THE EFFECT OF ACUTE PHENYLALANINE TYROSINE DEPLETION AND BRIGHT LIGHT EXPOSURE ON MOOD AND REWARD SENSITIVITY IN MILDLY SEASONAL WOMEN

by

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5-HT	serotonin
AA	amino acid(s)
AD	antidepressant
AMPT	alpha-methyl-*para*-tyrosine
APTD	acute phenylalanine tyrosine depletion
ATD	acute tryptophan depletion
B	balanced
BDI	Beck Depression Inventory
CNS	central nervous system
CSF	cerebrospinal fluid
DA	dopamine
GSS	Global Seasonality Scale
HVA	homovanillic acid
L-DOPA	L-dihydroxyphenylalanine
LNAA	large neutral amino acids
MAO	monoamine oxidase
MDD	major depressive disorder
MDE	major depressive episode
MIP	Mood Induction Procedure
NE	norepinephrine
PD	Parkinson's disease
PET	Positron Emission Tomography
Phe	phenylalanine
POMS	Profile of Mood States
PR	Progressive Ratio
PT-	phenylalanine/tyrosine-deficient
SAD	seasonal affective disorder
SCID	Structured Clinical Interview for DSM-IV

SD	standard deviation
SEM	standard error of the mean
SMAST	Short Michigan Alcoholism Screening Test
SPAQ	Seasonal Pattern Assessment Questionnaire
SSRI	selective serotonin reuptake inhibitor
SSS	Stanford Sleepiness Scale
TH	tyrosine hydroxylase
Tyr	tyrosine
VAS	Visual Analog Scale(s)

ABSTRACT

The monoamine neurotransmitter dopamine (DA) is thought to be involved in the pathophysiology of numerous mood disorders including major depression and seasonal affective disorder. To investigate these associations further, we tested whether reduced light might make individuals particularly vulnerable to the effects of lowered DA, as produced by the acute phenylalanine/tyrosine depletion (APTD) method. Healthy, mildly seasonal women without a personal history of psychiatric disorders were tested in either bright or dim light, and then ingested, on separate days in randomized counterbalanced order (i) a nutritionally balanced amino acid (AA) mixture, and (ii) a mixture deficient in the DA precursors phenylalanine and tyrosine. Mood was assessed at 5 different times throughout the day using the Profile of Mood States (POMS), a series of mood Visual Analog Scales (VAS) and the Stanford Sleepiness Scale (SSS). The motivation to seek monetary units of reward was assessed using a *Progressive Ratio* (PR) breakpoint task. Data analyses suggested two novel findings. First, in dim light, APTD decreased the overall POMS score and two subscale scores, as well as the SSS score, indicating lower mood associated with less energy and more sleepiness. These effects were not seen in bright light. Second, in dim light, there was also a trend toward an APTD-induced decrease in willingness to work for monetary reward. Together, these results raise the possibility of a direct relationship between bright light exposure and DA

functioning; bright light may act to prevent the functional depletion of DA as induced by APTD.

ABRÉGÉ

On considère que le neurotransmetteur monoamine dopamine joue un rôle signifiant dans la pathophysiologie de plusieurs désordres psychologiques (dépression grave et troubles affectifs saisonniers inclus). Pour mieux vérifier l'association entre la dopamine et ces désordres, nous avons examiné l'effet que produirait la réduction de la lumière : cette réduction de luminosité rendrait-elle les individus plus vulnérables aux effets malfaisants de la DA réduite, effectué par la déplétion aiguë de « acute phenylalanine/tyrosine » (APTD) ? Des femmes en bonne santé, touchées légèrement par les troubles affectifs saisonniers et sans historique de graves maladies psychiatriques, ont été exposées à un éclairage fort ou bien faible. Ces femmes ont ingéré, à des dates différentes et dans un ordre aléatoire et contrebalancé : i) un produit nutritionnellement équilibré contenant des acides aminés, et ii) une mixture pauvre en précurseurs DA, phenyalanine et tyrosine. L'humeur de ces femmes a été évaluée 5 fois par jour en utilisant les outils suivants : Profile of Mood States (POMS), une série de Visual Analog Scales (VAS) et le Stanford Sleepiness Scale (SSS). La motivation à la rétribution monétaire a été évaluée en fonction d'un Progressive Ratio (PR). Notre analyse a révélé deux nouveaux résultats. Premièrement, sous la lumière sombre, l'APTD a réduit le degré général de POMS et ses sous -échelons associés, ainsi que le degré

de SSS, un résultat qui indique une humeur basse associée à moins d'énergie et une envie de dormir accrue. De tels effets n'ont pas été trouvés dans le cas de celles qui ont été exposées à une lumière vive. Deuxièmement, nous avons remarqué que dans le cas d'une luminosité basse, les résultats s'inclinaient vers une réduction de la motivation à travailler en vue de récompenses financières, précipitée par l'APTD. Pour résumer, ces résultats mettent à jour la possibilité d'une correspondance étroite entre l'exposition à la lumière brillante et le fonctionnement de la DA ; la lumière vive peut, selon notre analyse, empêcher la déplétion fonctionnelle de DA, précipité par APTD.

1.0 Introduction

1.1 Overview

A series of pioneering experiments in the late 1950s led by Arvid Carlsson identified dopamine (DA) as a discrete chemical neurotransmitter. Since then, DA has been extensively studied, and subsequently implicated in a vast number of psychiatric and neurological disorders. These include, but are not limited to roles in illnesses such as Parkinson's disease (PD), schizophrenia and psychosis, as well as behaviours related to movement, attention, learning, reward-seeking and mood. DA is thought to be involved in the broad spectrum of depressive disorders which, according to one recent epidemiological study, affects approximately 9.5% of the population in the US with at least mild severity (Kessler, et al., 2005). The same study suggested that 6.7% of the population suffers from major depressive disorder (MDD). According to the DSM-IV (Spitzer, et al., 1992), a specifier of MDD is a subset of directional symptoms that characterize seasonal affective disorder (SAD); this typically presents as depressive-like symptoms in a circ-annual pattern, with the most severe period being the fall/winter months and a return to normal mood (euthymia) or hypomania in the spring/summer, though the converse exists as well (Rosenthal, et al., 1984). Moreover, it has been found that in northerner latitudes of North America (Mersch, et al., 1999), a significantly larger proportion of the population experiences subsyndromal symptoms of SAD. Depending on their severity, the symptoms associated with depression and SAD, including effects on sleep, energy and mood, can have broader influences on day-to-day functioning, including job

performance and interpersonal relationships. The physiological mechanisms by which SAD and subsyndromal SAD symptoms are induced remain unclear, though it has become widely acknowledged that environmental light exposure is likely involved. One recent study has indicated that at a directly neurological level, the light factor may act in part through the serotonergic system (aan het Rot, et al., 2008), however the underlying neurobiological mechanisms remain unresolved and could very well involve other monoaminergic systems such as DA. The broader aim of our study was to bring some elucidation to the relationship between environmental light and brain DA. To examine more closely the contribution of light on putative DA-related behaviours, 14 healthy women with mild self-reported seasonal changes in mood underwent acute phenylalanine tyrosine depletion (APTD), a manipulation that transiently decreases brain DA synthesis, under bright or dim light conditions, and completed a number of measures examining mood and behaviours related to reward-seeking.

1.2 The Neurotransmitter Dopamine

1.2.1 Synthesis and Biochemistry

Dopamine (DA) belongs to the biochemical group known as the catecholamines, so named because each contains the chemical structure known as catechol (a 3,4-dihydroxylated benzene ring). Included in this group along with DA, are norepinephrine (NE) and epinephrine; all three of these are derived from the amino acids phenylalanine (Phe) and tyrosine (Tyr).

DA is synthesized in both the cytosol and presynaptic terminal of catecholaminergic neurons in a two-step biosynthetic pathway. The first step is

the oxidation of tyrosine to the structure to L-dihydroxyphenylalanine (L-DOPA). This reaction is catalyzed by the enzyme tyrosine hydroxylase (TH), and is considered the slower, or rate limiting step in the synthesis of DA and NE. Phenylalanine is also thought to function as a cosubstrate with Tyr for TH, but is not an inhibitor of this enzyme in vivo at normal or high concentrations (Fernstrom & Fernstrom, 2007). In the second step, L-DOPA is decarboxylated in a reaction catalyzed by dopa decarboxylase to produce DA and CO_2. Since dopa decarboxylase is abundant in the cytosol of catecholaminergic neurons, the synthesis of DA is dependent on L-DOPA availability. DA can be further converted to norepinephrine in a reaction catalyzed by the enzyme, β-hydroxylase; in turn, norepinephrine can be further methylated by the enzyme phenylethanolamine-*N*-methyl transferase to form epinephrine.

The action of catecholamines is typically terminated by selective uptake back into the axon terminal from the synaptic cleft via Na^+-dependent transporters. Once in the axon terminal, the molecules are recycled back into synaptic vesicles for reuse, or destroyed by monoamine oxidases (MAO). In the frontal lobes, the reuptake of DA occurs via NE transporters into NE terminals. Outside of the cell, the DA signal is terminated by catechol-o-methyl transferase (COMT) and also two forms of monoamine oxidase (MAO-A and MAO-B). These enzymes act to catabolise DA into dihydrophenylacetic acid and 3-methoxytyramine intermediates before the final metabolite, homovanillic acid.

1.3 Dopamine and Depressive Mood Disorders

1.3.1 A role for dopamine in major depression

Brain monoamines such as serotonin (5-HT), NE and DA are thought to be involved in the regulation of mood and the pathophysiology of mood disorders. One of the earliest theories regarding the neurochemical basis of major depression, and one that remains dominant, is the monoamine deficiency hypothesis, which implicates all three of the aforementioned monoaminergic systems. During the past few decades, biochemical monoamine models of depression have focused on 5-HT and NE systems. This reflects two observations: (i) the well-demonstrated ability of near all antidepressants to increase 5-HT, NE, or both (Brunello, et al., 2003; Invernizzi, et al., 2001; Kreiss & Lucki, 1995; Savitz, et al., 2009; Stahl, 1994), and (ii) the ability of manipulations that decrease 5-HT or NE to lower mood (Ellenbogen, et al., 1996; McCann, et al., 1995; Verhoeff, et al., 2003; Young & Leyton, 2002; Young, et al., 1985a), reinstate depressive symptoms (Berman, et al., 1999; Smith, et al., 1997), and reverse the clinical efficacy of serotonergic (Bell, et al., 2001; Delgado, et al., 1999; Van der Does, 2001) and noradrenergic antidepressants (Delgado, et al., 1993; Delgado, et al., 2002; Miller, et al., 1996), respectively.

Recently, though, an interest in DA's role in depression has re-emerged due to two primary observations. First, although selective serotonin reuptake inhibitor (SSRI) treatments have become the standard as the first-line treatment for major depression, a large proportion of patients (28-55%) fail to fully recover or experience residual symptoms (Nierenberg & DeCecco, 2001; Nierenberg, et al., 1999). Second, major depressive episodes (MDE) are commonly

characterized by symptoms that suggest a role for hypo-functioning DA systems, including low energy, psychomotor retardation, decreased motivation and decreased "interest or pleasure in all, or almost all, activities most of the day, nearly every day" (Spitzer, et al., 1992), especially given DA's importance in motivation and reward (Roiser, et al., 2005). Moreover, the presence of residual symptoms, including sleep disturbances, diminished pleasure, loss of interest, fatigue or loss of energy and decreased motivation, is a predictor of relapse and recurrence, and may be associated with dysregulation of DA and NE transmission systems (Nutt, 2006).

1.3.2 Possibility of disrupted DA function in depression

There is some evidence to suggest that there is disrupted DA functioning in individuals with depression. Decreased levels of homovanillic acid (HVA), a DA metabolite and thus a marker of DA function, have been consistently found in the cerebrospinal fluid (CSF) of depressed patients, as compared to healthy controls (Willner, 1983). Additionally, several studies have shown an inverse relationship between concentrations of CSF HVA and the magnitude of response to certain antidepressants (AD) (Post, et al., 1978; Praag, et al., 1975; Van Scheyen, et al., 1977).

Brain imaging techniques have become increasingly prominent in the study of DA functioning in healthy and depressed populations. In some studies, increased post-synaptic DA receptor binding has been found in depressed humans as compared to healthy controls (D'Haenen H & Bossuyt, 1994; Shah, et al., 1997), possibly reflecting an increased number of D_2 receptors or an increased

affinity of the receptor for its substrate due to a decrease in presynaptic DA release or synaptic DA availability, while others observed no difference in binding (Klimke, et al., 1999; Parsey, et al., 2001). In another small scale study examining pre-synaptic DA functioning using PET techniques with the [^{18}F]fluorodopa marker found evidence for decreased DA functioning in the left caudate of depressed individuals with psychomotor retardation. Thus imaging techniques remain an interesting avenue for examining DA functioning in depression.

Finally, populations with Parkinson's disease (PD), in which the nigrostriatal DA system experiences neuron loss and is thus impaired, present a higher than expected prevalence of major depression and subsyndromal depressive symptoms. Approximately 5-10% of PD's patients experience major depression and an additional 10-30% present subsyndromal symptoms (Tandberg, et al., 1996).

1.3.3 Some antidepressant (AD) efficacy facilitated by brain DAergic systems?

Studies in both animals and humans examining the influence of antidepressant drugs on DA strongly suggest a role for DA in the regulation of mood and depression. In rats, it has been shown that repeated administration of certain antidepressants (fluoxetine, desipramine, and tranylcypromine) enhances DA agonist-induced behaviour (Ainsworth, et al., 1998a), as well as postsynaptic D_2 receptor expression and binding in the nucleus accumbens of rats (Ainsworth, et al., 1998b). This, and similar evidence in animal studies, support the hypothesis that the efficacy of chronic antidepressant treatments function by increasing the

sensitivity of postsynaptic D_2 dopamine receptors and thus potentiating dopamine transmission, particularly in the limbic system (D'Aquila, et al., 2000). Furthermore, it seems that the time course for the therapeutic effects and the effects of chronic treatments of antidepressants on dopamine transmission are similar (Gino Serra, et al., 1992).

In humans, DA agonists have been shown to have AD efficacy, especially in patients with low pre-treatment CSF HVA (Corrigan, et al., 2000; Post, et al., 1978). The effectiveness of three different ADs on depressed patients with psychomotor retardation has been correlated to the extent of their respective pro-dopaminergic effects (Rampello, et al., 1991). In this study, patients experiencing combinations of symptoms including hypokinesia, reduction of speech, and hypersomnia were administered either amineptine (dopamine uptake inhibitor), clomipramine (serotonin uptake inhibitor) or minaprine (increases both DAergic and 5-HTergic transmission); amineptine and minaprine were found to be more therapeutically effective than either clomipramine or placebo (Rampello, et al., 1991). Amineptine, which is thought to predominately prevent the re-uptake of DA with minimal NE and 5-HT activity has been found to have AD effects with comparable efficacy to that of other AD's nonspecific to DA such as monoamine oxidase inhibitors or selective serotonin reuptake inhibitors, but is not considered a particularly viable treatment option due to its abuse potential (Nutt, et al., 2007). Further bupropion, an antidepressant and smoking-cessation aid, has been shown to have antidepressant efficacy equivalent to several SSRIs; evidence in both animal (Ascher, et al., 1995; Cooper, et al., 1980) and human models suggests the mechanism of action occurs through prevention of the reuptake of DA and NE at

a synaptic level by occupation of DATs by bupropion metabolites (Damaj, et al., 2004; Learned-Coughlin, et al., 2003), but not through increases in striatal DA release (Egerton, et al., In Press)

1.3.4 Effects of DA manipulations on depressive symptoms

Early studies looking at drugs which deplete monoamine content, such as Reserpine and methyldopa, an inhibitor of dopa decarboxylase that lowers endogenous CNS stores of catecholamines and serotonin, found that these could induce depression in some populations of patients with previous histories of depression, though some of these findings are not consistent (Berman, et al., 1999; DeMuth & Ackerman, 1983). The α-methylparatyrosine (AMPT) method, used to temporarily decrease catecholamine levels, has been found to cause a temporary but clinically significant relapse in depressive symptoms in previously depressed individuals as compared to a control treatment and to healthy, never-depressed individuals (Berman et al., 1999). However, it should be noted that the target of AMPT is non-specific to either DA or NE (McTavish, et al., 1999c).

