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Effects of tryptophan manipulation on neuropsychological performance

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Effects of Tryptophan Manipulation on Neuropsychological Performance

by

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Abstract

The purpose of this study was to examine the effects of tryptophan, the amino acid precursor to serotonin, on neuropsychological performance. A dietary method of tryptophan manipulation was employed to temporarily raise, lower, or maintain circulating levels of tryptophan within the body, allowing the resulting cognitive and affective sequelae to be measured. A total of 73 participants (50 females, 23 males) completed this mixed-design, double-blind study. Participants were quasi-randomly assigned to one of three tryptophan conditions (augmented, depleted or balanced) and were provided with breakfast, lunch and a snack. A comprehensive neuropsychological test battery was administered 1.5 hours after completion of lunch. Analyses were conducted on each gender separately. No significant results were found for females. For the males, however, significant between-group differences were found for the Rey-O delayed recall, with those in the Depletion group scoring significantly higher than those in the Balanced or Augmented conditions. Males in the Augmented condition also performed better than those in the Balanced or Depleted condition on the speed component of the Ruff 2 & 7 Selective Attention Test. With regards to affect, males in the Augmented group demonstrated a near-significant difference on the PANAS positive affect scale on the fourth PANAS administration compared to those in the Depleted group. These differences in positive affect levels seem to be primarily driven by the trend in excitement levels between males in the Augmented and Depleted conditions over time. Results of this study support the hypothesis that dietary manipulations aimed at

altering tryptophan levels had an effect on some cognitive tests and positive affect, at least with regards to males.

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"If we knew what it was we were doing, it would not be called research, would it?"

- Albert Einstein

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Human behaviour is continuously influenced by factors stemming from the external environment and from internal systems. The reaction to these stimuli is mediated by the central nervous system (CNS), in part through chemical messengers known as neurotransmitters (see Appendix A for glossary of terms). Of all the neurotransmitters in the mammalian CNS, serotonin, one of the main metabolic products of tryptophan, has received a great deal of interest within the literature. In particular, the focus of most research studies on serotonin has been on the association between the neurotransmitter and mood (Benkelfat, Ellenbogen, Dean, Palmour, & Young, 1994; Flory, Manuck, Matthews & Muldoon, 2004; Heninger, Delgado, Charney, Price & Aghajanian, 1992; Smith, Pihl, Young, & Ervin, 1987; S. N. Young, Smith, Pihl, & Ervin, 1985). However, a growing body of evidence suggests that serotonin also plays a role in many cognitive functions (e.g., Lieberman, 2003). Given that serotonin and it's precursor (tryptophan) are derived from foods that are consumed (Sirek & Sirek, 1970), there is growing support for the notion that diet can produce reliable effects on tryptophan levels and thus, on cognitive performance (e.g., Dye, Lluch & Blundell, 2000), presumably through the serotonergic pathway (one of the major pathways resulting from tryptophan metabolism). In order to examine this notion, the neurochemistry behind tryptophan entering the body and becoming converted to serotonin will first be reviewed. Following this review, the role of serotonin innervation, function, and the neuroanatomical structures related to the serotonergic system, as well as those related to the other pathways of tryptophan metabolism, will be discussed. Finally,

this paper will examine the methods by which tryptophan levels can be manipulated. This study will investigate the effects of tryptophan on neuropsychological performance.

Neurochemistry Review

In order to fully understand the ways in which tryptophan can exert its influence on cognitive and affective responses to stimuli in the environment, it is first necessary to understand the neurochemical bases of serotonin synthesis via tryptophan metabolism. The entire process begins with the ingestion of nutrients that are metabolized in order to provide the necessary amino acids for neurotransmitter synthesis to occur. Thus, the first step in this process begins with the amino acids.

The Amino Acids

Three of the main components in the mammalian central nervous system (CNS) – peptides, amines, and non-essential amino acids (Wurtman, Hefti & Melamed, 1981) – are heavily dependent upon the ingestion and/or creation of amino acids. Amino acids are critical to proper nutrition and are the building blocks of both proteins and peptides. Although proteins are essential macronutrients responsible for the growth and development of the body, including bones, skin, muscle and blood, as well as for the repair of tissues (Blass, 1994), it is the peptides that are a major component of the mammalian CNS. Peptides are the chemical messengers of the CNS and are broadly distributed throughout the nervous system (Brownstein, 1994).

When amino acids are broken down, amines are the resulting product. Amines, which comprise the second main component of the CNS, are involved in the production of neurotransmitters. Based on their structure, amines are the building blocks of either

the catecholamines (e.g., epinephrine, norepinephrine, or dopamine) or the indolamine serotonin (Pinel, 2006). Serotonin in an example of a monoamine, meaning it is synthesized from a single amino acid. Compared to amino acids, monoamines are a group of small-molecule neurotransmitters that are produced by cell bodies that are mainly located in the brain stem (Pinel, 2006). Neurotransmitters are released more diffusely from monoamines than from amino acids as their axons are highly branched with many varicosities (i.e., swellings along the axon of the presynaptic neuron that release neurotransmitters), enabling more widespread release of neurotransmitters into the extracellular space (Bunin & Wightman, 1999) serving a neuromodulatory function.

The third main component of the mammalian CNS is that of the non-essential amino acids. There are twenty standard amino acids, of which twelve are classified as non-essential; the remaining eight are comprised of essential amino acids. The title of "essential" versus "non-essential" relates not to the importance of a given amino acid, as all are crucial for optimal health, but rather it relates to the means by which the body obtains the amino acids. Non-essential amino acids, such as glutamine, can be synthesized by the human body from essential amino acids or via the catabolism of proteins. Essential amino acids such as tryptophan, however, cannot be produced by the body and thus they must be obtained through the ingestion of food (Wurtman, et al., 1981). The distinction between the eight essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonin, tryptophan, and valine) (V. R. Young, 1994) and the three semi-essential amino acids (histidine, tyrosine, and arginine) is that the latter can be synthesized by the adult human body but not by infants and growing children whose metabolic pathways are in the process of development (V. R. Young,

1994). Both essential and non-essential amino acids are the major constituents involved in the creation of proteins (Barondes, 1974; Blass, 1994).

Although not strictly one of the three essential components of the mammalian CNS, essential amino acids play a major role in neurotransmitter synthesis. Based upon their chemical composition, essential amino acids can be classified into the aromatic amino acids (tryptophan, tyrosine, phenylalanine and histidine), the branched-chain amino acids (leucine, isoleucine, and valine), and the other amino acids (threonine and methionine) (Cooper, Bloom & Roth, 1996; Paul & Southgate, 1978; Wurtman et al., 1981). All three groups are examples of 'large neutral amino acids' (LNAAs), a term used to describe amino acids that compete with each other for uptake into the brain and subsequent neurotransmitter synthesis (Curzon, 1985; Wurtman et al., 2003). Brain levels of neurotransmitters will change depending on the levels of the precursor(s) in the blood plasma, as will changes in blood plasma levels of competing LNAAs (Fernstrom & Wurtman, 1972). Ultimately, however, it is the transport systems of the blood-brain barrier that determine which transmitters will gain access to the brain.

