The Effects of Acute Tryptophan Depletion and Psychological Traits on Cardiovascular and Mood Responses to Interpersonal Conflict

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ABSTRACT

The effects of acute tryptophan depletion and psychological traits on cardiovascular and mood responses to interpersonal conflict

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The present study investigated the effects of serotonin and psychosocial factors including Cook-Medley hostility, Trait anger, Trait anxiety, and Beck depression scores on cardiovascular and mood responses to interpersonal stress. Eighty-five males and females participated in either an acute tryptophan depletion, a procedure that lowers brain serotonin levels, or a sham tryptophan depletion. They were subsequently exposed to an interpersonal conflict stressor. Cardiovascular and mood measures were recorded at baseline, post-depletion pre-stress, during the stressor, and during recovery. All participants exhibited heightened cardiovascular responses as well as increased anxious, hostile, and depressed mood to the interpersonal conflict. Effects of depletion on cardiovascular reactivity were observed exclusively during recovery. Effects of depletion on negative mood were found at rest and during stress with increased negative affect in the depleted versus balanced condition. Interactions between tryptophan depletion and psychological factors other than hostility were also observed. Greater negative mood responses were found in depleted individuals with high scores on anger, anxiety, and depression factors. Overall, these findings suggest that the effect of serotonin on the stress response may be modulated by psychological factors. Implications for future research on the interaction between serotonin, psychological factors, cardiovascular reactivity, and mood are discussed.

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The Effects of Acute Tryptophan Depletion and Psychological Traits on Cardiovascular Responses to Interpersonal Conflict

Cardiovascular disease (CVD) includes hypertension, stroke, and chronic diseases of the heart muscles or membranes, as well as coronary heart diseases (CHD), such as angina pectoris and myocardial infarction, which are characterized by an inadequate supply of oxygen to the heart (Houston, 1988). CVD has been the leading cause of death in developed countries since the 1900's (Allen, 2000; Dembroski & Costa, 1987; Ferrari & Bianchi, 2000), and is expected to be so as well for most of the developing countries over the coming years. The World Health Organization predicts that by 2030 almost 25,000,000 people will die per year from CVD, remaining globally, the leading cause of death (WHO, 2011). Since the 1960's there has been a marked decline in the rate of CVD related deaths in Western countries. Notwithstanding this trend, heart and circulatory diseases remain the leading cause of death in the UK (Scarborough et al, 2010), Europe as a whole, where just under 50% of all deaths are from CVD (Allender et al, 2008), and continues to be the primary cause of death in the United States for both men and women (Xu et al, 2010). Also important to note is that the decline has been disproportionate between men and women, with the majority of the decline occurring in men. Death from CVD amongst women in the US has remained relatively stable (Lloyd-Jones, Adams, Brown, Carnethon, et al., 2010).

The downward trend of CVD as a cause of death in Canada has paralleled that of the US (Conference Board of Canada, 2009). A decade ago in Canada the CVD death rate was just under 36% (Statistics Canada, 1999) and in 2004, 32% (Public Health Agency of Canada, 2009). Nonetheless, CVD remains the leading cause of death in

Canada, and perhaps more significantly, in 2005 relative to 1994 there had been an increase in reported heart disease predominantly in males (19%), but also in women (2%; Lee et al, 2009; Tu et al, 2010). Additionally, and perhaps more ominously, CVD risk factors have risen as well during this period, even amongst younger people, with hypertension alone, rising 77% in men and women combined (Lee et al, 2009).

CVD is a multifactorial disease (Frolkis, 1999; Statistics Canada, 1999; Wimbush & Peters, 2000) with traditional medical risk factors, such as high blood pressure, elevated serum cholesterol, diabetes, habitual cigarette smoking, physical inactivity, and obesity, reportedly accounting for only 50% of or less of CHD cases according to research spanning 50 years (Brand, Rosenman, Sholtz, & Friedman, 1976; Everson-Rose & Lewis, 2005; Jenkins, 1971, 1976; Rosengren, Hawken, & Ounpuu, et al, 2004; Thom, Epstein, Feldman, & Leaverton, 1985; Williams, 2008; Yusuf, Hawken, & Ounpuu et al, 2004). Beaglehole & Magnus (2002) refer to the *only 50% known factors* as a blindly repeated myth and cite epidemiological evidence that the major CHD risk factors including cholesterol levels, high blood pressure, smoking, and physical activity account for at least 75% of CHD. Nonetheless, even 25% of the huge CHD toll is still an extremely large number and the etiology of the remaining cases continues to be a focus of investigation, with the potential influence of psychological factors hypothesized to be a mediating factor (Dembroski, Macdougall, Costa, & Grandits, 1989; Kaplan, Manuck, Williams, & Strawn, 1993; Williams, 2008; Williams, Barefoot, & Schneiderman, 2003).

Psychosocial factors have an impact comparable to other risk factors and can increase risk two to four fold (Frolkis, 1999). Psychological stress has been posited as a mediating factor in CVD, both in its role as a factor in the etiologically non-identified

cases as well as in relation to high blood pressure or hypertension. Individuals vary in their reaction to psychological stressors and research suggests that psychological stress may only confer additional risk on individuals who are susceptible either physically or mentally to the development of the disease (Brody et al., 1987). Williams (2008) points out quite strongly that psychosocial factors must work through biological pathways and other factors that influence biological pathways. Psychosocial factors may influence the traditional risk factor such as smoking and body mass. More directly they are factors in cardiovascular responses to in vivo and in vitro stressors (Suarez et al, 1998).

Psychological Mediators of CHD: The Role of Type A Behavior Pattern

Early research identified a constellation of behaviors that were linked to the onset of CHD. Friedman and Rosenman (1959, 1974) labelled their particular behavioral style construct "Type A Behavior Pattern" (TABP), which included success oriented behavior (such as hard-driving job involvement, intense ambition and competitiveness, impatience, and schedule inflexibility), hostility, and aggression (Friedman & Rosenman). Prior research and findings of the next decade were almost unanimously positive in upholding the epidemiological validity of TABP. Individuals with TABP had markedly increased prevalence of clinical CHD over those individuals ("Type B") that did not demonstrate these behaviors (Friedman & Rosenman, 1959). Type A behavior was assessed using the Structured Interview (SI), which challenged participants in a confrontational manner that "relied more on the presence of empathetic and vigorous voice stylistics than it does as on the content of responses as the major criteria" (Williams & Barefoot, 1988). A major prospective study utilizing this paradigm was conducted by Rosenman and his colleagues (Rosenman et al., 1964), the Western Collaborative Group Study (WCGS) that followed

more than 3,000 men between the ages of 30 and 59 for a period of 8-1/2 years. Type A participants, on follow-up, had CHD or its symptoms at twice the prevalence rate of the Type B's (Rosenman et al., 1964, 1975), even after controlling for traditional risk factors and, therefore, being a Type A individual was viewed as an independent risk factor. Additional studies on related CVDs and their symptoms, supported the hypothesis that TABP was a significant factor in the endpoint development of CVD, as did studies on patients undergoing coronary angiography (e.g. Blumenthal, Williams, Kong, Schanberg & Thompson, 1978; Frank, Heller, Kornfeld, Sporn, & Weiss, 1978; Zyzanski, Jenkins, Ryan, Flessas, & Everist, 1976). In the late 1970s, given the strong evidence, scientific review panels of the National Institute of Health (Siegman, 1989) and in 1981, the National Heart, Lung, and Blood Institute (Dembroski & Williams, 1989; Houston, 1988) concluded that TABP was an independent risk factor for CHD, comparable to traditional risk factors.

In the decades that followed, however, new and re-analyzed data began to contradict the findings of the previous 25 years, both in terms of prospective epidemiological studies and in cross-sectional studies on patients undergoing coronary angiography (Williams & Barefoot, 1988). In a re-analysis of the WCGS data with appropriate statistical controls, many of the predictive findings were no longer supported (Brand, 1978). One study showed that Type A individuals were actually at less risk for CHD mortality if their initial diagnosis was symptomatic myocardial infarction (Ragland, & Brand, 1988). Angiographic studies, as well, failed to support the link (Dembroski & Williams, 1989; Dimsdale, Hackett & Hutter, 1979; Siegman, Feldstein, Tommaso, Ringel, & Lating, 1987). More damaging evidence came from the Multiple Risk Factor

Intervention Trial (MRFIT), a major prospective study, which failed to replicate the findings of the WCGS though it used the same protocol and included more than 3,000 participants in the subset that specifically addressed this issue (Dembroski, MacDougall, Costa, & Grandits, 1989; Siegman, 1989). Although TAP was not associated with CHD, elements of hostility showed a small relation. In a reanalysis of the MRFIT data Matthews, Gump, Harris et al. (2004) recoded the participants' interviews that had been carried out during the initial study to examine TAP interactions. They reported that the earlier analyses had used hostile attitudes as the measure, whereas the reanalysis used additional measures of hostility including irritation, arrogance, uncooperativeness, and anger and reported that hostility prospectively predicted CVD in high-risk individuals. A particular subgroup of high hostile individuals had a five-fold risk compared to low hostile individuals. Myrtek in his 2001 review of prospective studies on CHD, TAP, and hostility reported that there was no association between TAP and CHD, and an extremely small statistically significant effect size for the association between hostility and CHD. He concluded that this association accounting for approximately 2% of the variability "has no practical meaning for prediction and prevention." Clearly not all researchers agree. Matthews, Brooks et al, for example, discuss several clinical implications of their findings including informing patients of health related consequences of hostility and anger so that they can take actions (e.g., stress and anger management training) to change their health trajectory. They cite several studies (Blumenthal, Jiang, Babyak, et al.1997; DiGiuseppe & Tafrate, 2003) indicating that there are interventions that can lead to healthier outcomes. As several studies above have shown, hostility is associated with health-adverse behaviors that occur during the early stages of CVD (Räikkönen &

Keltikangas-Jarvinen, 1991; Siegler, Peterson, Barefoot, et al. 1992).

Psychological Mediators of CHD: The Role of Anger and Hostility

TABP in itself is mostly a constellation of behaviors, rather than a psychological construct, and its multi-dimensionality led researchers to look at underlying factors or components of TABP that might better explain both the positive and negative findings. The role of anger and the construct of hostility began to be investigated as mediators or risk factors in CHD (Dembroski & Williams, 1989; Diamond, 1982) and have continued to be intensively studied over the past 4 decades. Hostility and anger "form the toxic core of the TABP", according to Williams (1989, p. 196). In a review paper on hostility and health, Smith (1992) noted that this research suffers from ambiguity and a lack of consensus concerning the basic concepts, with the least amount of agreement regarding hostility. Hostility is considered a stable personality trait that may lead to angry affect, in combination with behavioral aggression, in diverse situations (DiGiuseppe, Eckhardt, Tafrate & Robin, 1994). A similar definition, of a tendency to feel anger towards others and the desire to inflict harm on others, is offered by Chaplin (as cited in Smith, 1994; p.26). Others have defined hostility as a pervasive, complex set of feelings, negative attitudes, beliefs, and appraisals, concerning other individuals, which motivate aggressive behavior (Spielberger, Reheiser & Sydeman, 1995; Smith, 2003). A hostile attribution bias results in individuals viewing others as threatening and tends to produce reactive aggression (Dodge & Coie, 1987), as well as a belief that others are generally unworthy and not to be trusted (Smith, 1992).

One of the most widely used measures of hostility (Barefoot, 1992; Smith, 1992) is the Cook and Medley Hostility (Ho) scale (Cook & Medley, 1954), a 50-item true-false

questionnaire derived from the Minnesota Multiphasic Personality Inventory. Although the scale includes items that ostensibly measure both hostility and the admission that the respondent may act aggressively or view aggression as an appropriate instrumental tool, it is purported to tap into the cognitive component of hostility (Barefoot, 1992) and is relevant to interpersonal processes (Kamarck, Manuck, & Jennings, 1990; Prkachin, Mills, Kaufman, & Carew, 1991).

In 1976 researchers at Duke University began to use the Ho as an evaluative measure, alongside the SI and other psychosocial measures, with patients that were to undergo coronary angiography. Williams and his colleagues (1980) found that patients with high Ho scores were 1.5 times more likely to have clinically significant arterial occlusion that those with low Ho scores. Several other prospective studies found that the Ho scale predicted CHD and mortality rates (Barefoot, Dahlstrom & Williams, 1983; Barefoot, Williams, Dahlstrom, & Dodge, 1987; Shekelle, Gale, Ostfeld, & Paul, 1983). One 25-year follow-up study of almost 500 physicians who completed the MMPI prior to medical school admission, failed to predict CHD or mortality rates (McCranie, Watkins, Brandsma, & Sisson, 1986). Williams and Barefoot (1988) argue that the fact that the MMPI profile was to be used as part of the admissions process, makes it likely that applicants answered in a socially desirable way, thus under-reporting their hostility levels. Based on the accumulated data, Williams and Barefoot (1988) concluded, in their review of the Ho scale, that, as a valid measure in assessing adverse health outcomes, "the Ho scale must be considered a robust indicator of coronary-prone as well as of mortality-prone behavior" (p. 198).

Several studies have supported the construct validity of the scale (Barefoot,

Dodge, Peterson, Dahlstrom, & Williams, 1989; Bishop & Quah, 1998; Pope, Smith, & Rhodewalt, 1990; Smith, & Frohm, 1985) though others have challenged this (Costa, Zonderman, McCrae, & Williams, 1986). Subsequent to Barefoot and Williams' (1988) review, several other studies failed to support an association between Ho and CHD. Hearn, Murray, and Luepker (1989) failed to find a predictive link for CHD mortality, CHD morbidity, or total mortality in a retrospective study of more than 1,300 university students who had completed the MMPI more than 30 years earlier and a study by Leon, Finn, Murray, and Bailey (1988) arrived at a similar conclusion, based on 30-year data from close to 300 men. In a study looking at various diseases, all the components of hostility were associated with non-CHD disease, but not with CHD, and the authors concluded that the having the diseases resulted in high hostility scores (Ranchor, Sanderman, & Bouma, et al., 1997). Hemingway and Marmot in their review (1999) found that the evidence for the hostility and TAP association with CHD was less consistent than for other factors such as depression or anxiety, and a 2002 update reported that the inconsistency held for anxiety as well.

Notwithstanding these negative findings researchers have continued to look at associations between particular components or types of hostility and anger and CVD. Data from the Atherosclerosis Risk in Communities Study, a large prospective study of CVD and risk factors, was analyzed and reported on in several articles (Williams, Nieto, Stanford, Couper, et al., 2002; Willams, Nieto, Stanford, & Tyroler, 2001; Williams, Paton, Siegler, et al., 2000). They reported that various subtypes of anger differed in terms of their association with CHD mortality and this association differed depending on age. Strong angry temperament and proneness to anger was associated with CHD while

anger-reaction was not. Younger individuals, both male and female, who had higher trait anger scores were at greater risk for ischemic stroke than low-scoring individuals. Yan, Liu, Matthews, et al., (2003) reported an association in young adults between hostility and long-term risk of hypertension amongst more than 3,000 black and white adults who were in the Coronary Artery Risk Development in Young Adults (CARDIA), a study with 15 years of data. Coronary artery disease was found to be associated with the expression of anger in a sample of 97 males with already stenosed coronary arteries (McDermott, Ramsay, Bray, 2001; Ramsay, McDermott, & Bray, 2001). In a 16-year study of 259 males who had succumbed to CVD as well 259 matched still living controls, hostility was reported to be a risk factor for CVD mortality (Matthews, Gump, Harris, et al., 2004). In a study of 589 healthy black and white women (Everson-Rose, Lewis, Karavalos, et al., 2006) undergoing screening for subclinical atherosclerosis greater subclinical atherosclerosis was found to be statistically significantly associated with hostility, although the effect was small. In another study of 636 women with suspected CAD various components of anger and hostility were found to be associated with CAD and increased symptoms (Krantz, Olson, Francis, et al., 2006). Miller, Smith, Turner, et al., (1996) in their meta-analysis of 45 studies, reported a small but statistically significant consistent association between Ho and CHD, but stated that Ho was more predictive of all-cause mortality. Whiteman (2006) described the findings regarding hostility and CHD as exciting and at the same time frustrating given the inconsistencies between studies and the consistent yet small to moderate association between hostility and MI.

In a recent meta-analysis (Chida & Steptoe, 2009) of 44 prospective studies

looking at both healthy and already suffering with CHD individuals, they found that hostility and anger were independently associated with CHD outcomes. In a study of just under 100,000 healthy predominantly white women in the US, higher levels of Cynical Hostility were found to be associated with higher CHD (Tindle, Chang, Kuller, et al, 2009). Haukkala, Konttinen, Laatikainen, et al. (2010) looked at five different measures of hostility and anger for an association with CVD and ischemic heart disease in a prospective study of almost 8,000 men and women in Finland and found that low Anger Control predicted CVD events.

The role of psychological factors in disease pathways is a vast area of research. Wiebe and Fortenberry (2006) provide a discussion of the multiple pathways and models thought to link the two. These include stress-moderation models, health behavior models, illness behavior models, and biological models. The stress-moderation model is the most widely utilized and encompasses the organism's short-, medium-, and long-term response to acute and chronic, as well as chronically-acute, stressors of varying intensities. Stress results in the activation of multiple physiological processes with interacting and cascading effects including sympathetic, neuroendocrine, as well as immune system responses. Chronic stress, in particular psychosocial stress, is consistently associated with coronary atherosclerosis (Brotman, Golden, & Wittstein, 2007). Acute stress is also associated with myocardial dysfunction. Left-ventricular ballooning can occur following acute emotional stress (ibid). Left ventricular hypertrophy predicts CVD (Kapuku, Treiber, Davis, et al., 1999) and is one of the strongest predictors of negative CVD outcomes in hypertensive individuals (Gradman & Alfayoumi, 2006), and cardiovascular reactivity (CVR) following mental stress has been shown to be associated with left

ventricular mass (Allen, Matthews, & Sherman, 1997; Treiber, McCaffrey, Pfieger, et al, 1993). In a review of 21 studies looking at stress-related CVR and left ventricular mass, a moderate association was found (Taylor, Kamarck, & Dianzumba, 2003). Overall, CVR – the response of the circulatory system to stress – has been shown to be associated with the aetiology and course of CVD (Smith & Gerin, 1998). The authors discuss two different roles of CVR associated with the development of CVD. In the first role, relatively stable individual differences in the magnitude and particular aspects of the cardiovascular response to various types and levels of stress (e.g., heart rate and blood pressure) may be associated with different health trajectories. In the second, psychosocial risk factors including trait hostility or anger are brought into play to a greater or lesser degree based on the type of stressor – interpersonal conflict for example – and affect the intensity and duration of the CVR.

Psychological Mediators of CHD: The Role of Depression and Anxiety

Hostility on its own is considered a factor in CVD, but may also interact with depression. Suarez (2003) examined a sample of 90 healthy males and found that high hostiles with above average levels of BDI symptomatology were at increased risk for cardiac events, including myocardial infarction, based on their higher levels of plasma interleukin-6. Interleukin-6 has been shown to be a risk predictor of future cardiac events and contributes to the development of atherosclerotic CVD (e.g., Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000). Depression has been shown to complicate CVD conditions (e.g., Celano & Huffman, 2011; Lavoie & Fleet, 2000). Its independent contribution to the future onset of coronary disease in currently healthy individuals was examined in a review of 10 studies that met the inclusion criteria, which included at least

a 4-yr follow up period, for a meta-analysis (Wulsin & Singal, 2003). Seven of the studies found that depressive symptomatology was an independent risk factor, two showed mixed results, and one study found no association. Several issues were raised by Wulsin and Singal including the heterogeneity with regard to outcome variables, control factors, sample composition, definitions of the exposure variable. They also cite a publication bias against negative findings that may skew the picture; however they concluded that given the magnitude of the pooled effect, there would have to be almost 600 "file drawer" (authors' quotes) studies to negate the risk association. In the 10 studies, nine different measures of depression were used including seven self-report instruments. It was pointed out that the two studies that used a structured interview, which is considered the most rigorous and specific assessment method, found high levels of risk; however, the lone study that found no association, used a self-report measure designed by the study's researchers. The authors of the meta-analysis conclude that the risk finding underestimates the true impact of depression in the aetiology and course of CVD as it does not take into account the interaction between depression and other psychological factors. More recently van der Kooy et al. (2007) included 28 studies in a meta-analysis examining the association of depression to the broader domain of circulatory system disease, and not just CHD. Explicitly included in the aim of their analyses was an exploration of the heterogeneity and methodological quality of the studies. They applied rigorous criteria (18 elements related to internal reliability, precision, and generalizability), and culled the 28 longitudinal case control or cohort studies from more than 1,600 published articles that matched the search keywords. Of the 28 studies only 11 met criteria for high quality. The findings supported the association

amongst depression and various CVD's in a community-dwelling general practice based sample. Although clinical depression was most strongly associated with CVD, depressed mood had an effect of moderately increasing the risk for CHD, MI, and other CVDs.

In a discussion paper (Rosenman & Hjemdahl, 1991) regarding the casual relation between anxiety, stress, and CVR to hypertension, one of the world's leading cardiovascular researchers, Rosenman, concluded that:

> Cardiovascular responses constantly occur in the waking state, whether or not associated with perceived stress, and any type of emotional arousal similarly raises the blood pressure. Differences during all types of emotions are largely quantitative, and there does not appear to be any specificity of sympathoadrenal response to emotional stress. Perhaps it is time to view these responses as physiological and homeostatic, rather than as psychologically reactive,'' and to require firmer evidence to support old hypotheses about causal roles of anxiety, stress and cardiovascular reactivity in the pathogenesis of sustained hypertension. (p.156)

Contrast those words with the words of (Dimsdale, 2010) another world-renowned cardiovascular researcher, J. E. Dimsdale, just 20 years later in an editorial article for an issue of the Journal of the American College of Cardiology that contained articles and a meta-analysis finding anxiety as an independent predictor of cardiac events that occur many years later:

> It is odd that anxiety symptoms can be such a strong beacon, lighting the way to future coronary disease decades in advance. Cardiologists are certainly cognizant of anxiety's effects on transient physiology (blood pressure, palpitations, angina). But it is intriguing indeed to note that one single assessment of anxiety casts such a long shadow decades into the future. (p.47)

> The studies in this issue of the *Journal* suggest that, by the time patients with symptoms of CHD present themselves to a cardiologist, early-life anxiety might have already taken its toll. Anxiety hurts. It hurts subjectively, and these studies suggest that anxiety hurts physiologically. Physicians are frequently timid about assessing emotional symptoms. It is odd that we thread catheters, ablate lesions, and give rectal exams but are

> uncomfortable asking our patients about their lives. Assessment tools like the Prime-MD (8) are readily available, with their easy-to-ask questions such as "have you been bothered a lot by 'nerves' or feeling anxious or on edge?" Such questions open the door. Findings such as those described in these articles suggest that including this information in our clinical assessments might be relevant for the diagnosis (and prevention) of CVD. (p. 48)

Although not in full agreement (e.g., Lane, Carroll, Ring, Beevers, & Lip, 2000), there is ample recent evidence that anxiety, as is the case with depression, complicates the course of in progress CVD. In a 5-year follow up study of 440 coronary artery bypass patients, Tully, Baker, and Knight (2008) reported a statistical trend for a finding that showed preoperative levels of depression were associated with a higher risk of mortality; however, mild preoperative levels of anxiety were statistically associated with an independent, and nearly twofold increased level of mortality risk. Anxiety has also been shown to be an independent factor in the onset and course of CVD. Kawachi, Sparrow, Vokonas & Weiss, (1994) looked at over 2,000 male Bostonians over a 32-year period and found that men who reported two or more symptoms based on a 5-item anxiety symptom scale had a greater risk for dying of CHD and MI compared to men who did not report any anxiety symptoms. The authors point out that anxiety symptomatology had been assessed only once and on average the death occurred 17.5 years later. The authors also discussed two other cohort studies that had also found a strong association between anxiety and sudden cardiac death.

Denollet, Maas, Knottnerus, Keyzer, and Pop (2009) reported that anxiety was independently associated with a 77% increase of all-cause premature mortality including CVD, while depression was not associated with increased premature mortality. The study

examined over 5,000 healthy middle-aged women (*M* = 50.4 yr) in the Netherlands over a 10-year period. The depression and anxiety ratings were based on a self-report 10-item scale of which 7 were related to depression and 3 to anxiety (anxious/worried, scared/panicky, & ruminating about things that went wrong).

There have been several meta-analyses that have looked at anxiety and CHD. Kubzansky et al. (1998) reviewed the literature from between 1980 and 1996. They found too few studies that looked at chronic anxiety and CHD to conduct a formal meta-analysis; however, they conclude that "the magnitude, consistency, and dose-response gradient of the association lend support to the notion that anxiety may contribute to risk of CHD" (p.51). More recently, Roest, Martens, de Jong, and Denollet (2010) conducted a meta-analysis looking at anxiety and the risk of incident heart disease based on 20 studies that included the studies in the previous meta-analysis/review by Kubzansky et al. In all just under 250,000 healthy individuals with an average follow-up period of 11.2 yr were included in the meta-sample. Overall, they found a 26% increase in risk for incident CHD and a 48% increase in risk for cardiac death. The authors point out that almost all of the included studies covaried out traditional risk factors. Clinical depression and anxiety are often comorbid (e.g., Aina & Susman, 2006; Hirschfeld, 2001). The combination of depression and anxiety has been investigated in several studies for the individual or combined association with various types of CVD. Grimsrud, Stein, Seedat, Williams, and Myer (2009) studied a nationally-representative sample of almost 4,500 adults in South Africa, and examined the association amongst comorbid depression and anxiety, and hypertension. They failed to find an association between pure hypertension and anxiety or depression alone, or combined anxiety and depression; however, if another chronic

physical condition was present then the associations arose. When adjusted for other chronic conditions they found no hypertension and anxiety, depression, or combined effect. A similar lack of an association between anxiety or comorbid anxiety and depression was found in a sample of over 60,000 individuals from Norway. Depression was associated with CVD mortality to the same degree as its association with all cause morbidity. In a 37-year follow-up study (Janzky, Ahnve, Lundberg, & Hemmingsson, 2010) of almost 50,000 young (18 – 20 years old) Swedish men who were seen by a psychologist and participated in a structured interview as part of military conscription, anxiety but not depression was associated with CHD later in life.

Cardiovascular Reactivity: One Possible Link Between Stress and CVD

Stress – Reactivity component.

Cardiovascular reactivity, or 'hyperreactivity,' has been posited as a link between stress and CVD, as well as between hostility, and its related components, and CVD. Blascovich and Katkin (1993) reviewed the stress-reactivity-disease model and point out that, although the stress-reactivity component of the model has been well studied, the reactivity-disease component has received less attention. Over the past 20 years there has been a considerable increase in research in this area.

Animal and human studies have provided much evidence for a stress – reactivity link. In the laboratory, physical stressors elicit increased cardiovascular responses. One such stressor, the cold pressor test, in which a limb is immersed in ice water, results in relatively large increases in both systolic and diastolic blood pressure, as well greatly increased peripheral resistance, with lesser increases in heart rate (DeQuattro & De-Ping Lee, 1989). Psychological stressors, such as public speaking tasks, mental arithmetic

tasks, competitive games, and interpersonal conflict, also result in varying patterns of increased cardiovascular responding. Manuck, Kasprowicz, Monroe, Larkin, and Kaplan (1989) give an excellent review of studies utilizing both physical and psychological stressors.

Stressors exert variable CVR influences, as a function of personality characteristics. Hostility in particular appears to moderate the cardiovascular responses to stress. In a natural hospital setting, researchers found that high hostile ambulatory paramedics were more reactive in interpersonal conflict situations than their low hostile co-workers. Jamner, Shapiro, Goldstien, and Hug (1991) found that Cynical Hostility interacted with defensiveness and that individuals high on hostility and low on defensiveness had greater HR and DBP reactivity. A fairly large number of laboratory studies have looked at the hostility – stress interaction. A deception paradigm study, in which participants were told that unsolvable anagrams were easy to solve, found increased cardiovascular reactivity in high hostile women and men, with few differences between the sexes (Weidner, Friend, Ficarrotto, & Mendell, 1989). In our own laboratory, competitive games, mental arithmetic, and mental arithmetic with harassment - an interpersonal conflict - have clearly shown increased cardiovascular reactivity to psychological stressors, especially amongst individuals who are high on trait hostility (Miller, Dolgoy, Friese, & Sita, 1996, 1998; Miller et al., 1998). Other researchers have studied the effects of interpersonal challenge or conflict on cardiovascular reactivity of individuals with high trait hostility and have shown increased reactivity in that group (Davis, Matthews, & McGrath, 2000; Smith & Allred, 1989; Suarez, Kuhn, Schanberg, Williams, & Zimmerman, 1998); not all studies have supported this finding. In a study

utilizing an interpersonal conflict, Allred and Smith (1991) found that there was no association between level of Ho scores and CVR. A similar finding was reported by Smith and Houston (1987). Suls and Wan (1993) used a meta-analytic approach to review 28 research reports and concluded that the Ho scale was predictive of at least some CVR components and, furthermore, that interpersonal stressors were more likely to result in CVR than other forms of psychological stress.

Anxiety, Depression, and Cardiovascular Reactivity

Reactivity – Disease component

Hostility.

With regard to the reactivity – disease component of the model, there has been considerable research as well. Animal research has shown links from stress to CVR to disease endpoint. In a series of studies with cynomologous macaques, Kaplan et al. (1993) demonstrated that manipulation of psychosocial stress was associated with degree of atherosclerosis. Kaplan et al. posit that the primate model is relevant to humans and argue that, in fact, humans face even greater numbers and types of psychosocial stressors. In a study looking at a common daily event, Spitzer, Llabre, Ironson, Gellman, and Schneiderman (1992) found that simply meeting strangers in social situations resulted in increased CVR as compared to social situations with family members. Rodent models of hypertension are reviewed in Saab and Schneiderman (1993) and the authors conclude that the development of hypertension in rats is facilitated by the interplay between stress and CVR. Humans who respond to stress with increased total peripheral resistance (one of the CVR mechanisms) are at greater risk for hypertension (Girdler, Turner, Sherwood, & Light, 1990; Light & Sherwood, 1989). While the exact mechanisms leading from

cardiovascular hyperreactivity to CHD are not certain, various pathways are posited. Animal studies provide evidence that recurrent pharmacologically induced or behaviorally induced (utilizing various physical and social stressors) sympathetic activation leads to transient elevations in blood pressure and the development of endothelial damage, similar to that found in early atherosclerosis (Kaplan et al., 1993).

Stress-induced reactivity is also related to morphological changes, such as the narrowing of the arterial lumen (tubular cavity) in both coronary and peripheral arteries, hence increased vascular resistance (Blascovich & Katkin, 1993). This vasoconstriction is thought to occur partially due to arterial muscle hypertrophy and results in chronic blood pressure elevations and increased hemodynamic turbulence during reactive episodes (e.g. stressful encounter), leading to atherosclerotic lesions (Blascovich & Katkin). High CVR in response to both physical and psychological laboratory stress challenges predict hypertension in later life (Light, Dolan, Davis, & Sherwood, 1992; Light, Sherwood, & Turner, 1992) supporting the line of reactivity to disease endpoint. In a study of 100 healthy black and white children (Matthews, Salomon, Brady, & Allen, 2003) which used impedance cardiography, particular elements of CVR were found to be associated with 3-year follow-up blood pressure levels and the authors state that this supports the CVR and risk for hypertension link. In a study that looked at air traffic controllers, 20-year follow-up data showed that increased systolic blood pressure response to work stress was associated with long-term risk of hypertension (Ming, Adler, Kessler, et al., 2004). In addition to blood pressure, increased heart rate has also been shown to cause similar type of damage (Clarkson, Manuck, & Kaplan, 1986). While the exact mechanism of even this small portion of the physiology of CVD is not known, Julius (1987) has emphasized the

contribution of the central nervous system. Other pathways including neuroendocrine systems are likely involved as well, with the possibility that short term CVR to stress may mediate longer term neuroendocrine responses (Larson, Ader, & Moynihan, 2001).

Hostile individuals are likely to exhibit cardiovascular hyperreactivity (Engebretson, & Matthews, 1992; Williams, Barefoot, & Shekelle, 1985), especially in situations that elicit an anger response to an interpersonal conflict (Hardy & Smith, 1988; Lai & Linden, 1992; Miller, Dolgoy, Friese, & Sita, 1996, 1998; Miller, Friese, Dolgoy, et al., 1998; Smith & Allred, 1989) and this may be the pathway by which hostility confers additional risk for CVD. Findings by Brondolo, Rieppi, Erickson, et al., (2003) partially support this hypothesis. They examined the role of hostility and interpersonal interactions in a sample of just over 100 healthy males and females and found the higher the negative perception of the interaction, the higher the ambulatory blood pressure, with larger increases in diastolic blood pressure associated with high hostility. They did not find an association with heart rate and hostility. Studies that focused on the cardiovascular responses of women found that their greatest cardiovascular response was to anger eliciting stressors (Powch & Houston, 1996; Suarez, Harlan, Peoples, & Williams, 1993).