More selective manipulations of DA transmission have also implicated a role for this transmitter. Administration of the immediate metabolic precursor to DA, L-DOPA, increases DA concentrations in the brain. L-DOPA was used in early studies on unipolar and bipolar depressed patients to examine its effects on the manic- and depressive-associated symptoms. The strongest finding was that, following repeated administration of relatively high doses, some patients showed an increased activation of verbal and motor behaviour, particularly in those depressed patients with retardation symptoms (Bunney, et al., 1971; Murphy, et

al., 1973). These studies consistently found that increasing doses of L-DOPA resulted in corresponding increases in DA, L-DOPA and the DA metabolite HVA in urinary output. Additionally, relatively high doses of L-DOPA were associated with hypomania in bipolar patients; however, these same doses were incapable of outright reversing severe depressive symptoms (Bunney, et al., 1971; Murphy, et al., 1973). Overall, these studies suggest a role for changes in DA metabolism in hypomania, mania and the psychomotor retardation aspects of depression.

Though less direct in its action, certain psychostimulants such as amphetamine and cocaine induce DA release in the brain and were amongst the earliest agents used for treatment against depression and have been shown to induce mania. However, these were typically not found to be efficacious treatments. Of interest is the finding that those suffering from more severe MDD experienced greater subjective reward in response to oral ingestions of d-amphetamine as compared to healthy controls and mildly depressed individuals (Tremblay, et al., 2002). More recently, this same group performed a smaller scale study that confirmed and extended these results using imaging techniques (Tremblay, et al., 2005).

1.4 Seasonal Affective Disorder (SAD) and Catecholamine Involvement

1.4.1 Overview of SAD

SAD is a subtype of depression characterized by the onset of episodes closely resembling the clinical presentation of major depression but in a distinct circannual pattern; symptoms present in the autumn and winter, and then return to remission during the spring and summer (Berman, et al., 1999; Rosenthal, et al.,

1984). SAD patients typically experience increased appetite, weight gain, decreased energy levels and hypersomnia (Rosenthal, et al., 1984). Though this profile of symptoms characterizes a more 'atypical' depression, information obtained regarding the basis or etiology of SAD may have implications for non-seasonal depression as well (Neumeister, et al., 2001a).

According to recent epidemiological studies using criteria based on the *DSM*, estimates for the prevalence of SAD in North America range from less than 1.0%, as determined through a survey of the US population (Blazer, et al., 1998) and 2.9%, as determined through a survey of a Canadian population (Levitt, et al., 2000). These estimates are lower than those previously found using less conservative criteria, but the number of people affected remains large. Moreover, a substantially larger proportion of individuals (>10%) suffer from subsyndromal symptoms (Rosen, et al., 1990), which can negatively affect quality of life.

The most effective current treatment options for SAD are bright light therapy and antidepressants. The benefits of light therapy for individuals suffering from seasonal symptoms are both consistent and rapid, though short-lived, as relapses typically occur upon discontinuation of treatment (Lam & Levitan, 2000b). Equalling the efficacy of bright light treatment is the therapeutic use of antidepressants, of which SSRIs are most commonly used and have been most extensively studied (Lam, 2002). Recently though, bupropion, a norepinephrine-dopamine reuptake inhibitor (NDRI) antidepressant, has been shown to have prophylactic efficacy, preventing even the onset of winter depressive symptoms (Modell, et al., 2005).

Though these treatment avenues provide a measure of insight, the underlying pathophysiology of SAD remains unclear. The efficacy of light treatment suggests the involvement of circadian rhythms, since light is a powerful regulator of internal rhythms. There is evidence for the photoperiod hypothesis, which suggests that the shortened winter daylight hours in the light/dark cycle play a role, as well as for the phase-delay hypothesis, which suggests that internal circadian rhythms become desynchronized with external clock rhythms (Lam, et al., 2000b). However, the efficacy of antidepressant treatments suggests a role for neurotransmitters; of these 5-HT has been the most extensively studied and established as being dysregulated in SAD, though NE and DA have strongly been implicated as well.

1.4.2 DA functioning and SAD

There are several lines of evidence supporting a role for brain DA in the pathophysiology of SAD, though none are especially conclusive or consistent. A suggested marker of DA function is the secretion of prolactin. Both DA and 5-HT play important functional roles in the regulation of prolactin; serotonin is stimulatory whereas DA provides tonic inhibition (Harmer, et al., 2001; Zimmermann, et al., 1996). In humans, the acute phenylalanine tyrosine depletion (APTD) method, which acts to transiently decrease brain dopamine levels, increases plasma prolactin levels (Gijsman, et al., 2002; Lythe, et al., 2005; McTavish, et al., 2005). Conflicting results regarding prolactin secretion have been found in patients with SAD. Some studies report increased levels (Jacobsen, et al., 1987), while others show SAD patients have lower prolactin levels than

healthy controls in both winter and summer. Is has, however, been suggested that lower levels of prolactin may actually reflect an upregulation of pituitary D2 receptors due to reduced pre-synaptic DA function (Depue, et al., 1990a; Depue, et al., 1989). Similarly, other studies examining the circadian profiles of various biological factors such as melatonin, cortisol, and body temperature in populations of SAD patients and healthy individuals have found conflicting results (Sohn & Lam, 2005).

Central thermoregulation is thought to be mediated in part by DA cells located in the anterior hypothalamic preoptic areas, where they influence thermosensitive neurons involved in central homeostatic thermoregulation; also possibly through nigrostriatal pathways (Arbisi, et al., 1994; Cutler, et al., 1979; Lee, et al., 1985; Schwartz & Erk, 2004). A preliminary study found that SAD patients have a blunted thermoregulatory heat loss in the winter that can be normalized following light therapy and during the summer (Arbisi, et al., 1989) and these results were later replicated in a larger sample size by the same group (Arbisi, et al., 1994). Thus these results indicate a potential dysregulation of the DA system in individuals with SAD. Another putative measure of DA function is spontaneous eye blink rate. Decreased eye blink rate has been associated with impaired DA activity (i.e. in PD patients), while increased rates have been correlated to increased DA transmission (Karson, 1983). One study found an increased eyeblink rate in SAD patients (Depue, et al., 1990b), while another did not (Barbato, et al., 1993).

Perhaps providing more compelling evidence, a SPECT imaging study found decreased availability of striatal dopamine transporter (DAT) binding sites

in symptomatic SAD patients; this may suggest an underlying primary cause, or may be a compensatory response secondary to lowered DA availability in the synaptic cleft that occurs with the onset of symptoms (Neumeister, et al., 2001b).

1.4.3 Monoamine manipulation and SAD

In SAD patients in remission following phototherapy or during the summer, lowering 5-HT using the acute tryptophan depletion method (ATD) has typically, though not always (Lam, et al., 2000), been seen to produce a brief worsening of mood, suggesting a role for the 5-HT system and phototherapy on the regulation of mood (Lam, et al., 2000a; Lam, et al., 1996; Leyton, et al., 2000a; Neumeister, et al., 1997; Neumeister, et al., 1998a). However, an additional role for at least one of the catecholamine systems has been suggested by the mood worsening effect seen following administration of the tyrosine hydroxylase inhibitor, alpha-methyl-para-tyrosine (AMPT). In a study by Neumeister and colleagues (1998b), SAD patients in remission through light therapy underwent both the ATD procedure and the catecholamine-depleting AMPT method. It was found that both manipulations reversed the positive effects of light therapy as compared to placebo condition (Lam, et al., 2001; Neumeister, et al., 1998b). Further supporting these results, a small study found that SAD patients in full summer remission experienced a temporary but clinically significant relapse of depressive symptoms after undergoing the AMPT method (Lam, et al., 2001).

It should be noted that a limitation of the AMPT method is that it is non-specific to DA or NE, so it remains unclear which catecholamine is more

important. Moreover, the effects of ATD may not be directly comparable to those of AMPT because depletion rates are different. In ATD studies maximal depletion of brain 5-HT is thought to occur around 5-7 hours after the start of the experiment, whereas in AMPT studies maximal depletion occurs only after a night's sleep (Lam, et al., 2001).

1.5 Influence of Bright Light on Dopamine

The influence of light is inevitably a subject of interest in the study of SAD. Data on the interaction between bright light and DA functioning are somewhat limited and indirect. One small scale study found that successful phototherapy in female SAD patients translated to a decrease of NE and metabolites in urinary output (Anderson, et al., 1992), but a subsequent study measuring NE and DA metabolites in CSF of SAD patients following bright light treatment found no difference in metabolite levels post-treatment, or as compared to healthy controls (Rudorfer, et al., 1993).

Recently, a study examining the effect of light deprivation on the cell viability of 3 aminergic neuron systems related to a rat model of depression found that animals kept in complete darkness for 6 weeks showed both anatomical and behavioral features indicative of a depressed state as compared to control animals kept in a daily equal part light-dark schedule (Gonzalez & Aston-Jones, 2008). The three neuron systems studied, including 5-HTergic and DAergic systems, all showed apoptosis as a result of long-term complete darkness (Gonzalez, et al., 2008). When rats kept in darkness were also administered the antidepressant Desipramine, which primarily affects norepinephrine, both neural and behavioural

effects of the treatment were significantly reduced (Gonzalez, et al., 2008). This has implications in relation to the monoamine deficiency hypothesis of depression; however the possibility that the lack of light acted in an alternative method to induce depression and consequently a depletion of monoamines should be noted.

1.6 DA and Reward-Related Behaviours

DA's involvement in reward-related processes has been extensively studied over the past several decades, though not without controversy as the exact role has been the subject of much debate. An early hypothesis was that DA directly mediated the subjective pleasure associated with reward (Berridge & Robinson, 1998; Wise, 1982). Lending some support to the hedonia/pleasure hypothesis, studies have shown that extracellular DA release in limbic striatal regions and subjective measures of pleasure in humans can increase in response to a spectrum of rewards including drugs of abuse such as cocaine (Schlaepfer et al 1997; Cox et al 2009), tobacco (Barrett, et al., 2004), amphetamine (Leyton, et al., 2002; Volkow, et al., 2001; Volkow, et al., 1994) and alcohol (Boileau, et al., 2003), as well as natural rewards such as palatable food (Small, et al., 2003), and to both food- (Volkow, et al., 2002) and drug-related cues (Volkow, et al., 2006; Wong, et al., 2006). Typically, a correlation between the magnitude of this DA response and pleasure or positive affective states is identified (Barrett, et al., 2004; Boileau, et al., 2006).

Accumulating evidence, though, suggests that although DA influences responses to reward related stimuli, this is not due to an effect on pleasure *per se*.

Rather, DA is thought to have an effect on processes related to reward such as the incentive salience of reward-related stimuli; namely the ability of these to elicit and sustain interest and goal-directed behaviour, independent of the subjective pleasurable effects of reward (Berridge, 2007; Leyton, et al., 2007). For example, in a recent study, healthy men were administered APTD, given a dosage of *d*-amphetamine and then asked to perform a reward/punishment-related Go/No-Go task, were observed; compared to the control treatments, APTD increased the number of commission errors in conditions with cues that predicted reward but did not alter the subjective effects of the drug, suggesting that DA is important in the incentive salience of reward-related stimuli, and the ability to respond to them preferentially (Leyton, et al., 2007). Similarly it has been shown that APTD can act to reduce the self-administration of rewards such as nicotine and alcohol in button pressing tasks of progressively increasing ratios, so designed to examine an individual's willingness to work for units of reward (Barrett, et al., 2008; Venugopalan, et al., 2009; Venugopalan VV, 2009).

Further, evidence from studies looking at lowered DA transmission in humans has strongly suggested that decreased DA is associated with impaired selective attention (Kahkonen, et al., 2001); and transiently lowered DA has been shown to have the ability to lower the attentional biases towards smoking-related cues observed in cigarette smokers (Munafo, et al., 2007).

1.7 Acute Phenylalanine Tyrosine Depletion as a Method of Examining the Behavioural Effects of Lowered Brain Dopamine

1.7.1 The APTD method

APTD is a method for transiently decreasing brain catecholamine neurotransmission, and has been used in animals and humans to examine the effects of lowered dopamine function. Brain DA synthesis is partially dependent on the relative availability of Tyr in the plasma as compared to other free large neutral amino acids, as this affects the amount of Tyr able cross the blood-brain barrier, and the availability of the key enzyme tyrosine hydroxylase. In the APTD method, participants ingest a mixture of essential and non-essential amino acids (AA). Following intestinal absorption, the increased concentration of amino acids in the plasma induces protein synthesis in the liver. Because the APTD mixture lacks Tyr and Phe, the precursors to catecholamines, endogenous stores of these AA from tissues and plasma become depleted as they become incorporated in to new protein, effectively reducing the relative availability of both Tyr and Phe in the plasma (Leyton, et al., 2000c; Moja, et al., 1996; Sheehan, et al., 1996). The rate-limiting enzyme in catecholamine synthesis, TH, is not typically fully saturated with Tyr (approximately 75%) (Carlsson & Lindqvist, 1978), so the lowered availability of Tyr likely results in decreased catecholamine synthesis.

Evidence from both animal and human studies have shown that, while initially expected to reduce both DA and NE transmission, APTD seems to primarily decrease DA transmission (Booij, et al., 2003; McTavish, et al., 1999c). In animals, Tyr/Phe-free mixtures have been shown to reduce CSF concentrations of the DA metabolite HVA (Palmour, et al., 1998), the rate of TH (Fernstrom &

Fernstrom, 1995), and stimulated DA release (Jaskiw & Bongiovanni, 2004; Le Masurier, et al., 2004; McTavish, et al., 1999a); though one study in rats did not find any reduction in DA levels themselves following APTD (Biggio, et al., 1976). In humans, APTD has a non-linear dose-response relationship on plasma catecholamine precursor levels, with maximal depletion occurring approximately 240 minutes following the ingestion of at least a 30 g tyr/phe-free mixture, and a marked decrease remaining up to 360 minutes following ingestion (Moja, et al., 1996). More recently, PET [^{11}C]raclopride binding studies have shown that resting levels of DA, as well as amphetamine-stimulated DA release, in the striatum are significantly reduced following the APTD treatment (Leyton, et al., 2004; Montgomery, et al., 2003).

1.7.2 Behavioural effects of APTD

Behaviourally, APTD has been found to reduce alcohol ingestion in healthy female (Leyton, et al., 2000b) and male social drinkers (Barrett et al 2008), manic symptoms in bipolar patients (McTavish, et al., 2001; Scarna, et al., 2003), craving to cocaine and related cues (Leyton, et al., 2005), the ability to preferentially respond to reward paired cues (Leyton, et al., 2007), and some of the psychostimulant effects of amphetamines (McTavish, et al., 2001; McTavish, et al., 1999b). It has also been seen to alter the ability to adjust betting behaviour in a gambling task (McLean, et al., 2004; Roiser, et al., 2005; Scarna, et al., 2005). However, it does not lower the mood-elevating effects of drugs of abuse (Casey, et al., 2006; Leyton, et al., 2005; Leyton, et al., 2000c), the effects of short-term induced panic (Coupland, Zedkova, Sanghera, Leyton, & Le Melledo,

2001), or the effects of induced stress (Coupland, et al., 2001; Leyton, et al., 2000c).

1.7.3 Effect of APTD on mood

The effects of APTD on mood have not been particularly pronounced or robust. On its own, APTD does not cause a significant mood lowering in healthy people and does not reinstate depressive symptoms in individuals recovered from a history of major depression (Booij, et al., 2003; McTavish, et al., 2005; Ruhe, et al., 2007). However, significant mood lowering was found in healthy individuals subjected to APTD following a psychological stressor/challenge (Leyton, et al., 2000c). In other studies, APTD has only been found to elicit mild mood-lowering responses associated with boredom, lowered contentedness and apathy (Leyton, et al., 2000c; McLean, et al., 2004). Overall, APTD does not seem to result in explicit feelings of sadness or depression. However, disordered perception and valuation of future outcomes, especially rewards, are thought to be mediated in part by reduced limbic DA release and may contribute to the maintenance of depressive states (Pizzagalli, et al., 2008).

1.8 Primary Hypotheses

The primary hypotheses are that APTD, especially in conjunction with a negative mood procedure, will induce a mood-lowering effect, and this response will be greater in dim than bright light. These anticipated mood changes will primarily be measured by self-reported answers to the Profile of Mood States (POMS) questionnaire, and secondarily by self-reported ratings to positive and

negative Visual Analog Scale (VAS) mood items and the Stanford Sleepiness Scale (SSS). We expect that some subjective states (*e.g.,* sense of depression, energy levels) will be more affected than others (*e.g.,* anxiety, irritability). It is also predicted that APTD will impair reward-related decision-making behaviour and the ability to sustain interest in reward, as measured by a Progressive Ratio (PR) button-pressing task for monetary reward, particularly in those participants who tested in dim light.

