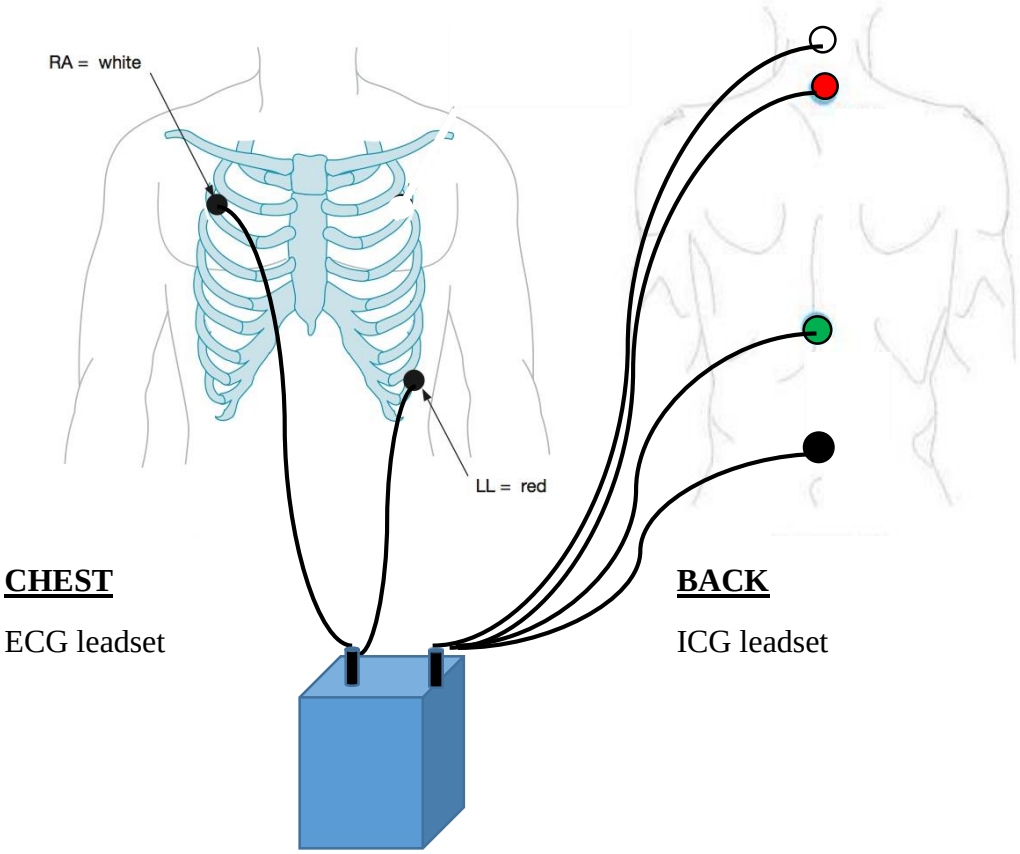
CP _____

Rate Pain Level on Task:



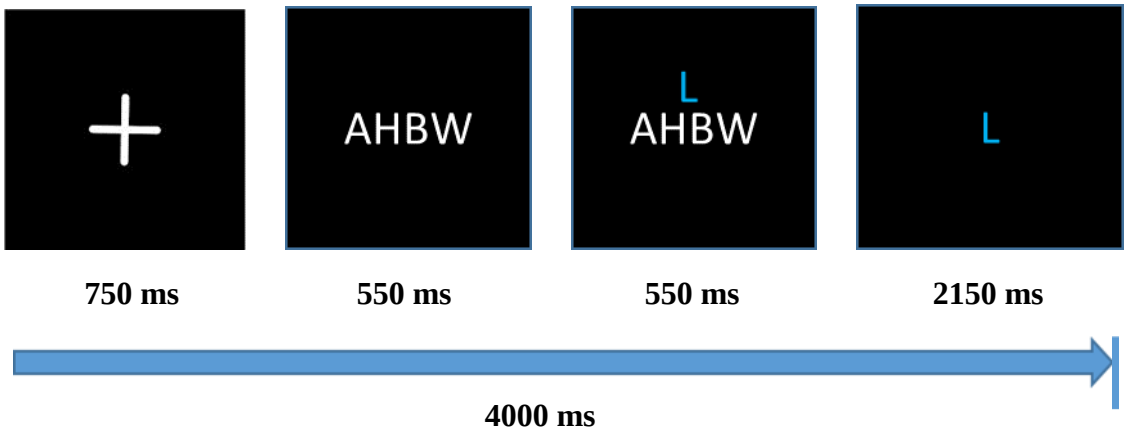
APPENDIX K

Electrode Placement Diagram



Note: Derived from an image on www.lifeinthefastlan

APPENDIX L



Memory task. Each trial consists of presentation of a fixation cross on a computer screen for 750 ms followed by a series of nonsense letters consisting of four capital letters presented for 550 ms. The letter series is then masked, with a single blue letter appearing above the masked series for the remainder of the trial. Subjects must then press one of two keys indicating whether or not the blue letter was presented in the preceding letter series. Each trial is a total of 4000 ms long. Subjects completed 14 practice trials before beginning the 3-minute task. Subjects were instructed to attempt to respond correctly for at least 90% of 44 total trials (Richter, Friedrich, & Gendolla, 2008).

APPENDIX M

Between-Session Journal

Please provide a very brief hour-by-hour account of your activities during the hours in between morning and afternoon sessions of the study.

Date: _____

Hour 1:
Time:_____ (e.g. 11:00 a.m. to 12:00 p.m.)
Activity:_____ (e.g. "Was in a class"; Or "ate a light meal")

Hour 2:
Time:_____
Activity:_____

Hour 3:
Time:_____
Activity:_____

Hour 4:
Time:_____
Activity:_____

Hour 5:
Time:_____
Activity:_____