The Blood-brain Barrier

The blood-brain barrier is comprised of capillary endothelia with tight junctions between cells that create a continuous membrane separating the brain from circulating blood and preventing many harmful or toxic substances from gaining access to the brain (Betz, Goldstein & Katzman, 1994; Brightman, Reese & Feder, 1970; Oldendorf, 1976; Pinel, 2006). According to Oldendorf, there are two ways in which molecules can cross the blood-brain barrier: 1) lipid-mediation, through which lipid-soluble molecules can easily pass; and 2) carrier-transport systems, in which special transport carriers are

needed to carry the molecules through the blood-brain barrier. As LNAAs are not lipid-soluble (Betz et al., 1994), Pardridge and others (Pardridge & Oldendorf, 1977; Wurtman, et al., 2003) posit that there is a special transport system for LNAAs that enables them to cross the blood-brain barrier. As many of the LNAAs serve as neurotransmitter precursors, they must compete with each other for access to the carrier molecules (Pardridge & Oldendorf, 1977). Thus, those LNAAs with the highest blood plasma concentrations are most likely to gain access to the carrier molecules, enabling them to cross the blood-brain barrier and thus to alter the levels of brain neurotransmitters (Cooper et al., 1996). Essential amino acids that serve as precursors to catecholamines or indolamine are readily admitted via the leucine-preferring system (L-system), whereas the alanine-system (A-system) serves to limit entry of the non-essential amino acids and actively transports them out of the brain (Betz et al., 1994; Oldendorf, 1971). In other words, the essential amino acids are rapidly and broadly swept into the brain from the blood, whereas the non-essential amino acids are not as readily exchanged (Oldendorf, 1971). It is for this reason that Pardridge and Oldendorf (1977) state that "… the blood-brain barrier amino acid transport probably is the limiting factor in determining availability of amino acids in the brain" (p. 8). Thus, even though the essential amino acids can cross the blood-brain barrier with greater ease, they must compete for access to the carrier systems. It is this function, in addition to the ratio of each amino acid, which serves to limit the synthesis of neurotransmitters.

Tryptophan:LNAA Ratios

An additional factor that influences the rate of amino acids crossing the blood-brain barrier is the ratio of each amino acid and whether or not there is competition with

other molecules for carrier transport. For example, tryptophan is the least abundant of the amino acids (Paul & Southgate, 1978; Wurtman, 1970; Wurtman & Fernstrom, 1972; Wurtman et al., 1981). As mentioned previously, the LNAAs (which include tryptophan) compete with each other for access to the blood-brain barrier transport molecules. Thus, the other LNAAs gain access to transport carriers and cross the blood-brain barrier in greater volumes than tryptophan simply because they are more abundant. The reasons for the greater abundance of the other LNAAs as compared to tryptophan are at least three-fold. First, although the main source of tryptophan is from dietary protein (Munro, 1974), tryptophan comprises only a fraction of the total amino acid content of most dietary protein (approximately 1 to 1.6% compared to 25% for the LNAAs) (Wurtman, 1970). For this reason, the ingestion of protein greatly increases levels of the LNAAs, thereby maintaining the lowered tryptophan:LNAA ratio. Second, half of all tryptophan that is ingested is excreted in the urine without being converted into serotonin (Sirek & Sirek, 1970). Finally, tryptophan is the only LNAA that is metabolized by the liver into kynurenine, which in turn is used in the synthesis of niacin; a key function of niacin is the metabolism of carbohydrates. Since enzymes found in the liver can also break tryptophan into smaller proteins, the actions of the liver result in less tryptophan being available for systemic circulation (Brown, 1980).

Thus, it is clear that there are many factors at work to maintain the tryptophan:LNAA ratio. According to Fernstrom and Faller (1978), "… competition among tryptophan and other large neutral amino acids in blood is a very important, and perhaps dominant, determinant of tryptophan uptake into the brain" (p. 1537). There are, however, two ways in which the ratio of tryptophan to LNAAs can be altered, which in

turn will alter the rate of transport of the different amino acids across the blood-brain barrier. The first method of raising plasma tryptophan levels is by ingesting greater quantities of tryptophan-containing foods or supplements. Although this might seem like a good technique in principle, it is in fact a poor method of altering the tryptophan levels. This is because ingesting dietary protein, albeit the main source of tryptophan, also raises the levels of LNAAs competing for transport across the blood-brain barrier. For example, consuming a meal that contains 10% protein will raise tryptophan in levels that are proportionate to the raise in LNAAs (Fernstrom & Faller, 1978; Fernstrom & Wurtman, 1972). Thus, brain tryptophan levels will actually decrease due to increased competition from the other LNAAs that utilize the same transport carrier (Blass, 1994). According to Teff, Young, and Blundell (1989), even 4% protein in an otherwise protein-free meal will prevent a rise in tryptophan levels compared to that of the LNAAs, while an amino acid mixture (100 g) that does not contain tryptophan will effectively reduce tryptophan plasma levels by 70 to 90% (S. N. Young, Ervin, Pihl & Finn, 1989). Thus, it is clear that simply raising tryptophan levels will not suffice in adequately altering the tryptophan:LNAA ratio.

The alternative method, which appears to be the most rapid and most effective means of altering the tryptophan:LNAA ratio, is by lowering levels of the other LNAAs through the ingestion of carbohydrates (i.e., “carb-loading”). The consumption of carbohydrates results in an increase in insulin levels, which in turn lowers plasma levels of the LNAAs by drawing them into the surrounding tissue (Wurtman et al., 1981). Tryptophan, however, is impervious to this effect, presumably because approximately 70 to 80% of plasma tryptophan is albumin-bound which prevents its penetration into

peripheral cells (Blum et al., 1992; Lyons & Truswell, 1988; McMenamy, Lund & Oncley, 1957). Thus, ingesting carbohydrates essentially clears the way for tryptophan to travel to the blood-brain barrier and gain priority access to the carrier transport molecules which deliver it into the brain where it can be synthesized into serotonin.

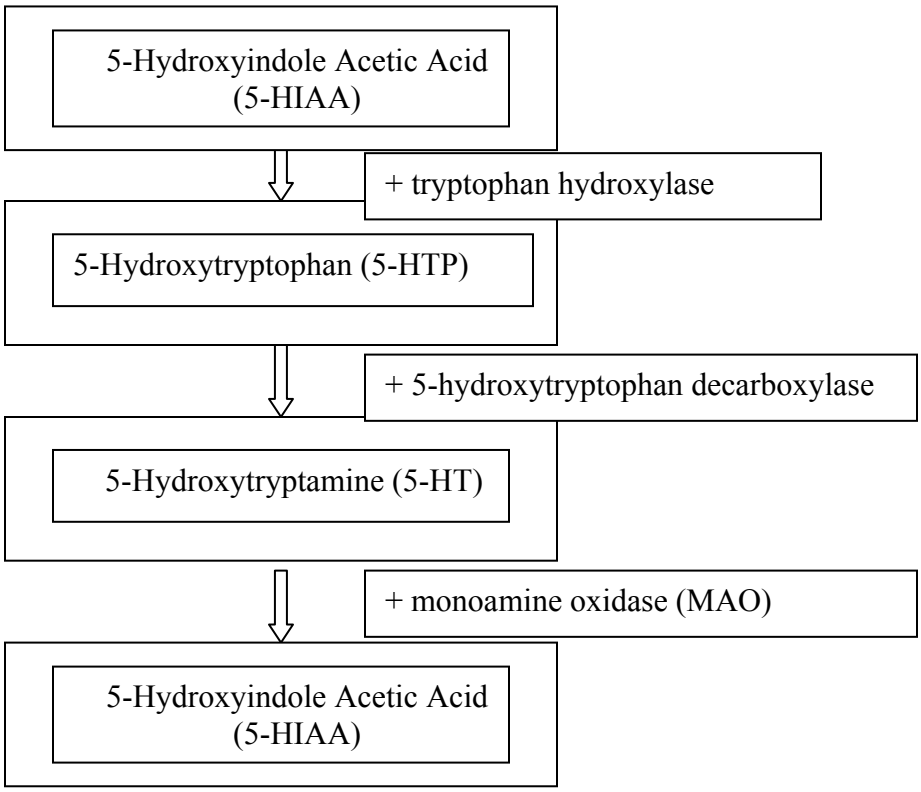
Serotonin Synthesis

All neurotransmitters are synthesized from precursors that are stored as tissue proteins until needed. The essential amino acid tryptophan serves as the precursor for the indolamine serotonin (5-hydroxytryptamine, 5-HT), a neurotransmitter found throughout the brain and body. Originally discovered due to its effects on smooth muscle contraction, serotonin was simultaneously yet independently discovered by Ersparmer (1940) in Italy and by Rapport, Green and Page (1948) in Cleveland, USA. Both groups noticed that following blood clotting a substance was found within the serum that appeared to cause vasoconstriction. The "tonic" effect of the serum substance was eventually termed "serotonin" and was subsequently found to have a wide array of effects on the body (Sirek & Sirek, 1970).