2.0 Methods

2.1 Participants

Ethical approval to conduct the study was obtained through the Institutional Review Board of the Faculty of Medicine at McGill University. Healthy, mildly seasonal women were recruited through advertisements in local newspapers and university classifieds asking for healthy females who feel less energetic in the winter than in the summer and who were interested in participating in a study involving the effects of a dietary factor on mood and behavior. Individuals were invited to contact the laboratory for more information. Following a more detailed description of the study over the telephone, interested individuals were given a brief telephone interview to assess their mood and behavior changes over time. This was done using the Global Seasonality Scale (GSS) of the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal et al., 1984), which asks individuals the degree, on a scale of 0 to 4, to which their sleep length, social activity, mood, weight, appetite and energy level change with the seasons. The inclusion criterion was a GSS score of 6 or higher, indicative of at least slight seasonal change in mood and behavior, with symptoms worsening in the winter and improving in the summer. According to one epidemiological study, this includes approximately 44% of the population (Kasper, et al., 1989b). Individuals reporting current major medical illness, i.e., underlying hormonal, cardiac, or digestive tract disorders (e.g. Celiac or Crohn's disease), current or past Axis I disorder, or current use of any psychotropic drugs or hormonal contraceptives were excluded at the time of the telephone interview, as were

individuals under the age of 18 or over 40. Participants over the age of 40 were excluded to reduce the variability associated with age-related changes in DA brain function. Increased age (>40) is correlated with decreased DA transporter availability (Volkow, et al., 1996), decreased DA receptor availability and function (Kaasinen, et al., 2000; Suhara, et al., 1991), as well as decreased cognitive and motor abilities (Bäckman, et al., 2000; Volkow, et al., 1998). Following the telephone screening based on these criteria, 80 women were interested and eligible.

Those candidates meeting these initial criteria were invited for a secondary assessment, at which time they were given a detailed description of the study and provided written informed consent. During this screening, individuals completed a number of questionnaires including the full SPAQ, the Short Michigan Alcoholism Screening Test (SMAST; Selzer et al., 1975), the 21-item Beck Depression Inventory (BDI; Beck, et al., 1979), and a self-rating version of the Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorder Version (SIGH-SAD-SR; Williams et al., 1994). Candidates were interviewed using the Structured Clinical Interview for DSM-IV, Non-Patient Edition (SCID-NP; Spitzer, et al., 1995) and their general physical health assessed through a routine check-up consisting of a standard physical examination, urine and blood analyses, and electrocardiography. Exclusion criteria were a current medical illness, current or past Axis I disorder according to the DSM-IV, and a BDI score of 15 or higher (indicative of possible depression of at least mild severity).

Participants were also asked to provide information regarding their menstrual cycle as testing only took place during the follicular phase to avoid potential variability from hormonal fluctuations experienced during the luteal phase of the women. Women were selected on the basis of a history of regular menses ranging in length from 26-35 days with a maximum of three days variation month-to-month. Women suffering from premenstrual dysphoric disorder were excluded.

Participants who passed the screening (n=38) were assigned to one of two light groups based on order of study entry. Of these women, 15 were lost to follow-up, and 3 became ineligible due to irregular menstrual cycle, health issues or scheduling constraints. Of the 9 women allocated to the dim condition, 2 dropped out after the first day (1 stated side effects, 1 stated illicit drug use post-test day). Of the 11 women allocated to the bright condition, 4 dropped out after the first test day (3 stated side effects, 1 stated time constraints). Four of the six dropouts had received the phenylalanine/tyrosine-deficient mixture. The study was completed by 14 women (dim: n=7; bright: n=7).

2.2 Study Design

Participants were randomly placed into one of two groups, either a bright or a dim light condition. All participants, irrespective of their light condition, received the two different amino acid mixtures, either nutritionally balanced or phenylalanine and tyrosine deficient, on two separate test days. Though neither participants nor experimenters were blind to the light conditions, the amino acid

mixtures were administered using a randomized, counterbalanced, double-blind design.

2.2.1. Testing facilities

Testing was performed at the Centre for Study and Treatment of Circadian Rhythms (Douglas Hospital Mental Health University Institute, Montreal, Quebec, Canada) during two consecutive winter seasons, starting and ending with the daylight savings time changes which occurred at the end of Fall and the beginning of Spring (November 4, 2007 to March 9, 2008 and November 2, 2008 to March 8, 2009). The participant test rooms were equipped with a private bathroom, bed, sofa, TV, DVD player, desk and chair. The rooms were free of time cues, windowless, air-conditioned, temperature-controlled (mean 23.5 °C, SD 2.0 °C), and soundproof. Contact with the experimenter in the adjacent control room was maintained through an intercom system. Light was administered by ceiling-mounted banks of cool-white fluorescent fixtures (4100 °K, F32T8/TL841, Philips Lighting, Somerset, NJ and F032/841, Sylvania, Danvers, MA) covered with lenses emitting less than 1% of radiant energy up to 400 nm. Light of lower wavelengths, including UV (100-400 nm), which could cause burns, is omitted using this procedure. Light intensity settings were established in the control room using an electronic device with setting ranging from 0 to 10,000 lux as verified with a calibrated light meter (IL1400A, International Light, Newburyport, MA).

2.2.2. Pre-study test day preparation

Participants were asked to maintain a self-reported regular sleep-wake schedule in the week before each test day. This was verified by daily phone calls to the laboratory's time-stamped voice mail and/or the maintenance of a written sleep-wake log. On the day preceding each test day, participants followed a low-protein diet (22.6 g of protein, 2212 kcal) to enhance the effect of the phenylalanine/tyrosine deficient amino acid mixture given on one of the test days. The diet was pre-packed and delivered to or picked up by the participant prior to both test days. Participants were asked to eat at regular hours. They were permitted water *ad libitum* and up to 3 cups of coffee or tea during the day, but were prohibited from consuming alcohol. For at least three weeks prior any test day, participants were asked to abstain from the use of any illicit drug and provided a urine sample for a drug screen (Triage™ Panel for Drugs of Abuse, Biosite Diagnostics, Inc., San Diego, CA) on the eve of each test day.

Participants arrived at the lab at the Centre for Study and Treatment of Circadian Rhythms in the evening before each test day, at least 1 hour before their normal sleep time, to spend the night in time isolation in the windowless test room. In this way, exposure to outdoor light the morning of the test day was avoided. Upon their arrival, participants handed in all personal devices indicating the time and were placed in isolation in their test room with light intensity set at 150 lux. At this time, they completed a set of mood questionnaires and provided a urine sample for the drug screen and a pregnancy test. The mood questionnaires consisted of the BDI, the Profile of Mood States (POMS; McNair, et al., 1982), a series of visual analogue scales (VAS; Bond & Lader, 1974) and the Stanford Sleepiness Scale (SSS; Hoddes, et al., 1973). At their normal sleep time, the light

intensity of the test room was reduced to complete darkness (<0.01 lux) and individuals were asked to go to sleep. A night technician monitored participants overnight in the adjacent control room, which is connected to the test room by a two-way intercom system. Participants were asked to fast from their sleep time until the beginning of the test day in the morning.

2.2.3 Test day procedures

For an overview of the testing day procedure and timeline, see Figure 1. Test days began at participants' normal wake time when the lights were turned on to 10 lux. Participant in the dim condition (L-) remained at this level of light intensity throughout the day. For participants in the bright condition (L+), the light intensity was increased to 3000 lux, the equivalent to the amount on a sunny day, in three 10-minute increments (150, 380 and 3000 lux) and remained at that level for the remainder of the day. Light exposure at eye level was generally 25-30% lower than at ceiling level. Morning baseline mood questionnaires (POMS, mood VAS, SSS) were completed by participants approximately 30 minutes following their wake time. Participants also had a blood sample taken to measure plasma levels of large neutral amino acids (LNAAs) including free and total phenylalanine and tyrosine.

Participants were then administered either a phenylalanine/tyrosine-deficient mixture (PT-) or a nutritionally balanced amino acid mixture (control test, B) and asked to ingest the mixtures as quickly as possible. During the subsequent 4-hour waiting period, participants were required to remain in the test room and permitted to read or watch videos of a relatively neutral nature, but not

permitted to sleep. They then filled in the mood questionnaires again, as well as a time estimation questionnaire simply asking the participants to write down how much time they thought had passed since waking up. These questionnaires were again administered at hours 5, 6, 7 and immediately prior to participant discharge. A second blood sample was taken between hours 4 and 5. Computer tasks consisting of the Facial Emotion Recognition task, the Cued- Reinforcement Reaction task, and the Progressive Ratio task were given between hours 4-6.

Following each task, participants were asked to indicate how well they thought they had performed using a VAS ("How well did you do?"). A negative mood induction procedure was administered between hours 6 and 7.

Upon completion of the test day, each participant was given a high-protein meal. Following approval from the physician on call, they were then sent home by public transportation or by taxi. All participants resumed their normal diet and sleep/wake cycle between the two test days.

.2.4 Treatment

The PT- mixture consists of 14 amino acids in powdered form: L-alanine 4.58 g, L-arginine 4.08 g, L-cysteine 2.25 g, glycine 2.97 g, L-histidine 2.67 g, L-isoleucine 6.67 g, L-leucine 11.25 g, L-lysine monohydrochloride 9.17 g, L-methionine 2.5 g, L-proline 10.17 g, L-serine 5.75 g, L-threonine 5.42 g, L-tryptophan 1.92 g, L-valine 7.42 g. The B mixture contains the same amino acids plus L-phenylalanine 4.75 g and L-tyrosine 5.75 g (Leyton, et al., 2000c). This procedure for APTD is based on that for ATD, which was first used more than

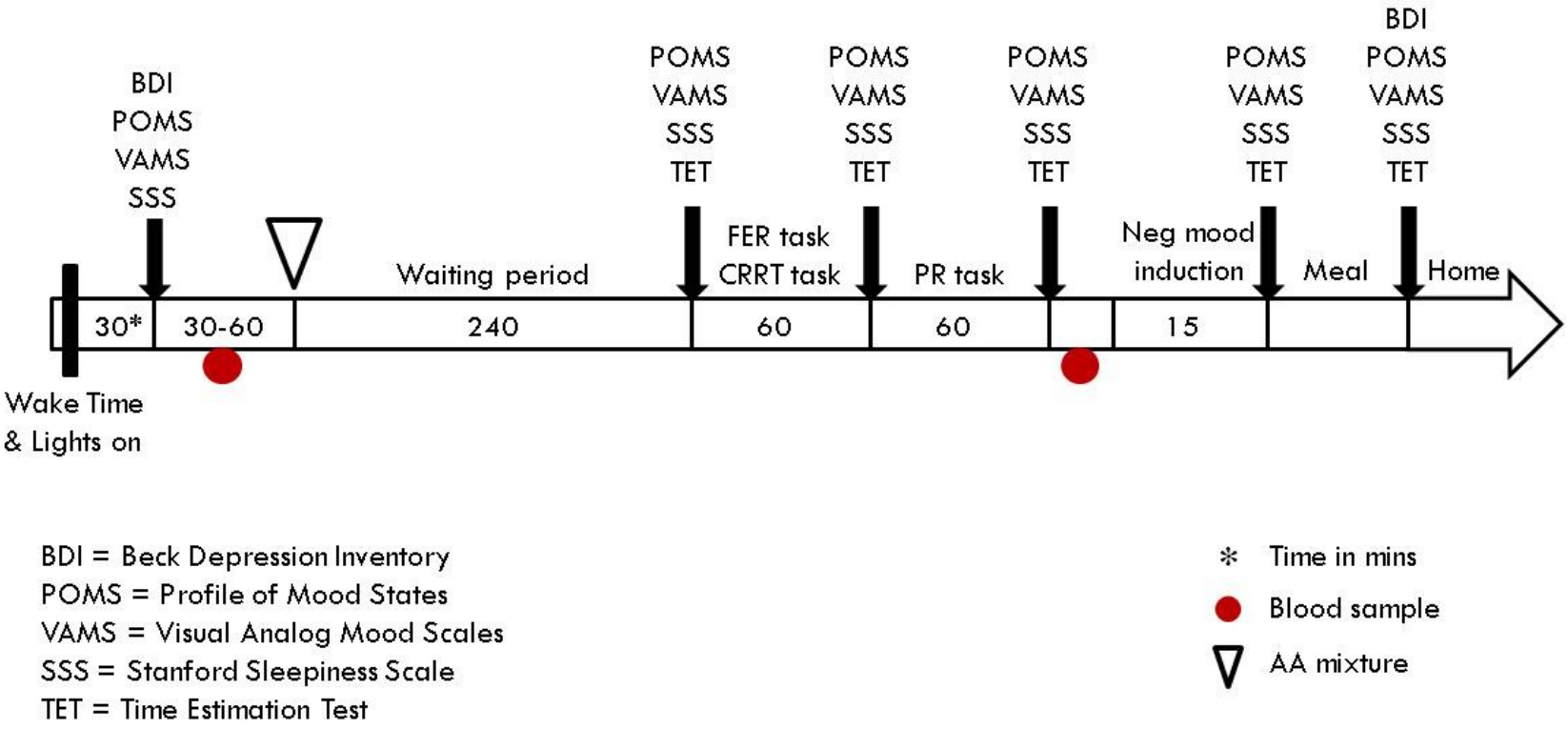

Figure 1. Schematic of the timeline on the two test days for the participants. The wake times varied between individual participants according to self-reported normal sleep and wake schedules. Numbers indicate minutes.

two decades ago (Young, et al., 1985b). The total of ~80 g of amino acid mixtures ingested is approximately the amount in a 400 g steak. The amino acids are given in the same proportion as they occur in human milk, except glutamate and aspartate, which are excluded because of concerns about their toxicity. This would not affect the ability of the PT- mixture to lower plasma and brain phenylalanine and tyrosine availability because glutamate and aspartate are not essential amino acids. Because of the unpleasant taste of L-methionine, L-cysteine, and L-arginine, these amino acids are administered separately in capsules at the time of ingestion. The remaining amino acids were prepared for oral administration by mixing with 150 ml water, and 50 ml chocolate syrup. Those who did not like the taste of chocolate were given the option of taking the amino acids in orange juice. Participants received the PT- and B mixtures at least three days apart under double-blind conditions. Order of administration (PT- first or second) was counterbalanced.

2.2.5 Computer Tasks

i) Facial Emotion Recognition

Participants were comfortably seated in a chair in front of a laptop computer on a large table. Faces with varying degrees (0% to 100%, in 10% steps) of emotional expressions (angry, disgusted, fearful, happy, sad, surprised) were shown on the screen and participants were asked to indicate which emotion they saw by clicking on the matching emotion word with a mouse. This task was

used as a measure of social perception. These results are not currently included; they are intended for future analyses.

ii) Cued-Reinforcement Reaction Time

This reward-based decision-making task was designed to assess goal-directed behaviour as guided by signals that predict the likelihood of rewards. During the task, subjects are presented with three circles surrounded by a coloured frame on a laptop computer monitor, and are required to identify, as fast as possible, the one circle that differs from the other two. If the response was correct but made slowly, the subject could win one point. Correct responses made quickly could win 100 points. Incorrect responses did not win points. The thresholds for fast *vs*. slow responses were based on individual differences in speed as determined during two 20-trial practice blocks. The cue, in the form of the coloured frame came in one of 3 different colours (blue, red, yellow), each representing a different probability of associated reward (i.e. 10%, 50% or 90%). These results are not currently included; they are intended for future analyses.

iii) Progressive Ratio

Participants completed a PR task "working" for financial rewards. They were informed that they would have the opportunity to earn extra money. Participants had the opportunity to earn $5.00 up to 10 times. To earn the first $5.00, individuals were required to complete 100 button presses. The number of button presses required to earn each subsequent $5.00 was then increased by a

factor of 2.3 (*i.e.,* 230, 529, 1217, 2799, 6438, 14 807, and etc). The session lasted until the participants wished to stop or the maximum time of one hour. The dependent variable is the "breakpoint"; *i.e.,* the maximum number of times that the subject is willing to press so as to obtain additional reward. The PR task parameters were determined during a pilot study (see Appendix 1)

2.2.6 Test measures

Mood state: Non-clinical fluctuations in mood may be assessed using the 72-item Profile of Mood States (POMS). It is composed of 6 bipolar scales (Agreeable-Hostile, Clearheaded-Confused, Composed-Anxious, Confident-Unsure, Elated-Depressed, Energetic-Tired), with each scale consisting of 12 adjectives rated on a 4-point scale. The 2 poles of each subscale respectively represent the positive and negative aspects. For each subscale, the sum of the positive items minus the sum of the negative items plus a constant of 18 is transformed into a T-score with a mean (SD) of 50(10). Both the total POMS score and the individual subscales are used as indicators of mood states.