Suarez and Williams (1990) argue that the experience of anger is, in fact, essential in eliciting a hyper cardiovascular response. Studies, such as those by Smith and Houston (1987) that did not elicit anger fail to show such responses. As with hostility, anger is a complex construct that has been heavily researched over the past 30 years. Abel, Larkin, and Edens (1995) looked at components of anger in a study of undergraduate females and found that in response to a mental arithmetic stressor, those with moderate levels of

Anger-Out showed lower levels of blood pressure than women with either low or high Anger-Out. Heart rate as well was lower for the moderate Anger-Out group than the group with high Anger-Out. No CVR effects were found for Anger-In. Compared to physical stressors and mental arithmetic tests, an anger interview was found to elicit larger and more sustained blood pressure increases (Prkachin, Mills, Zwaal, and Husted, 2001). Harassment was used to examine dispositional anger and CVR in a study of Chinese and Indian men living in Singapore (Bishop & Robinson, 2000). They found different patterns between the two groups; however, dispositional anger was related to systolic blood pressure increases under the harassment condition. Schum, Jorgensen, Verhaeghen et al., (2003) conducted a meta-analysis of 15 studies that explored trait anger and ambulatory blood pressure. They reported a modest association for trait-anger and anger-expression and blood pressure levels.

Depression.

Kibler and Ma (2004) reviewed the literature from 1887 to 2001 for peer-reviewed studies on depressive symptoms and CVR to a laboratory stressor that included at least one cardiovascular measure (e.g., HR, SBP, DBP, etc.,) and culled 11 relevant studies. As HR, SBP, and DBP were often the only CV measures, they were the only ones retained for the meta-analysis. Eight different measures of depressive symptoms were used; however; the authors of the meta-analysis reported that all the indices were treated as equivalent due to reported high concurrent validity. None of the CV measures were statistically significantly associated with depressive symptomatology though there was trend for statistical significance for HR. The authors conclude that although the statistical significance is debateable, the moderate (HR) to small (SBP & DBP) effect

sizes found should not be dismissed. Subsequent to the 2004 meta-analysis, several studies have continued to investigate the possibility of an association between depressive symptoms and CVR.

Matthews, Nelesen, and Dimsdale (2005) found an association between depressive symptoms based on the Center for Epidemiological Studies Depression Scale (a 20-item self-report measure) and increased systemic vascular resistance (equivalent to TPR). Based on this measure 91 participants were divided into high and low depressive symptoms groups. HR, HR variability, HI, SV, MAP, CO, and SVR measures were obtained during the 3-min stressor period that consisted of the participants tracing the outline of a star and its mirror image. The lone significant finding was for a marked increase in SVR during the stress period in individuals with high depressive symptoms. Baseline values for SVR were greater as well for this group, with the authors suggesting that higher baseline individuals react more strongly. Carroll, Phillips, Hunt and Der (2007) reported a negative association between CVR and depression scores in a sample of over 1,600 individuals of three age cohorts (24, 44, 63 years of age) during an auditory serial math task.

The link between anxiety and depression and CVR is less clear than that of anger and hostility; however, none of the studies described here had neither interpersonal nor provocative components similar to the studies utilized by hostility and anger researchers. Depressive symptoms and trait aggression and the interaction amongst them were the factors of interest in a study on CVR to a laboratory stressor that consisted of a speaking task (Betensky & Contrada, 2010). Female university students (n = 63) were asked to prepare for and recount an autobiographical episode that had occurred in the past 6 month

and had caused depressed feelings. HR, SBP, and DBP measure were collected every 60 s during baseline and the stressor period. Women with high trait verbal aggression showed a positive association between depressive symptoms and blood pressure reactivity to the stressor while women with low verbal aggression did not.

Serotonin: Psychological and Behavioral Effects

Serotonin (5-hydroxytryptamine; 5-HT) and its experimentally manipulated levels has been associated with the regulation of mood in both normal (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1996; Knott, Howson, Perugini, Ravindran, & Young, 1999; Ravindran, Griffiths, Merali, Knott, & Anisman, 1999; Smith, Pihl, Young, & Ervin, 1987; Young, Smith, Pihl, & Ervin, 1985) and clinical (Booij et al., 2005; Delgado et al., 1990, 1994; Leyton et al., 1997, 2000) or at risk populations (Benkelfat, Ellenbogen, Dean, Palmour, & Young, 1994; Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1999; Quintin et al., 2001).

Approximately half of the studies on healthy adults demonstrated a lowering of mood or depression-like affect following acute depletion of tryptophan (Trp), the precursor of serotonin (Young & Leyton, 2001). Since the studies reviewed in Young and Leyton, there have been several that have failed to find any mood effects purely as a function of acute tryptophan depletion (ATD; e.g., Hughes et al., 2003; Moreno et al., 1999, 2010; Sambeth et al. 2009; Shansis et al., 2000). Smith et al. (1987) carried out a study on 80 healthy young (ages 18 - 25) males, which had no pure wait period to assess the effect of ATD alone. Participants spent 5 h post-ingestion in either a positive (friendly & physically comfortable) or negative (unfriendly & physically uncomfortable) environment completing non-specified tests. They reported increased depression based on

the Multiple Affect Adjective Check List (MAACL; Zukerman & Lubin, 1965) Depression Scale, and on the overall scores of the Depression Adjective Check List (Lubin, 1981) as well as MAACL-based anxiety in the tryptophan depleted condition (Trp- condition) compared to the balanced condition (B condition). The friendly condition can be construed as a pure wait period as there was no active stressor and Trp- participants reported an increase in both hostility and depression; nonetheless the completion of unspecified tests makes this assumptive pure wait state questionable. Schmeck et al. (2002) found an effect on mood following ATD, but only for women, and not men, who scored high on trait aggression. Mortimore, Connell, and Van (1997) found no effect on anxiety based on ATD in a sample of 31 healthy participants exposed to aversive tones after a 4 hr wait period and then 30 min later participated in a simulated public speaking task. Anxiety was assessed using four different instruments including the Profile of Mood Scales (POMS; McNair, Lorr, & Droppleman, 1988), the State Trait Anxiety Inventory (STAI; Spielberger, Gorusch, & Lushene, 1970), and a Visual Analogue Scale (VAS).

Hughes et al. (2003) failed to find any effects on mood and anxiety based on a VAS following ATD in a sample of 20 healthy males, neither as a function of pure depletion (wait period) nor during tests of verbal and visuo-spatial learning and memory as well as attention and executive function. Other studies have found mood lowering effects of ATD in healthy women but not males as well (e.g., Ellenbogen et al., 1996; Smith, Clifford, Hockney, Clark, & Cowen, 1997). The latter study by Smith et al. reported a reduction in women only on a single VAS item, Happy, 1 hr after depletion. Given that the effects of depletion, if any, are usually only after 4 h and usually at 5 – 7

hr post depletion, it is possible that the reduction in Happy is more directly related to the ingestion of an 85.8 g bolus of amino acids that are rather unpleasant to ingest, not infrequently resulting in emesis.

Findings in both the clinical and at risk populations reported mixed findings as well, with a greater number of at risk studies demonstrating a mood lowering effect (e.g., Benkelfat et al., 1994) and most, but not all (e.g., Davies et al., 2006) clinical studies reporting lowered mood (Bell, Abrams, & Nutt, 2001; Klaassen, Riedel, van Someren, Deutz, Honig, and van Praag (1999). Van der Does (2001) examined several of the mood studies and suggested that the negative findings may be an artifact of incomplete manipulatory reduction of 5-HT levels. Booij, Van der Does, Benkelfat et al. (2002) reviewed six studies in their "mega-analysis" (authors' quotes) and reported that approximately 50% of previously depressed patients had a recurrence of symptoms. Neumeister, Nugent, Waldeck et al., (2004) reported a transient recurrence of depressive symptoms in 27 remitted major depressive disorder patients (18 women & 9 men) but not in the healthy control group (10 women & 9 men).

ATD has also been used to explore the role of 5-HT function on cognitive functions including memory, attention, and executive function, including decision making and reward appraisal. Clark, Roiser, Cools, et al., (2005) examined the effect of ATD on impulsivity using a stop signal response inhibition paradigm and found no association between ATD and stop signal reaction time. Decision making including reward/punishment appraisal has been examined using ATD and has been shown to impair the appropriate use of punishment information (Blair, Finger, Marsh, et al., 2008; Finger, Marsh, Buzas, et al., 2007). Mendelsohn, Riedel, and Sambeth (2009) reviewed

66 studies that used ATD to examine memory, attention, and executive function. Overall there was evidence that ATD impaired episodic memory consolidation for verbal information but there was little evidence of a similar effect on non-verbal learning. It had no effect on semantic memory, spatial memory, or affective working memory. ATD has also been used in combination with various neuroimaging techniques to study brain activation during cognitive (e.g., response control, memory, and selective attention, etc.) and cognitive/affective (e.g., emotional face processing and emotional words processing) information processing. Fusar-Poli, Allen, McGuire, et al. (2006) report on 26 ATD in combination with neuroimaging studies that had been carried out between 1997 and 2006. Evers, Sambeth, Ramaekers et al., (2010) report the results of 20 studies (several of which had been included in the Fusar-Poli review) and describe the areas of brain activation and issues related to the use of particular neuroimaging techniques.

Impulsivity and disinhibition, also, have been linked to 5-HT function in similar populations as described above, both in laboratory settings (LeMarquand et al., 1998) and in natural settings (Dolan, Anderson, & Deakin, 2001). Aggression is the most frequently studied behavior in relation to 5-HT dysfunction (Young & Leyton, 2001). As with mood and impulsivity, the association between 5-HT and aggression has been studied in diverse populations both in the laboratory (e.g. Bjork, Dougherty, Moeller, & Swann, 2000; Cleare & Bond, 1995, 1997, 2000; Dougherty, Bjork, Huckabee, Moeller, & Swann, 1999) and natural settings (Brown et al., 1982; Dolan, Anderson, & Deakin, 2001; Virkkunen, Nuutila, Goodwin, & Linnoila, 1987). Dougherty, Bjork, Marsh, and Moeller (1999, pp. 227-228) claim that "dysfunctional serotonin (5-HT) neurotransmission has been correlated with aggressive acts towards self and others and this is the most

replicated finding in the neurobiology of aggression."

Animal studies have shown that vervet monkeys with experimentally manipulated (lowered) levels of 5-HT exhibit increased levels of aggression (Chamberlain, Ervin, Pihl, & Young, 1987). Rat studies demonstrate 5-HT-deficit-induced increases in muricide (mouse killing behavior), filicidal (pup-killing) behavior, as well as other aggressive behaviors; similar effects are found with felines, effects which can be reversed through restoration of 5-HT function (Eichelman, 1979).

An inverse relationship between aggression and 5-HT levels in humans is the most frequently reported finding. The findings related to laboratory induced aggression have been mixed and it is important to understand the 5-HT manipulation paradigms and elicitor tasks used. Correlational studies have most often assessed the level of 5-HT through the analysis of the major 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid, obtained through a lumbar puncture. Golden et al. (1991) reviewed the literature relating to suicide, aggression, and 5-HT and reported that almost all studies of individuals who have attempted suicide and postmortem studies on successful suicides have revealed low 5-HIAA levels. Repeat violent offenders have lower levels of 5-HIAA than those who have committed a single violent crime (Golden et al.) and a study by Virkkunen et al. (1987) revealed that arsonists had lower levels than normal controls.

Studying Brain Serotonin Levels

There is no easy way to directly study brain 5-HT levels and the relation between central 5-HT and 5-HIAA is not always clear (Golden et al., 1991). Nishizawa et al. (1997) point out that there is spinal cord metabolism of 5-HT, catabolism rates of 5-HT vary, and there is transport of 5-HIAA into and out of the central nervous system. They

conclude that "CSF 5-HIAA levels are a poor index of dynamic changes in 5-HT synthesis in brain tissue" (Nishizawa et al., pp. 5310-5311). Carpenter et al. (1998) state that 5-HIAA in lumbar cerebrospinal fluid (CSF) only approximates central 5-HT release and utilization. In experimental studies, pharmacological challenges that either increase or decrease the availability of 5-HT have been used. The 5-HT agonist *d*-fenfluramine has been shown to be associated with decreases in aggression in a non-clinical population (Manuck, et al., 1998). This study utilized a point/money subtraction provocation (The Point Subtraction Aggression Paradigm – Cherek at al. 1996, 1997a, 1997b, 1997c) and measured the number of retaliatory subtractions made by the participant against the purported competitor who had caused money to be subtracted from the participant and compared a control group with a pharmacologically challenged group. Another *d*-fenfluramine study by Cleare and Bond (1997) that did not include any confrontational component, using only self report questionnaires assessing hostility and aggression, demonstrated the inverse relationship between 5-HT and aggression as well.

Acute Tryptophan Depletion and 5-HT Reduction

Tryptophan (Trp), a large amino acid, is one of the 10 essential amino acids and is the precursor to 5-HT, being first converted by the enzyme tryptophan hydroxylase into 5-hydroxytryptophan (5-HTP) and into 5-HT by the decarboxylizing action of the enzyme L-aromatic acid decarboxylase. As the enzyme tryptophan hydroxylase is, under normal physiological conditions, only approximately half saturated by its substrate, the rate of 5-HT synthesis is dependent on the availability of free plasma Trp. Most of the Trp in the blood stream is in an albumin-bound form that is unable to cross the blood-brain barrier; hence, it is not available for synthesis into brain 5-HT. Unbound, or free

plasma Trp, competes with the five of the other amino acids (isoleucine, leucine, phenylalanine, tyrosine & valine) in crossing the blood brain barrier, sharing a common transport system (Bell, Abrams, & Nutt, 2001;Young, Smith, Pihl, & Ervin, 1985). Once it enters the brain it is available to be acted upon by tryptophan hydroxylase. Tryptophan, in addition to being the precursor to 5-HT, is a rate limiter of all protein synthesis. The combination of these two properties provides a unique way to manipulate brain 5-HT levels.

Protein synthesis by the liver is a continual activity and requires a steady supply of amino acids including, Trp. Subsequent to an amino acid deprivation (through dietary restrictions, for example) the liver is primed to resume protein synthesis. In laboratory studies, participants are typically put on a 24-hr protein-Trp-restricted regimen. Following the 24-hr diet, the consumption of a balanced amino acid challenge will result in the resumption of normal protein synthesis. If, however, the post-diet challenge of amino acids lacks Trp, several events occur. The liver begins to synthesize protein and, in so doing, quickly scavenges any remaining Trp from the blood pool and tissues, incorporating it into new proteins. This prevents Trp from, potentially, crossing the blood-brain barrier. Additionally, the presence of a large quantity of amino acids competing with Trp for transport across the blood-brain barrier further reduces the chance that any Trp will become available in the brain for conversion into 5-HT (Olendorf, 1973). Approximately 5 hr after the ingestion of a Trp deficient amino acid challenge, free and total plasma Trp levels will have declined by approximately 80-90% (Bell, Abrams, & Nutt, 2001; Carpenter et al., 1998; Delgado et al., 1990; Reilly, McTavish, & Young, 1997; Williams, Shoaf, Hommer, Rawlings, & Linnoila, 1999). Following this

reduction there is a marked decline in brain 5-HT synthesis (Nishizawa et al., 1997; Young, 2002). Several studies (e.g., Firk & Markus, 2009; Marsh, Dougherty, Moeller, Swann, & Spiga, 2002) have utilized a Trp augmented challenge, in addition to the depletion and control, with findings that suggest inverse effects of the depletion paradigm (i.e. reduced aggression and impulsivity as well as mood enhancement).

Effectiveness and Specificity of Acute Tryptophan Depletion

Evidence that acute tryptophan depletion (ATD) reduces 5-HT levels comes from various sources. Nishizawa et al. (1997) measured 5-HT synthesis in the human brain using positron emission tomography imaging that demonstrated a marked lowering of 5-HT synthesis in all brain regions following an ATD. Carpenter et al. (1998) utilized a continuous sampling of CSF and found that Trp depletion does robustly lower central Trp levels, but "only modestly lowers central 5-HIAA levels and may reduce general 5-HT function" (pp. 33). Williams et al. (1999), sampled CSF and plasma levels of free and bound Trp as well as 5-HIAA during a Trp depletion protocol. Plasma levels of Trp began to decrease 2 hr post depletion and reached a nadir 4 hr later. CSF Trp began to decrease at 2.5 hr after depletion followed by 5-HIAA 1.5 hr later. CSF Trp reached its lowest point 8 hr post depletion and 5-HIAA at approximately 14 hr after the depletion. Animal studies have shown that an ATD can lower central nervous system levels of Trp, 5-HT, and 5-HIAA in the CSF (Williams et al.). That an ATD lowers CSF Trp and 5-HIAA levels in vervet monkeys as well as being specific in its actions, in that it did not lower metabolites of other amino acids, was demonstrated by Young, Ervin, Pihl, and Finn (1989). Decreased hippocampal 5-HT release was also shown in rats following an acute administration of a Trp-free amino acid mixture (Stancampiano, Melis, Sarais, et

al., 1997). Klaassen, Riedel, Deutz, Van Someren, and van Praag (1999) showed that lysine depletion did not result in the same mood and memory differences found in ATD, supporting the hypothesis that ATD effects brain 5-HT metabolism and not protein metabolism in general. Moore et al. (2000) point out that while there has been limited investigation of changes in other Trp containing regulatory proteins such as neurotransmitter receptors, ion channels, and enzymes that are involved in neurotransmitter synthesis and breakdown, 5-HT remains the "most parsimonious TRP "metabolite" capable of producing the spectrum of RTD effects" (Moore et al., pp. 618). Recent investigations using ATD in rats have supported the specificity of 5-HT reduction. Cahir, Ardis, Elliott et al, (2008) showed specificity of ATD in rats to lowering 5-HT in the central hippocampus but ATD did not lower brain-derived neurotrophic factor.

In a further rat study, free plasma Trp levels were reduced to levels similar to those reported in humans resulting in reductions in 5-HT and 5-hydroxyindolacetic acid, but there was no reduction in levels of dopamine or noradrenaline (Ardis, Cahir, Elliott et al., 2009). Jenkins, Elliott, Ardis et al., (2010) showed that chronic tryptophan depletion reduced central 5-HT in rats and impaired object-recognition memory. This effect was reversed by a single administration of risperidone but not reversed by a dopaminergic influencing antipsychotic, suggesting that particular serotonergic receptors (5-HT2A) play a role in the memory impairment, and that ATD selectively affects the 5-HT system.

Acute Tryptophan Depletion and Aggression Studies

Smith, Pihl, Young, and Ervin (1986) used a modified Buss (1961) paradigm

(shock delivery to a nonexistent partner), in conjunction with depletion and augmentation, and found no effect of Trp manipulation on aggressive behavior. LeMarquand, Benkelfat, Phil, Palmour, and Young (1999) reported no effects of depletion on aggression in healthy males without a positive family history of alcoholism who were engaged in a modified Taylor aggression task, similar to the Buss paradigm described above. Participants with a positive family history displayed more aggressive behavior, especially early in the task. They also reported evidence of increased impulsivity and decreased mood in all of the participants. A possible limitation of this study is the small sample size ($N = 36$). Previously, Pihl et al. (1995) used a similar paradigm in combination with the administration of alcohol to look at aggression in normal human males and found that both the depletion and alcohol ingestion increased aggressive responding. Using a point/money subtraction paradigm, such as that described above, in combination with depletion and augmentation, Marsh, et al. (2002) studied aggressive behavior in women and found that aggressive behavior increased with depletion and decreased with augmentation.

Studies have been conducted that explicitly examined the possible interaction between trait hostility or aggressivity and 5-HT levels in producing increased or decreased aggressive responding and related disinhibition or impulsivity. LeMarquand et al. (1998) reported an ATD disinhibition effect in non-aggressive adolescent males; however they found no effect in the aggressive group, possibly due to a ceiling effect. In a study by Cleare and Bond (1995), Trp depletion and augmentation caused a marked rise and fall, respectively, in subjective feelings of aggression, as well overt behaviors, in the high trait aggression group. The findings from the low trait aggression group were less

consistent, with little effect on subjective feelings of aggression and variable results on the measures of actual aggressive behavior. In a study with negative results, Salomon, Mazure, Delgado, Mendia, and Charney (1994) failed to find ATD related changes of self-reported hostility in a group of aggressive patients. The authors point out that their sample size was small ($N = 14$) and there was no provocational aspect to the study, with participants merely being observed for spontaneous acts of hostility in a "neutral, clinically sterile environment" (Salomon et al., pp. 571).

Two studies looked at the effects of Trp on aggression in hostile and non-hostile participants and supported the inverse aggression – 5-HT relation. Finn, Young, Pihl, and Ervin (1998) reported that ATD resulted in higher hostile mood on the Multiple Affect Adjective Checklist (Zuckerman & Lubin, 1965) in participants with both low and high trait hostility as assessed by the Ho scale. The increase, however, was greater for those high in trait hostility. High hostiles demonstrated decreases in hostile mood in the balanced condition, an unexpected finding that the authors attribute to the increase in Trp levels following the Trp loading which resulted from a balanced amino acid challenge. They hypothesize that individuals who are high on antisocial and hostile traits may be susceptible towards Trp induced changes in hostility. These results may not be generalizable to high hostiles in the population at large, as many of study participants had MMPI profiles indicative of a personality disorder and may form a distinct subgroup. Dougherty, Bjork, Marsh, and Moeller (1999) induced laboratory aggression using the Point Subtraction Aggression Paradigm described above. The 8 male participants were classified as high or low hostile based on a median split of scores on the Buss-Perry Aggression Questionnaire (Buss & Perry, 1992). In a repeated measures 3-condition plus

baseline design: baseline, depletion, augmentation, and a food restricted control day, high hostiles exhibited elevated aggression in the depletion condition.

Serotonin, Tryptophan Depletion, and Cardiovascular Effects

Serotonin's role in regulation of the cardiovascular system is extremely complex. In the early 1990s the number of positively identified main 5-HT receptor families had reached three and all appeared to mediate cardiovascular activity, with a proposed fourth type playing a hypothesized myocardial stimulation role as well (Saxena & Villalon, 1990). Ten years later, there were seven main 5-HT receptor types with more than 20 subtypes identified (Hamel, 1999; Glennon, 2003) distributed throughout the human body and brain (Côté, Fligny, Fromes, et al., 2004), though it is as yet unclear whether all have a differential function (Villalon & Centurion, 2007). Ramage and Villalon (2008) reported that all 5-HT receptors except the 5-HT_6 are involved in cardiovascular regulation. The modulating and mediating effects of both central and peripheral serotonergic systems, directly and indirectly through their effects on other physiological systems, is yet to be fully unraveled.

5-HT acting directly on the vasculature can cause both vasoconstriction (Chester, et al., 1990; Conner, Fenuik, & Humphrey, 1989; Dahm et al., 1996) and vasodilation (McFadden et al., 1991). In healthy individuals, direct infusion of 5-HT into coronary arteries results in vasodilation, whereas infusion in arterioscelrotic coronary arteries results in severe vasoconstriction (McFadden et al.); slow intravenous infusion of 5-HT will elicit tachycardia prior to any changes in blood pressure (Saxena & Villalon, 1990). Heart rate and blood pressure can be reduced through the administration of selective 5-HT_{1A} receptor agonists; however, 5-HT_3 receptors on the vagal nerve endings in the heart

can lower heart rate by inducing a short duration hypotension. This reduction in heart rate is reversed when the bradycardia reflex is suppressed, as in deep anesthesia, for example, and tachycardia ensues. The blood pressure response to 5-HT generally follows a three step sequence: an initial short duration fall in blood pressure (hypotension), a longer duration rise (hypertension), followed by a longer lasting hypotension. The initial hypotensive response is the result of a rapid bradycardia and subsequent decrease in cardiac output following the stimulation of 5-HT_3 receptors located on the afferent cardiac vagal nerves located in the heart and lungs. This cardiopulmonary reflex is referred to as the von Bezold-Jarisch reflex (Ramage & Villalon, 2008). The middle hypertensive phase, or pressor phase, results from 5-HT_2 mediated vasoconstriction. These receptors are located directly on blood vessels in certain species (e.g. rat and cat) and on the adrenal medulla in others (e.g. dog). The third phase, hypotensive effect or depressor effect, is caused by activation of CNS of 5-HT_1 receptors, that decrease sympathetic activity and stimulate vagal nerve activity, reduced transmitter release of sympathetic nerve terminals, vasodilatation (expansion of the smooth muscles of the vasculature system), and the release of a relaxant factor in the walls (the endothelium) of the vasculature system (Saxena & Villalon). Blood pressure increase in rats is presumed to be mediated by the activation of a particular central receptor subtype, 5-$HT_{2/1C}$, which then causes the release of vasopressin (Pergola, Sved, Voogt, & Alper, 1993).

5-HT injected directly into the CNS results in depressor, pressor, and biphasic responses. The particular effect depends on many factors including the dosage, the site, and the physiological state of the animal. For example, opposite effects can be observed depending on whether the animal is conscious or not, normotensive or hypertensive, etc.

Diaz, Ni, Thompson et al (2008) demonstrated a reduction in normotensive and hypertensive rat blood pressure following chronic exogenous 5-HT, a finding that was the opposite of their original hypothesis. Distinct receptor subtypes in particular brain sites, such as the dorsal and median raphe, anterior hypothalamus, and ventrolateral medullary raphe, produce mainly pressor effects, while other subtypes, located in the midline medullary raphe nuclei, can produce pressor or depressor effects, depending on which subtypes are activated (Jonnakuty & Gragnoli, 2008). CNS 5-HT stimulation of particular serotonergic pathways and receptor subtypes results in an integrated cascade of neuroendocrine and autonomic responses. The opposing effects which can occur simultaneously within a singular brain structure may also be observed in the vasculature. Whether constriction or dilation occurs depends, again, on many factors, such as the relative density of particular subtypes located in the same blood vessel areas, the dose of 5-HT, as well as the pre-administration vessel tone (Saxena & Villalon, 1990).

Across many species, the main effect of 5-HT administration is a rapid, short-lived, deep bradycardia (Saxena & Villalon, 1990). In human beings, an increase in CNS 5-HT, usually as a result of drugs aimed at boosting 5-HT function such as SSRI's, can lead to Serotonin Syndrome. This condition, though usually mild, can in extreme cases lead to death. Symptoms include tachycardia and, paradoxically, either hyper- or hypo-tension, with the former being more prevalent (Nolan & Scoggin, 1988). A possible explanation for the two opposing symptoms may be as described above, with each effect resulting from a different phase of the three phase process.

5-HT is also stored within the blood platelets and its release, possibly during ischemic (reduced blood-flow) periods, stimulates thrombus formation (similar to a blood

clot) and platelet aggregation. Selective serotonin reuptake inhibitors (SSRIs) reduce the level of platelet stored 5-HT and possibly have an anti-aggregation effect (Krishnan & Clary, 2000). Transient hemodynamic turbulence in severely stenosed canine arteries with endothelial injury can be eliminated with a 5-HT receptor antagonist and recreated through the administration of 5-HT. It is argued that similar effects can be created in humans and, therefore, may be involved in certain ischemic syndromes (van den Berg et al., 1989). The contraction of the muscular coat of the blood vessels, vasospasm or angiospasm, is thought to be caused by increased concentrations of 5-HT at the site of the coronary arterial stenosis (narrowing) in both animal models and humans (Ashton et al., 1986; Schmitz, Apprill, Buja, Willerson, & Campbell, 1985).

Autonomic activity modulated by the pattern of activity of central nervous system stimulated 5-HT_1 receptors, that includes decreased sympathetic activity, an increase in parasympathetic function, and vagal nerve activity, can be induced through 5-HT loading, agonist administration, as well as through Trp supplementation (Saxena & Villalon, 1990; Villalon & Centurion, 2007). This pattern of autonomic activity is seen in low hostile individuals and the converse pattern of increased sympathetic and decreased parasympathetic activity seen in high hostile individuals may thus be associated with serotonergic dysfunction. Reduced autonomic arousal and lower heart rate is associated with disinhibited temperament, socialized aggression, as well early adulthood criminal and violent behavior, whereas heightened levels of autonomic arousal and reactivity may confer a protection from criminal activity (Raine, 1997).

Acute Tryptophan Depletion Induced Cardiovascular Changes

There have been several studies that have reported on cardiovascular changes

induced by ATD. In a study of healthy males utilizing a cholecystkinin-tetrapeptide (CCK-4) challenge researchers looked at the possible interaction between this drug and 5-HT. CCK-4 is known to cause panic attack like symptoms and is thought to be associated with an interaction between the CCK-4 and 5-HT systems. Although they do not report pre-depletion cardiovascular measures, they report that there was no statistically significant cardiovascular effect of the ATD (Koszycki, Zacharko, Le-Melledo, Young, & Bradwejn, 1996). Williams et al. (2001), in a correlational type study (measurement of natural levels of 5-HIAA as opposed to 5-HT manipulation), collected cardiovascular measures during a 45-min mental stress protocol and carried out CSF 5-HIAA sampling. They analyzed their cardiovascular data using a two-way repeated-measures ANOVA and used a median split to assign participants into high or low 5-HIAA groups, comparing baseline to stress period cardiovascular changes. Based on the literature described above, they expected to find increased reactivity in the depleted condition. "The greater CV responses exhibited by persons with high CSF 5HIAA levels were surprising to us in light of our expectation that decreased CNS 5-HT function (i.e., *low* 5HIAA) would be associated with increased biological reactivity. "Because our results are quite robust statistically and internally consistent, it is unlikely they are due to chance" (Williams et al., pp. 304).

Thirty healthy men and women participated in a simulated public speaking task, a task that is known to induce anxiety Monteiro-dos-Santos et al., (2000). ATD was used under double blind conditions. The speaking task itself elicited increased anxiety; however, there was no general effect of the ATD. Additionally, the speaking task elicited increased heart rate and blood pressure, but there were no ATD group differences.

Nineteen remitted depression patients participated in an impulsivity task, the Continuous Performance Test, under high- or low- dose ATD conditions (Booij, Swenne, Brosschot, et al., (2006). Patients with a history of suicidal ideation only, showed increased impulsivity and anxiety under the high-dose condition. These symptoms correlated with increased heart rate during rest and an impulsivity task. Davies, Hood, Argyopoulos, et al., (2006) utilized ATD to study remitted anxiety disorders patient group that included remitted patients with social anxiety disorder or panic. Panic patients ($N = 27$) were stress challenged through the administration of flumazenil, a benzodiazepine antagonist, and the social anxiety group was stress challenged through the use of an autobiographical script of a significant anxiety episode. ATD resulted in statistically significantly higher anxiety responses to the stressors under the depletion condition. ATD resulted in increased systolic and diastolic blood pressure as a function of depletion condition. Stress-induced systolic blood pressure increases were greater under the depletion condition; however, only the panic patients had similar increases in stress-induced by trp condition diastolic blood pressure. Heart rate showed only pre-stress and stress-induced increases with no effect of trp condition. There was no correlation between the magnitude of the cardiovascular response increases and the magnitude of the stress-induced anxiety response increases when trp conditions were taken into account.

Healthy females ($N = 15$) participated in a study looking at conscious and incidental processing of emotional information – happy, angry, or sad faces (Beacher, Gray, Minati et al., 2011). ATD was not associated with mood; however, there were differential ATD effects with regard to attractiveness rating of happy faces but not angry or sad faces, and to arousal ratings for angry faces but not happy or sad faces. While there

were statistically significant systolic blood pressure effects based on the facial emotion, with decreases in systolic blood pressure in response to angry faces relative to happy or sad faces, there was no effect of ATD status on blood pressure.

Studies examining the role of psychosocial factors during interpersonal conflict and cardiovascular reactivity and mood continue to be carried out (e.g., Denson, Grisham, & Moulds, 2011; Neumann, Maier, Brown, et al, 2011) as well as studies looking at serotonergic systems, using various techniques including ATD to examine cardiovascular reactivity and mood in response to a multitude of human and animal processes that include cognition, sensation, physical behaviors including motoric behaviors, and affect, as well as stress responding (e.g., Geeraerts, Oudenhove, & Boesmans, et al., 2011; Hutchinson & Ruiz, 2011; Ohira, 2011). To the author's knowledge there have been no other studies using ATD to examine mood and cardiovascular responses in response to interpersonal conflict and to what extent psychosocial factors influence these responses.

The goal of this study is to examine the effects of low serotonin levels on cardiovascular and mood reactivity to interpersonal conflict. In particular this study will address the question of whether psychological factors including hostility, anger, anxiety and depression, which are known to play a role in both cardiovascular and mood reactivity, modulate, under stressful conditions, the response of individuals to low levels of serotonin, which in and of itself affects reactivity. Due to the difficulty of directly manipulating central nervous system serotonin levels, acute tryptophan depletion, a method which reduces the availability of serotonin production's rate-limiting precursor will be utilized. Previous research in our laboratory failed to find strong associations

between cardiovascular or mood measures and the interplay amongst tryptophan status and levels of hostility as assessed with the Ho scale. The present study will reexamine the potential role of hostility in a larger sample and additionally investigate the possible modulation of serotonin's role in cardiovascular and mood stress-related reactivity by trait anger, trait anxiety, and depressive symptomatology.