As shown in Figure 1, serotonin is created via a three-step process, starting with the precursor tryptophan. Through the utilization of a single oxygen atom, the rate-limiting enzyme tryptophan hydroxylase (which is only found in cells that produce 5-HT) converts tryptophan into 5-hydroxytryptophan (5-HTP) (Frazer & Hensler, 1994; Sirek & Sirek, 1970). Serotonin is almost immediately synthesized once tryptophan is converted to 5-HTP (Cooper et al., 1996) through the action of the aromatic amino acid decarboxylase, an enzyme that removes a carboxyl group (Sirek & Sirek, 1970). The resultant 5-HT is eventually degraded by monoamine oxidase through the removal of an

amine group (Sirek & Sirek, 1970). The main product generated through the catabolism of 5-HT is 5-hydroxyindole acetic acid (5-HIAA), which is excreted through the urine (Frazer & Hensler, 1994). It is estimated that the average human expels between 1 and 10 mg of 5-HIAA per day (Sirek & Sirek, 1970). As aromatic amino acid decarboxylase is not normally saturated in the human brain, levels of 5-HT can thus be boosted either through increasing 5-HTP itself (e.g., via supplementation), or by increasing tryptophan (e.g., via the ingestion of carbohydrates) which will in turn increase levels of 5-HTP, and thus of 5-HT (Frazer & Hensler, 1994). Furthermore, although 5-HTP is only found in trace amounts in the brain (Frazer & Hensler, 1994), it is lipid-soluble (unlike tryptophan), and is therefore able to cross the blood-brain barrier without a carrier transport. In this way, 5-HTP can act directly to increase brain serotonin levels (Murray, 1998). It is important to note, however, that of the approximate 10 mg of 5-HT found in an adult male (Sirek & Sirek, 1970), only about 1-2% of that total amount is found in the brain. Within the brain, however, the pineal gland acts as the factory for producing 5-HT as it contains the enzymes necessary to convert tryptophan into 5-HT (Frazer & Hensler, 1994). The metabolic activity of the pineal gland, which is located outside of the blood-brain barrier, is directly influenced by external factors such as light from the environment (via sympathetic innervation) that regulate the daily rhythmic cycle of 5-HT synthesis (Cooper et al., 1996).

Figure 1. Flow chart depicting the enzymes responsible for converting tryptophan into serotonin (5-HT).



In order for tryptophan, 5-HTP, or any other precursor to successfully change the rate of neurotransmitter synthesis, however, five conditions must be met: 1) food consumption must produce equivalent increases in the amount of neurotransmitter precursors in the plasma levels; in other words, there must be no mechanism working to keep plasma levels within a strictly controlled and constant range; 2) a mechanism must be in place to enable the precursor access to the brain via the blood-brain barrier; 3) raising plasma levels of the precursor must be able to further saturate transport carriers so as to increase the amount of a given precursor gaining access to the brain (i.e., transport carriers must maintain a sub-threshold level of saturation); 4) there must be a low affinity between the enzyme and the precursor, resulting in poor binding and inefficient catalysis; the precursor will act as the rate-limiting factor in the genesis of the neurotransmitter; and 5) the enzyme must be free of significant inhibitory feedback from the creation of its product (i.e., it must not "turn itself off" after producing a specific amount of its neurotransmitter) (Wurtman et al., 1981). With regards to these conditions, Carlsson and Lindqvist (1978) have found that the enzyme tryptophan hydroxylase is only 50 to 75% saturated with tryptophan, indicating that the enzyme is not normally saturated in the mammalian brain (Cooper et al., 1996; Curzon & Knott, 1974; Frazer & Hensler, 1994; Gessa & Tagliamonte, 1974; S. N. Young, 1993). Further, Lovenberg, Jequier, and Sjoerdsma (1968) posit that there is no evidence to suggest that 5-HT inhibits tryptophan hydroxylase, thereby decreasing its own rate of production. Of note, Gallager and Aghajanian (1976) did find evidence that the rate of firing of 5-HT neurons in the raphe nuclei slows after the administration of large doses of tryptophan, resulting in decreased release, and perhaps decreased generation, of 5-HT. However, since such instances do

not normally occur without external manipulation, it appears that there is minimal feedback control of 5-HT synthesis under normal conditions. Thus, according to Spring (1986), tryptophan meets all five conditions for ensuring successful alteration of the rate of serotonin synthesis.

Up until this point, we have discussed the process of metabolism by which the amino acid tryptophan gets converted into serotonin, the indolamine neurotransmitter involved in many different functions throughout the brain and body. This next section will detail the routes of serotonin innervation within the brain and will discuss the specific neuroanatomical structures innervated by these pathways that are implicated in various cognitive functions. There is, however, a gap in this literature that cannot fully be bridged as not enough is currently known about the effects of tryptophan manipulation on neuroanatomical function. A better understanding of these pathways would enable more precise and accurate predictions about the role of tryptophan on specific neuroanatomical structures and functions.

Serotonin Innervation and Functional Neuroanatomy

Serotonergic neurons have diffuse branching axons innervating much of the CNS (Frazer & Hensler, 1994; Tork, 1990). The majority of their somas can be found along the brainstem midline, especially in the raphe nuclei of the brainstem, the pons and medulla (Cooper et al., 1996; Frazer & Hensler, 1994). These latter nuclei act as "variable ratio sensors" (Wurtman et al., 1981, p. 323) by keeping the rest of the brain informed about the body's metabolic status. This information is in turn used to help determine which actions should be carried out next (e.g., deciding which foods should be consumed at the next meal). In more general terms, serotonin receptors are located

throughout the brain and spinal cord (e.g., the amygdala, hippocampus, hypothalamus, and substantia nigra, as well as all layers of the cerebral cortices) (Graeff, 1997; Molliver, 1987; Palacios, Waebar, Hoyer & Mengod, 1990), with each area being responsive to certain types of input and nonresponsive to others. Forebrain regions in control of physiological functions are the termination points for ascending projections, while the spinal cord is the end terminal for descending projections (Tork, 1990). Until its release into the synapse via exocytotic mechanisms (Sanders-Bush & Martin, 1982), vesicles in the terminal buttons of the pre-synaptic neuron store the serotonin molecules. The rate of release is determined by the rate of firing of serotonergic cells (Frazer & Hensler, 1994). Once released, the activity of 5-HT is terminated via reuptake at the serotonergic terminals. Generally speaking, the serotonergic pathways emerge from the dorsal and the medial raphe nuclei and project to various neuroanatomical structures (Takagi, Shiosaka, Tohyama, Semba & Sakanaka, 1980). The routes of serotonergic innervation throughout the brain, however, are extensive, influencing several different and, in many cases, overlapping neuroanatomical structures and functions. The specific contributions of these structures to cognitive functioning are the focus of this next section.

Memory and Learning. Many different regions of the brain are involved in different types of memory. The most well-known of these structures is the hippocampus, a pair of seahorse-shaped structures that line the inner fold of each side of the temporal lobes (Lezak, Howieson & Loring, 2004). The ventral hippocampus receives serotonergic innervation from dorsal raphe projections, whereas the dorsal hippocampus receives serotonergic innervation from the medial raphe nuclei (Graeff, 1997; Molliver, 1987; Steckler & Sahgal, 1995). The hippocampus plays a major role in normal learning

and retention, quickly associating and integrating information from multiple cortical areas (Eichenbaum & Cohen, 2001). Serving as an index of where each memory is stored in the neocortex (Schacter, Norman, & Koutstaal, 1998), the hippocampus encodes information about events and the context in which they occurred, as well as the thoughts and emotions that were triggered by them. These multiple pieces of information (i.e., "new" memories) are then consolidated and stored in the neocortex as "older" memories (Fuster, 1995). The hippocampus plays a particular role in spatial memory as the perceptual and memory systems are closely linked (Zola & Squire, 2000).