A list of Visual Analog Scales (VAS) included 7 positive mood items (Elated, Enthusiastic, Excited, Happy, Interested, Lively, Satisfied) and 6 negative mood items (Angry, Anxious, Bored, Depressed, Irritated, Restless). Items were rated on unipolar scales ranging from 0 to 100 with increments of 5. Composite Positive Mood and Negative Mood scores were calculated using the average of positive and negative item scores, respectively.

Two baseline measurements of mood state were obtained. The BDI, POMS, and mood VAS were administered 30-90 minutes before normal sleep time the evening before each test day (evening baseline). Then 30 minutes after normal wake time in the morning of each test day, the POMS and mood VAS were administered again before ingestion of the amino acid mixture (morning baseline). Effects of amino acid mixtures on mood were measured repeatedly in the afternoon of each test day, at 4, 5, 6, and 7 hours after amino acid mixture ingestion.

Side Effects: Dizziness, headache, nausea have been reported both following amino acid mixture ingestion and with bright light. Therefore, each mood assessment also included three VAS labeled "Dizzy", "Headache", and "Nauseous", with scaling identical to the mood VAS. When regurgitation occurred, a test day was discontinued if less than 45 minutes had passed since mixture ingestion.

Plasma Amino Acid Levels: Venous blood samples were collected 15 minutes before and between 4 and 5 hours after ingestion of the amino acid mixtures. Some of the samples could not be collected (3 morning, 1 afternoon). Samples were centrifuged immediately (10 minutes, 1500g, 0 °C) and stored at -80 °C, until further analysis.

Plasma amino acid levels were measured using high-pressure liquid chromatography (HPLC) with fluorometric detection on an Ultrasphere ODS reverse-phase column (Beckman Coulter, Fullerton, CA) with ophtalaldehyde pre-

column derivatization and aminoadipic acid as an internal standard. Total and free tryptophan levels were assessed by HPLC with fluorometric detection on a Bondpak® reverse-phase column (Phenomenex, Torrance, CA).

2.3 Statistical analyses

Data analyses were performed using SPSS v. 17.0 for Windows (SPSS, Inc, Chicago, Ill). Demographic and baseline screening characteristics were compared using T-tests (see Table 1).

For mood, POMS Total and subscale scores and mood VAS individual scales and negative/positive subscale scores were considered primary dependent variables. All data were analyzed using two-way mixed design ANOVAs. Drink treatment (PT-, B) was a repeated measures within-subjects factor. Light condition group (L+ or L-) was an independent between-groups factor. Time was a repeated measures within-subjects factor (5 levels: morning baseline, +4h, +5h, +6h, +7h, indicating time passed since amino acid mixture ingestion). All analyses were thus performed using between/within repeated measures ANOVAs (Treatment x Light x Time). The models considered the main effects of Treatment, Light and Time and their interactions. Type III tests were deemed significant at p-values of 0.05 or less. Significant interaction and/or main factor effects were analyzed post-hoc using pair-wise comparisons that were Bonferroni corrected for multiple comparisons. The possible influence of Order of administration of drink Treatment (PT- first or second) was also checked.

Findings are reported using estimated least-square means and standard errors of the mean (SEM).

For afternoon plasma LNAA analyses, models considered the between-subjects factor Light, the within-subjects factor Time (2 levels: morning and afternoon measurements), the within-subjects factor Treatment and the 2-way interactions.

3.0 Results

3.1 Baseline Measurements and Demographic characteristics

The demographic and baseline screening characteristics are summarized in Table 1. Baseline measurements were taken both the evening before each test day upon arrival at the testing centre, as well as in the morning prior the administration of the AA mixtures (see Figure 1). Measurements included the BDI, the POMS, the mood VAS, the side effects VAS, and the SSS. Both in the evening and the morning, there were no statistically significant differences revealed by Treatment and/or Light for any of the POMS *Total* or any of the subscale scores ($F_{1,12} \leq 3.035$, $p \geq 0.107$ for all), the combined *Positive* or *Negative* mood VAS ($F_{1,12} \leq 2.613$, $p \geq 0.132$ for all), the BDI ($F_{1,12} \leq 3.254$, $p \geq 0.096$ for all), or the SSS ($F_{1,12} \leq 4.167$, $p \geq 0.064$ for all). Similarly, there were no differences in baseline values for the VAS side effect symptoms of *Dizzy*, *Headache*, or *Nausea* in either the evening or the morning ($F_{1,12} \leq 2.380$, $p \geq 0.149$ for all). Those in the Bright condition tended to feel more *Stressed* in the evening prior their PT- day than the evening prior their B day (p=0.052), however this difference was resolved by the morning baseline measurement.

3.2 Plasma amino acids

The ingestion of the B and PT- AA mixtures produced the expected effects on plasma concentrations of Phe and Tyr as indicated by significant Treatment by Time interactions (Tyr: $F_{1,8}$=66.681, p=0.000; Phe: $F_{1,8}$=60.185, p=0.000). Tyr concentrations significantly increased from morning baseline concentrations six

	Group; mean (SD) where applicable	
	Dim (n=7)	**Bright** (n=7)
Age (years)	23.86 ± 6.817	20.14 ± 2.116
Body Mass Index (kg/m^2)	21.429 ± 1.6378	23.057 ± 2.6501
Beck Depression Inventory score at lab screening	3.86 ± 4.488	2.14 ± 2.193
Global Seasonality Scale score at lab screening	11.86 ± 3.976	10.14 ± 2.340

Table 1. Demographic and baseline screening characteristics for each light group. There were no significant group differences by t-test ($p>0.192$ for all).

hours following ingestion of the B mixtures (p=0.001), and significantly decreased following ingestion of the PT- mixtures (p=0.000), see Table 2. Similarly, Phe concentrations increased from morning baseline concentrations six hours following ingestion of the B mixtures (p=0.000), and decreased six hours following ingestion of the PT- mixtures (p=0.000), see Table 2. There were no effects of light alone (Tyr: $F_{1,8}$= 1.492, p=.257; Phe: $F_{1,8}$=0.060, p=.813) or interactions of light with condition (Tyr: $F_{1,8}$=.361, p=.565; Phe: $F_{1,8}$=.846, p=.385) or Time (Tyr: $F_{1,8}$=.148, p=.710; Phe: $F_{1,8}$=1.157, p=.313). There was no main effect of Time (Tyr: $F_{1,8}$= $F_{1,8}$=4.193, p=0.075; Phe: $F_{1,8}$=3.475, p=0.099).

Similar effects emerged when looking at the ratios of the concentrations of Tyr and Phe to that of total LNAA. There were Treatment by Time interactions for both Tyr:LNAA ($F_{1,8}$=50.271, p=0.000) and Phe:LNAA ($F_{1,8}$=198.260, p=0.000). There was no significant difference in Tyr: LNAA from the morning to the afternoon measurements following ingestion of the B mixtures (p=.270), but the anticipated decrease following the PT- mixtures was significant (p=0.000). Phe:LNAA significantly decreased following the B mixtures (p=0.000) and following the PT- mixtures (p=0.000). There were no effects of light (Tyr:LNAA: $F_{1,8}$=4.360, p=0.070; Phe:LNAA: $F_{1,8}$=0.160, p=0.700), no interactions of light with treatment (Tyr:LNAA: $F_{1,8}$=1.363, p=.277; Phe:LNAA: $F_{1,8}$=0.317, p=0.589), and no interactions of light with time (Tyr:LNAA: $F_{1,8}$=0.97, p=.763; Phe:LNAA: $F_{1,8}$=0.045, p=0.837).

	PLASMA CONCENTRATIONS (µmol/L ± SEM)			
	B		PT-	
	AM	PM	AM	PM
TYR	54.1 ± 3.3	*** 123.4 ± 14.8**	56.1 ± 2.5	*** 13.1 ± 0.9**
PHE	48.9 ± 1.3	*** 74.8 ± 1.7**	48.0 ± 1.7	*** 12.3 ± 1.2**
TYR:LNAA	0.13 ± 0.008	0.111 ± 0.014	0.137 ± 0.005	*** 0.012 ± 0.001**
PHE:LNAA	0.116 ± 0.002	*** 0.063 ± 0.004**	0.115 ± 0.002	*** 0.012 ± 0.002**

Table 2. Plasma concentrations of free large neutral amino acids (LNAA) before and 6 hours after AA mixture ingestion. Significant changes are indicated in bold and by the asterisk ($p<0.015$ for all). B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures. Tyr=Tyrosine, Phe=Phenylalanine.

3.3 Mood

3.3.1 POMS

The outcomes of the POMS mood measurements are summarized in Table 3. A mixed-effects (Treatment x Light x Time) ANOVA on the POMS Total scores yielded a significant three-way interaction ($F_{4,48}$=4.929, p=0.002) and a significant effect of Time ($F_{4,48}$=8.404, p=0.000). Post-hoc analyses indicated that the interaction was driven by a significant APTD-induced lowering of mood specifically in those individuals who were tested in the Dim light condition; at a time point 6 hours after initial ingestion of the mixtures (T=+6h), the Dim light group's mean POMS *Total* score on the PT- day lowered to 300.0±13.8 as compared to 333.4±15.7 on the B day (p=0.014). Post-hoc power analyses revealed that within the Dim group, there was an effect size of 0.85 and the power was 0.63. Using a further *a priori* power analysis, it was determined that a power of 0.80 could be achieved with the addition of 4 participants. Bright light prevented this mood lowering effect of the APTD, as these changes in mood were not seen in the Bright light group at this same time point (B: mean 320.7±15.7, PT-: mean 334.0±13.8, p=0.274). These effects are shown in Figure 2. In looking at the POMS *Total* scores of the Bright and Dim light groups on the PT- day, post-hoc power analysis revealed an effect size of 0.93 and a power of 0.50. It was determined through an *a priori* power analysis that the addition of 8 participants to each group, for a total n of 30 would generate a power of 0.80.

The same three-way interactions were seen for two of the six individual POMS subscales: *Energetic-Tired* ($F_{4,48}$=4.303, p=0.005) and *Clearheaded-Confused* ($F_{4,48}$=4.446, p=0.004). Post-hoc analyses indicated this was due to

APTD-induced mood-lowering effects at T=+6h when subjects were tested in the Dim light but not Bright light. Additionally, for both subscales, there was an effect of Time alone, with all participants generally becoming less energetic/more tired and less clearheaded/more confused as the day progressed, see Table 3 and Figures 3 and 4.

There was a significant Treatment x Time interaction seen on the *Agreeable-Hostile* subscale ($F_{4,48}$=2.684, p=0.042). All participants felt significantly less agreeable/more hostile following the PT- mixture than following the B mixture at T=+6h; however this effect was independent of any influence by the light factor, see Table 3.

There was a significant main effect of Time on the *Elated-Depressed* ($F_{4,48}$=14.070, p=0.000), *Composed-Anxious* ($F_{4,48}$=3.602, p=0.012) and *Confident-Unsure* ($F_{4,48}$=3.738, p=0.010), subscales, with a general trend of lowered scores with time. This was most notably at T=+7h on the *Elated-Depressed* subscale, immediately following the negative mood induction procedure, indicating this was effective in elucidating subjective feelings of depression or sadness (see Figure 5). However, as with the other two immediately aforementioned subscales, there were no significant effects and/or interactions of Treatment or Light condition. Interestingly, post-hoc pair-wise comparisons showed a significantly lowered score at T=+6h in the Dim group following the PT- mixture, but not the B mixture, on the *Composed-Anxious* subscale.

		Mood Scores (mean±SEM)			Outcomes of *F*-tests, corresponding *p*-values						
	LIGHT	**B**	**PT-**	***p*-value**	**Treatment x Light x Time $F_{4,48}$=**	**Treatment x Time $F_{4,48}$=**	**Treatment x Light $F_{1,12}$=**	**Light x Time $F_{4,48}$=**	**Treatment $F_{1,12}$=**	**Light $F_{1,12}$=**	**Time $F_{4,48}$=**
POMS											
Total score	L+	320.7±15.7	334.0±13.8	0.274	**4.929, *p*=0.002**						**8.404, *p*=0.001**
	L-	333.4±15.7	300.0±13.8	**0.014**							
Energetic-Tired	L+	46.8±2.5	51.4±3.0	0.144	**4.303, *p*=0.005**						**3.620, *p*=0.012**
	L-	52.2±2.5	45.7±3.0	**0.044**							
Clearheaded-Confused	L+	54.1±2.6	58.0±2.8	0.147	**4.446, *p*=0.004**						**4.592, *p*=0.019**
	L-	54.4±2.6	48.2±2.8	**0.030**							
Agreeable-Hostile	L+/L-	58.8±2.5	54.7±2.1	**0.007**		**2.684, *p*=0.042**					
Elated-Depressed	L+	52.1±3.1	52.5±2.5	0.906							**14.070, *p*=0.000**
	L-	55.4±3.1	49.2±2.5	0.109							
Composed-Anxious	L+	59.7±3.3	61.2±3.8	0.481							**3.602, *p*=0.012**
	L-	60.7±3.3	57.8±3.8	0.211							
Confident-Unsure	L+	53.4±2.9	52.2±2.7	0.675							**3.738, *p*=0.025**
	L-	53.7±2.9	47.4±2.7	**0.036**							

Table 3. Outcomes of POMS mood measurements. Mood scores and corresponding post-hoc pair-wise comparisons results (*p*-values) indicate those taken at T=+6h (from initial AA mixture ingestions). Outcomes of multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are also shown. Results of *F*-tests are shown with corresponding *p*-values for those with $p \leq 0.05$. Gray-scale indicates omitted non-significant outcomes of *F*-tests. Significant results are shown in bold. POMS=Profile of Mood States. L+=Bright light condition, L-=Dim light condition. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

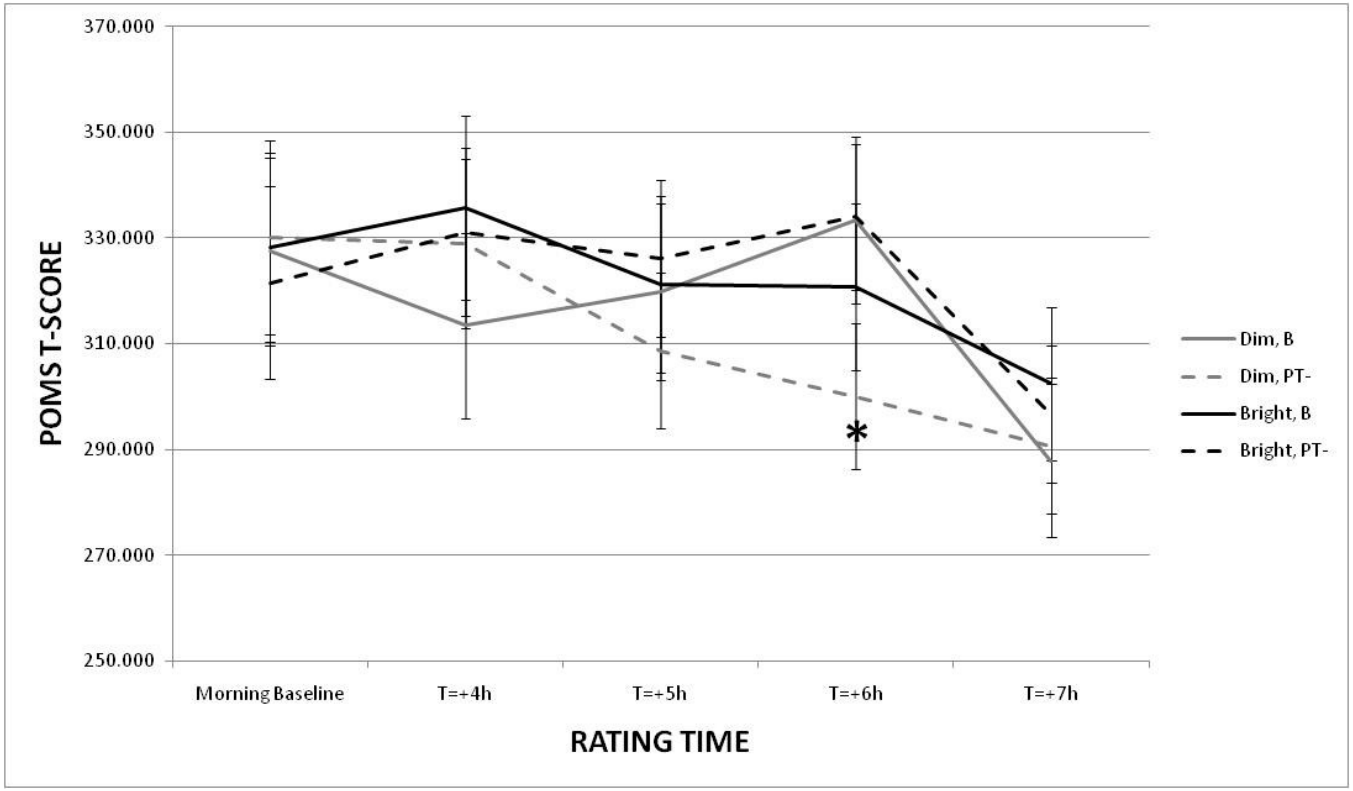

Figure 2. POMS *Total* scores per Light group and drink Treatment. Significant three-way interaction, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)), was analyzed post-hoc; significant result is indicated by asterisk (p=0.002). Mood score values are shown as mean corrected t-scores ± SEM. T=hours elapsed following initial ingestion of AA mixtures. POMS=Profile of Mood States. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