Hypotheses

Given the research described above, that 5-HT is implicated cardiovascular reactivity as well as in regulating or modulating mood and that trait hostility, trait anger, and trait anxiety as well as depressive symptomatology play a role in the cardiovascular and mood responses to interpersonal stress, it was hypothesized that:

A) changes in tryptophan levels would have little impact on cardiovascular reactivity during the waiting period,

B) during the active stress/harassment period participants in the tryptophan depleted condition would exhibit heightened cardiovascular reactivity compared to the participants in the balanced condition,

C) there would be an attenuation of the cardiovascular recovery response following the stress period in the tryptophan depleted condition,

D) there would be no differential decrease in mood during the 5.5 hr wait period,

E) during the math stressor/harassment period participants in the tryptophan depleted condition would show a greater increase in negative affect compared to the participants in the balanced condition,

F) during the math/harassment period, individuals with high scores on the four psychological factors would show a different response pattern in the tryptophan

depleted condition compared to participants with low psychological factor scores in the same condition, specifically that high psychological factor score individuals would show greater cardiovascular and mood reactivity compared to low psychological factor score individuals, while individuals in the balanced condition would not differ from each other as a function of psychological factor scores,

G) during the recovery period individuals with high scores on the four psychological factors would show a different response pattern in the tryptophan depleted condition compared to participants with low psychological factor scores in the same condition, specifically that high psychological factor score individuals would show a blunted cardiovascular and mood recovery compared to low psychological factor score individuals, while individuals in the balanced condition would not differ from each other as a function of psychological factor scores.

Method

Participant Selection

Forty-eight males and 37 females, aged 18-38.5, with sufficient command of English or French were recruited from Concordia University and McGill University. The stated purpose of the study was to look at the relation between 5-HT, cognition, and cardiovascular function. Pre-screening at the recruitment tables was conducted using the General Health Survey (see Appendix A), developed in our laboratory and used in previous studies. Potential participants, who did not report a systemic medical condition, were not smokers or recent drug users and, if female, were not using oral or injectable contraceptives, were invited to visit the laboratory for a more comprehensive interview. Potential participants who reported using Ecstasy (3-4 methylenedioxymethamphetamine,

MDMA) on more than three occasions were excluded, due to Ecstasy's serotonergic effects.

Measures and Apparatus

Psychological and medical screening.

During the comprehensive interview, potential participants underwent the Structured Clinical Interview for DSM-IV Axis I Disorders, Research Version, Non-patient edition (SCID -I/NP; First, Spitzer, Gibbon, & Williams, 1996). Applicants, who met the criteria for a psychiatric disorder, including alcohol or substance abuse/dependence, were excluded. Potential participants who had completed the SCID successfully were referred to a physician for a medical examination and health history. The examination included blood pressure measurement which, along with the health history, served to ensure that only normotensive participants were included. An electrocardiogram was obtained and evaluated by the physician. A urine analysis was conducted to determine the presence or absence of proteins or blood and verify pH levels. The physician recommended inclusion or exclusion in the study based on clinical judgement (see Appendix B) and completed a medical report (see Appendix C).

Demographic and Personality Trait Measures.

The Cook-Medley Hostility Inventory (Ho; Cook & Medley, 1954; see Appendix D), a 50-item true and false questionnaire derived from the Minnesota Multiphasic Personality Inventory, has been demonstrated to have relatively high levels of internal consistency, with an average Chronbach's alpha of approximately .80 (Smith & Frohm, 1985). Studies using the Ho in non-Western countries have also reported high internal consistency and test-retest reliability (Bishop & Quah, 1998). The State Trait Anger

Expression Inventory (STAXI; Spielberger, Johnson, Russell, Crane, Jacobs & Worden, 1985; Spielberger, Krasner, & Solomon, 1988; see Appendix E) consists of 44 items constituting six scales and two subscales. The STAXI has shown good internal consistency as well as good test-retest reliability (Bishop & Quah; Rule & Traver 1983). Spielberger's Trait Anxiety Inventory- Form Y-2 (STAI; Spielberger et al., 1970; see Appendix F) was used to assess trait anxiety. Test-retest reliability was .81 for trait anxiety and internal consistency ranged from .83 to .92 (Spielberger, 1983). The STAI also correlates with the Taylor Manifest Anxiety Scale, at .80 and the IPAT Anxiety Scale at .75. Test-Retest reliabilities range from .86 at 4 hr to .65 at 104 days. The Beck Depression Index (Beck, 1987), a 21-item self-report rating inventory measuring characteristic attitudes and symptoms of depression, was administered as part of the comprehensive interview during the screening process (see Appendix G). The BDI demonstrates high internal consistency, with average alpha coefficients of .86 (Devilly, 2001). The BDI was re-administered on the test day and the obtained scores were used as a measure, one of the psychological factors of interest, depression.

Outcome Measures

State affect measures.

State affect during the test period was assessed using the Bipolar Profile of Mood States - POMS (McNair et al., 1988) as well as with a Visual Analog Mood Scale - VAMS (see Appendices H & I respectively). The POMS is a 72-question instrument used to assess transient, distinct mood states and is composed of six bipolar mood scales such as agreeable–hostile, composed–anxious, etc., each comprised of 12 questions scored on a 4-point scale. There are various versions of the POMS and in general they appear to be

internally consistent with a relatively stable factor structure. The VAMS is similar to an analog affect scale, used previously in by Miller et al. (1998), which assesses changes in affective state. Participants indicate their present mood state on each of 13 affective variables, such as happiness, anger, irritation etc., by making a perpendicular mark, as instructed by the experimenter, along a continuous 120 mm horizontal line, representing the bipolar dimension of each mood state, e.g., Not at all Angry – Very Angry. The POMS and VAMS were administered in the morning – pre-ingestion of the amino acid mixture, 5.5 hr post-ingestion – prior to participating in the stressor math task phase, and immediately following the stressor phase. Both scales are considered highly sensitive to nonclinical changes in affective state. There are no mood measures associated with the recovery period, an unfortunate result of the experience with the first approximately 30 participants. An initial review of the data revealed that the participants did not appear to be accurately reporting their mood states at the end of the recovery period. Several participants were individually contacted and in brief interviews confirmed that subsequent to adhering to the preparation protocol for the lab portion of the study (special diet that wasn't particularly tasty, no caffeine, no alcohol etc.), plus the ingestion of the amino acid bolus, and the 6 hr wait period in the lab without being able to eat anything other than the snacks provided, and the math/harassment, they just wanted to receive their honorarium and leave. Several reported that they had just completed the POMS and VAMS two times (pre-math and post-math) in the previous 20 min or so, and had "had enough."

Cardiovascular measurements.

Systolic blood pressure (SBP) is the arterial pressure during the contraction phase

of the ventricles and diastolic blood pressure (DBP) is the arterial pressure during the relaxed phase of the ventricles. Measurements of SBP and DBP (in mm Hg) were obtained at 1 min intervals using the IBS Automated Blood Pressure and Pulse Rate Monitor SD- 700 A (IBS Corporation, Waltham, MA) and a blood pressure cuff placed on the participant's non-dominant arm. The blood pressure monitor uses a ruggedized sensor to detect arterial wall motion and audible as well as inaudible Korotkoff vibrations. A filter identifies specific vibrations associated with obstructed blood flow between the systolic and diastolic pressure range. The filtered signals correlate closely with those obtained by standard ausculatory methods using a stethoscope (Matthews et al., 1986, 1987).

Additional cardiovascular measures include mean arterial pressure (MAP: in mm Hg), heart rate (HR: in bpm), stroke volume (SV: in ml), cardiac output (CO: in l/min.), pre-ejection period (PEP: in msec), left ventricular ejection time (LVET: in msec), Heather Index (HI: ohms/sec/sec), and total peripheral resistance (TPR: in dyne-sec.cm $^{-5}$). MAP is defined as the mean arterial pressure during a single cardiac cycle. HR is the number of heart beats each minute. SV is the quantity of blood ejected by the heart during a single cardiac cycle. CO is the total volume of blood ejected by the heart during a period of time, typically, as is the case in the present study, 1 min. PEP is the time interval from the beginning of electrical stimulation of the ventricles to the opening of the aortic valve. LVET is the time interval from the opening to the closing of the aortic valve. HI is an estimate of myocardial contractility and TPR is the resistance to blood flow throughout the entire cardiovasculature. Values for these measures were obtained through non-invasive means using a Minnesota Impedance Cardiograph (Model 304A,

Instrumentation for Medicine, Greenwich, CT), an IBM compatible personal computer, EKG spot electrodes, and the Cardiac Output Program (C.O.P. Version 2.1, Bio-impedance Technology, Chapel Hill, NC). The impedance cardiography utilized a tetrapolar electrode-band configuration. The inner two recording electrode-bands were placed around the base of the participant's neck and around the thorax over the tip of the xiphoid process. The outer two electrode-bands were placed around the neck and the thorax at least 3 cm apart from each of the inner electrode bands.

The ECG signal was recorded independently using three spot electrodes. Two electrodes were placed on opposite sides of the rib cage at approximately the level of the seventh rib. The ground electrode was placed on the right hip bone. The ECG signal was filtered through a Coulbourn Instruments bandpass filter (Coulbourn Instruments, Allentown, PA) and then routed to the Minnesota Impedance Cardiograph. This bandpass filter is configured for optimal filtering of the EKG signal (low cutoff: 1 Hz, high cutoff: 150 Hz).Within every measurement minute, 55 s of recordings were obtained and processed by the C. O. P. system, yielding ensemble averaged values for HR, SV, CO, PEP, LVET, HI, and TPR.

Experimental Procedure

Overview.

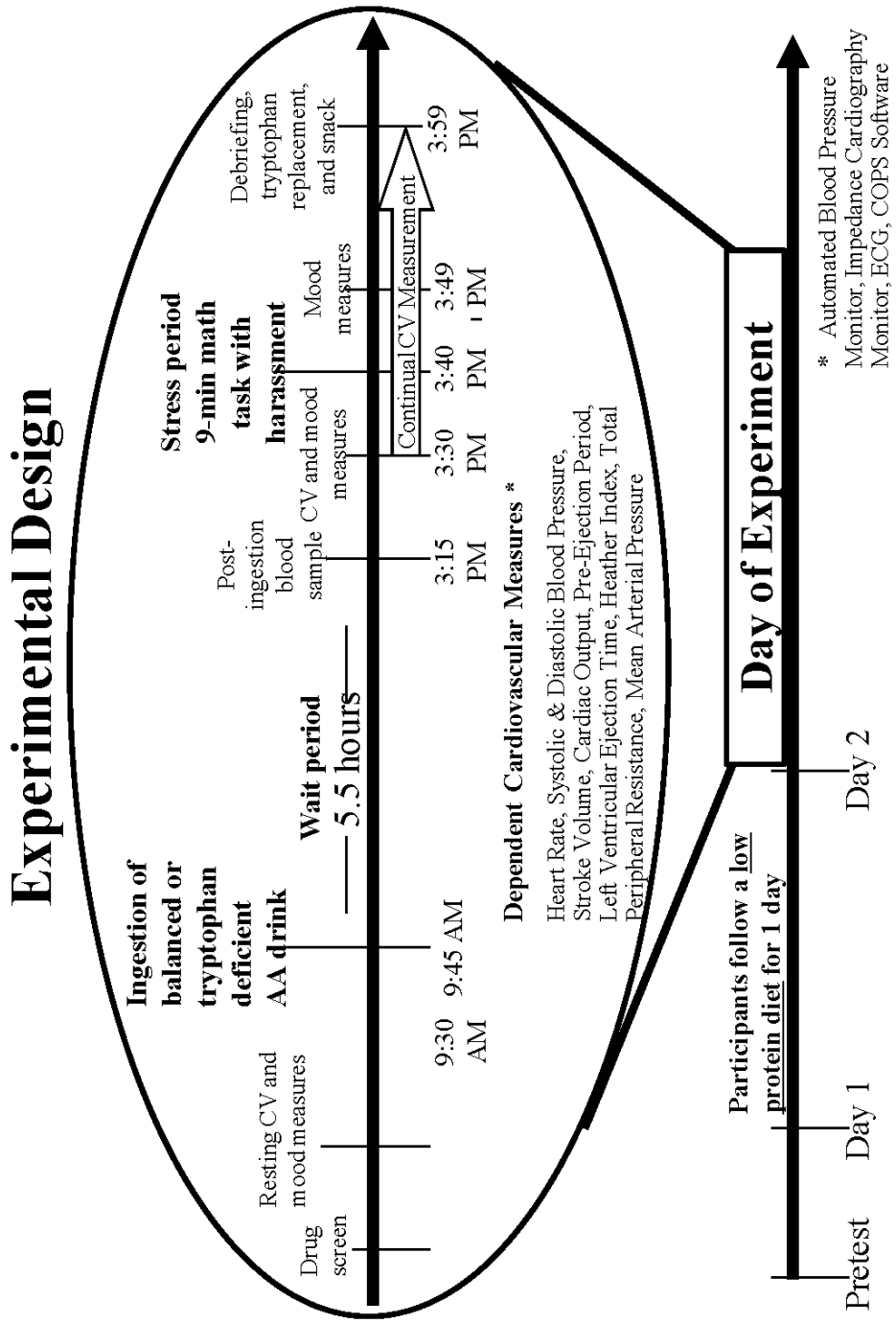
The experiment was a double-blind, placebo controlled study. The test period consisted of a preparatory day followed, 24 hr later, by the test day. The preparatory session was conducted outside of the laboratory, during which participants consumed a low protein diet and abstained from caffeinated coffee, cigarette smoking, alcohol, and drug consumption. The second session, the testing day, was conducted in the laboratory.

Participants were divided into two groups, based on a median split of the Ho scores of the first 50 potential participants screened (Low Hostility < 17), and randomly assigned to either the amino acid balanced control condition (B) or to the Trp deficient amino acid experimental condition (T-). All participants participated in a deception paradigm that consisted of a 9-min math task stressor and harassment by a confederate, 5.5 hr after ingestion of the amino acid mixtures. Venous blood samples were obtained prior to ingestion and 5 hr after ingestion. Cardiovascular measurements were obtained prior to ingestion, 5 hr post ingestion and during the 9-min stress period at 5.5 hr post ingestion. A diagram of the study protocol is presented in Figure 1.

Details of the procedure.

On the day (preparatory day) prior to the laboratory session, participants consumed a low protein diet (see Appendix J) which included pre-packaged, pre-cooked meals. These meals were similar to those used in previous ATD studies (Benkelfat, Ellenbogen Dean, Palmour, and Young, 1994; Delgado, et al., 1990; Ellenbogen, Young, Dean, Palmour, and Benkelfat, 1996). The meals provided adequate protein (22.6 g/24 hr) and caloric content (2212 kcal/24 hr), with minimal Trp content (160 mg/24 hr). The purpose of the low protein diet was two-fold: firstly, to ensure as much as possible similar dietary intake prior to the test day, secondly, to prime the participant to synthesize protein on the test day. Participants in both conditions were provided with the same diet in order to maintain the double blind aspect of the study. Participants were encouraged to eat at regular hours and were allowed water ad libitum. Participants were instructed to

Figure 1. Experimental Procedure.



fast from midnight until their arrival at the laboratory at 9:00 a.m. (see Appendix K).

Upon their arrival at the laboratory participants were screened for a broad range of drugs of abuse (including, phencyclidine, cocaine, amphetamines, tetrahydrocannabinol, and opiates). Urine samples were obtained and tested with a mouse monoclonal antibody competitive binding immunoassay based disposable test kit (Express Test® 5 Test Drug Panel, Biosite Diagnostics, San Diego California). Females were screened for possible pregnancy using a human chorionic gonadotropin (hCG) urine level detecting kit (ABBOTT Test Pack® +Plus hCG Urine, ABBOTT Laboratories Ltd., Mississauga, Ontario). Human chorionic gonadotropin is a hormone secreted by the developing placenta shortly after fertilization and can indicate pregnancy within 72-96 hr after implantation.

Accepted participants (three males and one female were rejected due to THC or amphetamine positive test results) were instrumented for cardiovascular measurements and 10 min of measurements were obtained. After the cardiovascular measurements recordings, participants provided a venous blood sample of approximately 15 ml for measurement of baseline total and free (non-albumin-bound) plasma Trp. Each participant was then given either a Trp-free amino acid drink or a balanced amino acid drink, both containing the same amino acids, with Trp added to the balanced drink. For the next 5 hr, participants remained in the test room and were monitored by video camera. They were not allowed to sleep, but were allowed to read affectively neutral material (EnRoute magazines) and watch affectively neutral videos (e.g. National Geographic: Titanic, Egypt, Gorillas of the Rainforest). Water, but not food, was available ad libitum.

After a delay of 5 hr, participants provided a second venous blood sample and, after a 15-min wait, baseline cardiovascular measurements were obtained. Immediately following the baseline measurements recording, participants underwent a 9-min math task stressor with harassment. The harassment procedure was a variation of a protocol developed by Suarez and Williams (1989) and is described in greater detail below. During this stressor period, cardiovascular measures were obtained and the participants were videotaped. At the completion of the math, task there was a 10-min recovery period during which cardiovascular measurements and videotaping were continued. Following the recovery period, participants were debriefed and provided with a snack and given a 1-g L-tryptophan tablet. This tablet served as a tryptophan replacement for those participants in the depleted, experimental condition and acted to maintain the double blind status of the study by being given to the non-depleted control participants as well. The tryptophan preparation is available by prescription in Canada and has not been associated with any cases of eosinophilia myalgia syndrome (Wilkins, 1990). As a means of ascertaining any negative sequellae from the test procedures, telephone contact was maintained during the evening and morning following the test session.

Amino acid drinks.

The amino acid mixtures are the same as those used by Young, Smith, Pihl, & Ervin (1985) previously in men, except that lysine monohydrochloride was used instead of lysine, to increase palatability, and the drinks given to females were adapted for the lower body weight of women (Ellenbogen et al., 1996). The AA mixture for males consists of L-alanine, 5.5 g; L-arginine 4.9 g, cysteine, 2.7 g; glycine, 3.2 g; L-histidine, 3.2 g; L-isoleucine, 8.0 g; L-leucine, 13.5 g; L-lysine monohydrochloride, 11.0 g; L-

methionine, 3.0 g; L-phenylalanine, 5.7 g; L-proline, 12.2 g; L-serine 6.9 g; L-threonine, 6.5 g; L-tyrosine, 6.9 g; L-valine, 8.9 g; and for the control (balanced) mixture, L-tryptophan, 1.92 g. The AA mixture for females consists of L-alanine, 4.58 g; L-arginine 4.08 g, cysteine, 2.25 g; glycine, 2.67 g; L-histidine, 2.67 g; L-isoleucine, 6.67 g; L-leucine, 11.25 g; L-lysine monohydrochloride, 9.17 g; L-methionine, 2.50 g; L-phenylalanine, 4.75 g; L-proline, 10.17 g; L-serine 5.75 g; L-threonine, 5.42 g; L-tyrosine, 5.75 g; L-valine, 7.42 g; and for the control (balanced) mixture, L-tryptophan, 1.92 g.

The drinks were prepared a few minutes before oral administration by mixing the powdered amino acids with either, (1) 150 ml water, 45 ml chocolate syrup, and 0.6 g of sodium cyclamate or, (2) 180 ml of orange juice and sodium cyclamate, according to the preference of the participants. Because of the unpleasant taste of methionine, cysteine, and arginine, these amino acids were encapsulated in gelatin-based capsules and administered separately. Due to dietary restrictions, two participants chose not to consume the gelatin capsules and the three amino acids were incorporated into the drink.

Determination of plasma tryptophan concentrations.

Plasma Trp was measured in all blood samples as an index of the extent of ATD. The free (non albumin bound) plasma Trp concentration was assumed to be equivalent to the concentration of Trp found in an ultrafiltrate of plasma prepared at $25^{O}C$ by centrifugal ultrafiltration (MPS-1, Amicon Inc, Beverly, Mass.) through YMT membranes (Millipore Waters, Bedford, Mass). Trp in the ultrafiltrate and in deproteinized plasma were measured by high performance liquid chromatography on a Waters Bondapak C18 (Millipore Waters) reverse phase column with fluorometric

detection (Anderson, Young, & Cohen, 1979).

Mathematical task.

The mathematical subtraction task (math-task) consisted of the Computerized Subtraction Task Version 1.21 computer program (Turner, Sherwood & Lutz, 1989), an IBM PC computer, and a computer mouse. The 9-min math task, divided into three 3-min trials, consisted of a series of mathematical subtraction equations presented with either correct or incorrect solutions. During each 3-min trial, 60 equations were presented for a task total of 180 equations. Each equation was presented for a duration of 3 s, first appearing as white characters against a black background and switching to yellow characters if the participant did not respond within the first 2 s. The participant responded by pressing the right computer mouse button if he/she thought the answer was correct or by pressing the left button if he/she thought the answer on the screen was incorrect. The participant was instructed to respond as rapidly as possible. If the participant's answer was correct, the computer emitted a high pitched tone indicating that the participant had responded accurately. If the participant's answer was incorrect, the computer emitted a low pitched tone indicating that the participant had responded inaccurately. Participants were informed that if they failed to respond within the 3 s, their non-response would be considered as an incorrect response, however, no tone would be emitted. The math task is designed in such a way that each participant attained a 50 to 60 percent correct response rate, that is, equations became easier or more difficult depending on each participant's performance.

Harassment procedure.

Following the second set of baseline measurements, Researcher A explained the

math-task instructions to the participant (see Appendix L). Researcher B, a male for male participants and a female for female participants, knocked on the door of the room adjoining the testing room, interrupting Researcher A's instructions, to tell Researcher A that she had a phone call from their supervisor. After completing the current sentence, Researcher A excused him or herself and exited to the adjacent room. In a loud voice, Researcher A pretended to engage in a telephone conversation in which they were being asked to leave the testing session. Researcher A then asked Researcher B to continue the testing for them. Researcher B voiced their opposition, stating angrily that he or she would not be responsible for any problems. Researcher A returned to the testing room, completed the instructions, and explained to the participant that Researcher B would be taking over, and then left the room. Researcher B, feigning anger, entered the testing room to start the math-task (see Appendix M for a more detailed script).

During the math-task, Researcher B delivered six anger-provoking statements to the participant, at predetermined times. Sample statements include: "Did you understand the instructions?!" and "Can't you do better than this?!" (see Appendix N). Participant comments were ignored, unless the participant wanted to discontinue the experiment. The testing session was stopped following a 10-min rest period. All participants were then debriefed about the deception (see Appendix O), the purpose of the harassment and the true rationale for the experiment. Participants who reported feeling suspicious about the harassment manipulation were excluded from the final data set.

Data Analysis

All statistical data analyses were conducted using PASW Statistics 18 Release 18.0.3 (Sep 9, 2010), running on a personal computer with the Windows 7 operating

system. Demographic characteristics of the participants were compared using a one-way analysis of variance (ANOVA), to verify equivalence of the experimental and control groups. Blood Trp levels were analyzed and compared using repeated measures ANOVA, to verify the effect of the ATD or control procedure on the experimental and control groups respectively. Mood and cardiovascular measurements were analyzed on an individual measure basis, as is the accepted practice in this area of research using change scores, which have been shown to be acceptable and perhaps preferred when studying change (e.g., Llabre, Spitzer, Saab, Ironson, & Schneiderman, 1991; Williams & Zimmerman, 1996). Change scores were calculated for each outcome measure for each of the three phases of the experiment. The first change scores, the post-manipulation 5.5 hr waiting period change scores, were calculated by using the outcome measure value at the end of the wait period and subtracting the outcome measure value at rest 5.5 hr earlier. The second set of change scores were calculated by subtracting the outcome measure value at the end of the 5.5 hr waiting period from the outcome measure value obtained during the math/harassment task, and the third set was calculated by subtracting the math/harassment outcome measure value from the recovery period outcome measure value. Change scores were used to control for baseline individual differences that are inherent in human cardiovascular measure levels. The use of change scores is frequently used in studies on cardiovascular reactivity and is also used in ATD studies (e.g., Leyton et al., 2000; Shansis et al., 2000).

Median splits for each of the four psychological factors were computed and this formed the basis for the four 2-level, high (Hi) and low (Lo) psychological factor group by Trp status, balanced (B) and depleted (Trp-) groups (e.g., Hi TAnger-B, Lo TAnger-B,

Hi TAnger-Trp-, Lo TAnger-Trp-).

The change scores were analyzed using 2 (B vs Trp-) x 2(Lo Psychological Factor x Hi Psychological Factor) ANOVA's. Follow-up comparisons (student's t-test) were carried out in investigating the source of any statistically significant group (Lo vs Hi) by condition (B vs Trp-) interactions.

Ethics

All participants who participated in the study gave written informed consent to their participation in the study (see Appendix P), as well as written consent for release of their medical information by the physician to the research team (see Appendix Q). The recruitment, selection, and study procedures were approved by the Research Ethics Board of the Department of Psychiatry, McGill University, Montreal, Quebec, Canada and the Ethics Review Board of Concordia University, Montreal, Quebec, Canada. Participants were paid $100 for their participation in the study.

Results

Demographic Characteristics and Pre-ingestion Measures of the Study Sample

Approximately 1,000 individuals were pre-screened, of whom approximately 550 were excluded, as their Cook-Medley scores fell within the central region of scores (14 - 22). An additional 140 individuals were omitted at this stage based on the results of the Prime MD interview and the General Health Survey, with approximately half for mental health reasons, such as depression and anxiety, and one quarter for physical health reasons, such as ulcers, asthma, etc. A smaller number were excluded based on substance use patterns, oral or injected contraceptives, language difficulties, a history of the study of psychology, etc.

In all, approximately 300 individuals were invited to participate in the comprehensive interview, of whom more than 100 withdrew of their own volition by either not returning calls or for personal reasons. In total, almost 200 potential participants were evaluated in person to assess mental health status. Twenty-one participants were excluded on the basis of a probable DSM-IV Axis 1 or Axis II disorder (American Psychiatric Association, 1994), five revealed a substance use pattern, five disclosed contraceptive use, four revealed a previous history of the study of psychology, and one was excluded based on physical health concerns. Approximately 160 individuals were invited to undergo the medical examination. One hundred and forty potential participants were seen by the physician and two were advised that they should not continue with the study. Subsequent to these steps, approximately 25 individuals chose not to continue with the study, with most simply not responding to the invitation to schedule the test day. An additional seven participants began the actual test day but did not complete the protocol. Reasons included three positive drugs screens, one individual fainting during the initial blood draw and one fainting during the preliminary blood pressure measurement. Two were asked to withdraw when they revealed a history of substance use not previously disclosed. In contrast to similar studies (Moore et al, 2000), there were relatively few individuals who reported feelings of nausea subsequent to the ingestion of the amino acid mixtures, and these who experienced symptoms reported that they were of short duration. Five individuals suffered emesis approximately 1 hr after ingestion, a lower number than is typically reported. None of these participants chose to withdraw from the study. Subsequent plasma analysis revealed no reason to exclude the data of these individuals, as their Trp levels were consistent with their group assignment.

Ninety-six participants completed the entire protocol. A total of 11 participants were excluded from the final analysis due to ambiguous depletion status (n = 1), questionable language competency (n = 2), problems with the data acquired (n = 5), and failure to be deceived with corroborating evidence from their data (n = 3).

Verification of the Construct Independence of the Four Psychological Factors

The utilization of four psychological factors required that the measures used to assess them be tapping into different constructs. The association between the measures was investigated using Pearson product moment correlations. All the correlations were significant r(76 to 85) = .297 to .541, $p < .01$ (See Table 1) but low enough to be considered as separate constructs.

Verification of Pre-Ingestion Homogeneity

For each of the psychological factors (Factor), a series of 2 (B vs. Trp-) x 2 (Lo Factor vs. Hi Factor) ANOVAs were conducted on the demographic data, as well as on post-instrumentation but pre-ingestion (Baseline) cardiovascular and mood measures.

Demographic measures did not differ between groups (see Tables 2-5). Pre-ingestion cardiovascular means and standard errors are presented for each of the Tryptophan Condition x Psychological Factor groupings (see Tables 6-9). Pre-ingestion mood means and standard errors are presented below for the POMS (see Tables 10-13), and for the VAMS (see Tables 14-17).

Baseline Analyses (Pre-Tryptophan Manipulation)

A series of 2 (B vs T-) x 2 (Lo vs Hi) ANOVAs were conducted on cardiovascular and state affect measures recorded post-instrumentation but pre-tryptophan manipulation while participants were resting.