The dorsomedial nucleus of the thalamus is also implicated in memory. It has significant reciprocal pathways to the prefrontal cortex (Graff-Radford, 2003), as well as receptive pathways with the amygdala, temporal cortex, hypothalamus and interthalamic nuclei (Afifi & Bergman, 1998). The dorsomedial nucleus of the thalamus receives serotonergic innervation via dorsal raphe projections (Cooper et al., 1996; Steckler & Sahgal, 1995). Damage to these projections or to the dorsomedial thalamic nucleus would presumably result in decreased availability of serotonin which would result in functional impairments in memory.

The neostriatum, part of the basal ganglia, is also involved in memory. More specifically, it is involved in procedural memory (Fuster, 1995), and it also plays a role in devising novel solutions to new situations (Saint-Cyr & Taylor, 1992). The neostriatum and the basal ganglia both receive serotonergic innervation via dorsal raphe projections (Cooper et al., 1996; Graeff, 1997; Steckler & Sahgal, 1995).

Several components of the limbic system, which is comprised of numerous structures including the amygdala, the cingulate gyrus, and the hippocampus

(Markowitsch, 2000), are also involved in learning and memory. The limbic system, which is spread through the brain stem (e.g., reticular activation system) and the cortex (e.g., olfactory bulb of the frontal cortex) (Lezak et al., 2004), are involved in memory, emotions, and motivation (Damasio, 1994; Markowitsch, 2000). The limbic system receives serotonergic innervation from both dorsal and medial raphe projections (Cooper et al., 1996; Graeff, 1997; Steckler & Sahgal, 1995). The mammillary bodies and the fornix, two other limbic components which are located within the posterior part of the hypothalamus, aid in the retention and consolidation of memories. The fornix connects the hippocampal areas of the forebrain with those of the mammillothalamic regions of the limbic system (Markowitsch, 2000); damage to the mammillary bodies impairs memory processing (Tanaka, Miyazawa, Akaoka & Yamanda, 1997). The amygdala, a small almond-shaped structure found deep in the anterior portion of the temporal lobe, is also involved in memory formation and consolidation by way of object recognition (Mishkin & Appenzeller, 1987) and by providing an emotional component to memories (Doty, 1990). It has extensive connections with many areas of the brain, including the nuclei in the brain stem, cerebral cortex, hypothalamus, thalamus, hippocampus, and basal ganglia (Lezak et al., 2004).

Lastly, the cerebellum, which is attached to the brainstem at the base of the brain, plays a role in memory and learning through its non-motor connections to the thalamus and via input received from the frontal, partietal and superior temporal cortices (Schmahmann & Sherman, 1998). In this way, the cerebellum influences learning and memory in general (Nyberg, 1998), as well as working memory (Desmond, Gabrieli, Wagner, Ginier, & Glover, 1997). Temporal, parietal, frontal and cerebellar cortices, as

well as all layers of the cerebral cortex, receive the majority of their serotonergic innervation from the dorsal raphe nuclei (Graeff, 1997; Molliver, 1987).

Overall, the literature on serotonergic influences on memory are inconsistent as Riedel, Eikmans, Heldens and Schmitt (2005) found that citalopram, a selective serotonin reuptake inhibitor (SSRI), results in impairments in delayed memory recall, whereas others have found that increased levels of serotonin improved memory and learning (e.g., Luciana, Burgund, Berman & Hanson, 2001; Park et al., 1994).

Attention. As with memory, many different neuroanatomical structures are employed in the process of arousal and attending to stimuli. The reticular formation is a network of reciprocal nerve fibers connecting the lowest part of the brainstem (the medulla oblongata) to all major cortical tracts. The reticular formation contains the reticular activating system which is involved in attention through its arousal of the cerebral cortex (Mesulam, 2000). The reticular activating system is responsible for alertness and for maintaining wakefulness (Mirsky, 1989). Lesions to this system result in disturbances in sleep and general responsivity (Lezak et al., 2004). Serotonergic innervation is mainly received from dorsal raphe projections (Steckler & Sahgal, 1995).

The thalamus also plays a role in attention (Corbetta, Miezin, Dobmeyer, Shulman & Petersen, 1991) by way of its connections with the reticular activating system (Steriade, Jones & Llinas, 1990). The thalamus, a pair of small, oval-shaped structures lying along both sides of the third ventricle within the forebrain and above the hypothalamus, is known as the "relay station" of the limbic system and of the brain as a whole as it is involved in many different functions and has extensive reciprocal and topographically organized cortical connections, including pathways to the premotor,

prefrontal, temporal and parietal cortices (Kolb & Whishaw, 1996; Sherman & Koch, 1998). The role of the thalamus in attention is illustrated by reductions in awareness to stimuli on the side opposite the damaged thalamus (Heilman, Watson, & Valenstein, 2003). As with the dorsomedial nucleus, the thalamus receives serotonergic innervation via dorsal raphe projections (Cooper et al., 1996; Steckler & Sahgal, 1995).

Components of the limbic system also play a role in attention. The ability to attend to stimuli is influenced by the amygdala (Eichenbaum & Cohen, 2001), but also by the cingulate cortex, a structure located deep within the brain above the corpus callosum. The amygdala has projections that terminate in the anterior portion of the cingulate cortex, influencing attention (Chelazzi & Corbetta, 2000), whereas the posterior portion is, in part, innervated by the hippocampus, thereby also implicating it in memory functions (Mesulam, 2000). Serotonergic innervation to the amygdala is via dorsal raphe projections (Steckler & Sahgal, 1995), whereas the cingulate cortex receives its innervation from medial raphe projections.

The cerebellum is also known to play a role in attention (Middleton & Strick, 2000a), and lesions to the white matter of the intercerebral conduction pathways that link the two hemispheres results in impairments in attention (Filley, 2001). As mentioned previously, the cerebellum is innervated by dorsal raphe projections (Cooper et al., 1996), whereas both the dorsal and the medial raphe projections are likely involved in innervating the white matter (Cooper et al., 1996; Graeff, 1997; Steckler & Sahgal, 1995).

Taken together, increasing serotonin would be expected to improve attention (Lieberman, Falco & Slade, 2002) through an effect on the reticular activation system,

resulting in increased arousal and vigilance. However, greater levels of serotonin may also impede certain aspects of attention (e.g. auditory and visual attention; Schmitt et al., 2000) and vigilance (Riedel et al., 2005) presumably by way of an inhibitory effect on the amygdala, the thalamic nuclei or cerebellar structures.

Executive Functioning. The term 'executive functioning' is a catch-all phrase pertaining to most higher-level cognitive functions, including such diverse functions as planning, cognitive flexibility, and abstract reasoning, among others. Thus, it is not surprising that, as with memory and attention, many different neuroanatomical structures are involved in these diverse functions. For example, several researchers have found that the cerebellum, though primarily thought of for its motor control, plays a role in abstract reasoning (Middleton & Strick, 2000a), planning (Dow, 1998), set-shifting (Le, Pardo, & Hu, 1998), and information processing speed (Botez, Gravel, Attig, & Vezina, 1985), as well as in more language-based functions such as linguistic processing (Leiner, Leiner, & Dow, 1989), and word generation (Raichle, 2000). It does this through its non-motor pathways. Lesions to these pathways would presumably decrease the amount of serotonin available to the structures innervated by these cerebellar pathways and, although it has been found that information processing slows as a result (e.g., Botez et al., 1985), it is unclear whether any effects on these cognitive processes result from decreases in brain tryptophan (and thus, presumably, serotonin) (e.g., Fischer, Colombani, Langhans & Wenk, 2002; Hughes et al., 2003; Luciana et al., 2001; Rogers et al., 1999; Schmitt et al., 2000).