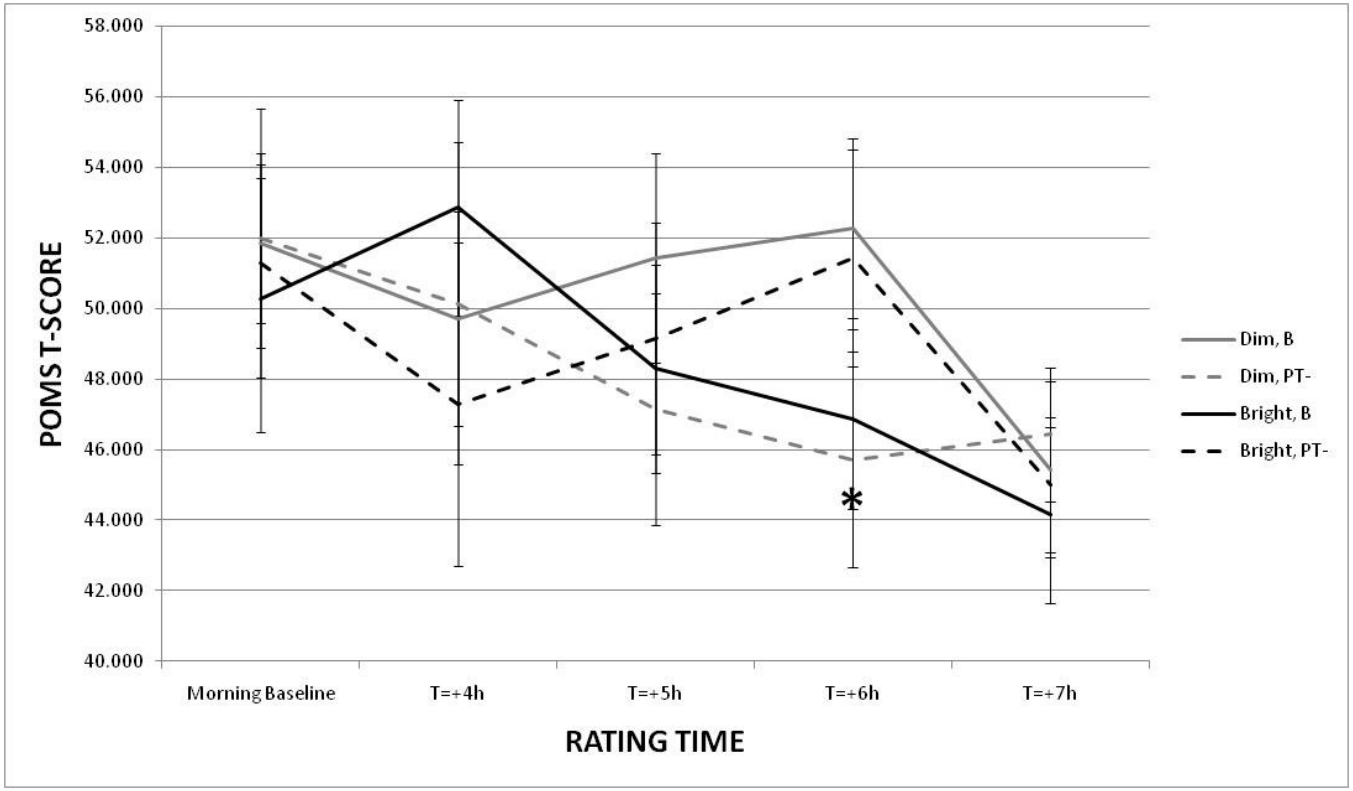

Figure 3. POMS *Energetic-Tired* scores per Light group and drink Treatment. Significant three-way interaction, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)), was analyzed post-hoc; significant result is indicated by asterisk (p<0.005). Mood score values are shown as mean corrected t-scores ± SEM. T=hours elapsed following initial ingestion of AA mixtures. POMS=Profile of Mood States. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

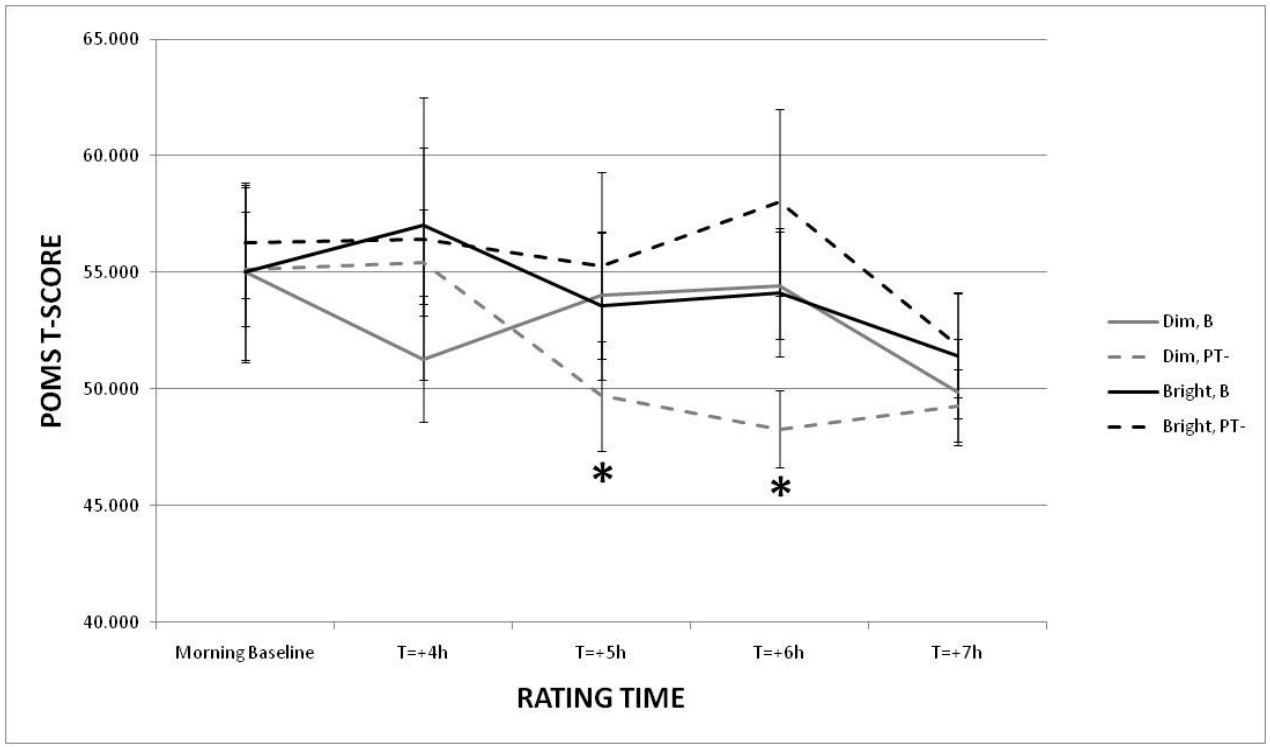

Figure 4. POMS *Clearheaded-Confused* scores per Light group and drink Treatment. Significant three-way interaction, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) was analyzed post-hoc; significant results are indicated by asterisk (p<0.004). Mood score values are shown as mean corrected t-scores ± SEM. T=hours elapsed following initial ingestion of AA mixtures. POMS=Profile of Mood States. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

3.3.2 Mood VAS

The primary results are summarized in Table 4. The three-way interactions seen on the POMS were not observed. Rather, three-way (Treatment x Light x Time) ANOVAs yielded significant Time by Light interactions on the *Depressed* ($F_{4,48}$=14.907, p=0.002) scale scores (see Table 4). Post-hoc analysis showed this was driven by an increase in scores in both groups at T=+7h, immediately following the negative mood procedure; also that the scores for those in the Dim light condition were significantly higher as compared to those in the Bright condition (p=0.038; see Figure 6). The same Time by Light interaction was shown on *Happy-Depressed* ($F_{4,48}$=4.563, p=0.003) and *Elated-Depressed* ($F_{4,48}$=3.427, p=0.015) subscales scores (created by adding reverse-scored *Depressed* ratings to *Happy* and *Elated* scores, respectively). Again, this was driven by a decrease in scores on each of these scales at T=+7h, with those in the Dim light condition having significantly lower scores as compared to those in the Bright (p<0.038). There was no influence of Drink Treatment.

There was a significant effect of Time on the *Depressed*, *Happy*, *Bored*, *Enthusiastic*, *Satisfied*, *Excited*, *Elated*, *Interested*, *Lively*, *Happy-Depressed*, and *Elated-Depressed* scale scores, see Table 4 for F-values.

3.3.3 SSS

A three-way (Treatment x Light x Time) ANOVA on the SSS scores yielded a significant three-way interaction ($F_{4,48}$=2.871, p=0.047, using Huynh-Feldt adjustment for non-sphericity). Post-hoc analyses indicated there was an APTD-

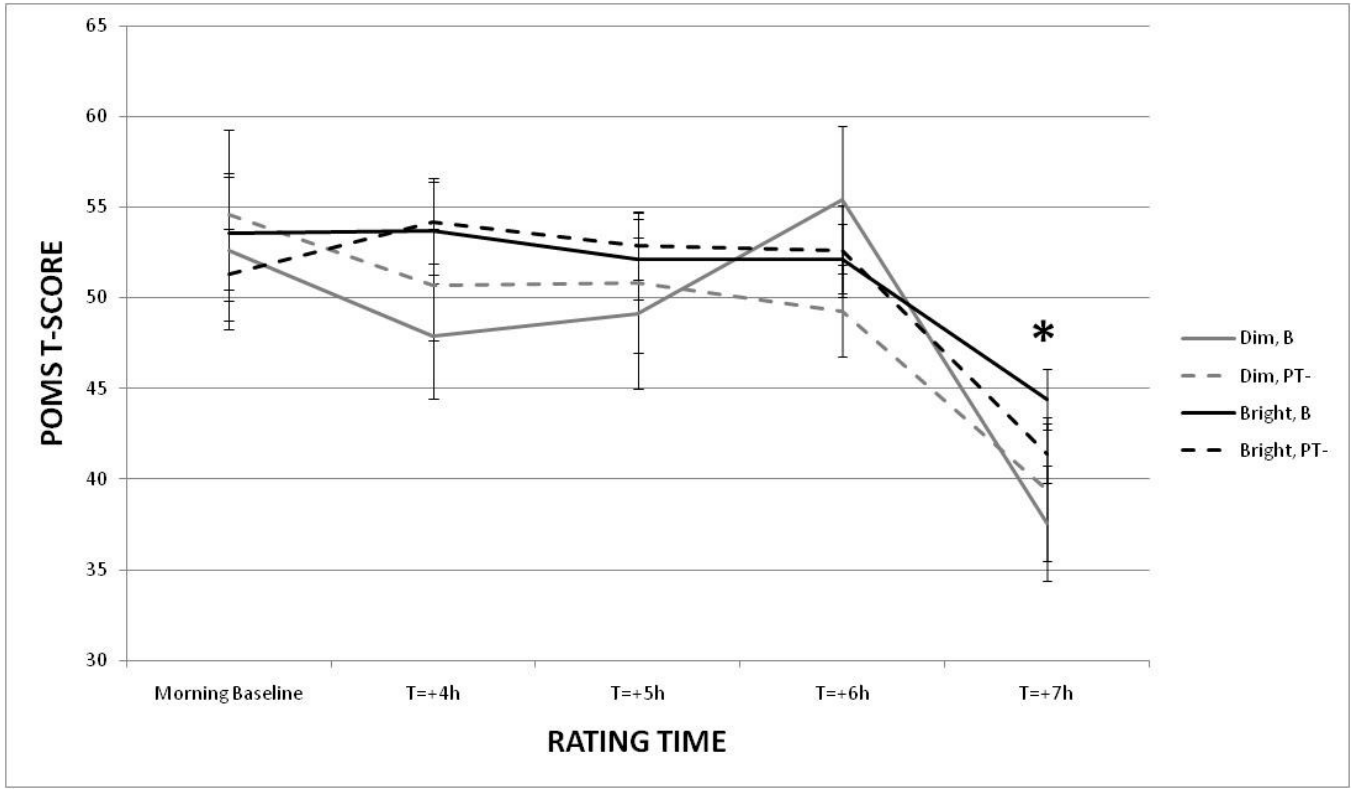

Figure 5. POMS *Elated-Depressed* scores per Light group and drink Treatment. Significant main effect of Time, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) is indicated by asterisk ($p<0.0001$). Mood score values are shown as mean corrected t-scores ± SEM. T=hours elapsed following initial ingestion of AA mixtures. POMS=Profile of Mood States. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

		Mood Scores (mean±SEM)					Outcomes of *F*-tests, corresponding *p*-values						
	LIGHT	T=Baseline		T=+7h		*p*-value	Treatment x Light x Time $F_{4,48}$=	Treatment x Time $F_{4,48}$=	Treatment x Light $F_{1,12}$=	Light x Time $F_{4,48}$=	Treatment $F_{1,12}$=	Light $F_{1,12}$=	Time $F_{4,48}$=
		B	PT-	B	PT-								
VAS													
Depressed	L+	5.0±5.7		*11.0±8.5		***0.38**				**5.089,**			**14.907,**
	L-	6.7±5.7		*39.2±8.5		**0.002**				***p*=0.015**			***p*=0.000**
Angry	L+	0.7±0.5		*0.3±3.7		***0.097**							
	L-	0.0±0.6		*10.0±3.7									
Stressed	L+	15.0±7.6		9.2±7.5		n/a			4.279,	2.249,			
	L-	10.3±7.6		17.5±7.5					*p*=0.061	*p*=0.104			
Happy	L+	67.1±7.7		*50.7±6.4		***0.042**				1.800,			**16.330,**
	L-	62.5±7.7		*30.0±6.4		**0.007**				*p*=0.144			***p*=0.000**
Bored	L+	20.9±5.7		24.6±6.7		n/a							**4.090,**
	L-												***p*=0.006**
Enthusiastic	L+	53.5±5.7		31.0±5.7		n/a							**11.093,**
	L-												***p*=0.000**

Table 4. Outcomes of VAS Mood measurements. Mood scores and post-hoc pair-wise comparisons *p*-values (significant results shown in bold) are shown. Asterisks within individual mood scales indicate pairs of significantly differing values and corresponding *p*-values. Outcomes of multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are shown. Results of *F*-tests are shown with corresponding *p*-values ≤ 0.05. Significant results are shown in bold. Gray-scale indicates omitted non-significant outcomes of *F*-tests. VAS=Visual Analog Scales. L+=Bright light condition, L-=Dim light condition. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures. T=Baseline is morning baseline measurement, T=+7h is relative to initial ingestion of AA mixtures.