Table 1

Correlations Between Psychological Factors

	CM HO	Trait Anxiety	Trait Anger	Beck Depression
CM HO	1	.32	.51	.38
Trait Anxiety	.32	1	.54	.42
Trait Anger	.51	.54	1	.37
Beck Depression	.38	.42	.37	1

Table 2

Demographic Information for Tryptophan (Trp) x Hostility Groups

		Balanced (B)				Depleted (T-)			
		LoHo		HiHo		LoHo		HiHo	
		M	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Sex(n):	M	5		16		16		11	
	F	13		6		11		7	
Age (YRS)		24.63	1.08	23.67	0.95	23.57	0.91	22.97	0.95
Weight (KG)		65.13	2.60	72.71	3.04	70.52	2.16	70.36	3.38
Height (M)		1.68	0.03	1.75	0.02	1.73	0.02	1.71	0.03
Education (YR)		16.00	0.46	15.03	0.43	15.26	0.23	15.06	0.37
Cook-Medley		9.78	0.65	23.77	1.23	10.56	0.72	26.11	1.23

Table 3

Demographic Information for Trp x Anger Groups

		Balanced (B)				Depleted (T-)			
		LoTAnger		HiTAnger		LoTAnger		HiTAnger	
		M	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Sex(n):	M	11		10		15		12	
	F	12		7		9		9	
Age (YRS)		22.77	0.67	25.90	1.29	23.49	0.99	23.13	0.86
Weight (KG)		66.38	2.61	73.24	3.31	70.49	2.44	70.40	2.87
Height (M)		1.70	0.01	1.74	0.02	1.72	0.02	1.73	0.03
Education (YR)		15.45	0.29	15.50	0.71	15.06	0.25	15.33	0.33
TAnger		19.62	0.50	28.51	0.57	19.79	0.58	29.13	0.81

Table 4

Demographic Information for Trp x Anxiety Groups

		Balanced (B)				Depleted (T-)			
		LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
		M	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Sex(n):	M	8		12		12		13	
	F	13		6		9		8	
Age (YRS)		23.45	0.90	25.06	1.13	23.33	1.11	23.57	0.87
Weight (KG)		66.62	2.81	72.13	3.26	67.84	2.92	74.68	2.25
Height (M)		1.71	0.02	1.72	0.02	1.68	0.02	1.76	0.02
Education (YR)		15.35	0.29	15.68	0.65	15.20	0.29	15.15	0.29
TAnxiety		28.00	0.50	37.44	0.78	25.04	0.63	39.47	1.30

Table 5

Demographic Information for TRP x Depression Groups

		Balanced (B)				Depleted (T-)			
		LoBDI		HiBDI		LoBDI		HiBDI	
		M	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Sex(n):	M	10		10		13		11	
	F	8		8		12		4	
Age (YRS)		23.57	1.00	24.71	1.19	24.28	0.96	22.63	1.02
Weight (KG)		68.10	2.98	71.15	3.11	69.63	2.21	74.28	3.95
Height (M)		1.71	0.02	1.72	0.02	1.70	0.02	1.77	0.03
Education (YR)		15.80	0.44	15.25	0.47	15.52	0.24	14.76	0.30
BDI		0.44	0.12	3.83	0.57	0.12	0.06	5.46	0.68

Table 6

Baseline Cardiovascular Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	60.46	2.28	64.23	2.30	62.08	2.01	64.10	2.74
DBP	66.12	1.67	66.64	1.59	67.34	1.69	68.73	2.00
SBP	100.23	1.55	106.48	1.37	104.99	1.92	107.29	2.40
MAP	77.49	1.27	79.92	1.22	79.89	1.41	81.58	1.86
SV	123.31	8.20	110.78	7.94	109.34	7.62	99.39	8.12
CO	7.35	0.52	6.83	0.34	6.66	0.42	6.16	0.40
PEP	135.26	4.29	135.08	4.14	127.03	2.75	144.34	4.97
LVET	303.31	5.24	286.03	7.35	285.93	6.78	290.70	5.92
HI	13.05	1.06	10.33	0.91	11.57	0.64	9.24	0.74
TPR	920.69	67.46	992.97	67.83	1138.98	127.42	1267.76	219.72

Note: LoHo=low hostility, HiHo=high hostility

Table 7

Baseline Cardiovascular Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	58.52	1.57	68.19	2.74	61.15	2.08	64.86	2.50
DBP	63.19	1.32	70.76	1.45	66.98	1.79	68.92	1.85
SBP	103.19	1.47	104.31	1.79	106.53	2.05	105.18	2.21
MAP	76.52	1.13	81.94	1.04	80.16	1.55	81.01	1.66
SV	126.95	5.63	101.61	10.48	115.68	7.73	93.57	7.47
CO	7.35	0.33	6.64	0.54	6.95	0.42	5.89	0.38
PEP	131.77	3.48	140.02	5.01	129.00	3.35	139.61	4.53
LVET	301.01	7.01	283.93	5.12	286.12	7.34	289.79	5.62
HI	12.60	1.01	10.11	0.89	11.11	0.67	10.09	0.77
TPR	872.06	41.93	1085.44	92.55	1055.32	108.57	1344.98	212.14

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 8

Baseline Cardiovascular Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	59.51	2.00	66.53	2.41	61.29	2.26	63.25	2.46
DBP	64.04	1.65	69.04	1.43	67.17	2.16	68.25	1.50
SBP	102.06	1.81	105.32	1.25	105.70	2.20	106.99	2.23
MAP	76.71	1.36	81.13	0.92	80.01	1.80	81.16	1.45
SV	114.28	7.97	117.02	8.63	107.63	8.46	106.52	8.41
CO	6.59	0.38	7.56	0.46	6.53	0.49	6.49	0.40
PEP	136.92	3.88	133.88	4.73	130.21	4.13	139.51	4.16
LVET	297.86	8.35	289.02	4.62	285.66	8.42	291.38	4.60
HI	12.76	1.05	10.37	0.975	11.52	0.63	9.54	0.76
TPR	1001.33	76.23	918.57	59.75	1191.02	160.00	1199.89	192.23

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 9

Baseline Cardiovascular Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	60.53	2.27	62.33	2.35	63.09	2.31	59.97	2.22
DBP	65.41	1.53	67.73	1.47	67.78	1.80	66.98	2.18
SBP	103.30	1.74	104.83	1.50	104.37	1.88	109.56	2.85
MAP	78.04	1.35	80.10	1.21	79.98	1.53	81.17	2.05
SV	116.07	6.88	125.31	9.66	105.21	7.68	115.58	9.58
CO	6.88	0.36	7.59	0.52	6.49	0.42	6.81	0.49
PEP	140.87	4.83	131.47	3.22	132.01	3.25	138.32	6.41
LVET	292.74	8.88	297.20	4.58	284.19	6.93	298.54	5.81
HI	11.19	0.99	11.57	1.05	11.31	0.69	9.91	0.87
TPR	956.70	57.98	919.72	82.09	1170.46	135.99	1192.28	266.96

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Table 10

Baseline POMS Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	31.88	.88	29.45	1.04	32.26	0.67	29.50	1.24
ED	27.24	1.18	26.50	1.04	27.11	1.04	26.22	1.14
ET	22.71	1.95	21.36	1.61	23.41	1.64	19.33	2.04
AH	28.59	1.25	29.00	0.88	29.22	0.97	29.44	0.91
CU	25.47	1.10	24.91	0.98	26.11	1.06	23.72	1.28
CC	28.65	0.94	27.86	0.91	30.26	0.80	27.67	1.04

Note: LoHo=low hostility, HiHo=high hostility, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 11

Baseline POMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	31.05	0.97	29.82	1.09	32.54	0.77	29.57	1.02
ED	27.18	0.95	26.35	1.29	27.92	1.09	25.43	1.03
ET	22.64	1.50	21.06	2.08	24.33	1.65	18.86	1.88
AH	29.45	0.86	28.00	1.25	30.00	0.92	28.52	1.00
CU	25.14	0.88	25.18	1.25	26.92	1.04	23.14	1.19
CC	28.55	0.87	27.76	1.01	31.08	0.71	27.10	0.96

Note: LoTAnger=low trait anger, HiTAnger=high trait anger, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 12

Baseline POMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	32.15	0.87	28.94	1.09	32.95	0.84	29.29	0.98
ED	27.85	0.99	25.78	1.23	28.57	1.03	24.57	1.03
ET	23.10	1.70	20.83	1.89	25.48	1.77	17.24	1.58
AH	29.95	0.85	27.39	1.20	30.57	0.92	28.05	1.08
CU	26.05	0.86	24.17	1.23	27.67	1.01	22.38	1.13
CC	28.95	0.83	27.44	1.06	31.52	0.78	26.95	0.91

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 13

Baseline POMS Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	31.64	0.96	29.22	1.15	32.68	0.71	28.13	1.23
ED	28.00	1.17	25.50	1.08	28.20	0.95	24.46	1.09
ET	23.76	1.73	19.55	1.82	24.44	1.52	17.40	1.96
AH	29.82	0.87	27.16	1.20	30.36	0.91	27.86	1.23
CU	25.70	0.97	24.55	1.18	27.32	0.89	21.60	1.23
CC	27.94	0.91	27.88	1.09	30.88	0.65	26.86	1.26

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 14

Baseline VAMS Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	1.81	0.28	2.96	0.48	1.83	0.25	2.33	0.37
Agreeable	10.64	0.52	10.00	0.53	10.96	0.35	10.22	0.62
Happy	10.00	0.44	9.64	0.45	10.11	0.38	9.39	0.57
Tense	2.36	0.44	3.05	0.51	2.67	0.40	3.83	0.64
Anxious	2.25	0.37	3.23	0.56	2.63	0.40	3.57	0.59
Relaxed	12.03	0.33	10.03	0.65	10.83	0.39	10.47	0.52
Discouraged	2.19	0.45	2.43	0.36	1.91	0.21	2.67	0.52
Annoyed	2.53	0.49	2.77	0.47	2.26	0.38	2.61	0.49
Sad	2.36	0.52	2.07	0.43	1.70	0.21	2.33	0.39
Irritated	2.33	0.53	2.41	0.43	2.15	0.37	2.61	0.48
Angry	1.72	0.26	1.96	0.40	1.96	0.34	1.44	0.11
Depressed	2.11	0.40	1.93	0.31	1.41	0.11	1.69	0.19
Guilty	1.56	0.18	1.91	0.36	1.48	0.20	1.72	0.23

Note: LoHo=low hostility, HiHo=high hostility

Table 15

Baseline VAMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.34	0.35	2.55	0.54	1.79	0.27	2.31	0.33
Agreeable	10.73	0.39	9.67	0.68	11.37	0.36	9.85	0.52
Happy	10.26	0.37	9.17	0.50	10.45	0.40	9.09	0.48
Tense	2.71	0.46	2.76	0.52	2.64	0.43	3.69	0.57
Anxious	2.69	0.46	2.91	0.57	2.87	0.52	3.14	0.42
Relaxed	11.65	0.34	9.94	0.80	11.20	0.34	10.09	0.51
Discouraged	2.45	0.37	2.14	0.42	2.04	0.36	2.40	0.32
Annoyed	2.58	0.39	2.76	0.59	2.39	0.48	2.40	0.34
Sad	2.47	0.48	1.82	0.42	1.77	0.28	2.16	0.28
Irritated	2.10	0.27	2.73	0.69	2.50	0.49	2.14	0.26
Angry	1.89	0.23	1.79	0.49	1.58	0.22	1.95	0.36
Depressed	2.21	0.31	1.73	0.37	1.27	0.09	1.81	0.16
Guilty	1.47	0.14	2.11	0.45	1.37	0.21	1.81	0.20

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 16

Baseline VAMS Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.31	0.40	2.33	0.41	2.00	0.40	2.00	0.19
Agreeable	11.04	0.42	9.33	0.60	11.54	0.36	10.02	0.52
Happy	10.45	0.39	9.00	0.46	10.71	0.37	9.04	0.50
Tense	2.35	0.43	3.11	0.55	2.40	0.45	3.78	0.53
Anxious	2.59	0.47	2.94	0.57	2.47	0.53	3.40	0.44
Relaxed	11.97	0.33	9.75	0.74	11.42	0.37	10.33	0.43
Discouraged	2.14	0.33	2.44	0.47	1.35	0.09	3.02	0.44
Annoyed	2.09	0.32	3.25	0.61	1.95	0.46	2.88	0.42
Sad	1.73	0.20	2.80	0.67	1.35	0.17	2.54	0.34
Irritated	1.92	0.28	2.80	0.64	2.04	0.47	2.69	0.39
Angry	1.64	0.21	2.08	0.49	1.64	0.24	1.85	0.37
Depressed	1.83	0.26	2.11	0.43	1.21	0.06	1.78	0.17
Guilty	1.47	0.15	2.11	0.43	1.14	0.06	2.02	0.28

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 17

Baseline VAMS Means and SE for Trp x DBI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	1.83	0.29	2.94	0.57	1.80	0.26	2.33	0.43
Agreeable	10.88	0.45	9.55	0.60	11.62	0.31	9.40	0.61
Happy	10.25	0.45	9.25	0.44	10.24	0.49	9.50	0.38
Tense	2.69	0.55	2.75	0.53	2.18	0.30	4.86	0.74
Anxious	2.47	0.56	3.08	0.56	2.54	0.45	3.86	0.61
Relaxed	11.77	0.34	10.52	0.66	11.40	0.32	9.53	0.64
Discouraged	2.38	0.36	2.36	0.50	1.58	0.14	3.16	0.59
Annoyed	2.16	0.33	3.38	0.62	1.94	0.40	2.93	0.42
Sad	2.25	0.39	2.30	0.62	1.40	0.18	2.70	0.39
Irritated	1.97	0.32	2.97	0.63	1.88	0.38	3.03	0.48
Angry	1.75	0.24	2.02	0.49	1.64	0.33	1.96	0.28
Depressed	1.97	0.30	2.16	0.44	1.24	0.08	1.83	0.19
Guilty	1.63	0.19	1.63	0.34	1.18	0.07	2.06	0.37

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Cardiovascular Measures

Tryptophan by Cook-Medley (Ho).

A main effect of Ho was observed for SBP [$F(1, 81) = 5.146$, $p = .026$]; PEP [$F(1, 80) = 4.668$, $p = .034$]; and HI [$F(1, 80) = 9.042$, $p = .004$]. Results indicated greater resting SBP, PEP, and lower HI for the Hi Ho participants compared to the Lo Ho participants. The main effect of Trp and the Trp x Ho interaction were not significant.

Tryptophan by trait anger (TAnger).

A main effect of TAnger was found for HR [$F(1, 80) = 9.054$, $p = .004$]; DBP [$F(1, 81) = 8.156$, $p = .005$]; SV [$F(1, 80) = 9.319$, $p = .003$]; CO [$F(1, 80) = 4.442$, $p = .038$;]; PEP [$F(1, 80) = 5.394$, $p = .023$]; HI [$F(1, 80) = 4.194$, $p = .044$] and MAP [$F(1, 81) = 4.827$, $p = .031$]. Results indicated higher resting HR, DBP, PEP, and MAP levels and lower levels of SV, CO, HI for the Hi TAnger compared to the Lo TAnger participants. The main effect of Trp and the Trp x TAnger interaction were not significant.

Tryptophan by trait anxiety (TAnxiety).

A main effect of TAnxiety was found for HI [$F(1, 76) = 6.42$, $p = .013$]. Results indicated lower resting HI for the Hi TAnxiety participants compared to the Lo TAnxiety participants. The main effect of Trp and the Trp x TAnxiety interaction were not significant.

Tryptophan by beck depression (BDI).

No significant main effects or interactions were found.

Affect Measures (POMS)

Tryptophan by Cook-Medley (Ho).

A main effect of Ho was found for Composed-Anxious [$F(1, 80) = 7.304$, $p = .008$] with Hi Ho participants on average reporting more anxiety than their Lo Ho counterparts. The main effect of Trp and the Trp x Ho interaction were not significant.

Tryptophan by trait anger (TAnger).

A main effect of TAnger was found for Composed-Anxious [$F(1, 80) = 4.771$, $p = .032$]; Energetic-Tired [$F(1, 80) = 3.952$, $p = .05$]; Clearheaded-Confused [$F(1, 80) = 7.285$, $p = .008$] and POMS Total [$F(1, 80) = 5.153$, $p = .026$]. On these measures the Hi TAnger participants consistently reported more negative mood than those in the Lo BDI group. The main effect of Trp and the Trp x TAnger interaction were not significant.

Tryptophan by trait anxiety (TAnxiety).

A main effect of TAnxiety was found for all measures of the POMS. Composed-Anxious [$F(1, 76) = 13.234$, $p < .001$]; Elated-Depressed [$F(1, 76) = 8.058$, $p = .006$]; Energetic-Tired [$F(1, 76) = 9.177$, $p = .003$]; Agreeable-Hostile [$F(1, 76) = 6.268$, $p = .014$]; Confident-Unsure [$F(1, 76) = 11.382$, $p = .001$]; Clearheaded-Confused [$F(1, 76) = 11.594$, $p = .001$] and POMS Total [$F(1, 76) = 14.060$, $p < .001$]. These results indicated that as a group Hi TAnxiety participants consistently reported more negative mood than the participants in the Lo TAnxiety group. The main effect of Trp and the Trp x TAnxiety interaction were not significant.

Tryptophan by Beck depression (BDI).

A main effect of BDI was found for all measures of the POMS. Composed-Anxious [$F(1, 71) = 12.175$, $p = .001$]; Elated-Depressed [$F(1, 71) = 8.145$, $p = .006$]; Energetic-Tired [$F(1, 71) = 10.073$, $p = .002$]; Agreeable-Hostile [$F(1, 71) = 5.755$, $p = .019$]; Confident-Unsure [$F(1, 71) = 10.141$, $p = .002$]; Clearheaded-Confused [$F(1, 71) = 4.48$, $p = .038$]

and POMS Total [$F(1, 71) = 12.453$, $p = .001$]. These results indicated that as a group Hi BDI participants consistently reported more negative mood than those in the Lo BDI group. The main effect of Trp and the Trp x BDI interaction were not significant.

Affect Measures (VAMS)

Tryptophan by Cook-Medley (Ho).

A main effect of Ho was found for Nervous [$F(1, 81) = 5.218$, $p = .025$] and Relaxed [$F(1, 81) = 5.594$, $p = .020$] with Hi Ho group members showing greater negative affect than Lo Ho group members. The main effect of Trp and the Trp x Ho interaction were not significant.

Tryptophan by trait anger (TAnger).

A main effect of TAnger was found for Agreeable [$F(1, 81) = 7.217$, $p = .009$]; Happy [$F(1, 81) = 7.764$, $p = .007$]; Relaxed [$F(1, 81) = 8.078$, $p = .006$] and Guilty [$F(1, 81) = 4.398$, $p = .039$]. For all of these measures Hi TAnger participants showed more negative affect than the Lo TAnger participants. The main effect of Trp and the Trp x TAnger interaction were not significant.

Tryptophan by trait anxiety (TAnxiety).

A main effect of TAnxiety was found for Agreeable [$F(1, 77) = 11.394$, $p = .001$]; Happy [$F(1, 77) = 11.598$, $p = .001$]; Tense [$F(1, 77) = 4.589$, $p = .001$]; Relaxed [$F(1, 77) = 12.037$, $p = .001$]; Discouraged [$F(1, 77) = 7.412$, $p = .008$]; Annoyed [$F(1, 77) = 5.154$, $p = .026$]; Sad [$F(1, 77) = 8.957$, $p = .004$] and Guilty [$F(1, 77) = 8.589$, $p = .004$]. For all of these measures Hi TAnxiety group members endorsed more negative affect than the Lo TAnxiety group members (e.g., more nervous & less relaxed). The main effect of Trp and the Trp x TAnxiety interaction were not significant.

Tryptophan by Beck depression (BDI).

A main effect of BDI was found for Nervous [$F(1, 72) = 4.293$, $p = .042$]; Agreeable [$F(1, 72) = 13.191$, $p = .001$]; Tense [$F(1, 72) = 6.413$, $p = .014$]; Relaxed [$F(1, 72) = 9.864$, $p = .002$]; Annoyed [$F(1, 72) = 5.504$, $p = .022$] and Irritated [$F(1, 72) = 5.191$, $p = .026$]. For all of these measures Hi BDI group members endorsed more negative affect than the Lo BDI group members (e.g., more nervous & less relaxed). The main effect of Trp and the Trp x BDI interaction were not significant.

Plasma Tryptophan Concentrations as a Function Time

The Trp deficient amino acid mixture resulted in a marked decline in total (see Figure 2) and free (see Figure 3) plasma Trp concentrations, with an almost 90% decline in total Trp in the depleted condition with similar results for free Trp. The balanced mixture resulted in an almost 60% increase in total Trp and an almost 100% increase in free Trp in the balanced condition. A mixed factorial ANOVA was conducted for total and free plasma Trp concentrations. The ANOVA for total Trp concentrations revealed a main effect of Time (baseline vs post-manipulation), F (1, 80) = 5.030, $p = .028$, with a statistically significant Trp x Time interaction, F (1, 80) = 272.806, $p < .001$. Follow-up analyses were conducted using pairwise comparisons that indicated no differences between the B and Trp- condition participants at baseline (pre-manipulation) *MD* -.197 *SE* 1.75 $p = .911$; however the groups differed as a function of the Trp manipulation *MD* -87.335 *SE* 5.322 $p < .001$.

The ANOVA for free plasma Trp concentrations revealed a main effect of Time (baseline vs post-manipulation), F (1, 75) = 3.074, $p = .536$, with a statistically

Figure 2. Total Plasma Tryptophan Levels

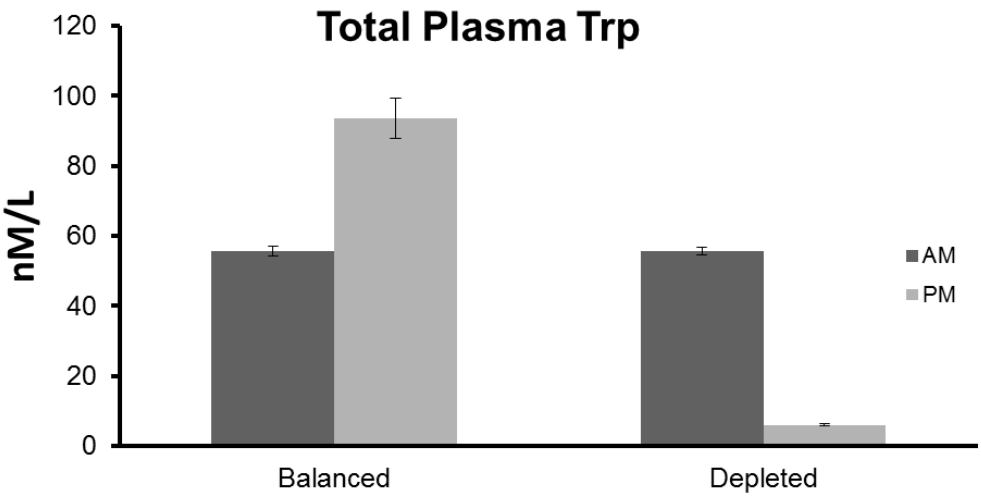
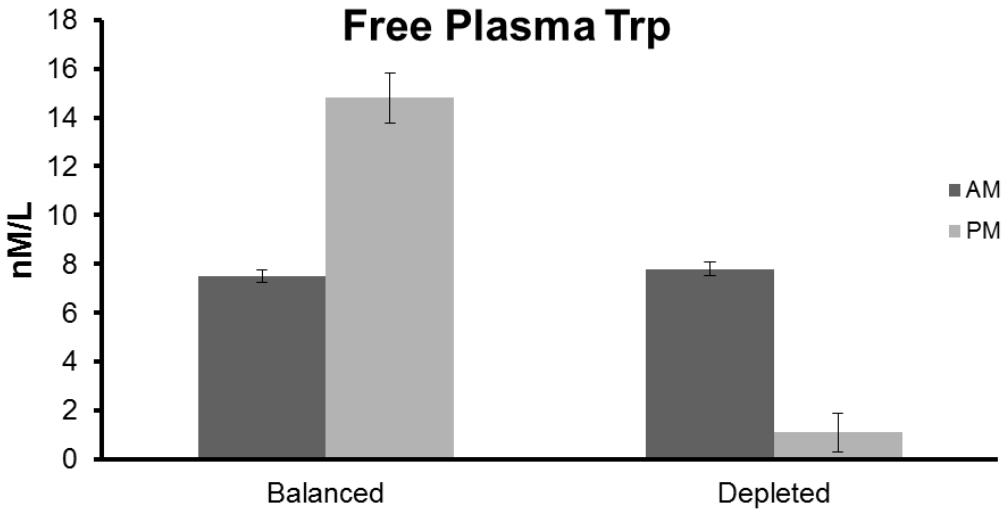


Figure 3. Free Plasma Tryptophan.



significant Trp x Time interaction, F (1, 75) = 1895.206, $p < .001$. Follow-up analyses were conducted using pairwise comparisons that indicated that there were no differences between the B and Trp- condition participants at baseline (pre-manipulation) *MD* -.329 *SE*.386 $p = .397$; however the groups differed as a function of the Trp manipulation *MD* -13.714 *SE* .973 $p < .001$, with the Trp- condition participants showing a marked effect of depletion.

Additional ANOVAs were carried out to verify that there were no between group differences (Trp- and B) for the other five LNAAs. No statistically significant group differences were found. (see Figures 4-8).

Additionally, given concerns (DeMyer, Shea, Hendrie, & Yoshimura, 1981) that the more accurate level of depletion reflects the competition between Trp and the other large neutral amino acids to cross the blood-brain barrier, post-5.5 hr ratios of both Total and Free Trp to the other large neutral amino acids were computed. Because the brain amino acid transporter LAT1/r4F2hc demonstrates a far higher affinity for free Trp than the albumin carrier, both free and bound tryptophan must be considered in modeling its transport across the blood–brain barrier (Fernstrom & Wurtman, 1972; Pardridge, 1979, 1998). Although there are differences in the affinities for each of the LNAAs and the competition with Trp is not equal between all the LNAAs, standard practice is to simply sum the concentrations of the other five LNNAs when calculating the Trp/∑LNAA ratio (e.g., Capuron et al., 2002). This simplified method was used in this study. Mixed factorial ANOVAs were conducted on these data and revealed a Trp x Time interaction [$F(1, 78) = 122.16$, $p < .001$]. Follow-up pairwise comparisons revealed that as expected

Figure 4. Plasma Tyrosine Levels.

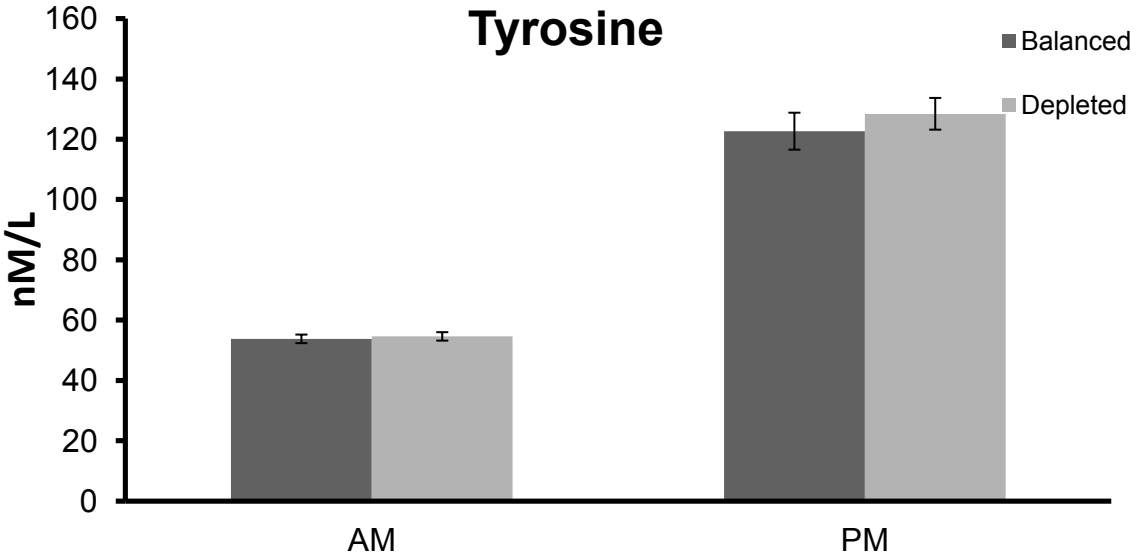


Figure 5. Plasma Phenylanine Levels.

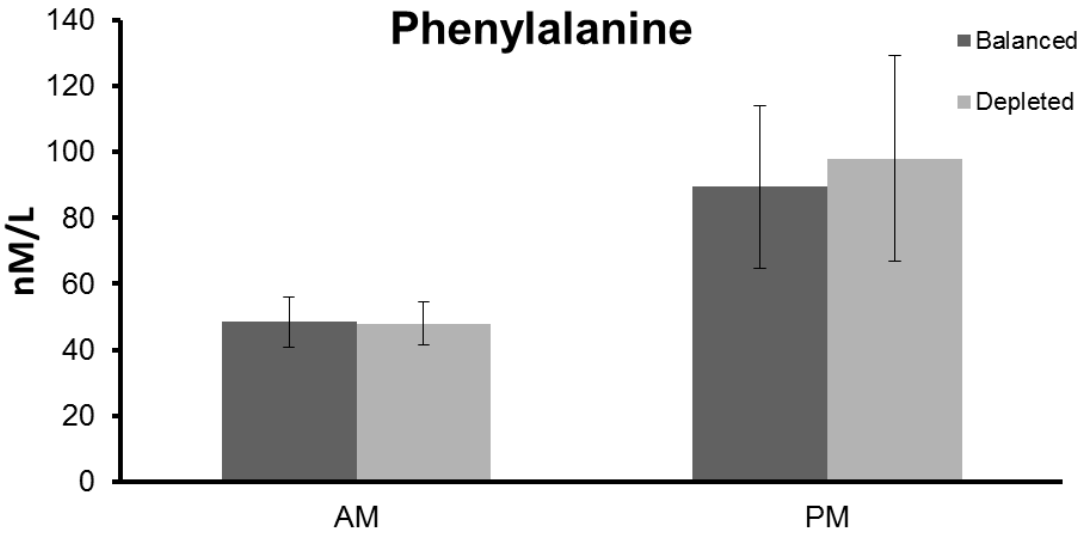


Figure 6. Plasma Valine Levels.

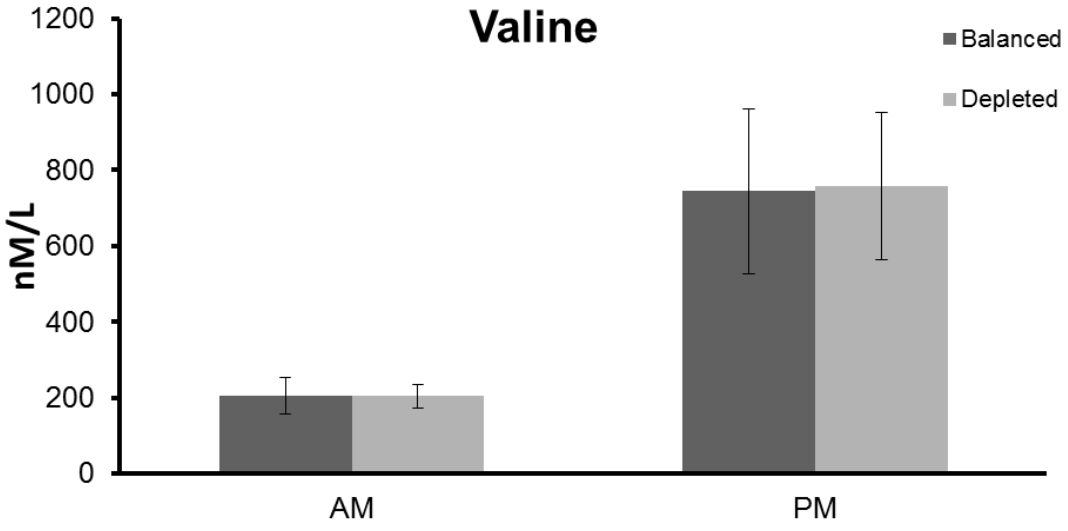


Figure 7. Plasma Isoleucine Levels.

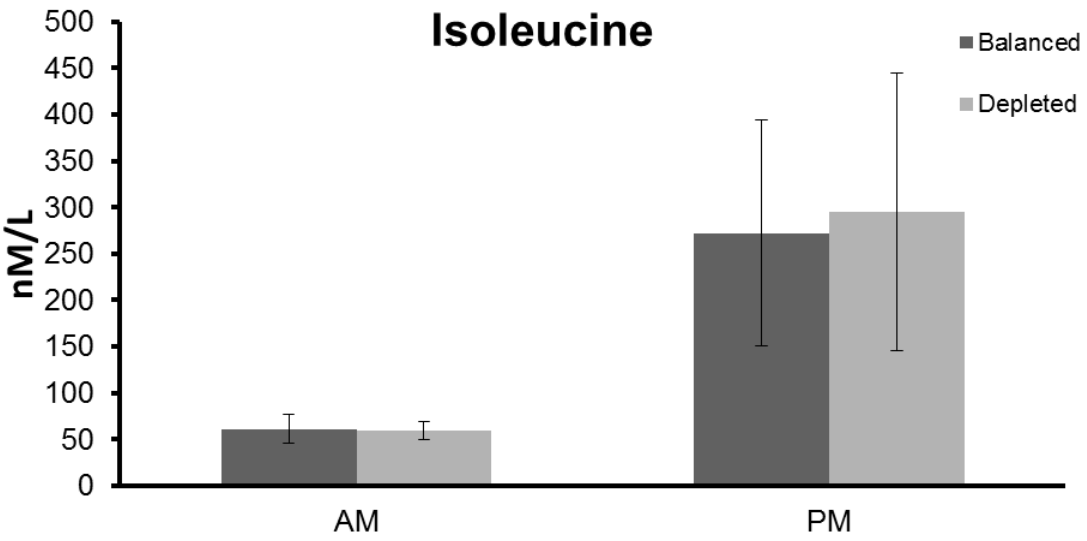
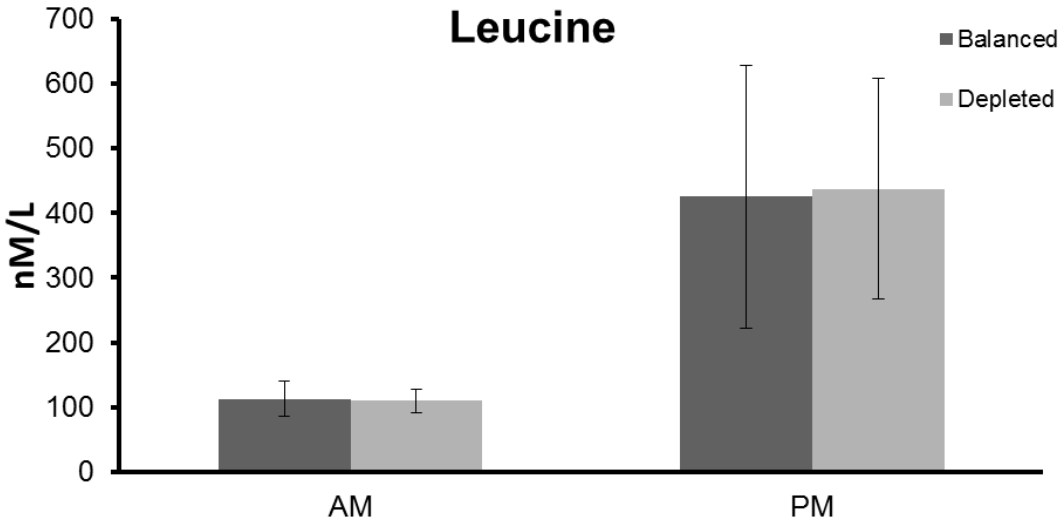


Figure 8. Plasma Leucine Levels.



there were no pre-manipulation differences between the Trp- and the B conditions *MD* -.003 *SE*.02 $p = .899$. Post-manipulation (5 hr), the groups differed substantially *MD* -.262 *SE*.016 $p < .001$ (see Figure 9).

Change from pre- to post-tryptophan manipulation.

A series of 2 (B vs T-) x 2 (Lo vs Hi) ANOVAs were conducted on changes from cardiovascular and state affect measures recorded 35 min prior to ingestion of the amino acid bolus to measures taken 5.5 hr post-ingestion.

Cardiovascular Measures

Means for CV measures taken 5 hr post-manipulation and pre-stressor are shown below (see Tables 18-21).

Tryptophan by Cook-Medley (Ho).

No significant main effects or interactions were found.

Tryptophan by trait anger (TAnger).

Main effects of Trp and TAnger were not significant; however, a Trp x TAnger interaction was found for DBP [$F(1, 79) = 5.046$, $p = .027$]. Follow-up analyses were conducted using independent sample t-tests which indicated that within the B group, Hi TAnger participants showed a greater decrease in DBP than Lo TAnger [$t(38) = 2.617$, $p = .013$]. Within the Trp- group, Hi and Lo TAnger participants decreased equally [$t(41) = -.342$, $p = .734$]. Amongst the Hi TAnger participants there were no differences as a function of depletion status [$t(35) = -1.038$, $p = .306$] with both groups showing a decrease. For those in the Lo TAnger group there were differences as a function of depletion status with those in the Trp- condition showing a greater decrease in DBP than those in the B condition who showed a smaller decrease [$t(44) = 2.173$, $p = .035$].