Each of the three main areas of the frontal cortex (premotor, motor and prefrontal cortices) is differentially innervated, albeit with some overlap. The premotor cortex,

which is responsible for selecting a movement, receives input from the prefrontal cortex and parietal areas, whereas the motor cortex, which is responsible for carrying out a movement, projects to the basal ganglia and red nucleus (involved in motor coordination). The prefrontal cortex, which receives its input from the parietal and temporal lobes, projects to the cingulate cortex, the basal ganglia, the amygdala and the hypothalamus (Kolb & Whishaw, 1996); this area is involved in the control of executive functions. The frontal cortex is innervated by both medial and dorsal raphe projections (Graeff, 1997). On a simplistic level, the frontal cortex is considered the primary location of executive functioning, although it is known that input into these functions are provided by most neuroanatomical structures in the brain (Lezak et al., 2004). Lesions to the frontal lobes tend to produce deficits in "reciprocal relationships between the major functional system" (Lezak et al., 2004, p. 78). This includes the limbic, sensory, motor and executive systems, resulting in deficits of integrating such varied constructs as memory, attention, drive, motivation, social behaviour and motor function (Barrash, Tranel & Anderson, 2000; Damasio & Van Hoesen, 1983; Lezak et al., 2004).

The basal ganglia, a bundle of nuclei comprised of the caudate, putamen and globus pallidus, as well as the amygdala, subthalamic nucleus and substantia nigra, sits at the base of the cerebral hemispheres. The caudate nucleus and putamen receive direct input from the cerebral cortex, whereas the substantia nigra and the globus pallidus project to the cerebral cortex via the thalamus (Lezak et al., 2004). Reductions in cognitive flexibility (i.e., the ability to change cognitive strategies to meet new demands) are noted after damage to the basal ganglia (Lawrence, Sahakian, Rogers, Hodge, &

Robbins, 1999). Collectively, these regions are innervated by serotonergic projections from the dorsal raphe nuclei (Graeff, 1997; Steckler & Sahgal, 1995).

Overall, certain aspects of executive functioning, such as novel solution generation, have been found to improve with increased serotonin levels (Kurup & Kurup, 2003), whereas other functions, such as verbal fluency and strategy planning, have been found to improve with decreasing serotonin levels (Park et al., 1994; Schmitt et al., 2000). It is thus unclear from the literature the exact ways in which increases or decreases in serotonin levels (as assessed via tryptophan manipulation) affect cerebellar, basal gangliar, and frontal cortical functioning.

Emotional Regulation. Many different neuroanatomical structures are involved in the processing, production and memory of emotionally-charged stimuli. The hypothalamus, which is located just above the brain stem but below the thalamus, influences the activity of the pituitary gland in order to control and coordinate such activities of the endocrine and autonomic nervous systems as circadian rhythms, hunger, thirst, fatigue and body temperature (Pinel, 2003; Rolls, 1999). Protective emotional reactions such as fear or rage are also controlled by this structure (Lezak et al., 2004). According to Flynn, Cummings and Tomiyasu (1988), depending on the location of the hypothalamic damage, a myriad of symptoms may emerge, such as obesity or reductions in drive or responsivity. Alterations in mood have also been implicated in damage to hypothalamic pathways (Andreasen, 2001). Serotonergic innervation to the medial and periventricular hypothalamus is received by dorsal raphe projections (Graeff, 1997; Steckler & Sahgal, 1995), whereas the lateral hypothalamus receives medial raphe

projections (Graeff, 1997; Molliver, 1987). Thus, damage to these pathways would presumably reduce the amount of serotonin available to the hypothalamus.

Through its role as a relay station for the limbic system, the thalamus exerts an influence on emotional responding. It has been found that damage to the thalamus results in apathy, emotional flattening and reductions in drive (O'Connor, Verfaellie, & Cermak, 1995). The cingulate cortex, part of the limbic system, has also been implicated in emotional responding (Rolls, 1999). As stated above, the thalamus and the cingulate cortex are innervated by dorsal and medial raphe projections, respectively (Graeff, 1997; Steckler & Sahgal, 1995).

The amygdala, which is also a component of the limbic system involved in emotional behaviour, has extensive innervation from the hypothalamus and is implicated in emotional responding (Lezak et al., 2004), processing and learning (Bechara, Damasio, Damasio, & Lee, 1999). Through its connection to the ventral hippocampus, the amygdala also plays a role in memories of emotional events (Pinel, 2003). Lesions to the amygdala dampen the expression of emotional states and aggressive behaviour (Aggleton, 1993; Brodal, 1981). Amygdalar lesions would also presumably decrease availability of serotonin in the brain, and it has been found that decreases in tryptophan, the precursor to serotonin, results in increased levels of aggression (Cleare & Bond, 1995; Pihl et al., 1995). The amygdala receives serotonergic innervation from dorsal raphe projections (Graeff, 1997; Steckler & Sahgal, 1995).

The basal ganglia are also implicated in emotional experiences, as it has been documented that bilateral damage to these structures results in flattening of emotional expression (Bhatia & Marsden, 1994). Damage to the circuitry of the non-motor

pathways of the basal ganglia are also implicated in several psychiatric disorders, including depression (e.g., Middleton & Strick, 2000b). As mentioned above, serotonergic innervation to the basal ganglia is via dorsal raphe projections (Graeff, 1997; Steckler & Sahgal, 1995).

Fine Motor Control. The last area of functional neuroanatomy to be discussed in this section pertains to motor control. The pons, which literally means "bridge", is a small structure on the brainstem above the medulla that is responsible for carrying sensory signal up to the thalamus, and for sending signals down from the cortex. It serves as a major pathway between the cerebellum and the cerebral cortex (Lezak et al., 2004). Together, the pons and cerebellum are responsible for kinesthetic and postural control, fine-tuning motor impulses from the cerebral cortex (Lezak et al., 2004). These areas are innervated by dorsal raphe projections (Steckler & Sahgal, 1995). Damage to either area results in difficulty with coordination and fine motor control (Barlow, 2002; Caplan, 2001). It has been reported within the literature that increased serotonin results in problems of motor coordination (e.g., Luciana et al., 2001) and slowed motor response time (e.g., Markus et al., 1998). Increased levels of serotonin may thus exert an inhibitory effect upon the pons and cerebellum, thereby reducing motor speed and response times (e.g., Luciana et al., 2001; Markus et al., 1998).

Serotonin, Mood and Cognitive Performance

As mentioned earlier, much of the research on 5-HT has focused on the role of serotonin in mental disorders and physiological disturbances. Indeed, serotonin has been implicated in many different disorders, such as depression (e.g., Sobczak et al., 2002), attention-deficit hyperactivity disorder (e.g., Barrickman, Noyes, Kuperman, Schumacher

& Verda, 1991), and sleep, arousal and insomnia (e.g., Lieberman et al., 1983), among others. Of the numerous disturbances in which 5-HT has been implicated, however, depression is a disorder commonly dealt with by clinical psychologists (Ormel, et al., 1994). Thus, it comes as no surprise that there is a growing literature on the effects of depression on cognitive performance. For instance, it has been reported that depression can result in lower scores on measures of executive functioning (e.g., Rogers et al., 2004; Veiel, 1997; but see Mitchell & Phillips, 2007), memory (e.g., Austin, Mitchell & Goodwin, 2001), working memory (e.g., Harvey et al., 2004), speed of processing and motor performance (e.g., Gualtieri, Johnson & Benedict, 2006; Sobin & Sackeim, 1997), intelligence (e.g., Hartlage, Alloy, Vazquez & Dykman, 1993) and attention (e.g., Porter, Gallagher, Thompson & Young, 2003). The mechanism behind this relationship between cognition and depression, however, is still somewhat unclear. It can be surmised that since depression is associated with reductions in serotonin levels (Frazer & Hensler, 1994), it could be that lower levels of tryptophan (or its metabolite, 5-HTP) are crossing the blood-brain barrier, resulting in lower levels of serotonin production and activity. This could in turn directly affect the neuroanatomical structures that are known to play a role in a given cognitive function. For instance, it has been reported in the literature that reductions in hippocampal volume are frequently noted in those suffering from depression (e.g., Campbell, Marriott, Nahmias & MacQueen, 2004; Videbech & Ravnkilde, 2004; but see Vythilingam, et al., 2004). Cortisol levels are often found to be elevated in those suffering from depression (e.g., Monteleone, 2001), and chronic elevation of cortisol is known to both reduce hippocampal activity and increase hippocampal atrophy (McAuley et al., 2009). Furthermore, stress is known to inhibit

hippocampal neurogenesis, a process that normally occurs throughout the lifespan, and to reduce dendritic branching (Jacobs, van Praag & Gage, 2000). Reductions in dendritic branching, as well as atrophy within the hippocampus, would both result in decreased hippocampal volume. Thus, it could be that the lowered levels of serotonin, such as are found in those with depressions (e.g., Wichers & Maes, 2002), affect memory via direct effect on the hippocampus (and possibly other neuroanatomical structures involved in this cognitive process).