		Mood Scores (mean±SEM)			Outcomes of *F*-tests, corresponding *p*-values						
	LIGHT	**T=Baseline**	**T=+7h**	***p*-value**	**Treatment x Light x Time $F_{4,48}$=**	**Treatment x Time $F_{4,48}$=**	**Treatment x Light $F_{1,12}$=**	**Light x Time $F_{4,48}$=**	**Treatment $F_{1,12}$=**	**Light $F_{1,12}$=**	**Time $F_{4,48}$=**
Anxious	L+	13.7±6.6	14.2±5.6	n/a							
	L-										
Satisfied	L+	59.1±5.1	38.5±5.1	n/a							**6.662, *p*=0.000**
	L-										
Excited	L+	46.1±6.7	27.1±5.8	n/a							**6.033, *p*=0.001**
	L-										
Elated	L+	25.1±6.9	7.5±4.0	n/a					3.935, *p*=0.071		**4.522, *p*=0.013**
	L-										
Interested	L+	55.0±5.4	36.9±6.1	n/a							**12.459, *p*=0.000**
	L-										
Restless	L+	9.2±4.6	11.9±4.2	n/a							
	L-										

Table 4…cont'd (2). Outcomes of VAS Mood measurements. Mood scores and post-hoc pair-wise comparisons *p*-values (significant results shown in bold) are shown. Asterisks within individual mood scales indicate pairs of significantly differing values and corresponding *p*-values. Outcomes of multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are shown. Results of *F*-tests are shown with corresponding *p*-values ≤ 0.05. Significant results are shown in bold. Gray-scale indicates omitted non-significant outcomes of *F*-tests. VAS=Visual Analog Scales. L+=Bright light condition, L-=Dim light condition. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures. T=Baseline is morning baseline measurement, T=+7h is relative to initial ingestion of AA mixtures.

		Mood Scores (mean±SEM)			Outcomes of *F*-tests, corresponding *p*-values						
	LIGHT	**T=Baseline**	**T=+7h**	***p*-value**	**Treatment x Light x Time $F_{4,48}$=**	**Treatment x Time $F_{4,48}$=**	**Treatment x Light $F_{1,12}$=**	**Light x Time $F_{4,48}$=**	**Treatment $F_{1,12}$=**	**Light $F_{1,12}$=**	**Time $F_{4,48}$=**
Irritated	L+	1.2±0.5	5.3±2.2	n/a							
	L-										
Lively	L+	45.1±6.7	26.7±6.3	n/a							**4.816, *p*=0.002**
	L-										
Happy-Depressed	L+	162.1±10.9	***139.6±13.9**	***0.029**				**4.563, *p*=0.003**			**22.885, *p*=0.000**
	L-	155.7±10.9	***90.7±13.9**	**0.000**							
Elated-Depressed	L+	113.2±11.5	***99.6±9.0**	***0.020**				**3.427, *p*=0.015**	3.999, *p*=0.069		**11.195, *p*=0.000**
	L-	125.3±11.5	***65.0±9.0**	**0.002**							

Table 4…cont'd (3). Outcomes of VAS Mood measurements. Mood scores and post-hoc pair-wise comparisons *p*-values (significant results shown in bold) are shown. Asterisks within individual mood scales indicate pairs of significantly differing values and corresponding *p*-values. Outcomes of multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are shown. Results of *F*-tests are shown with corresponding *p*-values ≤ 0.05. Significant results are shown in bold. Gray-scale indicates omitted non-significant outcomes of *F*-tests. VAS=Visual Analog Scales. L+=Bright light condition, L-=Dim light condition. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures. T=Baseline is morning baseline measurement, T=+7h is relative to initial ingestion of AA mixtures.

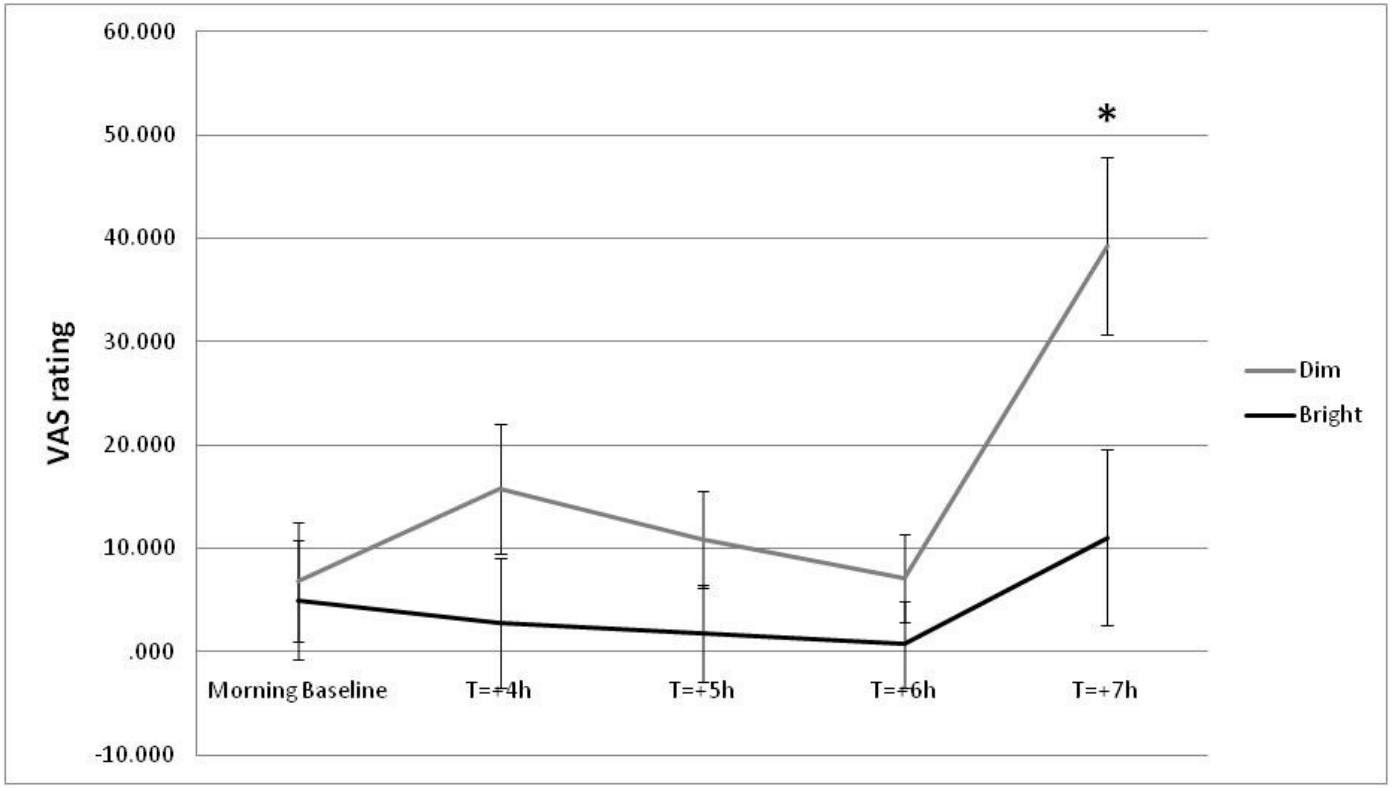

Figure 6. VAS scores for *Depressed* scale per Light group. Significant Light by Time interaction, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) was analyzed post-hoc; significant result is indicated by asterisk (p<0.038). Similar effects were seen on the *Happy-Depressed* and *Elated-Depressed* scales. Mood score values are shown as mean values ± SEM. T=hours elapsed following initial ingestion of AA mixtures. VAS=Visual Analog Scale.

induced increase in subjective measures of sleepiness in those individuals who were tested in the Dim light condition, at T=+6h, but not in the Bright light group. The Dim group's mean SSS score on the PT- day increased to 3.7±0.5, indicating increased sleepiness as compared to 2.2±0.3 on the B day (p=0.044).

There were also significant differences in scores between the two light groups at various time points on both the B and PT- days. On the B day, the Dim group reported higher levels of sleepiness than the Bright group at T=+4h (L+: 1.2±0.2, L-:3.0±0.2; p=0.002). On the PT- day, the Dim group reported higher levels of sleepiness than the Bright group at T=0h (L+: 2.0±0.2, L-: 3.0±0.2; p=0.021); T=+4h (L+: 1.5±0.3, L-: 3.0±0.3; p=0.012); and T=+6h (L+: 1.5±0.5, L-: 3.7±0.5; p=0.011). These findings are shown in Figure 7.

3.4 Side Effects Symptoms

VAS scores were obtained for the potential side effects of *Nausea*, *Dizziness*, and *Headache* as a result of AA mixture consumption. Three-way (Treatment x Light x Time) ANOVAs on these scores did not yield any significant interactions for any of these symptoms (see Table 5 for summary of F-values). There was a significant main effect of time for *Nausea* ($F_{4,48}$=5.533, p=0.012) and *Dizziness* ($F_{4,48}$=3.743, p=0.045), with the general pattern being an increase in these symptoms throughout the day as compared to morning baseline, and the peak being at T=+4h, just prior the afternoon questionnaires and computer tasks (see Figure 8). These effects were independent of any influence of Light or Treatment.

3.5 Influence of Seasonality

As a secondary analysis, the potential influence of seasonality on changes in mood scores in response to the APTD and Dim light was tested; the participants' GSS scores (at lab screening) were added as a covariate to the abovementioned mixed-effects analyses for the POMS *Total* and subscale scores. There was no influence of GSS scores, or interactions between the GSS scores and other factors (Treatment, Time, Light) on any of the POMS *Total*, *Composed-Anxious*, *Elated-Depressed*, *Confident-Unsure*, *Energetic-Tired*, or *Clearheaded-Confused* scales. The GSS scores also did not affect the three-way (Treatment x Light x Time) interactions previously seen on the POMS *Total*, *Energetic-Tired*, and *Clearheaded-Confused*.

The ANCOVA did reveal a significant Treatment by Light by Time interaction on the *Agreeable-Hostile* subscale ($F_{4,44}$=3.182, p=0.034). Post-hoc analyses indicated that at T=+6h, those participants in the Dim light felt significantly less agreeable/more hostile following the APTD than balanced AA mixtures (B: 56.143; PT-: 50.845, p=0.24). Nonetheless, this is still considered secondary analyses.

3.6 Progressive Ratio Task

The measures of *Completed Breakpoints* (i.e. 1, 2, 3, etc.) and *Money Earned* (i.e. $5, $10, $15, etc) showed Treatment by Light interactions that approached significance ($F_{1,12}$=1.778, p=0.207 and $F_{1,12}$=2.885, p=0.115, respectively). Post-hoc analyses using simple pair-wise comparisons with a Bonferroni adjustment in SPSS (v.17.0) on the *Money Earned* measure again

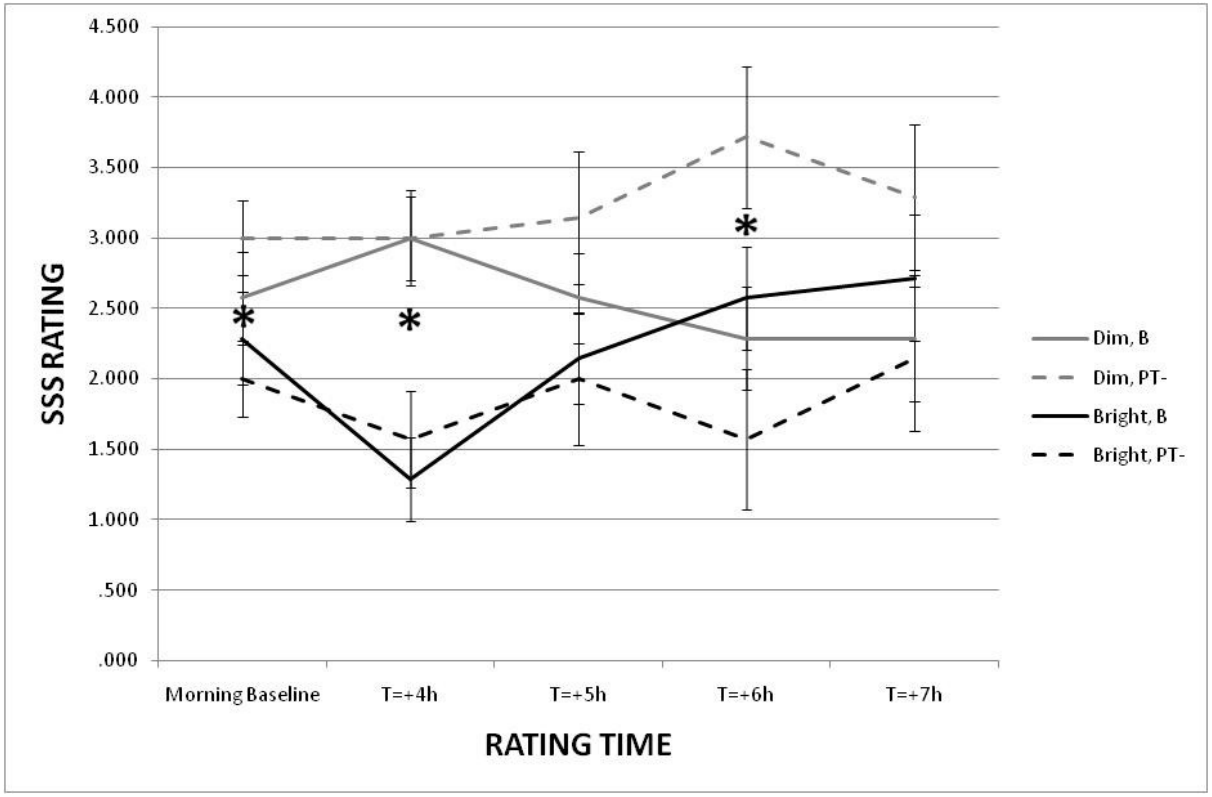

Figure 7. SSS ratings per Light group and drink Treatment. Significant three-way interactions, according to multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are indicated by asterisks (p<0.04 for all). SSS scores are shown as mean values ± SEM. T=hours elapsed following initial ingestion of AA mixtures. SSS= Stanford Sleepiness Scale. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

		Mood Scores (mean±SEM)			Outcomes of *F*-tests, corresponding *p*-values						
	LIGHT	**T=Baseline**	**T=+7h**	***p*-value**	**Treatment x Light x Time $F_{4,48}$=**	**Treatment x Time $F_{4,48}$=**	**Treatment x Light $F_{1,12}$=**	**Light x Time $F_{4,48}$=**	**Treatment $F_{1,12}$=**	**Light $F_{1,12}$=**	**Time $F_{4,48}$=**
Nausea	L+	2.3±1.5	7.8±3.1	n/a							**5.533, *p*=0.001**
	L-										
Dizziness	L+	2.5±1.7	7.7±3.1	n/a							**3.743, *p*=0.045**
	L-										
Headache	L+	7.6±5.0	16.0±4.6	n/a							
	L-										

Table 5. Outcomes of side effects VAS measurements. Mood scores and outcomes of multi-factorial ANOVA model (Treatment (2) x Light (2) x Time (5)) are shown. Results of *F*-tests are shown with corresponding *p*-values ≤ 0.05. Significant results are shown in bold. Gray-scale indicates omitted non-significant outcomes of *F*-tests. VAS=Visual Analog Scales. L+=Bright light condition, L-=Dim light condition. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures. T=Baseline is morning baseline measurement, T=+7h is relative to initial ingestion of AA mixtures.