Figure 9. Tryptophan to ∑Large Neutral Amino Acids Ratio.

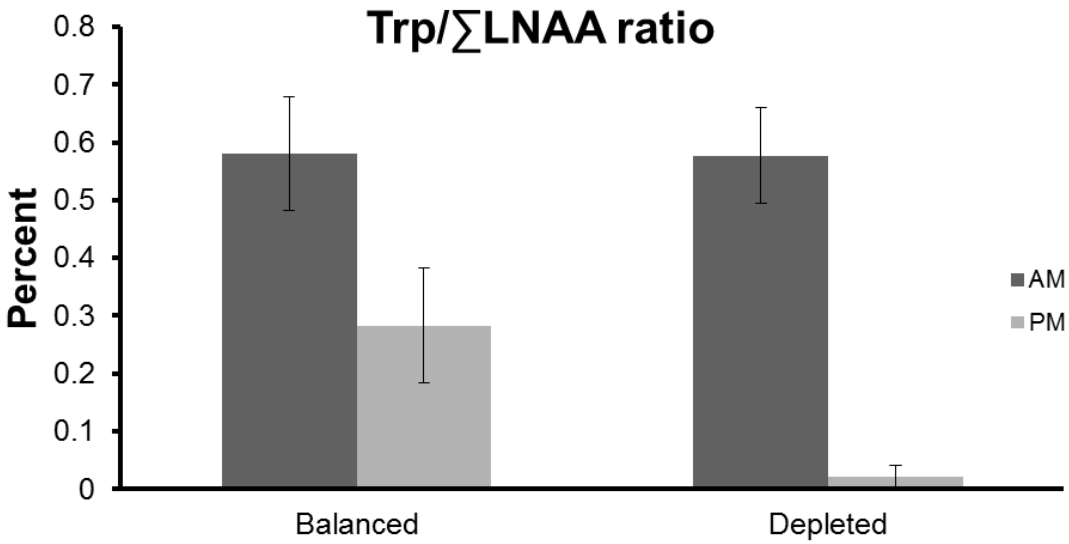


Table 18

Wait Period Cardiovascular Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	62.21	2.61	64.87	2.78	61.85	1.94	66.69	3.31
DBP	63.19	1.80	63.10	1.66	62.63	1.85	64.92	2.02
SBP	101.58	2.01	106.84	1.65	106.76	2.21	106.78	2.53
MAP	75.98	1.48	77.68	1.41	77.34	1.63	78.87	1.79
SV	131.27	6.44	121.62	8.72	126.21	8.79	108.00	9.53
CO	8.057	0.46	7.49	0.40	7.65	0.46	7.00	0.56
PEP	118.30	2.92	123.91	4.47	111.20	3.51	128.91	4.13
LVET	298.80	5.26	283.33	8.98	289.46	6.05	290.72	5.93
HI	15.91	1.23	12.27	1.08	14.46	0.76	11.91	1.22
TPR	805.23	47.53	922.35	95.10	953.26	109.34	1102.27	191.02

Note: LoHo=low hostility, HiHo=high hostility

Table 19

Wait Period Cardiovascular Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	60.21	1.97	68.57	3.40	62.07	2.127	65.47	2.86
DBP	61.75	1.42	65.02	2.05	62.37	1.81	64.88	2.09
SBP	104.31	1.83	104.69	1.99	108.13	2.26	105.20	2.44
MAP	75.94	1.32	78.24	1.58	77.62	1.58	78.32	1.90
SV	139.08	4.58	107.37	10.28	130.72	9.22	106.45	8.89
CO	8.31	0.34	6.94	0.49	7.96	0.48	6.77	0.49
PEP	119.29	3.47	124.24	4.55	111.52	3.98	125.01	3.94
LVET	299.13	4.53	278.02	11.19	287.22	6.62	293.04	5.53
HI	14.88	1.105	12.61	1.32	14.01	0.69	12.95	1.23
TPR	763.01	32.53	1019.64	120.18	878.70	84.32	1154.48	182.57

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 20

Wait Period Cardiovascular Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	61.68	2.36	66.65	3.03	62.72	2.47	63.89	2.63
DBP	60.48	1.34	66.29	1.96	62.30	2.08	64.67	1.60
SBP	103.31	2.07	105.46	1.68	106.23	2.71	107.67	2.03
MAP	74.76	1.27	79.35	1.55	76.94	1.81	79.00	1.46
SV	127.13	7.39	122.57	8.62	126.07	10.25	114.04	9.34
CO	7.66	0.42	7.82	0.46	7.76	0.56	7.05	0.48
PEP	123.93	4.18	119.33	3.69	113.53	5.15	124.26	2.68
LVET	297.21	5.46	282.43	10.09	288.56	7.11	291.45	5.22
HI	15.32	1.21	12.44	1.21	14.56	0.77	11.74	0.92
TPR	847.81	66.22	896.74	97.74	963.38	133.87	1078.67	162.34

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 21

Wait Period Cardiovascular Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	62.10	2.47	61.35	2.18	63.05	2.21	61.97	3.07
DBP	63.89	1.87	62.82	1.12	63.74	1.79	61.39	2.36
SBP	105.20	2.21	104.68	1.82	105.76	2.50	108.10	2.45
MAP	77.66	1.70	76.77	1.06	77.75	1.71	76.96	1.91
SV	132.86	5.21	130.33	9.03	121.37	8.74	126.66	11.97
CO	8.14	0.34	7.82	0.49	7.49	0.49	7.68	0.61
PEP	126.29	4.46	116.59	3.55	116.89	3.69	120.00	6.27
LVET	295.88	4.97	296.45	6.04	287.45	6.07	299.11	6.71
HI	13.97	1.33	13.61	1.16	14.08	0.85	13.04	1.37
TPR	792.30	40.29	858.02	74.74	993.99	120.71	997.27	215.84

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Tryptophan by trait anxiety (TAnxiety).

A main effect of TAnxiety was observed for CO [$F(1, 75) = 7.542$, $p = .008$]. Results indicated a greater increase in CO for the Lo TAnxiety participants compared to the Hi TAnxiety group. The main effect of Trp and the Trp x TAnxiety interaction were not significant.

Tryptophan by Beck depression (BDI).

A main effect of BDI was observed for DBP [$F(1, 71) = 4.416$, $p = .039$]; CO [$F(1, 70) = 4.112$, $p = .046$]; MAP [$F(1, 71) = 6.153$, $p = .015$]. Results indicated a larger decrease in DBP & MAP for the Hi BDI participants compared to the Lo BDI participants. Lo BDI participants showed a larger increase in CO compared to the Hi BDI participants.

Affect Measures (POMS)

Means and standard errors for post manipulation pre stress (wait period) POMS measures are shown below (see Tables 22-25).

Tryptophan by Cook-Medley (Ho).

A main effect of Trp was found for Composed-Anxious [$F(1, 80) = 13.011$, $p = .001$] and Agreeable-Hostile [$F(1, 80) = 9.238$, $p = .003$]. The Trp- group participants showed increased anxiety and hostility compared to the B group. Participants in the Trp- condition showed a larger overall increase in negative mood (POMS Total) compared to the participants in the B condition [$F(1, 80) = 4.734$, $p = .033$]. Main effects of Ho and the Trp x Ho interaction were not significant.

Tryptophan by trait anger (TAnger).

A main effect of Trp [$F(1, 80) = 15.154$, $p < .001$] and a Trp x TAnger interaction

Table 22

Wait Period POMS Means and SE for Trp x Hostility Groups 2

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	32.78	0.98	30.36	0.99	30.07	1.19	25.94	1.63
ED	28.00	1.32	25.36	1.22	26.15	1.14	23.61	1.79
ET	23.83	1.86	18.68	1.85	20.48	1.51	18.00	2.21
AH	30.50	0.96	28.59	1.02	27.33	1.42	25.67	1.63
CU	25.33	1.21	24.23	1.12	25.30	1.02	23.00	1.64
CC	28.78	1.17	27.05	1.10	28.26	1.12	25.67	1.78

Note: LoHo=low hostility, HiHo=high hostility, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 23

Wait Period POMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	31.52	0.96	31.35	1.12	31.54	0.91	24.86	1.57
ED	26.91	1.10	26.06	1.57	27.29	1.19	22.67	1.50
ET	21.52	1.42	20.29	2.61	20.96	1.73	17.81	1.84
AH	30.30	0.76	28.29	1.31	28.33	1.32	24.76	1.65
CU	25.09	0.95	24.24	1.46	26.04	1.03	22.48	1.45
CC	28.30	0.99	27.18	1.40	29.46	1.02	24.67	1.61

Note: LoTAnger=low trait anger, HiTAnger=high trait anger, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 24

Wait Period POMS Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	32.95	0.92	30.06	1.03	31.81	1.10	24.90	1.49
ED	27.29	1.27	25.83	1.38	28.71	0.95	21.24	1.49
ET	21.57	1.73	20.56	2.30	23.33	1.67	15.29	1.71
AH	30.71	0.89	28.00	1.13	30.10	1.19	23.10	1.65
CU	25.33	1.04	24.17	1.36	27.62	0.90	20.86	1.34
CC	29.10	1.03	26.28	1.26	30.38	0.92	23.81	1.58

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 25

Wait Period POMS Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	31.72	1.23	31.27	0.98	31.36	0.88	24.46	1.62
ED	27.88	1.42	25.05	1.35	26.76	1.19	23.26	1.51
ET	22.11	1.92	20.00	2.20	21.08	1.61	16.60	2.01
AH	30.33	1.02	28.05	1.11	29.44	1.04	23.26	1.80
CU	24.83	1.18	24.61	1.35	25.56	0.96	22.53	1.75
CC	27.61	1.34	28.33	1.04	28.80	1.10	25.46	1.78

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

was found for Composed-Anxious [$F(1, 80) = 6.628$, $p = .012$]. Follow-up analyses were conducted using independent sample t-tests which indicated that Hi TAnger participants in the Trp- condition showed increased anxiety compared to the Lo TAnger participants in the same group [$t(43) = 2.617$, $p = .012$]. In the B condition both Lo and Hi TAnger participants showed equal decrease in anxiety [$t(37) = -.991$, $p = .328$]. Amongst participants in the Hi TAnger group those in the Trp- condition showed increased anxiety (less composed) compared to those in the B condition [$t(36) = 3.740$, $p = .001$] (see Figure 10). Among the Lo TAnger participants no effect of tryptophan depletion was found [$t(44) = 1.169$, $p = .249$].

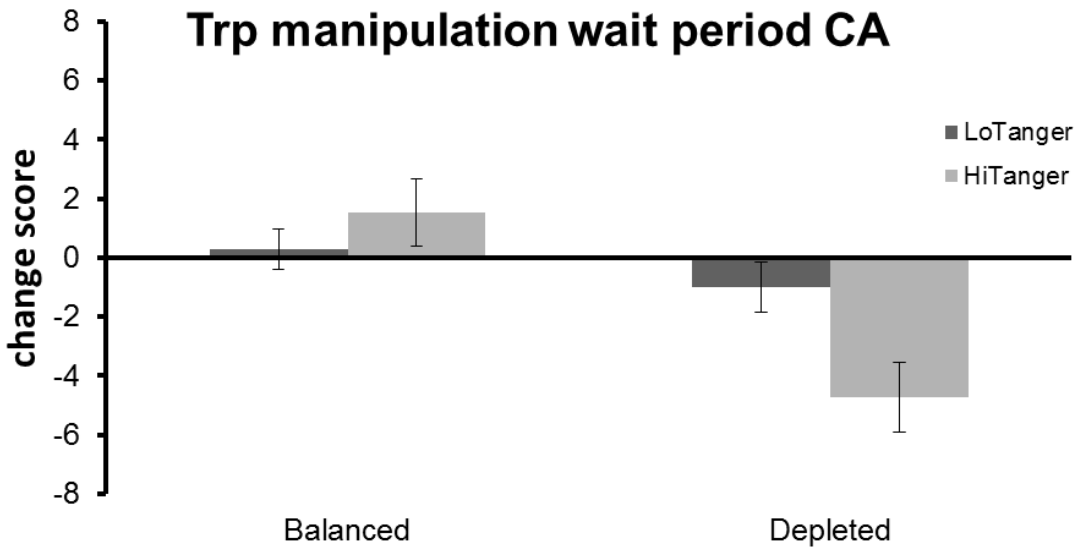
A main effect of Trp was found for Agreeable-Hostile [$F(1, 80) = 7.862$, $p = .006$] and for POMS Total [$F(1, 80) = 4.356$, $p = .04$]. On average participants in the B condition became more agreeable compared to the Trp- condition. The Trp- condition showed an overall higher negative affect than the participants in the B condition. The effect of TAnger and the Trp x TAnger interaction were not significant.

Tryptophan by trait anxiety (TAnxiety).

A main effect of Trp was found for Composed-Anxious [$F(1, 76) = 12.666$, $p = .001$] and POMS Total [$F(1, 76) = 4.1396$, $p = .045$]. The participants in the Trp- condition became more anxious compared to the B condition. In addition, the Trp- condition showed a greater decline in overall positive affect (POMS Total) compared to those in the B condition. The main effect of TAnxiety and the Trp x TAnxiety interaction were not significant for this variable.

There was a significant Trp x TAnxiety interaction found for Elated-Depressed [$F(1, 80) = 3.969$, $p = .05$]. Follow-up analyses were conducted using independent

Figure 10. Interaction Effect Wait Period Trp x Trait Anger POMS C-A.



sample t-tests which indicated that Hi TAnxiety participants showed an increase in depressed scores compared to Lo TAnxiety in the Trp- condition [$t(40) = 2.161$, $p = .037$] (see Figure 11) . There were no differences in the B condition [$t(36) = -0.613$, $p = .544$]. There were no other significant effects.

Tryptophan by Beck depression (BDI).

A main effect of Trp was observed for Agreeable-Hostile [$F(1, 71) = 8.449$, $p = .005$] with participants in the Trp- condition demonstrating higher hostility scores compared to the B condition. The effect of BDI and the Trp x BDI interaction for this variable were not significant.

A main effect of Trp was found for Composed-Anxious [$F(1, 71) = 14.87$, $p < .001$], but not for BDI. A Trp x BDI interaction was also found [$F(1, 71) = 6.613$, $p = .012$]. Follow-up analyses were conducted using independent sample t-tests which indicated that the Hi and Lo BDI participants in the Trp- condition did not differ from each other $t(38) = 1.789$, $p = .082$] nor did they differ in the B condition $t(33) = -1.883$, $p = .069$]. Amongst Hi BDI participants those in the Trp- condition showed an increase in anxiety compared to the B condition $t(31) = 4.158$, $p < .001$] (see Figure 12). Amongst the Lo BDI participants there were no differences as a function of depletion status $t(40) = .992$, $p = .327$]. There were no other significant effects.

Affect Measures (VAMS)

Means and standard errors for post manipulation pre stress (wait period) VAMS measures are shown below (see Tables 26-29).

Tryptophan by Cook-Medley (Ho).

A main effect of Trp was found for Nervous [$F(1, 81) = 4.374$, $p = .040$];

Figure 11. Interaction Effect Wait Period Trp x Trait Anxiety POMS E-D.

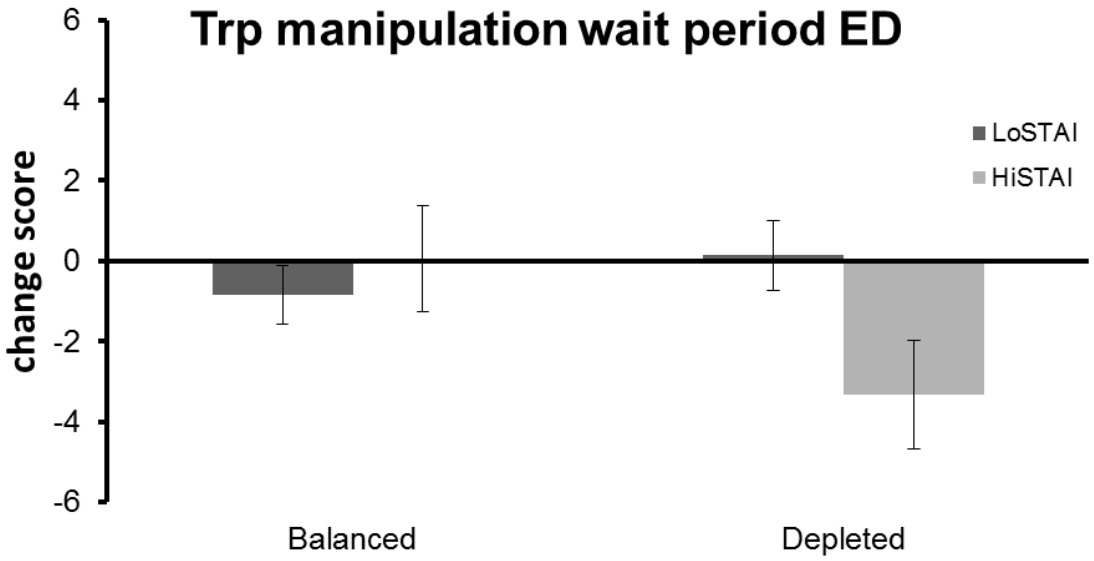


Figure 12. Interaction Effect Wait Period Trp x BDI POMS C-A.

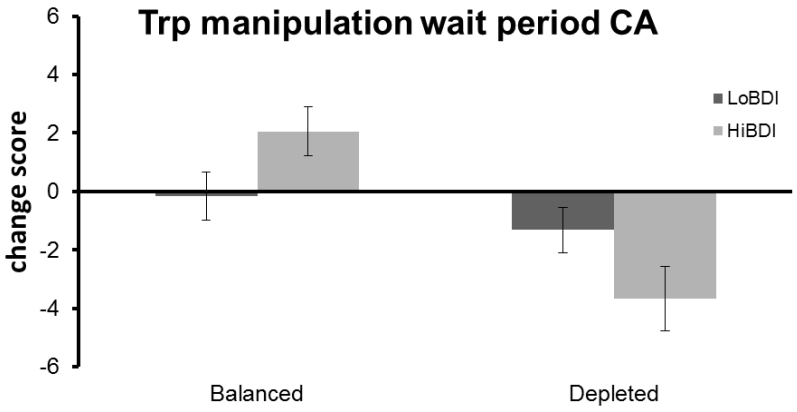


Table 26

Wait Period VAMS Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.67	0.61	1.80	0.23	2.30	0.49	2.67	0.61
Agreeable	11.03	0.61	9.82	0.57	9.89	0.66	9.58	0.70
Happy	9.97	0.65	9.70	0.48	9.89	0.61	8.92	0.70
Tense	2.78	0.63	2.82	0.52	2.35	0.47	3.28	0.70
Anxious	2.72	0.70	2.86	0.63	2.44	0.54	3.81	0.81
Relaxed	11.44	0.65	10.64	0.39	10.22	0.56	10.09	0.68
Discouraged	2.36	0.50	1.91	0.34	2.19	0.39	3.33	0.77
Annoyed	2.81	0.72	2.50	0.43	3.59	0.73	4.25	0.92
Sad	2.22	0.42	1.80	0.34	2.25	0.43	2.78	0.63
Irritated	2.25	0.40	2.57	0.43	2.98	0.58	3.94	0.87
Angry	2.03	0.34	1.73	0.34	2.52	0.50	2.42	0.53
Depressed	2.00	0.35	1.96	0.35	2.27	0.47	2.69	0.59
Guilty	1.81	0.31	1.68	0.30	1.85	0.35	2.22	0.48

Note: LoHo=low hostility, HiHo=high hostility

Table 27

Wait Period VAMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.43	0.47	1.85	0.32	1.93	0.37	3.02	0.67
Agreeable	10.95	0.44	9.55	0.76	10.43	0.61	9.00	0.73
Happy	9.71	0.47	9.91	0.67	10.56	0.52	8.28	0.72
Tense	2.91	0.58	2.64	0.52	2.00	0.35	3.54	0.72
Anxious	3.15	0.72	2.32	0.46	1.97	0.40	4.14	0.81
Relaxed	11.28	0.51	10.61	0.49	10.79	0.49	9.42	0.70
Discouraged	2.30	0.43	1.85	0.34	2.14	0.45	3.21	0.65
Annoyed	2.65	0.58	2.61	0.51	3.33	0.70	4.45	0.90
Sad	1.80	0.26	2.23	0.50	2.10	0.44	2.85	0.57
Irritated	2.26	0.32	2.64	0.53	2.77	0.50	4.04	0.87
Angry	1.67	0.17	2.11	0.51	2.27	0.47	2.71	0.56
Depressed	1.65	0.19	2.41	0.50	2.10	0.49	2.81	0.54
Guilty	1.43	0.11	2.14	0.46	1.58	0.32	2.47	0.45

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 28

Wait Period VAMS Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.14	0.44	2.25	0.45	1.90	0.36	3.07	0.70
Agreeable	11.21	0.44	9.38	0.72	11.38	0.47	8.42	0.74
Happy	10.35	0.50	9.30	0.60	11.21	0.46	8.04	0.66
Tense	2.71	0.57	2.94	0.58	1.57	0.21	4.02	0.74
Anxious	2.54	0.62	3.13	0.73	2.16	0.59	3.78	0.74
Relaxed	11.33	0.55	10.66	0.47	11.26	0.50	9.27	0.65
Discouraged	1.64	0.320	2.55	0.50	1.19	0.05	4.09	0.70
Annoyed	2.19	0.54	2.91	0.55	2.31	0.60	5.64	0.91
Sad	1.54	0.19	2.55	0.51	1.40	0.15	3.65	0.68
Irritated	1.66	0.18	3.16	0.54	1.76	0.22	5.16	0.88
Angry	1.61	0.17	2.13	0.49	1.64	0.31	3.40	0.66
Depressed	1.57	0.19	2.38	0.47	1.42	0.17	3.47	0.68
Guilty	1.35	0.09	2.22	0.43	1.19	0.05	2.76	0.55

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 29

Wait Period VAMS Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	2.52	0.54	1.86	0.38	1.48	0.18	3.16	0.66
Agreeable	10.63	0.52	9.80	0.76	10.94	0.52	8.26	0.83
Happy	9.83	0.58	9.41	0.61	10.58	0.47	8.10	0.84
Tense	3.02	0.69	2.69	0.55	1.66	0.21	3.73	0.77
Anxious	3.50	0.90	2.33	0.45	1.44	0.12	4.70	0.92
Relaxed	10.69	0.68	11.41	0.42	10.66	0.53	9.60	0.71
Discouraged	2.52	0.51	1.88	0.37	1.74	0.33	3.60	0.79
Annoyed	2.69	0.66	2.77	0.56	2.40	0.48	5.63	1.07
Sad	2.13	0.37	1.97	0.45	1.83	0.35	2.80	0.57
Irritated	2.36	0.39	2.69	0.51	2.50	0.48	3.90	0.81
Angry	1.88	0.34	1.94	0.41	1.82	0.39	3.03	0.67
Depressed	2.08	0.39	2.02	0.37	1.89	0.35	2.70	0.70
Guilty	1.75	0.30	1.69	0.34	1.36	0.14	2.26	0.50

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Annoyed [$F(1, 81) = 4.551$, $p = .036$] and Depressed [$F(1, 80) = 6.0111$, $p = .016$]. These results indicate that the B condition became less nervous, annoyed, and depressed compared to the Trp- condition. The effect of HO and the Trp x Ho interaction were not significant. There were no other significant effects.

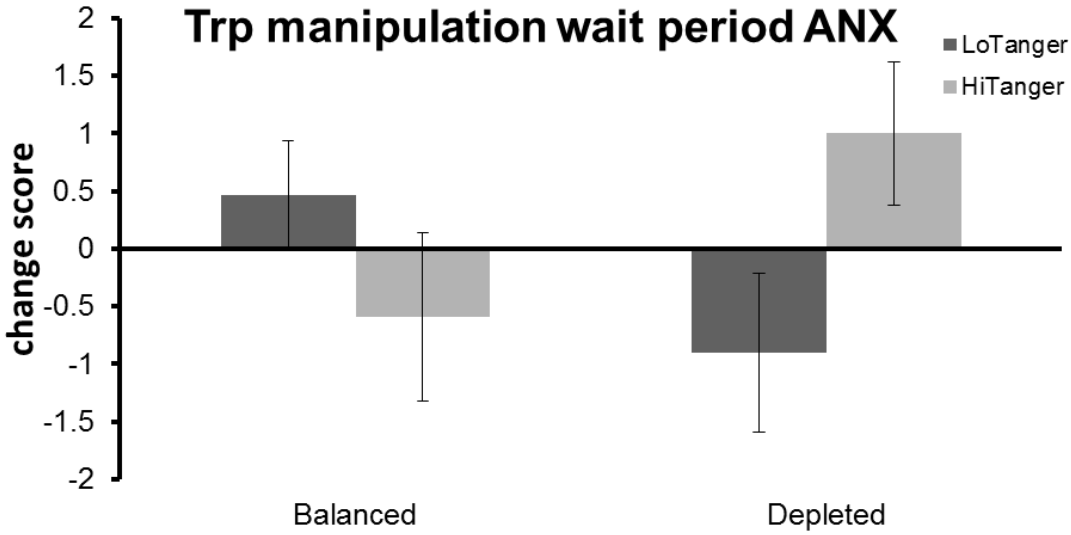
Tryptophan by trait anger (TAnger).

A main effect of Trp was found for Annoyed [$F(1, 81) = 4.976$, $p = .028$] with participants in the Trp- condition showing increased annoyance relative to the B condition. The effect of TAnger and the Trp x TAnger interaction for this variable were not significant. A main effect of Trp was also found for Depressed [$F(1, 80) = 5.059$, $p = .027$] with participants in the Trp- condition showing increased depression with a decrease in depression found for the B condition participants. The effect of TAnger and the Trp x TAnger interaction for this variable were not significant.

A Trp x TAnger interaction was found for Happy [$F(1, 81) = 4.214$, $p = .043$]. Follow-up analyses were conducted using independent sample t-tests which showed no differences between Lo and Hi TAnger in the Trp- condition [$t(43) = 1.065$, $p = .293$], . However, Hi TAnger participants in the B condition showed a greater decrease in happiness compared to the Lo TAnger participants [$t(38) = -2.178$, $p = .036$].

A Trp x TAnger interaction was also found for Anxious [$F(1, 81) = 5.356$, $p = .023$]. Follow-up analyses were conducted using independent sample t-tests which showed that the Hi TAnger participants demonstrated a greater increase in anxiety than the Lo TAnger group in the Trp- condition [$t(43) = -2.025$, $p = .049$] (see Figure 13) There was no group difference in the B condition [$t(38) = 1.247$, $p = .22$]. There were no other significant effects.

Figure 13. Interaction Effect Wait Period Trp x Trait Anger VAMS ANX.



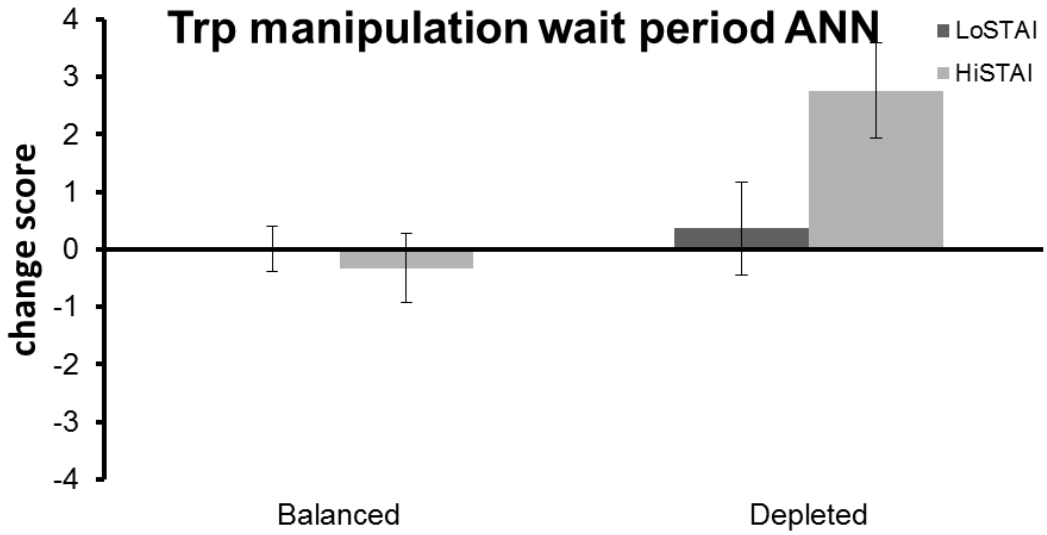
Tryptophan by trait anxiety (TAnxiety).

A main effect of TAnxiety was found for Disappointed [$F(1, 77) = 6.768$, $p = .011$] and Irritated [$F(1, 77) = 6.817$, $p = .011$] showing that Lo TAnxiety participants became less disappointed and irritated compared to the Hi TAnxiety participants. The main effect of Trp and the Trp x TAnxiety interaction for this variable were not significant.

A main effect of Trp was found for Depressed [$F(1, 76) = 5.85$, $p = .018$]. As a function of Trp, participants in the Trp- group became more depressed than those in the B group who became less depressed. As well a main effect of TAnxiety was found for Depressed [$F(1, 76) = 6.861$, $p = .011$]. As a function of TAnxiety participants in the Hi TAnxiety group became more depressed than those in the Lo TAnxiety group who became less depressed. The main effect of TAnxiety and the Trp x TAnxiety interaction for this variable were not significant.

A main effect of Trp, but not TAnxiety was found for Annoyed [$F(1, 77) = 5.953$, $p = .017$]. A significant Trp x TAnxiety interaction was also found for Annoyed [$F(1, 77) = 4.24$, $p = .043$]. Follow-up analyses were conducted using independent sample t-tests which revealed that Hi TAnxiety in the Trp- group became more annoyed than the Lo TAnxiety participants in the same group [$t(40) = -2.080$, $p = .044$] (see Figure 14). Lo and Hi TAnxiety participants in the B group did not differ from each other [$t(37) = .616$, $p = .541$]. Amongst the Hi TAnxiety participants those in the Trp- group showed increased annoyance while those in the B group showed a decrease $t(37) = -2.922$, $p = .006$]. For Lo TAnxiety participants there was no difference amongst them as a function of depletion status $t(40) = -.294$, $p = .770$].

Figure 14 Interaction Effect Wait Period Trp x Trait Anxiety VAMS ANN.



No main effects were found for Relaxed; however, a Trp x TAnxiety interaction was found [$F(1, 76) = 4.064$, $p = .047$]. Follow-up analyses were conducted using independent sample t-tests which revealed no group differences within the Trp- condition, or in the B condition. There were no other significant effects.

Tryptophan by Beck depression (BDI).

A main effect of BDI was found for Relaxed [$F(1, 72) = 5.713$, $p = .019$] revealing an increase in relaxed mood for the Hi BDI group compared to the Lo BDI group. No main effect of Trp or interaction for this variable was found.

No main effects were found for Nervous; however, a significant Trp x BDI interaction was found [$F(1, 72) = 9.501$, $p = .003$]. Follow-up analyses were conducted using independent sample t-tests which indicated that Hi BDI group becoming more nervous than the Lo BDI group in the B condition [$t(34) = 2.285$, $p = .029$]. The same pattern was found in the Trp- condition [$t(34) = 2.285$, $p = .029$]. Amongst Lo TAnxiety participants, those in the Trp- condition showed increased nervousness than those in the B condition $t(31) = 2.051$, $p = .036$]. A similar pattern was found amongst the Hi TAnxiety participants $t(41) = -2.192$, $p = .047$].

No main effects were found for Happy; however, a significant Trp x BDI interaction was found [$F(1, 72) = 4.145$, $p = .045$]. Follow-up analyses were conducted using independent sample t-tests which indicated no group differences in the B condition or in the Trp- condition.

No main effects were found for Anxious; however, a significant Trp x BDI interaction was found [$F(1, 72) = 8.437$, $p = .005$]. Follow-up analyses were conducted using independent sample t-tests which revealed no effect of BDI in the B condition;

however, a group difference emerged in the Trp- condition, as the Hi BDI participants became more anxious compared to the Lo BDI participants [$t(38) = -2.090$, $p = .043$] (see Figure 15) . Amongst the Hi BDI participants, no group differences emerged; however, amongst the Lo BDI group, participants in the Trp- condition reported a decrease in anxiety compared to those in the B group $t(41) = 2.753$, $p = .009$].

A main effect of Trp, but not of BDI was found for Annoyed [$F(1, 72) = 5.199$, $p = .026$].A significant Trp x BDI interaction was also found [$F(1, 72) = 5.643$, $p = .020$]. Follow-up analyses were conducted using independent sample t-tests which indicated no group differences in the B condition or in the Trp- condition. Amongst the Hi BDI participants, those in the Trp- condition showed an increase in annoyance compared to the B condition [$t(31) = -2.746$, $p = .010$]. No differences emerged in the Lo BDI group. There were no other significant effects.