According to Cook (1991), there is growing evidence to support such interactions between serotonin, mood and cognitive performance. These processes likely occur in the limbic system (described above), areas of the brain heavily innervated by serotonergic projections. Further evidence of the relationship between serotonin and cognitive performance lies within the hippocampus, a structure known to play a major role in long-term memory (LTM). As described above, if the serotonergic pathways projecting to the hippocampus are compromised, or if there is a change in the amount or rate of firing of serotonergic cells, hippocampal functioning could be altered as a result. The thalamus, which receives serotonergic projections from the dorsal raphe nuclei, is known to play a role in attention (Corbetta et al., 1991) and thus, alterations in the activity of this pathway or region could result in disruptions in information processing due to poor attention.

Taken together, the discussions in the previous two sections on functional neuroanatomy and the relationship between mood, serotonin and cognition provide evidence that many cognitive and affective processes are, in fact, affected by serotonin and the serotonergic pathways known to affect neuroanatomical structures.

Relationship between Serotonin, Depression and Antidepressants

As discussed above, depression is one of the most commonly seen psychological disorders dealt with by clinicians. One way in which depression is treated is via pharmacological intervention with antidepressant medications. There are three main classes of antidepressant medications used in the treatment of depression. The oldest class is known as the tricyclic antidepressants and they exert their effects by blocking the reuptake of serotonin and norepinephrine. These actions on the serotonergic system are thought to regulate mood (Murray, 1998). Monoamine oxidase inhibitors (MAOIs) work by decreasing the rate at which the MAO enzyme breaks down monoamine neurotransmitters, thus increasing the availability of serotonin, dopamine, epinephrine and norepinephrine in the synapse. A third class of medication is known as the serotonin reuptake inhibitors. These drugs exert their influence by nonselectively inhibiting the uptake of serotonin and other monoamines, thereby increasing their concentrations in the synapse. The selective serotonin reuptake inhibitors (SSRIs), however, only block the reabsorption of serotonin, thus making them of the greatest interest here. Since the SSRIs work by blocking the reuptake of serotonin into presynaptic neurons, greater amounts of serotonin are available in the synapse (Murray, 1998). This increase in serotonin availability increases the inhibitory activity of presynaptic receptors, thereby decreasing the firing rate of the serotonergic neuron (Blier & de Montigny, 1994; Nutt et al., 1999). This negative feedback mechanism results in an initial inhibition of the firing rate of the raphe nuclei. After this initial inhibitory period, the concentration of serotonin in the synapse begins to increase over the course of several weeks until serotonin levels reach therapeutic levels within the cortex (Nutt et al., 1999). There is documentation within

the literature that SSRIs are an effective treatment for depression (e.g., Kraft, Slager, McGrath & Hamilton, 2005; Pallanti, & Sandner, 2007; Rossi, Barraco & Donda, 2004; Zohar, & Westenberg, 2000; but see Hotopf & Barbui, 2004; Kirsch, Moore, Scoboria, & Nicholls, 2002) as they have generally been found to improve negative mood states in both normal, non-depressed persons, and in patients with depression (e.g., Barge-Schaapveld, Nicolson, van der Hoop, & DeVries, 1995; Barton, et al., 2008; Bell, Abrams, Nutt, 2001; Delgado et al., 1990). Of interest, however, is that there is research documenting the efficacy of serotonergic precursors, such as 5-HTP, as being equal to those of SSRIs in treating depression. For instance, Poldinger, Calanchini and Schwarz (1991) conducted a clinical trial wherein patients diagnosed with depression where given either 100mg of 5-HTP three times a day, or 50mg of the SSRI fluvoxamine three times a day for a period of six weeks. At the end of the six-week trial, 5-HTP was not only found to be as effective as the SSRI at treating depressive symptoms, but it also produced fewer side effects. These results echo those of van Praag (1981) who similarly found in his study that daily doses of 200mg of 5-HTP were as effective as the tricyclic antidepressant clomipramine at alleviating symptoms, again with fewer side effects. Further support for these findings comes from the literature on acute tryptophan depletion and SSRI use. For instance, Delgado and colleagues (1990) induced acute tryptophan depletion in a group of patients diagnosed with depression who were being successfully treated with SSRIs. Of the 21 patients in this study, two-thirds of them experienced a relapse in their depressive symptoms following tryptophan depletion. Upon consuming a normal meal, it took between 24 and 48 hours for remission to return. The authors found that free plasma tryptophan levels were negatively correlated with depression scores during the depletion,

with more tryptophan in the plasma resulting in lesser degrees of depression. These results have been replicated in the literature by several other researchers (e.g., Booij, Van der Does, Haffmans, & Riedel, 2005; Nutt et al., 1999; O'Reardon et al., 2004) which, when taken together with the literature on SSRIs, indicates that reductions in serotonergic activity may be implicated in depressive symptoms in certain groups of people (Cooper et al, 1996).

The relationship between serotonin and depression, however, is far from clear as there is documentation within the literature that selective serotonin reuptake enhancers (SSREs) such as tianeptine, which act to increase the reuptake of serotonin at the synapse, also result in improvements in symptoms of depression (Defrance, Marey & Kamoun, 1988; Mennini, Mocaer & Garattini, 1987). Thus, although it is unclear whether increases or decreases in serotonergic activity have greater implications for the treatment of depression, it does appear that serotonin is in some way involved in depressive symptoms.

Effects of Other Tryptophan Metabolites on Brain Function

There is also growing evidence, however, that other metabolites of tryptophan affect neuroanatomical function and thus, cognitive performance. Such metabolites are created when tryptophan circulating in the bloodstream passes through the liver, which can result in one of four possible outcomes: 1) tryptophan is returned, unchanged, into the bloodstream for use elsewhere in the body; 2) liver enzymes break tryptophan down into smaller proteins; 3) tryptophan gets converted into 5-HTP; or 4) tryptophan is converted into kynurenine (Murray, 1998). As kynurenine is a convulsant responsible for proper muscle function, an excess of kynurenine can result in damage to the muscles. Normally,

the liver produces greater quantities of kynurenine (used in the production of niacin) than 5-HTP. However, heightened or chronic stress can upset the balance even more by decreasing the supply of the enzyme necessary to produce 5-HTP, resulting in even less 5-HTP being produced and thus lowering the production of 5-HT in the brain (Murray, 1998). Kynurenic acid, which is produced by the kynurenine pathway and released by astrocytes, is critical for memory and learning (Potter, 2009). This is because the hippocampus is heavily innervated by glutamatergic neurons and glutamatergic neurotransmission is mediated by NMDA (N-methyl-D-aspartate) receptors. Kynurenic acid acts as an NMDA receptor antagonist, meaning that it reduces the chances that the calcium ion channel (which is controlled by NMDA) will open. The opening of this channel is essential for long-term potentiation (i.e., the protracted increase in communication that occurs between the pre- and the post-synaptic neuron when they are stimulated at the same time) (Carlson, 2007), which is, in turn, thought to be a major underlying mechanism in learning and memory (Bliss & Collingridge, 1993). Thus, greater amounts of kynurenine result in less 5-HTP in the brain, leading to less serotonin production in the brain, but more kynurenic acid, an NMDA antagonist the makes it less likely that long-term potentiation will occur. Together, these processes reduce both learning and memory within the hippocampus.