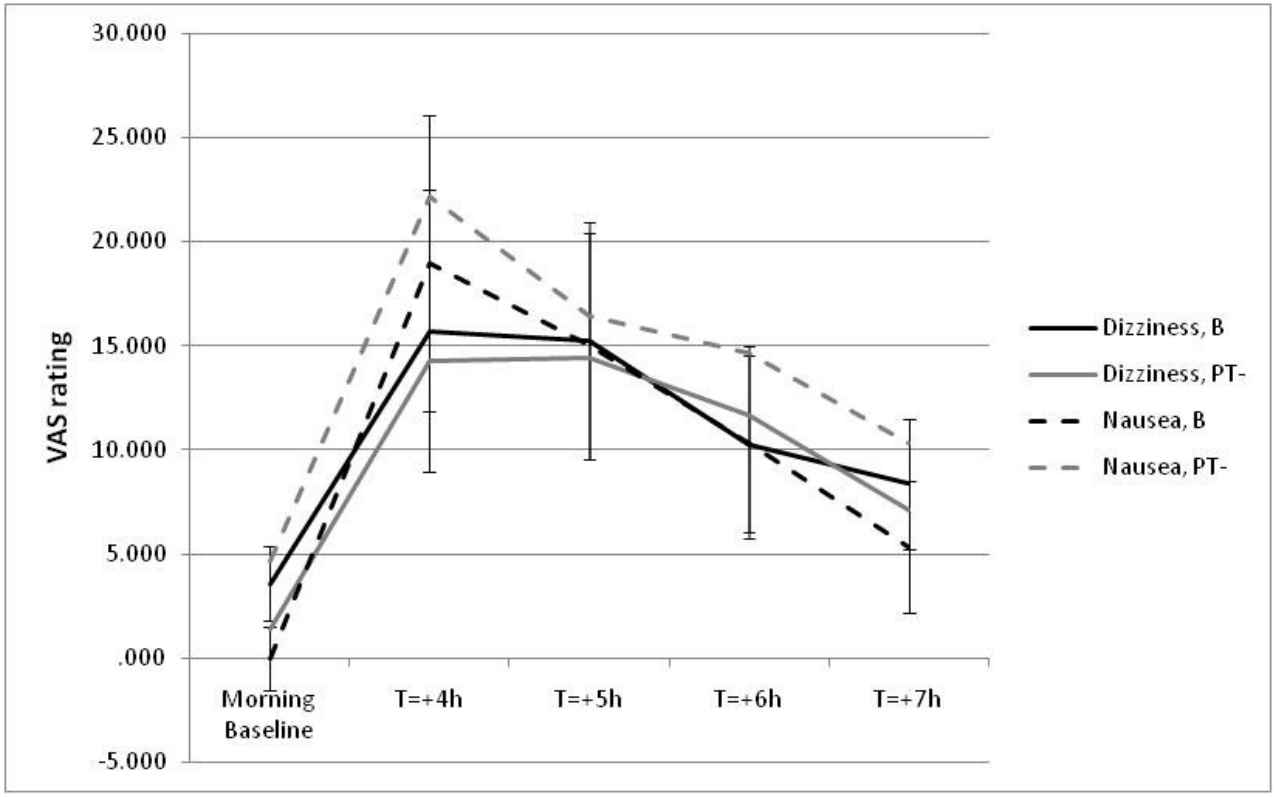

Figure 8. VAS ratings for side effect symptoms. Mood ratings are shown as the mean value ± SEM. T= hours elapsed following initial ingestion of AA mixtures. For Nausea and Dizziness, a significant main effect of Time was found ($F \geq 3.743$, $p \leq 0.01$ for both), however there were no differences between the drink Treatment conditions. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

showed a significant influence of the APTD in reducing the units earned by those individuals in the Dim light group, as this relationship was only seen following the PT- mixtures and not following the B mixtures (p=0.033; see Figure 9). Similarly, using the same analysis, this relationship approached significance for the *Completed Breakpoints* measure (p=0.084). Post-hoc power analyses showed an effect size of 0.64 and a power of 0.44 for the *Money Earned* measure, and an effect size of 0.55 and a power of 0.36 for the *Completed Breakpoints* measure. Further *a priori* power analyses determined that 10 and 16 additional participants, respectively, are required to attain a power of 0.80.

Contrastingly, the Treatment by Light interactions for the measures of *Final Breakpoint Presses* and *Total Presses* were not significant ($F_{1,12}$=0.179, p=0.680 and $F_{1,12}$=0.179, p=0.680, respectively; see Figure 10 for an example). Power analyses showed that a prohibitive number of additional participants (>30) would be required to attain a power of 0.80 for these two measures.

3.7 Order of Mixtures Administration

The above mixed-effects analyses were repeated with the additional between-subjects factor of Order, that is whether participants received the B or PT- mixture first.

POMS: There was a Treatment by Time by Order interaction on the *Energetic-Tired* axis ($F_{4,40}$= 3.061, p=0.027); however, post-hoc analyses showed that this difference was specifically at T=+7h and did not affect the previously seen interaction. Additionally, there was a Treatment by Light by Order interaction on the *Elated-Depressed* axis ($F_{1,10}$=4.962, p=0.050); post-hoc

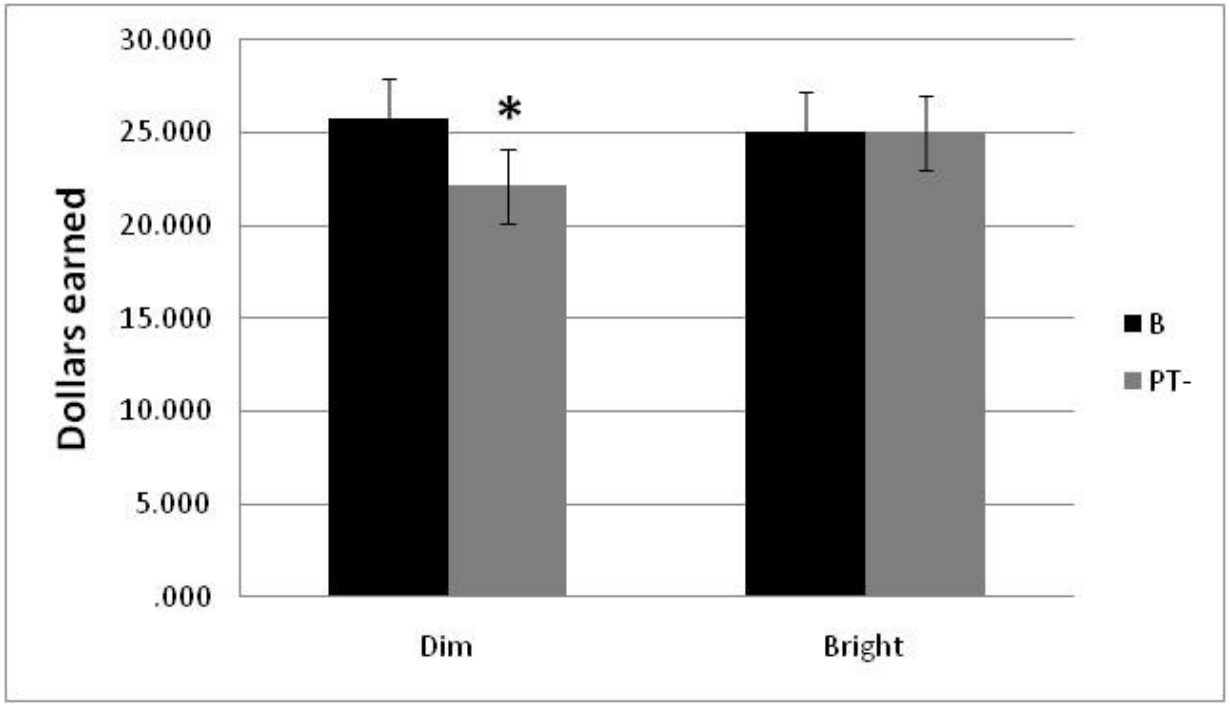

Figure 9. Amount of Money Earned as per drink Treatment and Light group. According to the two-way, mixed-effects ANOVA model there was no significant interaction between Treatment and Light ($F_{1,12}$=2.885, p=0.115). However, post-hoc analysis using pair-wise comparisons with a Bonferroni correction in SPSS (v.17.0) indicated a significant decrease in Money Earned by those individuals in the Dim light condition after undergoing APTD (as compared to taking the B mixtures); significant result is indicated by the asterisk (p=0.033). Values are shown as mean dollars earned ± SEM. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

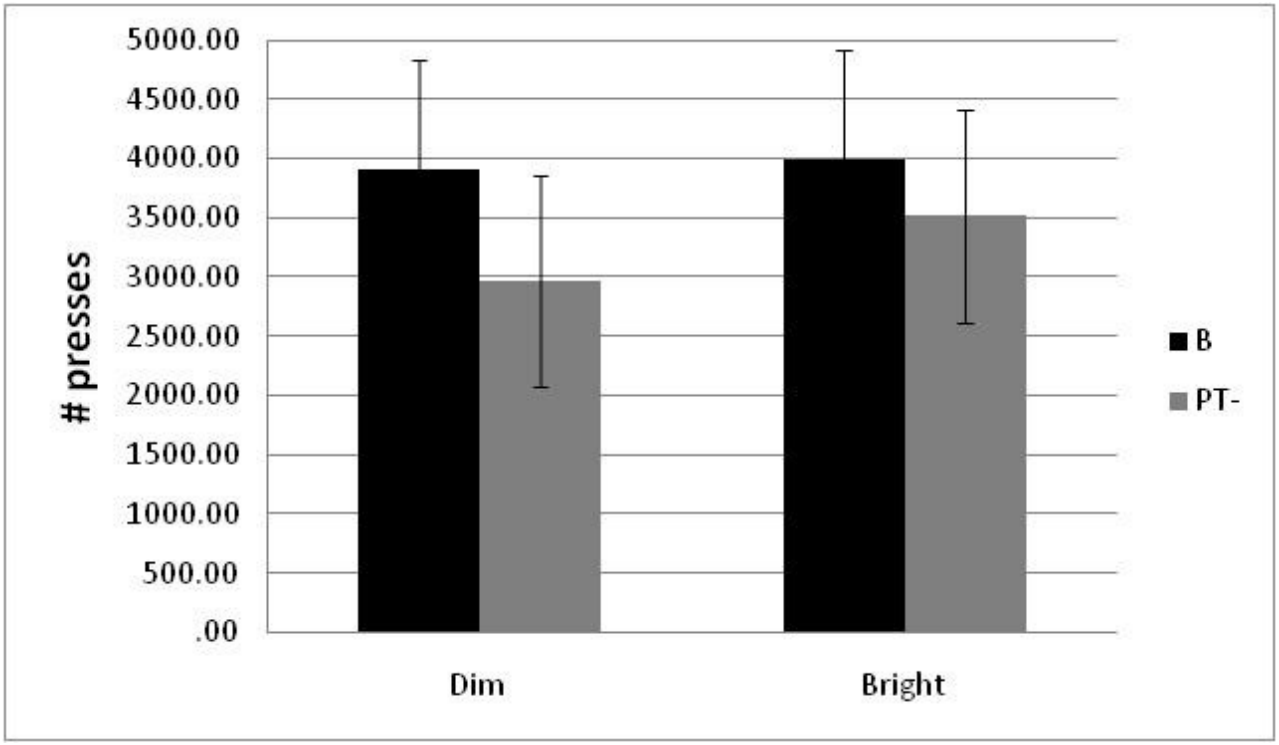

Figure 10. The number of button presses corresponding to the Final Breakpoint as per drink Treatment and Light group. According to the two-way, mixed-effects ANOVA model there was no significant interaction between Treatment and Light ($F_{1,12}$=0.179, p=0.680). Post-hoc analysis did not yield any further differences between any of the Treatment or Light groups (p>0.200 for all pair-wise comparisons). Values are shown as mean button presses ± SEM. B=balanced AA mixtures, PT-=phenylalanine/tyrosine-deficient AA mixtures.

analyses showed that in the Dim light group, those who received the B drink on the first day had higher ratings on both B and PT- day (p=0.037 and p=0.016, respectively) than those who received the PT- drink first.

VAS: There was a main effect of Order for the *Happy* ($F_{1,10}$=7.235, p=0.023) and *Enthusiastic* ($F_{1,10}$=5.314, p=0.044) scales, as well as various two- and three-way interactions with Order for the *Satisfied* ($F_{1,10}$=7.406, p=0.022), *Angry* ($F_{4,40}$=2.756, p=0.041), *Excited* ($F_{4,40}$=5.116, p=0.002), and *Restless* ($F_{4,40}$=3.871, p=0.021) scales. Again, post-hoc analyses showed these did not affect the previously seen results.

There were no interactions with or effects of Order observed for the *SSS* or any of the measures examined in the *PR* task (analyses not shown).

4.0 DISCUSSION

The most compelling finding was that an APTD-induced mood lowering in mildly seasonal, healthy women was prevented by bright light exposure. This interaction between APTD and bright light could not be attributed to baseline mood differences between the two light groups, to any effects of order of the administration of the AA mixtures, or to differing changes in plasma amino acid levels across these groups. Nor can these differences be attributed to various demographic or baseline screening factors tested.

Previous studies have found that the effects of APTD on mood lowering are not typically robust on their own (Booij, et al., 2003; McTavish, et al., 2005; Ruhe, et al., 2007), but at least one study has found that following a psychological stressor, healthy women can experience a mood lowering in response to APTD (Leyton, et al., 2000c). Our findings are in agreement with this literature. First, there was no mood-lowering effect of APTD when subjects were tested in bright light. Second, the APTD related mood-lowering effect that was seen in subjects tested in dim light occurred specifically following participation in a series of challenging neurocognitive tasks. Though the greatest effects on mood were initially expected to occur following the negative mood induction procedure (MIP), which was intended to act as a potential mood stressor, the negative MIP had a fairly potent effect on most of the participants, potentially overwhelming the more modest effects of APTD on mood.

Analysis of mood following the series of computer tasks, which depending on the participant took between 1-2 hours, revealed an effect of APTD on various

aspects of mood, such as energy/tiredness, alertness, motivation to seek reward and attention. Indeed, in addition to seeing effects on the POMS *Total* or overall score, there were interactions of Light and drink Treatment on the *Energetic-Tired* and *Clearheaded-Confused* axes. These findings also complement the results obtained in a recently completed study of very similar design, though using ATD in place of APTD (aan het Rot, et al., 2008). In that study, an ATD-induced mood lowering effect was prevented by bright light exposure on 5 of 6 of the POMS subscales, including *Agreeable-Hostile*, *Clearheaded-Confused*, *Composed-Anxious*, *Confident-Unsure*, *Elated-Depressed*, but interestingly not *Energetic-Tired*, as was seen in the present study (aan het Rot, et al., 2008). 5-HT is more strongly associated with the regulation of mood, anger and aggression (Siever, 2008; Young, et al., 2002), while the DA system has been associated with locomotor activity as well as with energy (Beninger, 1983; Carlsson, 1959; Nutt, et al., 2007).

The effect of catecholamine depletion in the context of mood and seasonal affective disorders has also been examined using alpha-methyl-*para*-tyrosine (AMPT). AMPT, a competitive inhibitor of the enzyme tyrosine hydroxylase, affects both DA and NE brain systems indiscriminately (McTavish, et al., 1999c) and has been used frequently to examine the effects of temporary catecholamine dysfunction (Booij, et al., 2003). Among patients with SAD, AMPT has been shown to cause a temporary reinstatement of depressive symptoms in patients that have entered remission either by bright light therapy or natural summer remission (Lam, et al., 2001; Neumeister, et al., 1998a; Neumeister, et al., 1998b). AMPT has also been shown to induce a temporary relapse of depressive symptoms in

remitted, drug-free individuals with a previous history of non-seasonal MDD (Berman, et al., 1999). This finding was recently replicated in a PET study. Remitted MDD patients administered AMPT were subsequently scanned with the fludeoxyglucose F18 marker; though this can only be used to non-specifically measure changes in cerebral blood flow and tone, the results nonetheless implicated the limbic system and ventral striatum (Hasler, et al., 2008). Though the results from AMPT and APTD studies are not directly comparable due to their different time-course and specificity, our findings do support the notion of a role for impaired DA in the regulation of certain aspects mood and the pathophysiology of depressive disorders. Furthermore, the present study, though somewhat preliminary in its findings, more directly implicates a potential relationship between short-term bright light exposure, central brain DA and associated cognitive functions than in previous studies.

The effects on mood observed on the POMS were not seen in the mood VAS ratings. The mood VAS were composed of 15 one-dimensional, unidirectional scales that participants were asked to rate from 0 to 100. This may be attributable to the relatively small sample size, or to a decreased sensitivity of the VAS in detecting mood changes as compared to the POMS, which has been shown to be able to detect subtler changes (Leyton, et al., 2000c). Indeed, the VAS detected the effects of the negative MIP and even a significant influence of bright light exposure on mood following the negative MIP, but did not detect any effects of APTD. According to the mood VAS, there was a significant increase in *Depressed* scores across both light groups, but the effects of mood were seemingly exacerbated by Dim light or conversely modulated by the Bright light

as those in the Dim group had significantly higher scores than those in the Bright group. This same effect appeared on the combined *Elated-Depressed* and combined *Happy-Depressed* VAMS subscales. These findings are in alignment with current findings regarding bright light therapy and mood. In the context of non-seasonal mood disorders, including unipolar and bipolar MDD (Kripke, 1998b; Tuunainen, et al., 2004), eating disorders (Lam, et al., 1994) and subsyndromal SAD symptoms (Avery, et al., 2001; Kasper, et al., 1989a; Norden & Avery, 1993) bright light therapy has been shown to be therapeutically efficacious. Indeed, it has been found that the effects are of similar magnitude to AD treatments and can produce a 12-35% reduction of depressive mood symptoms (Golden, et al., 2005; Kripke, et al., 1983; Yamada, et al., 1995); evidence has also suggested the two act to complementarily produce additive or enhanced effects (Kripke, 1998a; Levitt, et al., 1991). In the general population, light therapy has not been shown to have an effect on healthy individuals but has a beneficial effect on those suffering from at least subsyndromal seasonal symptoms (Kasper, et al., 1990; Kasper, et al., 1989a; Rosenthal, et al., 1987). In the present study, where the mood-lowering effects of the negative MIP were diminished by light exposure, all participants had histories of mild seasonal symptoms.