Change from post-tryptophan manipulation to math task.

2 (B vs. T-) x 2 (Lo vs. Hi) ANOVAs were conducted on cardiovascular and state affect stress minus post-Trp rest change scores. These analyses were conducted to identify the effect of Trp and psychological factors. Means and standard errors for CV measures during the stressor are shown below (see Tables 30-33).

Cardiovascular Measures

Tryptophan by Cook-Medley (Ho).

No significant main effects or interactions were found.

Tryptophan by trait anger (TAnger).

No significant main effects or interactions were found.

Figure 15. Interaction Effect Wait Period Trp x BDI VAMS ANX

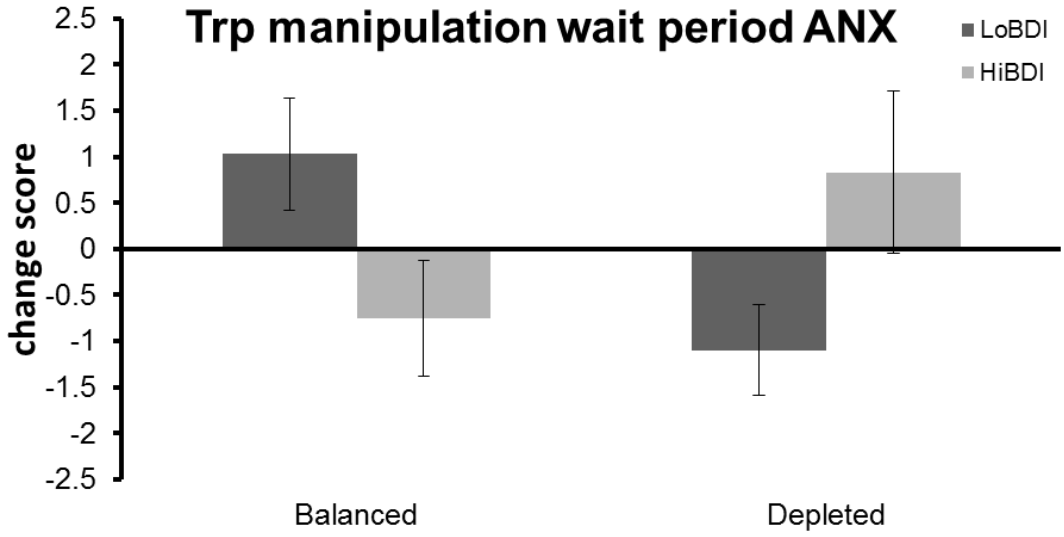


Table 30

Stressor Cardiovascular Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	81.15	4.10	80.03	3.31	76.53	3.19	82.16	4.64
DBP	71.55	1.85	71.75	1.81	68.10	1.92	74.01	1.98
SBP	116.14	2.63	123.24	2.18	121.82	2.89	122.61	2.80
MAP	86.41	1.64	88.91	1.75	86.01	1.93	90.21	1.79
SV	121.97	6.95	111.02	7.47	124.62	8.88	108.13	8.65
CO	9.93	0.85	8.63	0.55	9.45	0.71	8.76	0.79
PEP	99.80	3.96	106.30	4.94	93.49	3.62	107.38	5.91
LVET	284.41	6.26	269.47	9.79	280.71	6.19	278.28	6.83
HI	18.57	1.98	13.45	1.21	16.58	0.96	14.15	1.69
TPR	781.75	63.40	952.42	111.30	907.22	102.39	1069.88	196.76

Note: LoHo=low hostility, HiHo=high hostility

Table 31

Stressor Cardiovascular Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	76.91	3.44	86.15	3.48	76.87	3.59	80.98	4.03
DBP	69.58	1.47	74.47	2.12	69.05	2.12	72.12	1.94
SBP	118.61	2.22	121.98	2.85	124.68	3.01	119.36	2.66
MAP	85.92	1.51	90.31	1.87	87.59	2.08	87.87	1.82
SV	130.97	5.18	93.58	7.19	130.48	8.83	104.07	8.44
CO	10.14	0.68	7.86	0.58	9.98	0.75	8.28	0.71
PEP	100.58	4.47	107.26	4.32	94.03	4.74	104.81	4.53
LVET	284.05	6.42	265.04	11.21	279.02	6.16	280.47	6.90
HI	17.04	1.66	14.09	1.59	16.35	1.20	14.76	1.36
TPR	752.26	55.44	1054.53	135.03	845.77	83.05	1111.02	185.62

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 32

Stressor Cardiovascular Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	77.02	3.44	86.18	3.54	76.40	3.79	80.25	4.24
DBP	69.20	1.51	74.30	2.09	70.19	2.48	70.65	1.81
SBP	117.40	2.62	122.77	2.29	122.30	3.53	122.49	2.60
MAP	85.26	1.63	90.46	1.71	87.56	2.29	87.92	1.80
SV	117.79	6.83	113.34	8.34	122.38	10.96	114.45	8.43
CO	8.95	0.57	9.69	0.90	9.33	0.90	8.98	0.71
PEP	106.77	4.47	98.82	4.79	96.89	5.38	102.83	4.76
LVET	282.46	7.51	268.55	9.96	281.60	6.70	279.52	6.43
HI	16.96	1.40	14.88	2.10	16.26	1.31	14.21	1.28
TPR	846.69	74.71	893.34	122.11	991.25	136.67	991.87	168.03

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 33

Stressor Cardiovascular Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	78.10	3.60	80.19	3.99	77.98	3.62	77.70	5.16
DBP	73.17	1.96	71.24	1.35	70.70	1.98	68.34	2.70
SBP	119.07	2.77	121.78	2.61	122.39	3.32	121.68	2.48
MAP	88.47	1.83	88.08	1.55	87.92	2.12	86.12	2.05
SV	119.13	5.14	121.67	8.79	121.03	9.07	123.40	10.36
CO	9.34	0.59	9.68	0.92	9.38	0.77	9.43	0.87
PEP	110.29	5.36	95.72	3.60	96.18	4.48	103.91	6.52
LVET	275.67	6.93	288.25	6.50	279.37	5.96	287.28	8.40
HI	15.19	1.49	15.79	2.08	16.77	1.15	14.24	1.73
TPR	836.19	70.95	821.55	74.96	944.07	109.96	974.52	234.12

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Tryptophan by trait anxiety (TAnxiety).

No significant main effects or interactions were found.

Tryptophan by beck depression (BDI).

No significant main effects or interactions were found.

Affect Measures (POMS)

Means and standard errors for stressor POMS measures are shown below (see Tables 34-37).

Tryptophan by Cook-Medley (Ho).

No significant main effects or interactions were found.

Tryptophan by trait anger (TAnger).

No main effects were found. However, a significant Trp x TAnger interaction was found for Composed-Anxious [$F(1, 79) = 4.161$, $p = .045$]. Follow-up analyses were conducted using independent sample t-tests which revealed no group differences within the Lo TAnger group; however, the Hi TAnger group in the B condition demonstrated a larger increase in anxiety relative to the Lo TAnger group [$t(38) = 2.062$, $p = .046$]. There were no other significant effects.

Tryptophan by trait anxiety (TAnxiety).

No main effects were found; however, a significant Trp x TAnxiety interaction was found for Agreeable-Hostile [$F(1, 75) = 4.688$, $p = .034$]. Follow-up analyses were conducted using independent sample t-tests which indicated that Hi TAnxiety participants became more hostile than the Lo TAnxiety participants in the Trp- condition [$t(38) = 2.067$, $p = .046$] but not in the B condition (see Figure 16). There were no other significant effects.

Table 34

Stressor POMS Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	24.33	2.21	23.09	1.55	21.08	1.68	21.17	2.09
ED	24.17	1.63	22.14	1.42	21.36	1.37	21.22	1.59
ET	25.83	1.69	19.09	1.66	21.24	1.63	20.56	1.80
AH	23.17	2.42	21.32	1.84	19.36	2.33	19.83	1.74
CU	25.17	1.60	22.45	1.27	23.20	1.45	21.61	1.80
CC	26.00	1.63	23.36	1.33	23.84	1.65	21.56	1.66

Note: LoHo=low hostility, HiHo=high hostility, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 35

Stressor POMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	25.57	1.80	21.06	1.68	23.17	1.61	18.75	1.99
ED	23.43	1.39	22.53	1.72	23.04	1.40	19.30	1.41
ET	23.57	1.51	20.18	2.22	22.52	1.51	19.15	1.86
AH	22.09	2.11	22.24	2.04	21.00	2.34	17.90	1.86
CU	24.09	1.40	23.12	1.48	24.04	1.50	20.80	1.65
CC	25.48	1.37	23.29	1.60	24.96	1.50	20.50	1.77

Note: LoTAnger=low trait anger, HiTAnger=high trait anger, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Table 36

Stressor POMS Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	25.05	1.91	22.22	1.80	26.05	1.14	15.75	1.94
ED	24.83	1.57	21.56	1.49	25.35	1.04	16.85	1.38
ET	22.86	1.83	21.67	1.93	23.80	1.69	18.40	1.75
AH	22.38	2.20	21.94	2.10	25.50	1.62	12.90	2.01
CU	24.14	1.51	23.33	1.43	26.10	1.06	18.75	1.80
CC	25.38	1.43	23.61	1.63	27.05	1.08	18.25	1.79

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

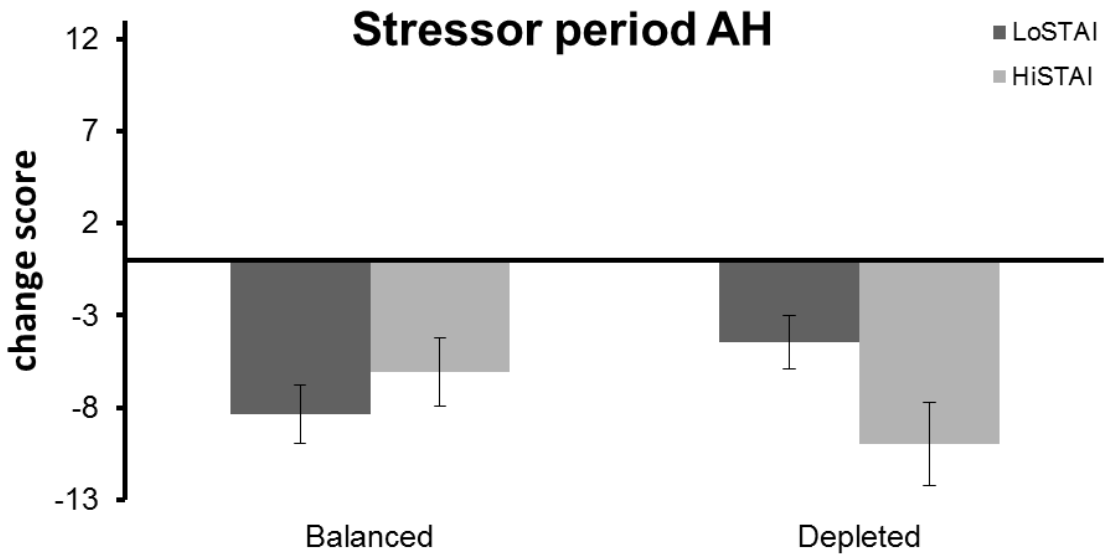
Table 37

Stressor POMS Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
POMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
CA	25.33	2.07	23.00	1.60	22.78	1.58	19.33	2.26
ED	23.50	1.70	23.00	1.37	23.39	1.33	18.73	1.54
ET	22.94	1.64	22.33	1.94	23.04	1.53	18.66	1.92
AH	21.83	2.24	22.05	2.28	22.60	2.22	16.06	2.01
CU	23.11	1.53	24.83	1.27	24.65	1.43	19.00	1.75
CC	24.05	1.57	25.83	1.42	25.17	1.51	19.40	1.90

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory, CA=composed-anxious, ED=elated-depressed, ET=energetic-tired, AH=agreeable-hostile, CU=confident-unsure, CC=clearheaded-confused

Figure 16. Interaction Effect Stressor Trp x Trait Anxiety POMS A-H.



Tryptophan by beck depression (BDI)

No significant main effects or interactions were found.

Affect Measures (VAMS)

Means and standard errors for stressor VAMS measures are shown below (see Tables 38-41).

Tryptophan by Cook-Medley (Ho).

No main effects were found; however, a significant Trp x Ho interaction was found for Agreeable [$F(1, 79) = 4.362$, $p = .040$]. Follow-up analyses were conducted using independent sample t-tests which indicated no group differences in the B or in the Trp- condition.

A Trp x Ho interaction was also found for Happy [$F(1, 79) = 5.816$, $p = .018$] . Follow-up analyses were conducted using independent sample t-tests revealing no group differences in neither B nor Trp- condition; however, amongst Lo Ho participants, those in the Trp- condition showed a greater decrease in happiness than those in the B condition [$t(41) = 2.151$, $p = .036$]. There were no differences as a function of depletion status amongst the Hi Ho participants [$t(38) = -1.353$, $p = .184$]. There were no other significant effects.

Tryptophan by trait anger (TAnger).

No significant main effects or interactions were found.

Tryptophan by trait anxiety (TAnxiety).

No main effects were found. However, a significant Trp x TAnxiety interaction was found for Anxious [$F(1, 79) = 4.479$, $p = .038$]. Follow-up analyses were conducted using independent sample t-tests which revealed no group differences in the B condition;

Table 38

Stressor VAMS Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	3.62	0.75	3.93	0.72	3.52	0.63	3.83	0.86
Agreeable	9.32	0.86	6.41	0.79	7.26	0.84	8.33	0.88
Happy	9.44	0.71	7.16	0.70	7.71	0.69	7.97	0.86
Tense	5.21	0.93	5.21	0.72	5.48	0.77	5.25	0.93
Anxious	4.47	0.82	3.55	0.74	4.78	0.79	5.50	0.95
Relaxed	8.79	0.95	7.59	0.76	6.29	0.79	7.17	0.95
Discouraged	3.71	0.85	3.82	0.72	4.25	0.77	4.91	0.97
Annoyed	5.21	0.89	5.98	0.94	6.15	0.83	6.00	1.09
Sad	3.06	0.72	2.43	0.56	2.44	0.46	2.92	0.57
Irritated	5.44	1.03	5.61	0.84	5.58	0.83	6.78	1.02
Angry	3.62	0.88	3.73	0.68	4.98	0.80	4.72	0.86
Depressed	2.12	0.48	2.30	0.41	2.27	0.44	2.72	0.67
Guilty	1.83	0.23	2.23	0.51	2.08	0.47	2.56	0.64

Note: LoHo=low hostility, HiHo=high hostility

Table 39

Stressor VAMS Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	3.31	0.59	4.41	0.90	2.979	0.6193	4.45	0.81
Agreeable	8.56	0.82	6.52	0.90	8.32	0.87	7.00	0.83
Happy	8.27	0.72	8.00	0.78	8.56	0.67	6.92	0.81
Tense	5.20	0.83	5.20	0.76	4.56	0.71	6.37	0.93
Anxious	4.09	0.78	3.76	0.77	4.34	0.77	5.92	0.93
Relaxed	8.68	0.90	7.38	0.70	6.77	0.84	6.50	0.88
Discouraged	3.75	0.71	3.79	0.86	3.79	0.69	5.42	1.00
Annoyed	5.70	0.89	5.55	0.98	5.81	0.90	6.42	0.96
Sad	3.02	0.64	2.29	0.58	2.35	0.48	2.97	0.53
Irritated	5.45	0.91	5.64	0.91	5.08	0.84	7.25	0.94
Angry	4.04	0.81	3.20	0.65	4.08	0.75	5.82	0.87
Depressed	2.36	0.45	2.02	0.40	1.91	0.40	3.10	0.64
Guilty	1.86	0.30	2.29	0.58	1.50	0.23	3.20	0.73

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 40

Stressor VAMS Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	3.17	0.69	4.36	0.79	2.28	0.41	5.04	0.87
Agreeable	8.32	0.89	7.00	0.90	9.95	0.67	5.64	0.85
Happy	8.30	0.80	8.13	0.71	9.95	0.52	5.59	0.71
Tense	5.20	0.83	5.11	0.82	3.35	0.58	7.59	0.84
Anxious	3.87	0.74	4.11	0.86	2.92	0.54	7.45	0.90
Relaxed	8.97	0.86	7.30	0.83	8.16	0.87	5.07	0.79
Discouraged	3.77	0.74	3.58	0.83	2.33	0.43	6.97	0.93
Annoyed	5.75	0.92	5.27	0.96	4.31	0.84	8.07	0.91
Sad	2.30	0.57	3.25	0.70	1.57	0.18	3.81	0.63
Irritated	5.35	0.91	5.63	0.97	3.54	0.70	8.81	0.80
Angry	3.52	0.76	3.77	0.81	2.88	0.60	6.95	0.85
Depressed	1.80	0.40	2.52	0.46	1.40	0.14	3.57	0.69
Guilty	1.57	0.16	2.63	0.61	1.38	0.14	3.21	0.73

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 41

Stressor VAMS Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
VAMS	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
Nervous	3.20	0.69	3.80	0.74	2.92	0.57	4.14	0.99
Agreeable	8.67	0.88	7.27	0.91	8.50	0.84	6.34	0.96
Happy	8.85	0.81	7.63	0.70	8.52	0.76	6.32	0.71
Tense	5.11	0.93	4.91	0.75	4.04	0.67	7.03	1.01
Anxious	3.97	0.90	3.44	0.66	3.29	0.61	7.50	0.96
Relaxed	8.61	0.96	8.47	0.78	7.38	0.83	5.46	0.99
Discouraged	2.85	0.71	3.91	0.72	3.52	0.68	5.42	1.08
Annoyed	4.94	0.92	5.36	0.96	4.66	0.79	7.57	1.10
Sad	2.94	0.70	2.27	0.52	1.92	0.36	3.42	0.67
Irritated	4.91	0.86	5.86	1.01	4.50	0.81	7.96	0.91
Angry	3.76	0.91	3.61	0.77	3.86	0.75	5.60	0.89
Depressed	2.55	0.54	1.77	0.28	1.74	0.35	3.10	0.73
Guilty	1.91	0.38	1.91	0.39	1.38	0.13	3.10	0.86

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

however, in the Trp- condition, Hi TAnxiety participants demonstrated a larger increase in anxiety than Lo TAnxiety [$t(39) = -2.611$, $p = .013$] (see Figure 17). There were no other significant effects.

Tryptophan by Beck depression (BDI)

A main effect of Trp was found for Anxious [$F(1, 69) = 4.548$, $p = .037$], indicating that the participants in the Trp- condition demonstrated a greater increase in anxiety than the participants in the B condition. The main effect of BDI and the Trp x BDI interaction were not significant.

Change from math task to recovery period.

2 (B vs. T-) x 2 (Lo vs. Hi) ANOVAs were conducted on changes in cardiovascular and state affect measures from the math task to the 10 minute rest/recovery period. These analyses were conducted to identify the effect of Trp and psychological factors.

Cardiovascular Measures

Means and standard errors for recovery CV measures are shown below (see Tables 42-45).

Tryptophan by Cook-Medley (Ho).

A main effect of Trp was observed for DBP [$F(1, 78) = 5.520$, $p = .021$]; LVET [$F(1, 78) = 5.578$, $p = .021$]. Results indicated a greater decrease in DBP for the B condition participants compared to the Trp- condition participants. The results for LVET indicated a greater increase (which means a lowering of stress reactivity) in the B condition participants than in the Trp- condition participants. The main effect of Ho and the Trp x Ho interaction were not significant.

Figure 17. *Interaction Effect Wait Period Trp x Trait Anxiety VAMS ANX.*

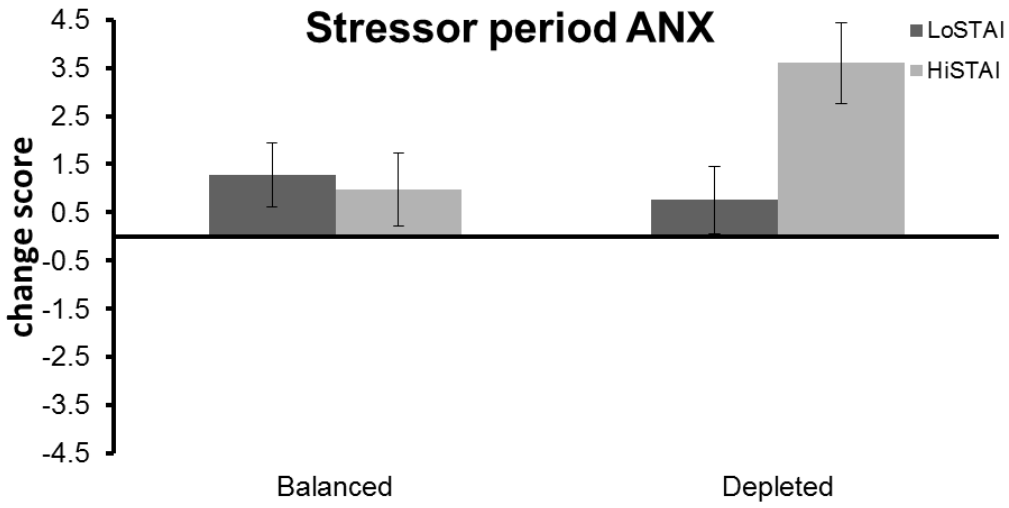


Table 42

Recovery Cardiovascular Means and SE for Trp x Hostility Groups

	Balanced (B)				Depleted (T-)			
	LoHo		HiHo		LoHo		HiHo	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	72.44	3.70	72.82	3.02	71.88	2.51	76.59	4.04
DBP	66.12	1.87	65.66	1.71	67.48	2.09	70.38	2.55
SBP	112.88	2.18	121.37	2.60	120.81	3.22	118.63	2.91
MAP	81.71	1.22	84.23	1.77	85.26	2.04	86.46	2.07
SV	127.58	6.96	118.45	8.03	124.09	9.87	106.06	8.72
CO	9.18	0.68	8.34	0.53	8.76	0.66	7.87	0.63
PEP	105.91	3.75	108.73	5.06	98.36	3.66	115.91	5.43
LVET	294.38	6.11	279.80	9.56	281.69	5.83	277.57	7.76
HI	17.58	1.61	13.37	1.23	15.39	0.75	12.63	1.47
TPR	773.19	50.82	915.05	98.38	939.16	102.24	1079.67	169.72

Note: LoHo=low hostility, HiHo=high hostility

Table 43

Recovery Cardiovascular Means and SE for Trp x TAnger Groups

	Balanced (B)				Depleted (T-)			
	LoTAnger		HiTAnger		LoTAnger		HiTAnger	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	68.79	3.01	78.98	3.06	71.77	2.74	76.05	3.55
DBP	64.73	1.64	67.50	1.91	67.14	2.08	70.32	2.48
SBP	116.82	2.46	118.90	2.89	122.20	3.07	117.48	3.19
MAP	82.09	1.58	84.63	1.57	85.49	1.85	86.04	2.31
SV	136.59	5.09	99.73	8.61	129.88	9.83	102.29	8.83
CO	9.39	0.55	7.64	0.58	9.24	0.70	7.47	0.56
PEP	104.62	4.30	112.07	4.57	100.45	4.56	111.10	4.69
LVET	296.25	5.25	270.47	12.20	281.46	5.66	278.41	7.62
HI	15.90	1.34	14.33	1.69	15.02	1.08	13.43	1.08
TPR	754.34	43.10	1006.82	129.89	866.74	81.98	1135.46	164.84

Note: LoTAnger=low trait anger, HiTAnger=high trait anger

Table 44

Recovery Cardiovascular Means and SE for Trp x TAnxiety Groups

	Balanced (B)				Depleted (T-)			
	LoTAnxiety		HiTAnxiety		LoTAnxiety		HiTAnxiety	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	69.26	3.22	78.14	2.97	71.64	2.90	75.30	3.71
DBP	63.63	1.52	69.00	1.94	67.86	2.37	69.35	2.23
SBP	115.69	2.30	120.19	3.16	121.48	3.50	119.57	3.02
MAP	80.98	1.36	86.06	1.79	85.73	2.03	86.09	2.13
SV	122.93	6.67	120.16	9.19	124.64	11.65	111.39	8.71
CO	8.40	0.53	9.17	0.73	8.68	0.73	8.20	0.69
PEP	111.67	4.18	102.19	5.08	103.53	5.31	109.23	4.69
LVET	292.32	6.48	278.45	10.99	281.30	6.26	283.19	7.40
HI	16.30	1.32	14.26	1.75	15.24	1.07	12.74	1.14
TPR	841.61	64.03	865.22	111.72	984.99	129.44	1032.35	152.45

Note: LoTAnxiety=low trait anxiety, HiTAnxiety=high trait anxiety

Table 45

Recovery Cardiovascular Means and SE for Trp x BDI Groups

	Balanced (B)				Depleted (T-)			
	LoBDI		HiBDI		LoBDI		HiBDI	
CV	*M*	*SE*	*M*	*SE*	*M*	*SE*	*M*	*SE*
HR	72.63	3.66	69.30	2.63	73.37	2.98	71.85	4.15
DBP	66.71	2.03	66.29	1.20	68.78	2.17	66.78	3.03
SBP	117.60	3.43	118.77	2.38	120.84	3.64	117.94	2.50
MAP	83.67	1.87	83.79	1.23	86.13	2.22	83.83	2.33
SV	123.82	5.75	130.23	8.77	120.11	9.32	121.14	12.85
CO	8.95	0.59	8.93	0.68	8.67	0.69	8.41	0.77
PEP	112.87	5.67	102.64	3.70	102.23	4.74	112.31	5.85
LVET	284.57	6.26	298.91	5.91	281.38	6.38	282.59	8.37
HI	14.89	1.45	14.89	1.58	15.50	1.07	12.75	1.26
TPR	810.14	55.39	819.59	68.26	971.96	113.70	1007.12	199.96

Note: LoBDI=low score on Beck's Depression Inventory, HiBDI=high score on Beck's depression Inventory

Tryptophan by trait anger (TAnger).

A main effect of Trp was observed for DBP [$F(1, 78) = 7.021$, $p = .010$]; LVET [$F(1, 76) = 4.65$, $p = .034$]. Results indicated a greater decrease in DBP for the B condition participants compared to the Trp- condition. The results for LVET indicated a greater increase in the B condition participants than in the Trp- condition. The main effect of TAnger and the Trp x TAnger interaction were not significant.

Tryptophan by trait anxiety (TAnxiety).

A main effect of Trp was observed for DBP [$F(1, 74) = 5.379$, $p = .023$]; LVET [$F(1, 72) = 4.528$, $p = .021$]. Results indicated a greater decrease in DBP for the B condition participants compared to the Trp- condition participants. The results for LVET indicated a greater increase in the B condition participants than in the Trp- condition participants. The main effect of TAnxiety and the Trp x TAnxiety interaction were not significant.

Tryptophan by Beck depression (BDI).

A main effect of Trp was observed for DBP [$F(1, 69) = 5.408$, $p = .023$] and LVET [$F(1, 67) = 5.917$, $p = .018$]. Results indicated a larger drop in DBP for the B condition participants and greater increase in LVET for participants in the same group compared to the Trp- condition participants. The main effect of BDI and the Trp x BDI interaction were not significant.

Discussion

The goal of this study was to examine the effects of low serotonin levels on cardiovascular and mood reactivity to interpersonal conflict. In particular this study addressed the question of whether psychological factors including hostility, anger,

anxiety and depression, which are known to play a role in both cardiovascular and mood reactivity, modulate, under stressful conditions, the response of individuals to low levels of serotonin, which in and of itself affects reactivity. The study examined a relatively young, physically and psychologically healthy sample of male and female, racially representative, university students. Due to the difficulty of directly manipulating central nervous system serotonin levels, acute tryptophan depletion, a method which reduces the availability of serotonin production's rate-limiting precursor was utilized. Research previously conducted in our laboratory failed to find strong associations between cardiovascular or mood measures and the interplay amongst tryptophan status and levels of hostility as assessed with the Ho scale. The present study reexamined the potential role of hostility in a larger sample and additionally investigated the possible modulation of serotonin's role in cardiovascular and mood stress-related reactivity by trait anger, trait anxiety, and depressive symptomatology.

Tryptophan

Cardiovascular.

Rest.

Given the results found in several studies (Drummond & Quah, 2001; Koszycki et al. 1996; Williams et al. 2001) and preliminary findings from the current study (Neumark et al., 2002) it was hypothesized that changes in tryptophan levels would have little impact on cardiovascular reactivity during the waiting period. Based on the results obtained, it appears that the tryptophan manipulation had no effect on cardiovascular functioning during the wait period. This lack of effect is consistent with the results found in other studies. Koszycki, et al. (1996) failed to find any effect of Trp- on HR, SBP, or

DBP following a 5 hr wait period. Monteiro-dos-Santos et al. (2000) examined the effects of ATD on a healthy relatively young sample of males and females prior to, and during, a public speaking task. They obtained measures of HR, SBP, and DBP during baseline, wait, stressor anticipatory period (preparing the speech), stressor, and recovery period. They found no CV effects as a function of Trp condition. Beacher et al. (2011) also failed to find any effects of ATD on cardiovascular function during the wait period. Leyton et al. (2000) found no differential effects of Trp- compared to B for HR and SBP; however, they found that DBP in the B condition decreased whereas there was no similar decrease in the Trp- condition. The studies by Leyton et al. and Monteiro-dos-Santos et al. are similar to the current study, in that the wait period precedes the stressor period and there may be an anticipatory component yet overall none of the studies found effects of ATD on CVR. The Leyton et al. study is not directly comparable to the current study in that participants carried out cognitive tasks during the wait period, and more importantly perhaps, their sample consisted only of females. A single study (Booij et al., 2006) with patients who had suicidal ideation showed an association between Trp and cardiovascular function. The researchers reported that in patients with a positive history of suicidal ideation increased HR followed a Trp loading. It appears that tryptophan levels, in the absence of other factors, do not effect cardiovascular functioning in healthy adults.

Stress.

The combination of the mental math task coupled with harassment resulted in a typical cardiovascular stress response amongst participants in both the Trp- and B conditions. Tryptophan levels were not associated with any between group differences, a finding that did not support the hypothesized increase in CVR as a function of Trp

depletion. This finding was consistent with the findings in Monteiro-dos-Santos et al. (2000), as well as in Leyton et al. (2000), described above. An ATD study that used a simulated public speaking stressor (Shansis et al., 2000) failed to report the results of the cardiovascular measures, which may be an oversight, but is more likely indicative of null findings. Another possible explanation for the lack of findings in the current study has to do with the nature of the stressor. Bjork, Dougherty, Moeller, Cherek, and Swann (1999), found that moderate levels of provocation resulted in increased aggressive responses as a function of an ATD, whereas a high level of provocation did not produce these differences. Wingrove, Bond, and Cleare (1999) examined whether high trait hostility males would exhibit different outcomes under Trp- or Trp+ conditions when engaged in an ostensibly cooperative game with an unknown partner that is rigged to cause failure in the study participants. Increased CVR as measured by SBP and DBP was observed in the Trp- condition with a decrease observed in the Trp+ condition. The level of "provocation" in this study was in all likelihood lower than that used in the current study.

Serotonin may be viewed as a mediator of mood and cardiovascular responses and, as such, the lack of significant findings is difficult to explain, especially in light of the ATD plasma results indicating a successful differentiation between the groups. However, as noted above, many biological systems are involved in the regulation of mood, cardiovascular function, and the response to stress. If one views serotonin as a modulator of these other systems, as opposed to a direct mediator, the findings of this and other studies may be more understandable. The capacity of serotonin to modulate the responses of the other systems, such as neuroendocrine systems, may be overwhelmed during extremely stressful and provocative situations, allowing more direct unmodulated

effects of the underlying mechanisms, resulting in varied responses.

Recovery.

During the recovery period DBP decreased to greater degree in the B condition than in the Trp- condition. This blunted recovery effect was also found for LVET, which increased to a greater degree in the B condition compared to the Trp- condition. Leyton et al. (2000) report that HR, SBP, and DBP returned to baseline following the stressors with no between Trp condition effects. They did not specify the latency to baseline return. Monteiro-dos-Santos et al. (2000) also report no effects of ATD on CV recovery, and they too do not provide specifics as to the duration of the recovery period. The definition and duration of recovery periods varies widely between studies and can have significant impact on results (Gregg et al., 1999). It is possible that the differences between the findings in the current study are related to the length of time over which recovery CV measures were obtained. As described above the current study employed a very provocative stressor. Recovery from an extremely provocative stressor may engage the 5-HT stress response differently than a lesser stressor and result in the blunted response observed under depleted Trp conditions. Although these latter two findings are consistent with the hypothesis it is important to be cautious with regard to over-interpreting these cardiovascular findings in light of the non-significance of most of the cardiovascular measures as well as the as yet unclear effects of serotonin in the vasculature.

Mood.

Rest.

Tryptophan depletion resulted in participants becoming more anxious and hostile as well as an overall increase in negative mood, based on the POMS. Based on the

VAMS a similar pattern was observed, Trp- participants reported being more nervous, annoyed, and depressed.