The NMDA receptors are also thought to play a role in developmental neural plasticity (Cooper et al., 1996). These receptors have agonist receptor sites for glutamate and for glycine, both of which must be activated if the ion channel is to open (Dingledine & McBain, 1994). However, kynurenic acid by-products, such as quinolinic acid, act as antagonists of the glycine receptor site (Dingledine & McBain, 1994), working to

decrease the chances of the NMDA-regulated ion channel opening, and thereby decreasing the likelihood of long-term potentiation occurring. Quinolinic acid is an excitatory neurotoxin, meaning that it binds to a neuron and causes damage or cell death due to its stimulation. In this way, quinolinic acid may play a role in neurotoxicity induced by NMDA (Cooper et al., 1996). Quinolinic acid has also been implicated in neurodegeneration within the rat hippocampus, resulting in cognitive impairment (O'Neill, Morgan, & Brioni, 1998). Fortunately, glial cells release kynurenate, a by-product of kynurenine, to serve a neuroprotective role within the brain (Cooper et al., 1996). It can thus be seen that the different by-products of tryptophan metabolism have differing effects on the neurochemistry of the brain. Quinolinic acid, a neurotoxin, may produce memory impairments, whereas kynurenate works to protect the brain from these neurotoxic effects. If there is an imbalance in these neurochemicals, the chances of long-term potentiation and thus learning and long-term memory, may be decreased.

Lastly, there is some evidence that melatonin, another tryptophan metabolite, plays a role in cognitive functioning. Melatonin is created in the pineal gland, which houses all the necessary enzymes to turn tryptophan into serotonin; serotonin is then converted into melatonin (Frazer & Hensler, 1994). Aside from its role in circadian rhythms, melatonin has been implicated in the inhibition of long-term potentiation in the rat hippocampus (Soto-Moyano et al., 2006) and in deficits in learning and memory in mice (Larson et al., 2006). Thus, if there are alterations in the amount of tryptophan reaching the brain, a ripple effect could be seen wherein less serotonin is created, resulting in less melatonin, which in turn could lead to decreased performance on learning and memory tasks.

Taken together, it is clear within the literature that there are many different pathways in which tryptophan can affect the neurochemical balance in the brain which may in turn produce changes in cognitive performance by way of influencing neuroanatomical functioning.

Effects of Tryptophan Augmentation or Depletion on Neuropsychological Performance

Amino Acid Protocol

One method of assessing the effects of tryptophan metabolites on affect and cognitive performance is via acute tryptophan depletion. As discussed previously, depletion works by decreasing the tryptophan:LNAA ratio, resulting in marked decreases of tryptophan compared to the LNAAs competing for transport across the blood-brain barrier. By depleting tryptophan levels, researchers can quantify and measure the effects of lower tryptophan levels on cognition. The most common means of accomplishing depletion is by ingesting a mixture of amino acids (prepared as a drink) containing all the essential amino acids except for tryptophan. The balanced, or placebo, condition in such studies sees the addition of L-tryptophan to the depletion mixture. In studies that include acute tryptophan augmentation, a larger dose of tryptophan is added to the mixture than that added for the balanced drink. At this point, a note on the terminology used within the literature is necessary as there are several terms that are used almost interchangeably when referring to acute tryptophan depletion (e.g., ATD, protein-loading) or to acute tryptophan augmentation (e.g., ATA, carbohydrate-loading). For parsimonious reasons, the remainder of this paper will employ the term ‘depletion’ to denote ‘acute tryptophan depletion’ or ‘protein-loading’, and the term ‘augmentation’ will be used to refer to

'acute tryptophan augmentation' or 'carbohydrate-loading'. The term 'balanced' will be used in reference to the 'balanced condition'.

Among the first to examine the effects of acute tryptophan depletion were S. N. Young and colleagues (S. N. Young, Smith, Pihl & Ervin, 1985), who found marked decreases in free and total plasma tryptophan levels following ingestion of the depletion mixture, and drastic increases following ingestion of the balanced drink. This method has since been employed by numerous researchers who have found similar results when examining the effects of depletion on total and/or free plasma tryptophan levels (e.g., Hughes et al., 2003; Schmitt et al., 2000). With evidence that this protocol produced reliable alterations in plasma and total free tryptophan levels, attention was turned to the effects such manipulations would have on mood and cognition given that alterations in plasma tryptophan levels would presumably result in an alteration in brain serotonin production and release.

In order to examine the effects of depletion on memory and learning, Park and colleagues (1994) recruited 12 male participants and randomly assigned them to a depletion or to a balanced group. Both researchers and participants were blind to the drink composition, and all participants took part in both conditions separated by a minimum of 7 days between test sessions. On both occasions, participants were provided with a 52 g amino acid drink (S. N. Young and colleagues (1985) employed a 100 g protocol), and blood samples were drawn both before ingesting the drink and approximately 4 hours later, just prior to commencing the neuropsychological testing. The domains of executive functioning, visual/visuospatial pattern learning and memory, attention, and working memory were tested using the Cambridge Neuropsychological

Test Automated Battery (CANTAB), a computerized neuropsychological test battery. Participants also completed visual analogue scales to measure their perceived level of sadness, anxiety, irritability, concentration, and energy levels, along with a computerized test of autobiographical memory. Researchers found that total free and plasma tryptophan levels were lowered following the depletion condition, but remained the same following the balanced condition. Overall, it was found that although neither mood nor autobiographical memory was affected by depletion, certain types of learning and memory (i.e., learning and remembering new rules and visuospatial pairings) were reduced by depletion. In addition, neither attention nor working memory showed any effect of depletion. The authors concluded that low tryptophan levels result in poor consolidation of information in memory and thus lower scores on measures of long-term memory (LTM) and learning.

The finding that depletion results in transient decreases in learning has been corroborated by several others. For instance, using the same protocol as S. N. Young's group (S. N. Young et al., 1985), Rogers and colleagues (1999) found reductions in visual discrimination learning following depletion (compared to a balanced condition), with participants demonstrating marked difficulty learning new stimulus-reward pairings. In this study, participants' ability to shift attention to now-relevant stimuli dimensions remained intact. In addition, both total and free tryptophan levels were lowered by the depletion but not by the balanced protocol. These results were interpreted to suggest that cortical and subcortical regions undergo a change in neuromodulation that is affected by serotonin (Rogers et al., 1999).

Transient decreases in LTM have also been noted by Riedel and colleagues (1999), who examined mood and cognition in tryptophan-depleted participants with and without a family history of depression. Using S. N. Young's protocol (1985), Riedel and colleagues found that, compared to a balanced condition, depletion resulted in lower scores on tests of LTM. More specifically, reductions in delayed recall and recognition response time and sensitivity were apparent 6 hours after ingesting the drink. No effects were found on perception, motor function, or short-term memory 6 or 24 hours after the initial drink, despite participants receiving 10 g booster portions 7, 11 and 13 hours after the initial ingestion of the drink and the low-tryptophan meals at lunch and dinnertime. Further, although no differences were found between groups with and without a family history of depression on the neurocognitive tests, depletion did produce lower moods in those with a family history of depression, a finding similar to that of Benkelfat and colleagues (1994). It should be noted, however, that 7 female participants (four of whom were in the depletion condition) withdrew from Riedel's study due to nausea induced by the amino acid drink (three of whom vomited). This point highlights the fact that the amino acid mixture is not only extremely potent, but also highly unpalatable, which increases the odds of protocol-induced confounds within the study. For instance, it is possible that even mild or unreported nausea may affect cognitive performance, making it more difficult to discern true effects from those produced by objectionable testing procedures. The disagreeable taste of the amino acid mixture has been reported by several other researchers (e.g., Greenwood, Lader, Kantameneni, & Curzon, 1975; Hughes et al., 2000; Park et al., 1994; Riedel et al., 1999), many of whom have tried to improve its taste by mixing flavoured concentrates or chocolate syrup into the drink.