There was no statistically significant influence of an interaction between APTD and light on any dependant variables of the monetary reward *Progressive Ratio* task tested; however, it was a clear *a priori* hypothesis that bright light exposure would attenuate the expected APTD-induced decrease in monetary breakpoints (or other analogous dependant variables). It was for this reason that

exploratory analyses were conducted. The resultant findings were that there was a significant effect of APTD in reducing the measure of *Money Earned* in those individuals in the Dim light condition. This finding is in agreement with recent literature in which APTD has been found to decrease the self-administration of alcohol in healthy, social female drinkers (Leyton, et al., 2000b), as well as the progressive ratio breakpoints for self-administered alcohol (Barrett, et al., 2008) and cigarettes (Venugopalan et al 2009). A non-significant tendency for APTD to decrease PR breakpoints in both groups of subjects was seen for one variable, but not for the others, at least consistent with the proposal that the effects are greater in Dim light. A further complicating factor may be that money is inherently a much more abstract type of reward than drugs or alcohol; while these often involve craving, pleasure and/or euphoria, the associations with money, and the brain processes involved, may be different. There is, however, a body of evidence supporting a role for DAergic systems in processes related to monetary reward. It has been found that anticipation for monetary reward, particularly that which is actively sought, is capable of eliciting activation of the nucleus accumbens and other aspects of the ventral limbic striatum, as measured using fMRI, possibly indicating increased DA release (Knutson, et al., 2001; Montague & Berns, 2002; Zink, et al., 2004). Further, imaging studies examining [^{11}C]raclopride binding potential have found that DA transmission increases during monetary reward tasks (Koepp, et al., 1998; Zald, et al., 2004). It may also be of interest that in PD patients, the use of DA agonist drugs used for treatment have been linked to the development of pathological gambling (Dodd, et al., 2005).

There is still much to be elucidated about the relationship between bright light and central DAergic systems as it is yet unknown how DA synthesis or transmission is affected. In the retina, DA is the primary transmitter involved in the light response and is mutually inhibitory with melatonin. The inhibitory actions of DA are thought to be exerted at this level by D_2 and D_4 receptors (Cahill & Besharse, 1991; Tosini & Dirden, 2000; Zawilska & Iuvone, 1992). The secretion of melatonin is determined by central circadian rhythms; melatonin secretion is related to the duration of subjective night and suppressed by light, so it is largely involved in the regulation of the photoperiod (Arendt, 2000; Checkley, et al., 1993). Various hypotheses postulate that the dysregulation of the photoperiod, and/or melatonin, or other circadian rhythms may in part be responsible for the pathophysiology of SAD (Lam, et al., 2000b), so the relationship between retinal DA and melatonin is of interest. Proposed hypotheses for both retinal hyposensitivity and hypersensitivity exist (Beersma, 1990; Reme, et al., 1990). One recent study supports the notion of retinal hyposensitivity; using electroretinography techniques, a reduced sensitivity in SAD patients was found as compared to healthy controls (Hébert, et al., 2004). However, the relationship between retinal and central DA remains unclear. A retinal and central melatonin-dopamine dysregulation hypothesis has been proposed, suggesting the possibility of a cascade of chemical or neuronal events beginning with retinal DA, through the retinohypothalamic tract and ultimately affecting central DA so further exploration to examine this idea would be of merit (Oren, et al., 1996). Indeed, in some PD patients, who suffer nigrostriatal DA loss, retinal DA deficiencies have

also been found (Bodis-Wollner, 2003; Harnois & Di Paolo, 1990; Nguyen-Legros, 1988).

4. 1 Limitations

The most important limitation of the present study was the small sample size. The intended *n* was 30 individuals, based on that used by aan het Rot and colleagues (2008). There were a number of reasons for the recruitment of a sample size that was less than desired. The stringent entry criteria ensured a consistent and high quality of desired participants, but slowed recruitment. Compounding this difficulty was that our testing season began and ended with Daylight Savings Time (DST); since the Canadian government adopted the changes to the start/end dates of DST implemented by the US government in the Energy Policy Act of 2005 (United States. Congress. House. Committee on Energy and Commerce., 2005), our potential testing season was shortened by several weeks from what it would have been in previous years. In the scope of this study, which could subsequently be executed only four months out of a year, this loss was significant. Once past the screening process, there were a number of difficulties that prevented the retention of some participants, including medical issues, loss of interest or aversion (typically resulting from vomiting) to procedures following the first test day, and significant irregularities with the menstrual cycle. The recruitment of additional participants could result in more robust results.

Though the recruitment of female participants comes with its additional set of specifications, there were a number of reasons the present study exclusively

examined this particular gender. Females tend to be more susceptible to seasonal mood changes, which were criteria for participation, especially since the effects of bright light therapy have typically been seen to be most beneficial to only those individuals experiencing at least subsyndromal seasonal symptoms (Kasper, et al., 1990; Kasper, et al., 1989a). Since the influence of APTD on mood has not been shown to be pronounced, the testing of females was also in part to increase the likelihood of observing any possible effects or interactions of the APTD or the light on the test measures. However, one disadvantage of testing solely females is that the observed effects cannot be generalized across gender and as of now are fairly specific to the population tested.

The APTD method putatively affects the central brain DA system; indeed PET imaging techniques have shown decreased DA release in response to APTD (Leyton, et al., 2004). Various studies have also shown effects of this method on a number of behaviours associated with brain DA systems (Barrett, et al., 2008; Leyton, et al., 2007; Leyton, et al., 2000c; McTavish, et al., 2001; Roiser, et al., 2005; Scarna, et al., 2003), as well as the consistent and expected effects on free plasma concentrations of LNAA (Moja, et al., 1996; Sheehan, et al., 1996). The efficacy of the depletion is also well documented in rat studies (Fernstrom, et al., 1995; McTavish, et al., 1999a). However, since we did not directly measure *in vivo* brain DA synthesis, and only used peripheral measures, it is impossible to definitively quantify or confirm the depletion effects. Depending on the different individuals, the effects could be comparatively strong, or weak. In the same way, it is impossible to confirm, at least in the present study, whether the effects of APTD on DA are direct, proximal or distal and whether the behavioural effects

are in fact mediated directly by the DA system, or by DA's effect on other brain monoamine systems, such as 5-HT. In animals, it has been shown that there are anatomical and functional connections between the 5-HT and catecholamine systems and that 5-HT transmission in particular is influenced by catecholaminergic systems (Guiard, et al., 2008; Kaehler, et al., 1999). However, supporting APTD's specificity on DA, it has been more directly shown using CSF measurements that APTD in monkeys did not have a significant effect on levels of Trp, the precursor to 5-HT (Palmour, et al., 1998).

As with nearly every study involving bright light, neither the tester nor the participant was blind to the condition. This inevitably raises the possibility that between the two light groups, the participants' expectations regarding the effects of the different light conditions influenced their responses, especially those associated with mood. A study by Wehr and colleagues (1987) examining the effects of eye versus skin phototherapy found that the patients' individual expectations were predictive of their responses and found phototherapy via the eyes most effective. Such findings may suggest something akin to a placebo-type effect when using bright light therapy, though it is difficult to distinguish between this and actual bio-physiological effects as any action of light must occur through some external entry point and there is evidence to suggest the eye as a strong candidate (Hébert, et al., 2004; Wehr, et al., 1987). Interestingly though, a study examining bright light therapy in non-seasonal depressive individuals found that while participants' expectations with regards to both the bright light treatment and a dim red light placebo were the same, the bright light therapy was significantly more effective (Kripke, et al., 1992). Nonetheless, it is possible that participant

expectations may inextricably be linked to the ability of bright light to act therapeutically. Having each participant undergo the APTD in both dim and bright conditions could more clearly distinguish any positive attributions to the bright light, but given individuals tend to develop a conditioned taste aversion to repeated exposures of the AA mixtures and the time commitment involved, this was not a realistically feasible option. Furthermore, these concerns are diminished in the present study as both the phenylalanine tyrosine-deficient mixtures and the balanced mixtures were administered in a double-blind, counterbalanced method. In this manner, any individual expectations of the light treatment should be present on both test days; in all of our analyses, there were no effects found of light alone.

Similar concerns regarding the effect of the artificiality of the environment should be lessened. It is possible that the construct of the testing environment (e.g. the long hours spent primarily in isolation and the stress of being a test subject) had an effect on mood, as it was not a natural setting under which to study the effects of light. However, it would be expected that the participants' perceptions or expectations of the surroundings would be present on both testing days. Further, it can be strongly argued that the parameters of the testing room did much to reduce the potential variability of the surrounding environment in terms of controlling for elements such as visual cues, temperature, and light exposure.

The lack of a main effect of drink Treatment on the *Progressive Ratio* task breakpoints was somewhat unexpected, though here too the *a priori* prediction was that bright light would diminish APTD's effects. The high ratio value used may also have been a factor; this value was chosen based was based on the results

of a pilot study conducted prior to the present study. The inclination was to choose a higher value than those previously used in drug and alcohol studies; the desire to seek money is not impeded or impacted by the aversive physical effects that can be caused by such substances and while monetary reward does not necessarily produce the same pleasurable or euphoric effects, it is nonetheless a salient reward. Indeed, it was found that participants were willing to invest substantial effort into earning money this way (see Appendix 1); however, it may have been the case that the factor by which the ratio value increased was set too high and was subsequently not sensitive enough to detect the effects of the depletion. A study by Johnson & Bickel (2006) found that individuals working for nicotine or money in a plunger-depressing PR task completed significantly higher PR breakpoints for nicotine than for monetary reward. This study also employed relatively high breakpoint values as participants executed up to 6000 depressions for cigarette puffs. The completed breakpoints for monetary reward were much fewer, possibly attributed to the increased saliency of nicotine for these particular individuals (dependent cigarette smokers) and relatively low value of the units of monetary reward ($0.05 and $0.25) (Johnson, et al., 2006).

4.2 Future Directions & Conclusions

An immediate future direction for the present study is to increase the sample size, ideally to double the current number. In doing so, the aim would be to increase the statistical robustness of the findings. Power analyses of the POMS mood results support current plans to double the experimental *n;* in comparing the values on the PT- day of the two light groups, the addition of 8 participants to

each group would increase the currently modest statistical power to a more acceptable value of 0.80 and in comparing the B versus the PT- condition within the Dim group itself, the addition of even 4 participants would increase the power to a level of 0.80. While much greater numbers would be required to attain the similar statistical power with the PR task results for the *Final Breakpoint Presses* and *Total presses* measures, compromise power analyses (with β=0.1 and α=0.05) did reveal that within the Dim group, the addition of 8 participants would increase the power of the B versus the PT- comparisons for the *Money Earned* and *Completed Breakpoints* measures to 0.80 and 0.71, respectively.

It would also be of interest to extend the present findings to psychiatric populations, both to symptomatic and remitted SAD patients with the expectation that APTD would attenuate the effects of the acute bright light exposure. Using imaging techniques immediately following the APTD and bright light exposure to study DA transmission could further corroborate these findings. Finally, an exploration using retinographic techniques may also be of interest in order to examine any differences in retinal activity and the individual responses to the testing parameters.

In conclusion, our findings suggest a direct relationship between acute bright light exposure, DA transmission and their influence on mood. This has broader implications in the mechanism of action in light therapy and the pathophysiology of SAD, as well as the regulation of mood and related behaviours.

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APPENDIX 1: *Progressive Ratio* Task Pilot Study

Aim: To examine the willingness of participants to button press in progressively increasing increments for units of monetary reward; more specifically, the willingness to work harder for higher versus lower values of reward ($1 vs. $2 vs. $3).

Participants: 21 healthy women between the ages of 18-40 and with no current or past history of any Axis I disorders as determined by the Structured Clinical Interview for the DSM-IV.

Experimental design: The participants completed 2 experimental sessions on 2 separate days to perform a Progressive Ratio task on the computer. The participants were randomly assigned to one of three reward conditions and given the opportunity to button press for $1, $2, or $5 units of reward (3 groups of 7 participants each). The dependent variable was the "breakpoint", which is equivalent to the final ratio value of button presses a participant is willing to complete in order to obtain a single unit of reward. The parameters of the task were set to increase by a factor of 2.3 to prevent the estimation of the button pressing pattern.

During their first trial, participants performed the task either to the point where they were no longer interested in continuing, or a time limit of 1 hour (unbeknownst to the participants) was reached. During their second trial, the

participants were given the additional option of discontinuing the task partway through a ratio value to earn half the value of a single monetary unit, in addition to what they had already earned (i.e. the participants were given the opportunity to earn a smaller reward without having to finish the full breakpoint).

Analysis: The dependent variables of Final Breakpoint ratio value, but the number of Completed Breakpoints, amount of Money Earned, and Total Number of Presses executed over the entire course of the task were all examined using repeated measures, two-way ANOVA models. Reward Condition ($1, $2, or $5) was an independent, between-groups factor and Day was a within-subjects factor. All analyses were performed on SPSS v. 17.0 for Windows.

Results: For all the dependent variables tested, there was a significant main effect of reward condition ($p<0.029$ for all). Post-hoc analyses using pair-wise comparisons with a Bonferroni adjustment showed that for Final Breakpoint and Money Earned, this was primarily due to a significant increase in both measures by the $5 group as compared to the $1 group on the first ($p<0.048$ for both), but not the second day ($p>0.067$ for both); see Figure A-1 for Final Breakpoint results, data not shown for Money Earned. For the Completed Breakpoints measure, there was a significant increase in completed breakpoints by the $5 group as compared to the $1 group on both days ($p<0.045$ for all; data not shown). There were no significant differences in the Total Number of Presses between or within groups ($p>0.091$ for all; data not shown).

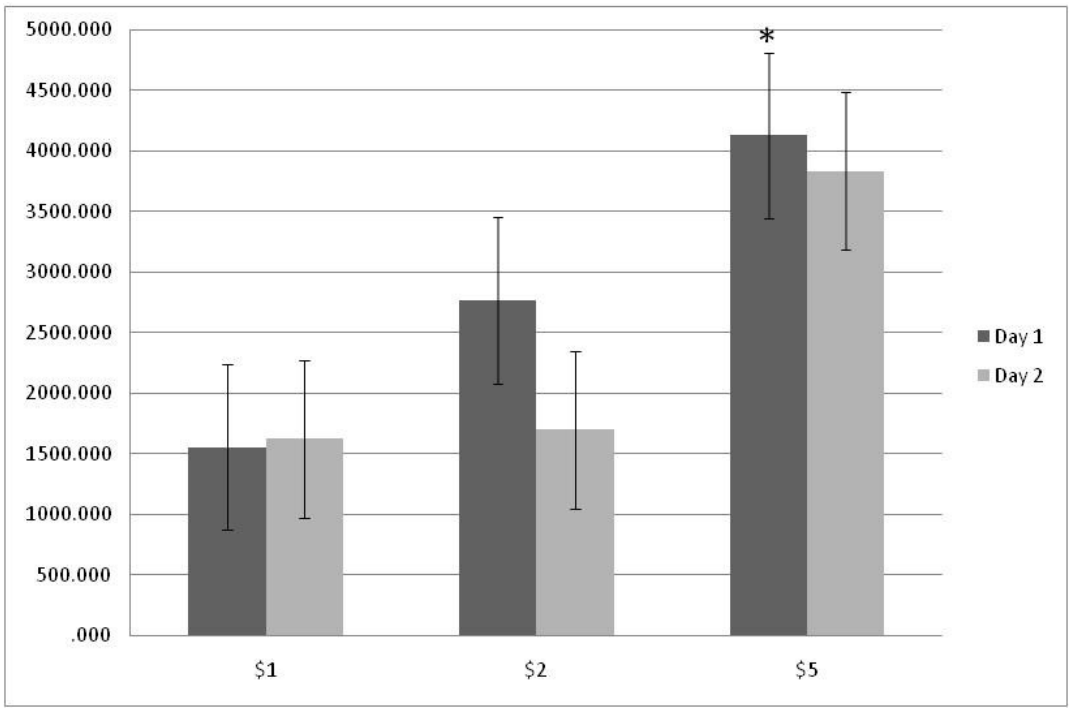

Figure A-1. Final average breakpoint value (shown as the number of button presses corresponding to the final ratio value) for the $1, $2, and $5 reward conditions. There was a significant main effect of Reward Condition ($p<0.029$), and a significant increase in the $5 condition as compared to the $1 condition during the first day ($p=0.048$). Similar trends were seen for the money earned and completed breakpoints measures.

Conclusion: Participants were willing to work to complete Progressive Ratio breakpoints in order to earn units of monetary reward. Further, those in the higher earning ($5) reward condition worked significantly harder at the task than those in the lowest earning ($1) reward condition.