Ellenbogen et al. (1996) investigated a sample of females only and reported Trp-related increases in POMS-based depression, anxiousness, tiredness, confusion, as well as a decrease in sureness with similar VAMS-based results. While the results of Ellenbogen et al. are partially consistent with the results of the current study (with a caveat in that the mood findings in the Ellenbogen et al. study did not show temporal stability), the findings in the current study are at odds with the findings of several studies, which found no mood effects of ATD. Benkelfat et al. (1994) that failed to detect any differential effects on mood as a function of Trp levels in a sample of males who did not have any family history of mood disorders. Moreno et al. (1999) did not find any ATD-based mood differences in 8 healthy females and 4 healthy males with no history of affective disorders. Schmeck et al. (2002) did not find any differential effects based on Trp condition in a mixed sample of healthy males and females. Neumeister et al. (2004), and Moreno et al. (2010) reported null findings as well. A possible explanation for the contrast between the findings in these studies and the findings in the current study may be found in the design of the studies. In the current study participants were aware that following the 5.5 hr wait and shortly after completing the mood ratings they would be performing a computerized mental math task. The anticipation of an upcoming stressor may in and of itself act as a low-level stressor.

There are two problems with this tentative explanation. Firstly, if the wait period is indeed a milder stress period there should have been cardiovascular effects; however, as reported above there were none. It may be, however, that there are different thresholds

at which effects of cardiovascular and mood reactivity are triggered. The lack of parallelism between CVR and mood has been found in several studies (e.g., Davies et al., 2006; Gregg, James, Matyas, & Thorsteinsson, 1999; Maron, Shlik, & Nutt, 2008; Ohira et al., 2009). Secondly, the lack of the anticipatory effect in other studies as a reason for the different finding in the current study may not suffice as an explanation. Monteiro-dos-Santos et al. (2000) failed to find any mood effects as a function solely of Trp-manipulation; even though, as in the current study, participants were aware that they would soon be participating in a stressor. There are two differences between their study and the current one. The stressor tasks are different and it is possible that a simulated public speaking task does not elicit the same anticipatory stress. This difference may interact with the differences between the sample populations. In the current study participants were almost exclusively young (M = 23.7 years of age) university students, whereas the participants in the public speaking study were somewhat older (M = 28 years of age) and employees of the hospital where the study was conducted. It is possible that differences in life experience may account for the discrepancy in that the participants did not find the upcoming task stressful. Though the authors reported that there were significant increases in anxiety and CV measures that are consistent with a stress response, they did not report the CV data; therefore a comparison between the response levels in their study and the current study could not be made. Additionally the anxiety scales differed between the two studies making any comparisons difficult. There have been two other ATD studies that have used a simulated public speech stressor. Shansis et al. (2000) failed to find any mood effects and neither did Mortimore et al. (1997). The latter study was reported on only in abstract form with no details as to the sample, no data

were provided, and it is unclear whether any wait period measures were obtained. In the former study, although mood measures were obtained prior to the simulated public speaking task, they were obtained prior to memory and attention tests that preceded the speaking task. It is unclear as to the duration of the test period. The design differences render comparisons between the studies difficult as the anticipation of the simulated public speaking may not have yet occurred at the time that mood measures were obtained.

Leyton and Young (2000) reported two statistically significant findings based on a 16-item VAMS during a wait period prior to participants engaging in a 10-min preparation for a 5-min speech and a 5-min mental math task. Participants in the Trp- condition, reported decreased liveliness and blunted increase in restlessness (those in the B group reported more restlessness). Direct comparisons between the Leyton and Young study and the current study cannot be made, as the Leyton and Young study, similar to the study by Ellenbogen et al. (1996) was carried out with females only.

Overall it appears that the Trp- related increases in negative mood in the current may be a function of a low-level stressor, the anticipation of an upcoming stress.

Stress.

Contrary to expectations of increased negative affect as a function of lower Trp levels during the stress period, only a single VAMS-based finding of a greater increase in anxiousness in the Trp- compared to the B condition was found.

Evers et al. (2005) found no effects of Trp condition following an hour-long series of cognitive tests including a reversal learning tasks during which participants had to choose between two presented stimuli that were probabilistically associated with reward

or punishment. As soon as the participants reached 80% correct predictability the stimulus-reward contingencies were reversed. This paradigm was expected to be somewhat stressful for the participants. There were no effects of Trp-levels on reported VAMS-based levels of mood that included depression, anger, fatigue, vigor, and tension. From the data presented in the report it appears that the reversal learning paradigm coupled with the other cognitive assessments failed to elicit lowering of mood in either Trp condition, a result that may indicate that the tasks were not experienced as stressors.

Abbott et al. (1992) failed to find any differential effects of Trp levels following exposure to a cold pressor. The authors posit that the lack of findings may be related to the participant selection criteria. As the study involved the administration of morphine extensive screening of the participants resulted in a sample that had "unusually low ratings for all aspects of affect" (p.64) based on low POMS baseline scores. The participants may have been relatively low in susceptibility to any further mood lowering. It is more likely that there are other reasons for the findings. In the current study exclusion criteria were quite stringent (see Appendix) resulting in a sample population that was quite low as well; however, mood decreases were nonetheless observed as an effect of ATD condition in the wait period.

Leyton et al. (2000) reported greater increased negative mood in the Trp- versus the B condition following the mixed speaking and mental math stressor. Trp- condition participants compared to B condition participants reported greater negative mood on all of the POMS scales as well as on the VAMS-based Happy, Irritated, Lively, and Clearheaded. The authors refer to this combination stressor as "a mild threatening psychological challenge" (p.54). Two aspects of this study differentiate it from the

current study. Firstly, as mentioned previously, these results were obtained in an all-female study, making it difficult to compare to the results of the current study. Secondly, the nature of the stressor was quite different. None of the ATD stress studies utilized an interpersonal conflict. In general the stressors were relatively mild and did not elicit high levels of hostility or anger. The current study utilized a paradigm that resulted in very high levels of negative affect on POMS-based anxiousness, hostility, depression, confidence, confusion and overall negative mood. Similar results were obtained based on the VAMS, with increased negativity on nervousness, agreeableness, tenseness, anxiousness, relaxedness, discouragement, annoyance, irritation, and anger (Neumark, 2002). Although partial η^2 values must be treated with caution due to the additive effect, nonetheless, given values on POMS measures of .544 for anxious, .444 for hostility for example, the effect size is significant indicating a strong mood response.

Too high levels of provocation though, can mask between group differences due to ceiling effects. Bjork, Dougherty, Moeller, Cherek, and Swann (1999), found that moderate levels of provocation resulted in increased aggressive responses as a function of an ATD, whereas high levels of provocation did not produce these differences. It is a strong possibility that the failure to find between ATD-condition differences in mood responses to the stressor is a result of a ceiling effect.

Overall, based on the results in the current study, within the context of the findings of other researchers, the differential mood lowering effects of Trp- in healthy individuals is unlikely to be observed in situations of either too low or too high stress.

Pihl et al. (1995) posed the question of how independent or interdependent, mood, depression, and aggression are and what the effects of this relation might be over the

long-term, they called for further research into factors such as personality that could account for the altered susceptibility to heightened aggression in Trp- conditions.

Tryptophan x Psychological Factors

Tryptophan x Cook-Medley hostility (Ho).

Cardiovascular and mood.

The general lack of findings related to hostility's influence on Trp's effects is somewhat surprising. During the wait period perhaps less so; however, findings by Finn et al. 1998 appear to be at odds with the findings in the current study. Their study examined the influence of trait hostility as assessed with the Ho as in the current study. A unique feature of their study was that they used five different levels of manipulation, a 25 g Trp-, 50 g Trp-, 75 g Trp-, and a 100 g Trp- mixture as well as a 102.3 g B mixture. The three latter Trp- mixtures resulted in approximately the same level of depletion that was greater than with the 25 g mixture; however the four levels did not statistically differ from each other. All Trp- differed from the B condition. Hostile mood as an outcome measure was inversely proportional to Trp plasma levels, while associations for anxiety and depressive although in the right direction, did not reach statistically significant levels. In terms of the interaction between hostility and Trp, they reported that in the Hi Ho group only, levels of plasma were inversely proportional to hostile mood as well as anxiousness, evidence of a modulatory effect and inconsistent with the findings in the current study.

There are several important differences between the two studies. The sample in the current study was composed of males and females whereas the Finn et al. sample was an all male sample. On the one hand this should make the inconsistent findings more so,

as in the current sample approximately half of the participants were women who as discussed previously, appear to be more susceptible to effects of aTrp- on mood, on the other hand and probably more validly, the levels of Ho in the two studies differed dramatically. In the study by Finn et al. (1998) the mean and median values were 23.9 and 24, respectively, while in the current study, the means and median were 17.1 and 17, respectively. The levels in the current study are not surprising given the exclusion critera and intensive screening. The higher levels make it more likely that there would be different findings especially in light of results in the Finn et al. study that showed that higher trait hostility levels were associated with greater increases in hostile mood. A second difference between the studies based on the screening criteria and the resultant characteristics of their sample. There were no substance usage exclusions other than participants being asked to not use substances for 24 hr prior to the study and there was no verification of this on the testing day other than observation, "There was no evidence that subjects had been drinking or consumed dugs the morning of their laboratory session (i.e., none smelled of alcohol or appeared intoxicated" (p.177). This is in direct contrast with the rigorous screening in the current study based on structured interviews, and day-of-testing urine analysis that in fact resulted in several last-minute exclusions. Though there were no observable signs, all the excluded participants in the current study admitted consuming substances within the prior 24 hr when shown the results of the substances screening. Higher levels of hostility in the Finn et al. study make it even likelier that some of the participants had not respected the 24 hr prohibition, as higher levels of hostility have been shown to be associated with higher levels of substance use (e.g., Gossop & Roy, 1976).

Likely related to this issue, Finn et al. who had assessed levels of anti-social traits in their sample (based on the MMPI) reported that the combination of high trait hostility and anti-sociality in 1/3 of their sample suggested that a significant number of their participants were personality disordered. In the current study, the rigorous screening process made it unlikely that the sample contained any personality disordered participants. Personality disorders in and of themselves likely effect mood responses in general, and increase the likelihood that participants in the Finn et al. study had consumed substances that may have exacerbated their hostile mood. Personality disorders especially anti-personality disorder are highly associated with substance use and substance use disorder (e.g., Messina, Wish, & Nemes, 1999; Verheul, van den Bosch, & Ball, 2005). In fact Messina et al. report that 90% of anti-personality disordered persons have a comorbid substance use disorder.

Even more germane to the current study a third difference is of importance not only in comparing the results of these two particular studies but of the broader field as a whole. Finn et al. (1998) in the discussion of their findings, report that contrary to their hypothesis, a substantial source of the Trp-induced mood change finding was as a result of the decrease in negative mood due to augmentation of Trp plasma levels following the "control" groups ingestion of the Trp-containing B mixture. The implications of this augmentation of Trp in the control group will be discussed later in the dissertation.

During the stress period as well, differences based on trait hostility were not significant. Wingrove, Bond, and Cleare (1999) examined whether high trait hostility males would exhibit different outcomes under Trp- or Trp+ conditions when engaged in an ostensibly cooperative game with an unknown partner. The game is rigged to cause

failure in the study participants thereby heightening their arousal and frustration. There were statistically significant differences of increased CVR as measured by SBP and DBP observed in the Trp- condition with a decrease observed in the Trp+ condition. Additionally, although there were no Trp-based differences on the main variables of interest in their study, anger and verbal aggression, they did find Trp-based group differences with increased restlessness and incompetency associated the lower level of Trp. As discussed above with regard to the wait period and the findings of Finn et al. (1998) the differences were likely a result of the contrast between Trp- and the augmentation of Trp, rather than as a function strictly of Trp-. It is even more likely that this was the case in the Wingrove et al. study as their augmentation consisted of 10.3 g of Trp compared to the 2.3 g in the Finn at al. study. A second difference that may account for the discrepant findings between Wingrove et al. and the current study is as mentioned before is that the level of "provocation" in their study was in all likelihood much lower than that used in the current study, and therefore a ceiling effect in the current study may account as well for a portion of the inconsistent finding between the two studies.

Generalization as to the presence or lack of associations amongst hostility and Trp as well the possible modulation of the serotonergic stress response by hostility should not be made solely on the basis of the relative lack of findings in this study based on the Ho. As described previously, various measures have been utilized in assessing hostility and have been hypothesized to tap into different elements of what increasingly appears to be a multidimensional construct. Expressive and neurotic (suppressed) hostility have been shown to differentially effect cardiovascular responses to harassment (Miller, Dolgoy, Friese, & Sita, 1996). Studies using similar stressors have found or failed to find

differences based on the hostility measure used (Mills, Schneider, & Dimsdale, 1989).

The Ho scale has been characterized as a measure of hostility, however, Costa, Zonderman, McCrae, and Williams (1986) argue that this is misleading since anger items are not represented in the Ho. According to the authors, "cynical mistrust" might better describe the dimension that the Ho taps, as two main factors, cynicism and paranoid alienation, emerged from their analysis. According to Schill & Wang (1990) the Ho is not an index of aggression, but rather suspiciousness, resentment, frequent anger and cynical distrust of others, which may not be unique to or the encompass the complexity of the various components of hostility as a construct. Prkachin, Mills, Kaufman, & Carew (1991) discuss findings related to the use of the Ho and conclude that Ho may be interpreted as cynical hostility. Sallis, Johnson, Trevorrow, Kaplan, and Hovell (1987) failed to find an association between hostility and blood pressure during their mental arithmetic and cold pressor studies, concluding that the Ho and cynical hostility are unrelated to blood pressure reactivity. As discussed earlier, this may have more to do with the lack of elicitation of hostility-related affect during stressors that are less related to the interpersonal domain (in which hostility and anger are more likely to be factors). In the Suarez et al. (1993) study on cardiovascular and emotional responses in women, an increase in CVR was reported, including blood pressure increases as a function of higher Ho scores and harassment, with no differences in a non-harassment unsolvable anagram task.

Tryptophan x trait anger (TAnger).

Cardiovascular and mood.

As with hostility there were no modulatory effects of trait anger on Trp for

cardiovascular measures; however, in contrast to hostility, trait anger did modulate Trp effects on mood during the wait period. Contrary to the hypothesis that predicted no effects of mood during the wait period Hi TAnger participants in the Trp- condition reported an increase in POMS-based anxiousness compared to the Lo TAnger participants in the same condition. Under the balanced Trp condition, both Hi and Lo TAnger participants reported decreased anxiousness. A similar result was found based on VAMS Anxious with Hi TAnger in the Trp- condition showing a greater stress response than their Lo TAnger counterparts. In the B condition no between TAnger group differences were found. A somewhat anomalous result was found on VAMS Happy, with no TAnger-based differences reported in the Trp- condition; however, in the B condition, Hi TAnger participants showed a larger decrease in happiness.

To the authors knowledge there are no other studies that have looked at trait anger in an ATD study; however, there are a few studies that have examined aggression. Salomon, Mazure, Delgado, Menida, and Charney (1994) reported on an ATD study with aggressive intermittent explosive disorder patients. In their study there were no differences in level of expressed hostility between the depleted or balanced conditions. One lone finding of decreased rather than increased irritability in the Trp- condition was found. This study; however, was conducted in a "neutral, clinically sterile environment" during a 7 hr wait period with no stressor tasks in the protocol. As there were no specific triggers of their anger it is not surprising that there were no effects found. Similarly, Schmeck et al. (2002) categorized participants as high or low on trait aggression. The study found no differences in anger in the Trp- condition between high- and low- trait aggression males but did report a significant finding in the Trp- condition of decreased

mood and increased anger and arousal in high- but not low- trait aggressive women.

Effects of trait anger on Trp for mood were not found during the stress period possibly due to the ceiling effect described above.

Tryptophan x trait anxiety (TAnxiety).

Cardiovascular and mood.

As with hostility and trait anger there were no modulatory effects of trait anxiety on Trp for cardiovascular measures. The results of the current study are at odds with those of Davies et al. (2006) who reported an increased pressor response (SBP and DBP rise without a concurrent rise in HR) in a group of 14 (of a total of 27 mixed anxiety disorders recovered patients) recovered from panic disorder patients as a function of Trp depletion compared to non-depletion and the administration of the benzodiazepine antagonist flumazenil. In the group of 6 recovered anxiety disorder patients whose stressor consisted of recalling an autobiographical script of a meaningful anxiety episode there were no statistically significant differential effects of Trp status.

To further complicate any generalizable inference with regard to Trp and cardiovascular reactivity it is unclear from the report on the study as to whether recovered meant currently untreated or recovered due to current use of SSRI's which clearly affect the serotonergic system.

There were also no modulatory effects of trait anxiety on Trp for mood during the wait period; however, consistent with the study's hypothesis Hi TAnxiety participants in the Trp- condition reported higher levels of POMS-based hostility than Lo TAnxiety participants. There were no differences based on TAnxiety levels in the B condition. The same pattern was found based on VAMS anxiousness. This is consistent with the Davies

et al. (2006) study who similarly found an effect of trait anxiety and Trp on anxiousness.

Tryptophan x beck depression (BDI).

Cardiovascular and mood.

As with all the other psychological factors there were no modulatory effects of BDI on Trp for any cardiovascular measures in any time period. BDI did however modulate Trp in relation to mood during the wait period.

Hi BDI participants in the Trp- condition differed from Hi BDI participants in the B condition, reporting higher levels of POMS-based anxiousness. Lo BDI participants did not differ from Hi BDI participants as a function of Trp status. Hi BDI participants in the Trp- condition reported more anxiousness than Lo BDI participants under the same condition. In the B condition there were no differences as a function of BDI levels. Hi BDI participants became more annoyed in the Trp- condition as well, with the pattern paralleling the findings with anxiousness.

Wingrove et al. (1999) found no BDI-based effects of mood in their "trait hostility" study described above; however, they found a correlation between the BDI scores and changes on the Bond and Lader (1974) Mood Rating Scale, a scale that contains bipolar items, for a single item Happy-Sad. More depressed participants in T+ condition becoming happier and a trend for more depressed participants to become sadder in the Trp- condition. These results are consistent with those of the current study. Wingrove et al. (1999) failed to find any BDI-based effects during the stressor portion of their study also consistent with the current study.

Bell, Hood, and Nutt (2005) report that early studies with positive findings of reduced mood in healthy participants had participants whose baseline depression scores

were at the upper end of the normal range while later studies examined participants with lower depression scores. The results of the study that this dissertation is based on are at odds with this explanation, as participants in the current study had relatively low scores (any evidence of depression or even moderate-to-high levels of non-clinical depressive symptomatology were grounds for exclusion from the current study), and nonetheless showed effects based on the variability within even the low range.

General Issues

CV measures in the current study did not parallel the mood findings. There is a general finding of a lack of parallelism amongst the CV responses and the psychological responses. Ohira et al. (2009) reported acute stressor related increased CVR in the Trp- condition as described above but without any concomitant between groups psychological effects; however, their only mood measure was a measure of subjective stress. Maron, Shlik, and Nutt (2008) found no correlation between blood pressure and reported levels of anxiety in their study although both BP and anxiety increased in the Trp- condition, leading to suggest that 5-HT has "distinct and different effects on these 2 stress domains, and that the cardiovascular responses are not merely a secondary consequence of the psychological changes (p.417)."

The finding that different psychological traits or factors can lead to similar stress responses effects has been reported by others. Flaa, Ekeberg, Kjeldsen, and Rostrup (2007) used an instrument designed to examine the biological basis of personality traits (Karolinska Scale of Personality) that is composed of 15 subscales including anxiety, guilt, suspicion, several types of aggression, as well as several others, in a study of 87 young males selected for the bipolar extremes and center of blood pressure screening.

Participants were exposed to a 1-min cold pressor task (ice-water hand immersion) followed 30 min later by a mental math task, in counterbalanced order. Two psychological variables, verbal aggression (shouting, arguing, etc.,) and detachment (no need for close relationships) were positively associated with DBP in the mental math task. In the cold pressor task both SBP and DBP were positively associated with detachment. They conclude that cardiovascular stress reactivity "is dependent on different personality traits, without any single trait being clearly dominant."

An additional issue worth noting is that the balanced condition of a tryptophan manipulation study may not be a neutral condition. Results of the analysis of Trp levels showed that both total and free levels of Trp rose significantly in the balanced condition during the wait/anticipatory period. Total Trp levels doubled and free Trp almost doubled during this period. While the balance group has traditionally been operationalized as a null change reference for the Trp- condition the increase in Trp levels necessitates an examination of potential issues. Trp loading has shown both positive and negative mood changes. While most studies demonstrate positive mood changes (e.g., Wingrove et al., 1999) or no effect (e.g., Luciana, Burgund, Berman, & Hanson, 2001), there are several that have demonstrated an increase in negative affect. Dougherty et al. (2008) reported increased negative mood ratings following the consumption of a 100g Trp+ bolus but not after a 50g Trp+ bolus. Sobczak et al. (2002, 2003) compared a 7g Trp intravenous challenge to placebo and found increased negative affect including increased anger, depression, fatigue, tension, and lower vigor and alertness. While the intravenous challenge resulted in an increased plasma Trp/∑LNAA ratio of 1500%, the Trp+ conditions of 100g and 50 g in the study by Dougherty et al. resulted in ratios of 570%

and 372% respectively which is much higher than the ratio found in the study that this dissertation is based on. Silber and Schmitt (2010) in their review of the effects of Trp loading on cognition, mood, and sleep, conclude that the mood effects of Trp loading are inconsistent, with positive, negative, and no effects being reported. The study by Pihl et al. (1995) included a Trp+ condition and found lower levels of aggressive responding in the Trp+ condition than in the Trp- condition. Bjork et al. (1999) point out that there may be other explanations for some of the ATD findings that are due to confounds of Trp loading. In the study by Pihl et al. (1995) increased aggression may have been due in part to irritability reported in the study, but with the irritability due to food deprivation, while in the Trp+ condition a sedative effect may have led to reduced aggression. Increased levels of Trp have been shown to have a sedative effect (e.g., Leathwood, 1987; Yuwiler, et al. 1981). Firk and Markus (2009) reported mixed findings based on which mood measurement instrument was used during a Trp loading study with a wait and an acute stress period that consisted of a 5-min speaking task and a 5-min mental math task, both in front of an audience. Based on the POMS, positive mood increased on Vigour as a function of Trp+. Based on the Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988) there were no Trp related effects.

Study Limitations

Participants in this study were excluded if they had a history of substance abuse or an Axis I disorder. Previous studies have often failed to exclude individuals based on past substance abuse, either implicitly as in the Miller et al. (1998) study or explicitly as in the Dougherty, Bjork, Marsh, and Moeller (1999) study. In order not to exclude aggressive/hostile individuals, studies such as theirs do not use substance usage history as

a criterion. While many recent studies assess recent substance use on the testing day through blood or urine samples, several, such as the study by Finn et al. (1998) described above. The Suarez et al. (1998) study did not report any participant evaluative criteria that included substance usage pattern issues, neither did they mention the utilization of either urine analysis or self-report on the day of the laboratory testing.

Although no statistical analysis was conducted, it became apparent to the researchers involved in this study that the higher hostile individuals, both men and women, who applied to this study, were more likely to indicate having histories of elevated substance use. It was extremely difficult to locate, recruit, and include in this study higher hostile individuals who were not disqualified based on these exclusion criteria. It is thus possible that the studies with higher hostility means may have included individuals who would have been excluded from this study. For women, the hypothesized serotonergic effects of oral and injected contraceptives resulted in this being an exclusionary criterion. Most females, particularly those who appeared to be high hostiles, were excluded on this basis.

Another potential limitation related to participant characteristics is race due to the unequal representation of particular racial groups in the Trp conditions. Both Blacks and Asians were over-represented in the Trp- condition. Differences between Asian and Caucasian individuals, with regard to mood, behavior, and anger expression, have been reported. Friesen's study (as cited in Drummond & Quah, 2001) reported that Japanese individuals attempted to mask negative emotions by smiling, whereas their North American counterparts did not typically do so. In the current study, three participants of Asian descent were the only ones to describe the harassing confederate as pleasant and

helpful and only when pressed for details did they admit that the confederate was "difficult". In the study by Drummond and Quah, Asians reported a smaller increase in anger-related feelings, when describing situations that would typically elicit such feelings, than their Caucasian counterparts although this difference failed to reach a statistically significant level.

Future Directions

Standardization of protocols and measures.

Previously it was pointed out there are ongoing difficulties with standardization, clarity, and precision in cardiovascular research to a large degree the same is true in the area of ATD research. Reilly et al. (1997) stated the following in the conclusion of their ATD review:

> The inconsistent findings and failures of replication evident in the literature, are the inevitable result of the use of widely varying protocols for TRP depletion. These have differed in respect of pre-test diet, composition of active drink and the nature of the control preparation. No consensus has yet emerged on the use of a standardized protocol to resolve these difficulties. The main components of such a standardized protocol would be, firstly an adequate dietary baseline (attained by low-TRP diet or overnight fast alone); secondly a sufficiently potent (yet tolerable) TRP-depleting drink, such as Young's original 100 g preparation; and finally, the inclusion of an inactive control. The free and total TRP/ LNAA or TYR/LNAA ratios need to be measured for test and control procedures and sufficient care taken to ensure that the control procedure is as inactive as possible. The validity of such a standard TRP-depleting protocol could be tested by attempted replication of some of the key findings we have outlined. The application of such a standard protocol would, undoubtedly, increase the overall validity and generalizability of studies (p.389).

Given questions regarding the effectiveness of ATD due to various factors including the competition for Trp to cross the blood brain barrier, and the varied findings between studies, researchers (e.g., Carpenter et al., 1998; Salomon et al., 2003; Williams

et al., 1999) have begun to use techniques that include direct measurement of CSF Trp and 5-HIAA levels, notwithstanding Nishizawa and colleagues' reservations as to the accuracy and meaning of CSF levels described above. Moreno et al. (2010) report a statistical trend of an inverse relation between CSF-Trp and depressive ratings in a sample of remitted major depressive episode patients who were either medication free or still being treated with SSRI's but no similar finding in the 7 healthy control participants. There was no association with anxiety, neither in the healthy nor in the patient groups. The study did not include any active stressor component and as such is more likely a purer measure of the effects of ATD. The authors conclude that ATD studies should include direct measurements of Trp as CSF Trp levels did not correlate as highly with plasma Trp as did CSF Trp and the Trp/∑LNAA. The caveats with regard to the research tools described above need to be addressed in nearer rather than further future. The vast amount of resources devoted to studying the issues in these domains needs to be used wisely and efficiently.

Emerging methodologies.

Williams et al. (2001) examined the CSF levels of 5-HIAA the metabolite of 5-HT. Contrary to their expectations individuals higher CSF 5-HIAA showed increased CVR compared to individuals with low CSF 5-HIAA. Williams and his colleagues speculated that this finding was the effect of the 5-HTTLPR genotype influencing the cardiovascular responses to stress, independent of CSF 5-HIAA level. 5-HTTLPR is a polymorphism of the promoter region of the serotonin transporter gene. The long (*l*) allele is associated with double the activity of the short (*s*) allele. This increased transcriptional efficiency and homoallelism (ss or ll) and heteroallelism (sl) is associated

with between race, gender, and gender x race differences on personality dimensions such as anxiety, anger, hostility, depression, and impulsiveness. Differences in CVR have been reported with individuals with an *l*-*l* or *l*-*s* genotype having higher levels of CSF 5-HIAA than those with the *s*-*s* genotype and both these factors, of genotype and CSF 5-HIAA levels, are independently associated with greater cardiovascular responses to stress. Race is associated with different 5-HTTLPR genotypes, showing significant variation in the frequency of the *l* allele. Blacks exhibit this allele in more than 70% of their population, Whites exhibit it in 50% to 60% of individuals, and less than 30% of Asians have the *l* allele.

In the future it may not be sufficient to merely categorize individuals based on depletion paradigms and phenotypic expression alone, but also accounting for the genotype in interaction with race and gender. These factors not only influence outcome but may also play an important role in defining individual psychological traits and states.

Serotonin as a precursor to traits, states, and the stress response.

There is a growing body of research that shows genetic and in particular serotonin-related genetic influences on CVR. Serotonin-related genetic differences also appear to be associated with psychological factors. Over a 4-yr period, 361 university students participated in a study by Conner et al. (2009) and responded daily for 30-day period once per year to a web-based survey that contained questions about their emotional states. The Likert-type 5-point scale asked participants how they felt at the moment (not at all to extremely) and included two items for each emotion/mood which were averaged together yielding a single score for each emotion/mood. Items included sad and dejected, cheerful and happy, jittery and nervous, as well as angry and hostile.

The latter two yielded a hostility score. Males only, showed significant differences in hostility on 4 of the 5 genotypes that were examined. Males only, as well, had genotypes related to sadness and anxiety; however, to a lesser degree than hostility. Happiness was not associated with genotype for either gender. Men who had two low-activity HTR1B chromosomes (the HTR1B genes code for the 5-HT_{1B} receptors) reported the highest levels of anger and hostility, those who had two high-activity chromosomes reported the lowest levels and the heterogeneous chromosome males reported an intermediate level. The authors raise several interesting points with regard to their findings. The finding that sadness, anxiety, and hostility are associated with the same genotype was not expected; however, it is consistent with findings from behavioral and psychological research that show links between all three, as well as the finding that serotonergic antidepressants and anxiolytics are effective in treating depression and anxiety but also aggressive behavior.

As mentioned above the with regard to hostility, polymorphisms of the 5-HTT gene (SKC6A4) may be related not only to mood outcomes but are also associated with the personality characteristics or traits themselves. The Conner study described above showed not only outcome measures but human phenotype related to aggression is associated with 5-HTT polymorphisms and that depression and anxiety are also associated with these polymorphisms. Lesch et al. (1996) reported on two studies with over 500 participants in total and reported that the s allele accounted for 7-9 % of inherited variance in anxiety-related personality traits. Sen, Burmeister, and Ghosh (2004) performed a meta-analysis of 26 studies on over 5,500 participants and found a trend for the association between the s allele and anxiety-related personality trait scores. Only one study found an association with the l allele. Upon further analysis they found

that different assessment instruments effected the association. Based on studies using the NEO (ref) and NEO neuroticism showed a strong association with s allele $p = 0.000016$.

Nordquist & Oreland reviewed research on early brain development that may help explain the complex and too frequently contradictory findings both within healthy and at risk populations, as well as guide future research in early identification, prevention and treatment of a range of psychological disturbances. “Despite overwhelming amounts of data supporting a robust association between these polymorphisms and shaping of behavioral traits and vulnerability to develop psychiatric disorders, the molecular and biological events leading to observed associations are still not fully understood” (p.3). That the low-expressing variants which are related to disorders that are associated with reduced 5-HT activity is at first glance odd, in that the extracellular levels should be higher in the presence of these low-expressing transporter polymorphisms. As well, as described above, in vivo human research using PET has found that these polymorphisms do not correspond to adult brain 5-HT levels. Further as alluded to before, sex differences seem to show contradictory effects of the polymorphisms. Serotonergic pathways are brain ubiquitous, and particularly heavy projections are found in the limbic system. The authors point out that 5-HT is one of the earliest neurotransmitters during embryonic development and 5-HT CNS neurons are present in human embryos within 5 weeks of gestation.

Elevated embryonic levels of 5-HT in monoamine oxidase A and 5-HTT knock-out mice lead to increased impulsiveness, aggressivity and possibly reduced stress responding in the former and reduced aggressivity and higher anxiety in the latter. A difficulty of using knock-out models is the complete lack of the proteins during the entire

life-cycle. A different knock-out mouse model (Tph2) is a mouse lacking the Type 2 variant of the enzyme tryptophan hydroxylase, the enzyme that is essential for 5-HT synthesis from Trp (as described earlier in the dissertation). Additionally there are mutant forms of the Tph2 gene that cause a reduction of 80% in enzyme activity. Pups with these 5-HT deficiencies develop into aggressive and anxious mice. Treating mice with 5-HTT inhibitors at different points in development (E8-18 or P4-21) that correspond in humans to the first and third trimesters, results in mice that display depressed and anxious type behaviors later in their life-cycle. Embryonic presence of 5-HT in a negative feedback loop based on the $5HT1_A$ receptor present on serotonergic neurons and target areas of the limbic system modulates the outgrowth of terminals from these neurons. High levels of embryonic 5-HT not only can affect the circuitry but according to the authors the size and capacity of the serotonergic system. In summary, low expression variant of 5-HTT can result in excess levels of CNS 5-HTduring early development leading to increased risk for psychological disturbances later in life, by altering circuitry and the architecture of the 5-HT system.

This atypical "wiring," as referred to by the review's authors, of the 5-HT circuitry, has implications across the human life-cycle. To complicate matters even more, yet in a way that may possibly explain the disparate CVR and ATD findings, sex conditioning and life events such as early-childhood maltreatment interact with the various polymorphisms resulting in multifinality, equal genotypes notwithstanding.

Notwithstanding all the caveats and limitations of the field and this study, it is likely that 5-HT plays a role in modulating many of the interconnected pathways of the human mind and body. It may play a role in the formation of psychological traits and

factors as well as respond to and modulate the influences and actions of those very factors. The activation and expression of 5-HT systems and subsystems is a complex multi-causal process. The data from this study suggest that minimally, psychological factors interact with serotonin under varying conditions ranging from neutral contexts, to mildly arousing and perhaps somewhat threatening situations, to severely challenging social interactions, and in all likelihood, in a complex interplay of environmental, psychological, and genetic elements.

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Appendix A.

GENERAL HEALTH SURVEY

Please answer the following questions to the best of your ability. All of the information will be kept confidential.

Date: _____

1. Name: _____

2. Address: _____

3. Telephone Number (s): _____ (home) _____ (work)

4. Date of Birth: _____

5. Sex: Male _____ Female _____

6. If university student or university graduate: Year of Study _____

Program of Study - Major _____ Minor _____

7. Please list any prescription medications (including birth control) you take regularly or occasionally: _____

8. Please describe any major illnesses, health problems or hospitalizations you have had during your adult life: _____

_____

9. Has a doctor ever told you that your blood pressure was high?

Yes _____ No _____

If yes, please give the date it was found to be high: _____

If yes, what is your blood pressure? _____

10. Have you ever been prescribed medication to lower your blood pressure?

Yes _____ No _____

If yes, specify drug name: _____

Are you currently taking such medication? Yes _____ No _____

11. When was the last time you were examined by a physician or other health care provider? Within the last month _____ Within the last 6 months _____
Within the last year _____ More than one year ago _____

12. Have you ever been told that you have had: (check yes or no)

	Yes	No
Chest pains or angina	_____	_____
High blood pressure	_____	_____
Coronary artery disease	_____	_____
Rheumatic heart disease	_____	_____
Hardening of arteries (arteriosclerosis)	_____	_____
Heart attack	_____	_____
Blood disorders (hemophilia)	_____	_____
Any other heart or circulatory problem	_____	_____
Kidney disease (other than stones)	_____	_____
Diabetes (high blood sugar)	_____	_____
Respiratory disorder	_____	_____
Gastrointestinal disorder	_____	_____
Ulcer	_____	_____
Nervous or Mental disorder	_____	_____
Asthma	_____	_____
Allergies	_____	_____
History of high/marginally high cholesterol	_____	_____
Reproductive system disease or disorder	_____	_____
Cancer	_____	_____
Arthritis or joint condition	_____	_____

Appendix B.

Exclusion Criteria

1) **History of Cardiovascular Disease or other systemic disease:**
   Coronary heart disease, hypertension, asthma, thyroid, angina, etc.
   Hypertension - High Blood Pressure:
   Above 130/80 mmHg (determined by 3 measurements).

2) **Chronic or current use of medications which affect Cardiovascular Hemodynamics:**
   Allergy medications, Steroid based bronchodilators, etc.

3) **Coronary Heart Disease/Hypertension**

4) **Pregnancy & Birth Control:**
   Oral and injectable forms of birth control. (estrogen/progestogen or progestogen alone, and Depo-Provera)

5) **Alcohol and other substance abuse including non-clinical elevated levels:**
   More than 5-6 drinks on a regular basis.
   A history of substance usage pattern that is atypical.
   Over 3 MDMA uses, if whole pills, (5 uses if some were ½s,) at least 6 months ago.

6) **History of psychiatric illness:**
   Depression – history of depressive episodes (more than mild "blue" periods).
   OCD – current or in remittance.
   Anxiety – current anxiety at more than mild levels or past history.

7) **Body Morphology**:
   15 % overweight for height

8) **Chronic heavy smokers:**
   Over 1 pack / day, or if they are irritated if they cannot smoke for 24 hours.

9) **Heavy coffee drinkers:**
   If they will become irritated or agitated by not drinking caffeine for approximately 48 hours.

Appendix C.

PHYSICAL EXAMINATION REPORT

Family Name: _____ **Given**: _____ **Sex**: F/M

DOB: _____ **Height**: _____ **Weight**: _____

Blood Pressure _____ **Pulse:** _____ **Respiration**: _____

HEENT: _____
_____

PERL:_____ **EOM:**_____
_____

Thyroid: _____ **Lymph:** _____

Respiration: _____
_____
_____

Cardiovascular: _____
_____
_____

GI: _____
_____

GU: _____ **U/A**: _____
_____

Central Nervous System: _____
_____

Skin: _____
_____

Musculo Skeletal: _____
_____

Pregnancy: Yes: ___ No: ___ **L.M.P**: _____

ECG: _____

HPI: _____

_____

Allergies: _____

_____

Previous Medical History: _____

_____

_____

Family History:

Mother: _____

_____

Father: _____

_____

Sibling(s): _____

_____

Functional Inquiry:

HEENT: _____

Thyroid: _____ **Lymph**: _____

Resp: _____

CVS: _____

GI: _____

GU: _____ **CNS**: _____

SKIN: _____ **MUSKO**: _____

I see no medical contraindication to his/her participation in your tryptophan depletion – serotonin and stress - study.

Signature: _____ **Date**: _____

Appendix D.

Cook Medley Hostility Inventory

Subject# _____

Date _____

This questionnaire consists of numbered statements. Read each statement and decide whether it is true as applied to you or false as applied to you. If the statement is TRUE or MOSTLY TRUE, circle the (T). If it is FALSE or NOT USUALLY TRUE, circle the (F). Remember to give your own opinion of yourself. Do not leave any blank spaces if you can avoid it; please answer every statement.

(T) (F) 1. When I take a new job, I like to be tipped off on who should be gotten next to.

(T) (F) 2. When someone does me a wrong I feel I should pay him back if I can, just for the principle of the thing.

(T) (F) 3. I prefer to pass by school friends, or people I know but have not seen for a long time, unless they speak to me first.

(T) (F) 4. I have often had to take orders from someone who did not know as much as I did.

(T) (F) 5. I think a great many people exaggerate their misfortunes in order to gain the sympathy and help of others.

(T) (F) 6. It takes a lot of argument to convince most people of the truth.

(T) (F) 7. I think most people would lie to get ahead.

(T) (F) 8. Someone has it in for me.

(T) (F) 9. My relatives are nearly all in sympathy with me.

(T) (F) 10. Most, people are honest chiefly through fear of being caught.

(T) (F) 11. Most people will use somewhat unfair means to gain profit or an advantage rather than to lose it.

(T) (F) 12. I commonly wonder what hidden reason another person may have for doing something nice for me.

(T) (F) 13. It makes me impatient to have people ask my advice or otherwise interrupt me when I am working on something important.

(T) (F) 14. I feel that I have often been punished without cause.

(T) (F) 15. I am against giving money to beggars.

(T) (F) 16. Some of my family have habits that bother me and annoy me very much.

(T) (F) 17. My way of doing things is apt to be misunderstood by others.

(T) (F) 18. I can be friendly with people who do things which I consider wrong.

(T) (F) 19. I don't blame anyone for trying to grab everything they can get in this world.

(T) (F) 20. No one cares much what happens to you.

(T) (F) 21. It is safer to trust nobody.

(T) (F) 22. I do not blame a person for taking advantage of someone who lays himself open to it.

(T) (F) 23. I have often felt that strangers were looking at me critically.

(T) (F) 24. Most people make friends because friends are likely to be useful to them.
(T) (F) 25. I am sure I am being talked about.
(T) (F) 26. I am not likely to speak to people until they speak to me.
(T) (F) 27. Most people inwardly dislike putting themselves out to help other people.
(T) (F) 28. I tend to be on my guard with people who are somewhat more friendly than I had expected.
(T) (F) 29. People often disappoint me.
(T) (F) 30. I have often met people who were supposed to be experts who were no better than I.
(T) (F) 31. It makes me feel, like a failure when I hear of the success of someone I know well.
(T) (F) 32. I am not easily angered.
(T) (F) 33. People generally demand more respect for their own rights than they are willing to allow for others.
(T) (F) 34. I am quite often not in on the gossip and talk of the group I belong to.
(T) (F) 35. I have often found people jealous of my good ideas, just because they had not thought of them first.
(T) (F) 36. I have sometimes stayed away from another person because I feared doing or saying something that I might regret afterwards.
(T) (F) 37. I would certainly enjoy beating a crook at his own game.
(T) (F) 38. I have at times had to be rough with people who were rude or annoying.
(T) (F) 39. There are certain people whom I dislike so much that I am inwardly pleased when they are catching it for something they have done.
(T) (F) 40. I am often inclined to go out of my way to win a point with someone who has opposed me.
(T) (F) 41. The man who had most to do with me when I was a child (such as my father, stepfather, etc.) was very strict with me.
(T) (F) 42. I like to keep people guessing what I'm going to do next.
(T) (F) 43. When a man is with a woman he is usually thinking about things related to her sex.
(T) (F) 44. I do not try to cover up my poor opinion or pity of a person so that he won't know how I feel.
(T) (F) 45. I strongly defend my own opinions as a rule.
(T) (F) 46. I frequently ask people for advice.
(T) (F) 47. I have frequently worked under people who seem to have things arranged so, that they get credit for good works but are able to pass off mistakes onto those under them.
(T) (F) 48. People can pretty easily change me even though I thought that my mind was already made up on a subject.
(T) (F) 49. Sometimes I am sure that other people can tell what I am thinking.
(T) (F) 50. A large number of people are guilty of bad sexual conduct.

Thank you for your cooperation. Your answers will be kept confidential!

Appendix E.

SELF-ANALYSIS QUESTIONNAIRE (TA)

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you generally feel. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which seems to describe how you generally feel.

	Almost Never	Sometimes	Often	Almost Always
1. I am quick tempered.....	1	2	3	4
2. I get annoyed when I am singled out for correction……	1	2	3	4
3. I am a hotheaded person……	1	2	3	4
4. I have a fiery temper……	1	2	3	4
5. I feel angry……	1	2	3	4
6. I feel irritated……	1	2	3	4
7. I get angry when I'm slowed down by others' mistakes……	1	2	3	4
8. I feel annoyed when I am not given recognition for doing good work……	1	2	3	4
9. I fly off the handle……	1	2	3	4
10. When I get mad, I say nasty things……	1	2	3	4
11. People who think they are always right irritate me……	1	2	3	4
12. When I get frustrated, I feel like hitting someone ……	1	2	3	4
13. I feel infuriated when I do a good job and get a poor evaluation……	1	2	3	4
14. It makes my blood boil when I am pressured……	1	2	3	4
15. It makes me furious when I am criticized in front of others……	1	2	3	4

Appendix F.

Spielberger Trait Anxiety Inventory (STAI)

Participant # _____

DIRECTIONS: A number of statements which people have used to describe themselves are given below. Read each statement and then blacken in the appropriate circle to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

1 = Almost Never 2 = Sometimes 3 = Often 4 = Almost Always

21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am calm, cool, and collected	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind ...	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Appendix G.

BECK DEPRESSION INVENTORY

DATE OF VISIT: _____SUBJECT NUMBER: _____

On this questionnaire, are groups of statements. Please read each group of statements carefully. Then pick out the one statement in each group which best describes the way you have been feeling the PAST WEEK, INCLUDING TODAY! Circle the number beside the statement you picked. If several statements in the group seem to apply equally well, circle each one.

Be sure to read all the statements in each group before making your choice.

1	0	I do not feel sad
	1	I feel sad
	2	I am sad all the time and I can't snap out of it
	3	I am so sad or unhappy that I can't stand it
2	0	I am not particularly discouraged about the future
	1	I feel discouraged about the future
	2	I feel that I have nothing to look forward to
	3	I feel that the future is hopeless and that things cannot improve
3	0	I do not feel like a failure
	1	I feel I have failed more than the average person
	2	As I look back on my life, all I can see is a lot of failures
	3	I feel I am a complete failure as a person
4	0	I get as much satisfaction out of things as I used to
	1	I don't enjoy things the way I used to
	2	I don't get real satisfaction out of anything anymore
	3	I am dissatisfied or bored with everything
5	0	I don't feel particularly guilty
	1	I feel guilty a good part of the time
	2	I feel guilty most of the time
	3	I feel guilty all of the time
12	0	I have not lost interest in other people.
	1	I am less interested in other people than I used to be
	2	I have lost most of my interest in other people
	3	I have lost all of my interest in other people
13	0	I make decisions about as well as I ever could
	1	I put off making decisions more than I used to
	2	I have greater difficulty in making decisions than before
	3	I can't make decisions at all anymore
14	0	I don't feel I look any worse than I used to
	1	I am worried that I am looking old or unattractive
	2	I feel that there are permanent changes in my appearance that make me look unattractive
	3	I believe that I look ugly
15	0	I can work about as well as before
	1	It takes an extra effort to get started
	2	I have to push myself very hard to do anything
	3	I can't do any work at all
16	0	I can sleep as well as usual
	1	I don’t sleep as well as I used to
	2	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
	3	I wake up several hours earlier than I used to and cannot get back to sleep

6	0	I don't feel I am being punished
	1	I feel I may be punished
	2	I expect to be punished
	3	I feel I am being punished
7	0	I don't feel disappointed in myself
	1	I am disappointed in myself
	2	I am disgusted with myself
	3	1 hate myself
8	0	I don't feel I am any worse than anybody else
	1	I am critical of myself for my weaknesses or mistakes
	2	I blame myself all the time for my faults
	3	I blame myself for everything bad that happens
9	0	I don't have any thoughts of killing myself
	1	I have thoughts of killing myself, but I would not carry them out
	2	I would like to kill myself
	3	I would kill myself if I had the chance
10	0	I don't cry anymore than usual
	1	I cry now more than I used to
	2	I cry all the time now
	3	I used to be able to cry, but now I can't cry even though I want to
11	0	I am no more irritated now than I ever am
	1	I get annoyed or irritated more easily than I used to
	2	I feel irritated all the time now
	3	I don't get irritated at all by the things that used to irritate me
17	0	I don't get more tired than usual
	1	I get tired more easily than I used to
	2	I get tired from doing almost anything
	3	I am too tired to do anything
18	0	My appetite is no worse than usual
	1	My appetite is not as good as it used to be
	2	My appetite is much worse now
	3	I have no appetite at all
19	0	I haven't lost much weight, if any, lately
	1	I have lost more than 5 pounds.
	2	I have lost more than 10 pounds.
	3	I have lost more than 15 pounds
		I am purposely trying to lose weight by eating less Yes _____ No _____
20	0	I am no more worried about my health than usual
	1	I am worried about physical problems such as aches and pains; or upset stomach; or constipation
	2	I am very worried about physical problems and it is hard to think about anything else
	3	I am so worried about my physical problems that I cannot think about anything else
21	0	I have not noticed any recent change in my interest in sex
	1	I am less interested in sex than I used to be
	2	I am much less interested in sex than I used to be
	3	I have lost interest in sex completely

Appendix H.

Profile of Mood States (POMS)

NAME: _____ DATE: _____

Below are words that describe feelings and moods people have. Please read **EVERY** word carefully. Then fill in **ONE** space under the answer which best describes how you feel. Suppose the word is happy, mark the one answer which is closest to how you have been feeling right now. The numbers refer to the phrases indicated below.

0 — much unlike this 1 — slightly unlike this 2 — a little like this 3 — much like this

1. Composed	0 1 2 3	25. Peaceful	0 1 2 3	49. Calm	0 1 2 3
2. Angry	0 1 2 3	26. Furious	0 1 2 3	50. Mad	0 1 2 3
3. Cheerful	0 1 2 3	27. Lighthearted	0 1 2 3	51. Jolly	0 1 2 3
4. Weak	0 1 2 3	28. Unsure	0 1 2 3	52. Uncertain	0 1 2 3
5. Tense	0 1 2 3	29. Jittery	0 1 2 3	53. Anxious	0 1 2 3
6. Confused	0 1 2 3	30. Bewildered	0 1 2 3	54. Muddled	0 1 2 3
7. Lively	0 1 2 3	31. Energetic	0 1 2 3	55. Ready-to-go	0 1 2 3
8. Sad	0 1 2 3	32. Lonely	0 1 2 3	56. Discouraged	0 1 2 3
9. Friendly	0 1 2 3	33. Sympathetic	0 1 2 3	57. Good-natured	0 1 2 3
10. Tired	0 1 2 3	34. Exhausted	0 1 2 3	58. Weary	0 1 2 3
11. Strong	0 1 2 3	35. Powerful	0 1 2 3	59. Confident	0 1 2 3
12. Clearheaded	0 1 2 3	36. Attentive	0 1 2 3	60. Businesslike	0 1 2 3
13. Untroubled	0 1 2 3	37. Serene	0 1 2 3	61. Relaxed	0 1 2 3
14. Grouchy	0 1 2 3	38. Bad tempered	0 1 2 3	62. Annoyed	0 1 2 3
15. Playful	0 1 2 3	39. Joyful	0 1 2 3	63. Elated	0 1 2 3
16. Timid	0 1 2 3	40. Self-doubting	0 1 2 3	64. Inadequate	0 1 2 3
17. Nervous	0 1 2 3	41. Shaky	0 1 2 3	65. Uneasy	0 1 2 3
18. Mixed-up	0 1 2 3	42. Perplexed	0 1 2 3	66. Dazed	0 1 2 3
19. Vigorous	0 1 2 3	43. Active	0 1 2 3	67. Full of pep	0 1 2 3
20. Dejected	0 1 2 3	44. Downhearted	0 1 2 3	68. Gloomy	0 1 2 3
21. Kindly	0 1 2 3	45. Agreeable	0 1 2 3	69. Affectionate	0 1 2 3
22. Fatigued	0 1 2 3	46. Sluggish	0 1 2 3	70. Drowsy	0 1 2 3
23. Bold	0 1 2 3	47. Forceful	0 1 2 3	71. Self-assured	0 1 2 3
24. Efficient	0 1 2 3	48. Able to concentrate	0 1 2 3	72. Mentally alert	0 1 2 3

Appendix I.

Subject Code: _____

Visual Analog Mood Scale (VAMS)

Indicate on each of the scales below, by starting a **vertical stroke** through the line at the appropriate point, **how you are feeling right now**.

Not at all Nervous	_____	Very Nervous
Not at all Agreeable	_____	Very Agreeable
Not at all Happy	_____	Very Happy
Not at all Tense	_____	Very Tense
Not at all Anxious	_____	Very Anxious
Not at all Relaxed	_____	Very Relaxed
Not at all Discouraged	_____	Very Discouraged
Not at all Annoyed	_____	Very Annoyed
Not at all Sad	_____	Very Sad
Not at all Irritated	_____	Very Irritated
Not at all Angry	_____	Very Angry
Not at all Depressed	_____	Very Depressed
Not at all Guilty	_____	Very Guilty

Appendix J.

LOW PROTEIN DIET

	Weight (g)	Protein (g)	Fat (g)	Carbohydrate (g)	kcal
BREAKFAST					
Banana 2	228	2.4	2	54	210
Orange juice 1/2 cup	120	0.8	0	13	52
White toast 2 slices	42	4.0	2	24	128
Margarine	10	0	8	0	68
Jelly 16ml	42	0	0	30	116
Decaf coffee or tea		0	0	0	
1/2 & 1/2 cream 15ml	20	0.5	2	1	27
Sugar 2 x 800mg	8	0	0	8	32
LUNCH					
Shredded lettuce	80	0.7	0	2	10
Raw carrots	55	0.6	0	5	23
Raw celery (1 stalk)	40	0.3	0	2	6
Tomato (1)	123	1.3	0	6	27
Cucumber (1/2 cup)	52	0.3	0	2	7
Oil (1 tbsp)	15	0	14	0	129
Vinegar (1 package)	20	0	0	0	1
Raisins 42g	45	1.5	0	36	136
Apple (1)	140	0	0	21	82
Peach (1)	90	0.6	0	10	38
Twix	48	1.0	6	16	118
Decaf coffee or tea		0	0	0	
1/2 & 1/2 cream 15ml	20	0.5	2	1	27
Sugar 2 x 800mg	8	0	0	8	32
DINNER					
Stir fried vegetables:					
Onions (4 tbsp)	40	0	0	3	12
Carrots	55	0.5	0	4	17
Celery (1 stalk)	40	0.3	0	1	6
Broccoli (1/2 cup)	44	1.4	0	2	11
Cauliflower (1/2 cup)	50	1.2	0	2	11
Mushrooms (1/2 cup)	35	0.9	0	1	39
Green pepper (1/2 cup)	50	0	0	3	13
Oil (3 tbsp)	45	0	44	0	386
Applesauce (1/2 cup)	128	0.2	0	25	97
1/2 & 1/2 cream 15ml	20	0.5	2	1	27
Sugar 2 x 800mg	8	0	0	8	32
Peach (1)	90	0.6	0	10	38
SNACK					
Raisins 42g	45	1.5	0	36	136
Twix	48	1.0	6	16	118
TOTAL		**22.6**	**88**	**351**	**2212**

Appendix K.

PREPARATORY DAY INSTRUCTIONS

Laboratory Telephone: 398-7156
Emergency Telephone (Erwin): 781-4626

On the day preceding our dietary experiment, we would like you to follow a low protein diet that begins at breakfast and ends at 12 AM.

This diet includes:

1. Breakfast
2. Lunch
3. Dinner
4. Snacks

If any of the items listed below are missing or if you have any questions, please call our lab at 398-7156. If there is no answer, please call Erwin at 781-4626.

Please Note: Underlined items are provided in your meals package.

1. Breakfast

Your breakfast consists of 2 bananas, orange juice, and 2 slices of bread. You may toast the two slices of bread, and eat them with margarine and/or jam. You may not eat any peanut butter or drink any milk (except a small amount in your coffee). You may have one cup of decaffeinated coffee or tea, with the milk and sugar.

2. Lunch

Your lunch consists of salad, celery, carrots, fruit, raisins, and a chocolate bar. You will be provided with salad dressing.

3. Dinner

Your dinner consists of stir-fried vegetables, which are to be kept frozen until needed. The frozen dinner container can be heated in the oven with the lid removed for about 35 to 45 minutes at 325^0F. It can also be heated in a microwave oven by removing it from the aluminum container and placing it in a microwave safe container, and heating it as required. You may have one cup of decaffeinated coffee or tea, with the milk and sugar.

4. Snacks

The remaining items (fruit, raisins, applesauce, and chocolate bar) are meant to be eaten at any time during the day, but we suggest that you save them for the evening.

Important!!

You do not have to eat everything listed on this sheet of paper, **but you cannot replace one food item for another, or eat anything outside the diet** (the same applies to beverages, and most importantly, no alcohol!)

You may drink water throughout the day.

You do not have to eat the food in the order presented here; you can eat the food in any order you wish.

Please stop eating at 12:00 AM the night before the experiment day at McGill.

Do not eat any breakfast.

If you are a smoker, do not smoke on the morning you are to come to McGill.

Appendix L.

MATHEMATIC SUBTRACTION TASK INSTRUCTIONS

A series of mathematical subtraction equations are going to be presented on the monitor. You must respond by pressing the right button (the one with the C on it) if you think that the answer on the screen is correct, or by pressing the left button (the one with the I on it) if you think the answer on the screen is incorrect.

If your response is accurate, in that you say the answer on the screen is correct and it is, or you say the answer on the screen is incorrect and it is, then you will hear a high-pitched tone indicating that you have responded correctly.

If you are inaccurate in your response, that is, you say the answer on the screen is correct and it is incorrect, or you say it is incorrect and it is correct, you will hear a low-pitched tone indicating that you have not responded accurately.

The task is nine minutes long and during that time the machines in the other room will continue to take readings. They will also take further readings after you have completed the math task.

Also, when you are done the math task I will be asking you to complete this questionnaire and to do two saliva collections. Please don't move too much while completing the questionnaire as the machines will still be taking readings.

Anyways, you have three seconds to respond to every equation. When the color of the mathematical equation on the monitor turns yellow, you have one second left to respond. If you are unsure at this point, guess, because a non-response is considered an incorrect response.

*** harasser's cue, request harasser to enter at this point.**

Appendix M.

Confederate Introduction Protocol

While R.A. is explaining the math-task instructions to the subject, the phone rings. Confederate knocks on the door and enters the testing room to tell R.A. that her supervisor is on the phone.

Conf: "Dr. Miller is on the phone."
R.A: "Just a minute please."

R.A. completes the instructions, excuses herself and exits to the adjacent room, leaving the door ajar. In a loud voice R.A. pretends to talk on the phone.

Conf: "Hello Dr. Miller. Right now? Well, I'm running a subject right now. Oh, okay, I'll ask if "Confederate" can take over for me. Okay, thanks, bye bye".

Confederate pretends to be angry with R. A.

Conf: "Now what?!" (angrily)

R.A: "Shhhh! (pause) that was Dr. Miller."
Conf.: "And?"

R.A: "He wants to see me right away."
Conf: "Now? - but you have a subject in there!"

R.A: "I know - but it sounds really important - would you mind taking over for me?!"
Conf: "Look - I won't be responsible if your results screw up!"

R.A: "Don't worry - nothing will go wrong - everything is set up in there - just follow the instructions."
Conf: "I don't normally deal with the subjects - that's your job you know!"

R.A: "You know I wouldn't ask you if I didn't have to -everything will be fine! (pause) okay? Thanks, I'll be back as soon as I can."

R.A. returns to the testing room and tells the subject that she must leave and that another researcher will be taking her place. R.A. then leaves the testing room and Confederate soon enters it, pretending to be angry.

Appendix N.

Anger Induction Statements

The 9-minute subtraction task stressor consists of three 3 minute trials. During each trial, two anger-provoking statements are delivered, one at 30-45 seconds into each trial when participant gets two answers wrong in a row, and the other 2/3 of the way through each 3-minute trial, again when the participant gets two in a row wrong.

Trial 1

1. Did you understand the instructions?
2. The right button is correct, the left button is incorrect.

Trial 2

3. Could you try harder this time?
4. Can't you do better than this?

Give participant saliva container at end of second trial before

Trial 3

5. It isn't that hard you know.
6. I can do better than that.

At end of task turn off computer screen and hand participant last set of questionnaires. Leave room (or pretend to) closing door with sounds.

Appendix O.

Post-Stressor Debriefing Instructions

RA: Oh, I guess "Confederate" left... Well, you're done… Here, could you please do this last saliva collection, I'm just going to go check the machines.

Go into other room and make machine-checking-like noises.

RA: Sorry I had to leave so quickly. How did it go? Did "Confederate" help you? Did you have any trouble with the instructions?

1. If P mentions trouble with "Confederate" ask : 'What do you mean?', What happened?', mention '"Confederate" didn't seem pleased to replace me.', 'Some people don't like "Confederate"', '"Confederate" is sometimes hard to get along with.'

2. If P states they knew she was part of the study find out: at what point they realized they were being deceived and how they figured it out.

(Video tape is running, however, note anomalies in the participant log).

DEBRIEF

RA: "Confederate" is part of the study. This is the only deception in the study; everything else I told you about the study is true. Everyone goes through the same thing. Everyone gets read the same comments about their performance on the math task. The math task is not an IQ test, it is designed to become harder every time you get a correct answer such that you cannot get more than 50% correct.

The reason we had to use deception for this study is that a math task is not a realistic stressor. The math task is hard, and it is programmed so that you get no more than 50% right so that "Confederate" can deliver the statements, but it is not a stress you would encounter regularly. More often than not, stress is inter-personal. The situation created by the deception is meant to be a more realistic stressor. The "Confederate" isn't really a nasty person. Invite the participant to meet the "Confederate".

Do you have any questions? (If you are unable to answer the question, then let the participant know someone will call them with the answer.)

Appendix P.

GENERAL CONSENT FORM

RESEARCH PROJECT CONDUCTED AT

Department of Psychiatry, McGill University

Chawki Benkelfat M.D.
Syd Miller Ph.D. Department of Psychology, Concordia University
Dr. Simon N. Young, Department of Psychology, McGill University
Dr. Robert Pihl, Department of Psychology, McGill University
Dr. Blaine Ditto, Department of Psychology, McGill University

The Effects of Gender, Emotion, Stress and Serotonin on Mood and Blood Pressure

We would like to invite you to participate in a study investigating the effects of gender, emotion, stress and serotonin on mood and blood pressure. In this study, you will undergo a brief interview to determine your current and/or past psychiatric history, a more detailed physical, mental and family history interview (approximately 2 hours) as well as a general medical exam in which physical and mental health will be assessed. You will be provided with a 24 hour low protein diet. You will spend approximately 8 hours at our laboratory, where you will first supply a urine sample for an illegal drug screen, a urine pregnancy screen for female, and a registered nurse will then take a blood sample. There are minimal risks associated with blood draws, which include the possibility of bruising, minor bleeding, and transient pain, however these effects are rare. Physiological measurements such as heart rate and blood pressure will be taken for a ten minute baseline period. You will ingest a 100g amino-acid drink containing the same proportion of amino-acids as found in human breast milk, together with either no tryptophan or 2.3g of tryptophan. Tryptophan is one of the essential amino acids found in the body, and is not unnatural or harmful. The amino acids will be mixed with water, chocolate syrup, and sweetener. Due to the constituents and consistency of the mixture you may experience slight nausea and/or vomiting following its ingestion. However, this occurs very rarely and passes very quickly (1.5 hrs.). It is also possible that you may experience some changes in mood (e.g., lowering of mood, sadness) as a result of ingesting this mixture. However, these changes are only temporary and will return to normal by the end of the experiment.

After ingesting the mixture, there is a 5 hour waiting period. During this time you will

complete a questionnaire package, watch a movie, and be permitted to read the magazines provided. At the end of the 5 hours a registered nurse will take a second blood sample. Physiological measures will again be taken and physiological changes will be examined while you play a computerized math task. Your performance on the math task will also be measured. During the experiment, changes such as an increase in heart rate and blood pressure will occur. These changes will be only temporary, returning to normal after the experiment and causing no harmful effects. These changes will be recorded through safe, painless, and non-invasive means, which will only require the placement of transducers on the skin. Salivary samples will be collected in order to measure the levels of various stress hormones. During these procedures you will be monitored via a video camera and recorded during the math task. These videotapes will be stored in a locked desk accessible only by study personnel, coded, analyzed for use in the current study and then erased within 3 months.

All subjects will undergo a urine sample based illegal drug screen. Subjects will be excluded from the study if the urine screen is positive. If a subject is excluded for this reason, the results of the urine test any data collected will be destroyed. Females will be screened for pregnancy and excluded if there is a positive result.
Participating in this study has no direct benefit for the subject.
You should not participate in this study if you are or think you might be pregnant.

To summarize, your participation will require you to:

1) Undergo a brief interview regarding your psychiatric history, as well as a more detailed (approximately 2 hours) personal interview regarding your physical, mental and family history.
2) Undergo a medical examination.
3) Eat a 24 - hour low protein diet.
4) Attend a laboratory session that will last approximately 8 hours.
5) Undergo a urine screen for illegal drugs.
6) Undergo a urine pregnancy screen (females only).
7) Ingest an amino acid drink.
8) Provide 2 blood samples.
9) Engage in a mathematical subtraction task during the laboratory session. The session will be videotaped.

10) Permit the measurement of several physiological responses including heart rate and blood pressure and salivary stress hormones.
11) Complete a series of questionnaires.

You will be paid $100.00 for your participation. This remittance will be paid to you right after you have completed the experiment. All information we obtain about you is strictly confidential and will not be seen by anyone who is not a member of the research team. Ultimately, all data will be coded using subject numbers rather than names. All data will be kept confidential unless otherwise required by law. Your participation in this study is completely voluntary and you will be free to withdraw from the study at any time. You have the right to ask questions at any time. You will receive payment proportionate to your time spent should you decide to withdraw before completion of the study.

Once you have carefully studied and understood this form, you may sign it in indication of your free consent to participate in the study.

Contacts:

Serotonin Testing Laboratory	398-7156
Regarding your rights as a subject:	
MUHC Ombudsman - Lamy, Danielle	934-8306
Concordia University Ombudsman	848-4964

In the case of an adverse event following your participation:

Chawki Benkelfat M.D.	398-6732
Sydney Miller Ph.D.	848-2183

NAME (PLEASE PRINT) _____

SIGNATURE _____

DATE _____

INVESTIGATOR'S SIGNATURE _____

Appendix Q.

CONSENT FOR RELEASE OF MEDICAL INFORMATION FORM

Release of Subject's Medical Information from Evaluating Physician to Researchers

RESEARCH PROJECT CONDUCTED AT

Department of Psychiatry, McGill University
Chawki Benkelfat M.D.
Syd Miller Ph.D. Department of Psychology, Concordia University
Dr. Simon N. Young, Department of Psychology, McGill University
Dr. Robert Pihl, Department of Psychology, McGill University
Dr. Blaine Ditto, Department of Psychology, McGill University

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I hereby authorize Lawrence Morris M.D. to release to the researchers and or their representatives, the results from the medical (physical) examination that I underwent as a preparatory step to my inclusion in this research project.

NAME (PLEASE PRINT) _____

SIGNATURE _____

DATE _____

PHYSICIAN'S SIGNATURE _____