Nonetheless, regardless of the palatability of the protocol, the literature seems to suggest that depletion results in reductions in LTM and has the potential to influence mood, particularly in those who are susceptible to depression (Benkelfat et al., 1994).

The effects of depletion on attention, however, are not as clear. For instance, using the same amino acid mixture employed by Park's group (1994), Coull and colleagues (1995) examined the effects of depletion on emotional selective attention as measured by a focused attention task and an attentional searching task. On both tasks, the words "left" or "right" served as targets, and the distractors were words selected from four groups of words with emotional valence (e.g., "choke" or "panic"). The attentional searching task added a component of compatibility such that compatible trials were those in which the target word "left" (or "right") appeared on the left side (or the right side, respectively) of the computer screen; incompatible trials were those in which the targets did not match the side of the screen on which they were presented (i.e., the word "left" appeared on the right-hand side of the screen). As with other studies employing acute tryptophan depletion, participants took part in both the depletion and the balanced conditions, with no less than 1 week between testing sessions. On both occasions, testing began 4 hours after ingesting the drink. The authors concluded that depletion did not affect focused attention, but they reported shorter response latencies when stimuli were incompatible on the attentional search task; emotional valence did not affect this relationship (Coull et al., 1995).

These findings are at odds with those of Schmitt and colleagues (2000), who administered a comprehensive neuropsychological battery to determine the effects of depletion on executive functions, memory, language, processing speed, mood, and

attention. They used the 100 g protocol and administered a 10 g maintenance dose 6 hours later. All participants took part in both the depletion and balanced conditions, which were separated by at least 7 days; blood samples were collected at multiple times on both days. Overall, Schmitt's group found that although depletion did not affect planning (bolstering the findings of Park et al., 1994), processing speed, or divided attention, it did produce significant improvements in both auditory and visual focused attention. A possible reason for the divergence in findings is that whereas Coull and colleagues (1995) utilized the same amino acid mixture as Park's group (52 g), Schmitt's group utilized the protocol employed by S. N. Young and colleagues (100 g) and included maintenance doses. It is likely that the greater dose of each amino acid, as found in the 100 g mixture, results in greater "depletion" of tryptophan as the levels of the other LNAAs increase and crowd out tryptophan for transport across the blood-brain barrier. The higher dose of amino acids ostensibly results in greater cognitive changes. Schmitt's group reports that, compared to the balanced condition, plasma tryptophan levels dropped 64% from baseline and the tryptophan:LNAA ratio fell by 33% from baseline 9 hours after ingesting the drink. Coull's group, on the other hand, did not report the drop in plasma tryptophan levels attained by their manipulation. However, as Coull employed the same protocol as Park and colleagues, it is likely that plasma and total free tryptophan was similarly drastically decreased. Of note, however, is that neither Park's, Coull's, nor Schmitt's group altered the amount of the amino acid mixture based on participants' body mass. Indeed, it seems that the norm within the literature is to provide all participants, regardless of gender or body mass, with the same quantity of the amino acid mixture (but see Fischer et al., 2002). While it is not doubted that these variables

may affect the degree to which tryptophan levels are altered, the fact that most studies do not control for these confounds makes generalization of these findings quite difficult.

Schmitt's group also found that verbal fluency scores improved 5 hours following ingestion of the depletion mixture (compared to baseline measures), supporting the notion that serotonin is important for proper frontal lobe functioning. This conclusion is based on the fact that verbal fluency tasks result in increased activation in the frontal lobes during imaging studies (Brannen et al., 2001). Further, Schmitt's results corroborated Park's (1994) assertion that serotonin reduces certain aspects of learning and memory as they found evidence of decreased LTM memory in light of spared short-term memory and retrieval abilities. An interesting point, however, is that Schmitt and Park both found reductions in LTM despite using different volumes of the amino acid mixture (100 g versus 52 g, respectively), suggesting that decreases in LTM are robust findings regardless of the degree of serotonin depletion. Schmitt and colleagues point out, however, that although Park's group did find selective reductions in learning and memory (as measured by the number of trials needed to learn spatial locations on a paired associates learning task), they failed to find such effects on any of the actual memory tests that were employed. Schmitt's group suggests that this is due to the fact that the memory tests utilized by Park's group did not measure memory for material learned and consolidated after a considerable delay. For this reason, Schmitt's group makes the point that, "[M]emory tests which do not incorporate delayed assessment or do not require long-term storage of new information are likely to fail to detect memory deficits of acute tryptophan depletion" (p. 27).

Few of the studies discussed thus far have included a neuropsychological test battery as comprehensive as that employed by Hughes and his colleagues (2003) in their efforts to examine the effects of tryptophan depletion on mood and cognitive functioning. In contrast to Schmitt's group (2000), whose battery contained several computerized tests and experimental tasks, Hughes' battery assessed a wide range of domains, including attention and executive functioning, verbal learning and memory, visuospatial learning and memory, and mood, using both paper-and-pencil and CANTAB tests. A group of 20 males were tested using the 52 g drink protocol; each participant took part in both the depletion and the balanced conditions, with no less than 7 days between testing sessions. On each test day, blood samples were drawn at four separate times, and testing began 4 hours after ingesting the initial drink. Analysis of the blood assays showed that there were no significant between group differences in baseline measures of either total plasma or free tryptophan levels. For the depletion condition, both total plasma and free tryptophan decreased over time, remaining at depletion levels of 60.7% and 56.9% at 330 minutes, respectively. For the balanced condition, however, total tryptophan increased by 33.6% at 240 minutes, but was depleted to a level of 6.8% below baseline by 330 minutes. Free tryptophan levels were increased by 71.8% at 240 minutes, but had reduced to 28.5% by 330 minutes time. Taken together, these findings demonstrate that the manipulation successfully altered total plasma and free tryptophan levels. Nonetheless, test results showed that the tryptophan manipulation failed to produce any decline in cognitive functioning or any changes in mood. These findings stand in stark contrast to the literature discussed previously in that several researchers have detected poorer performance within these same domains. Bolstering Hughes's findings, however,

are those of Shansis and colleagues (2000) who similarly found no effect of depletion on mood, attention, and memory using a healthy male sample. Shansis' team suggested that the discrepancy between their findings and those of others (e.g., Park et al., 1994) could be due to the testing instruments employed. That is, Park's group used the Cambridge Neuropsychological Test Automated Battery, which is known to be sensitive to subtle neuropsychological (i.e., cognitive) changes (Shansis et al., 2000), whereas Shansis used paper-and-pencil tests. However, unlike Shansis' group, Hughes' group employed three of the same measures from the battery employed by Park's group: the Tower of London, Attentional Set-shift, and the Paired Associates Learning tests. Thus, while it is possible that Shansis did not select measures that are sensitive enough to detect subtle cognitive changes brought on by depletion, it does not explain why Hughes, who administered the same protocol as Park and who also had a larger sample size than Park, found no significant results. As Hughes and colleagues point out, the failure to measure delayed memory or recognition, the degree of tryptophan (and thus serotonin) depletion or disruption, and any individual differences in the degree of sensitivity to depletion, including gender or body mass, or some combination of these factors may be responsible for the inconsistency of findings.

Regardless of the contradictory findings, the common factor between all of the studies discussed thus far is that they have only examined depletion in comparison to a balanced condition; that is, not a single one of these studies employed an augmented condition. The administration of an augmented condition is important as it enables us to learn about what happens when we raise tryptophan concentrations to abnormally high levels and the effects that this has on cognitive functioning. To overcome this gross

oversight, Luciana and colleagues (2001) conducted one of the only studies found for this review that examined cognitive functioning by employing both a depletion and an augmentation condition (no balanced condition was used). Using S. N. Young's (1985) 100 g amino acid protocol, participants took part in both conditions, separated by a minimum of 7 days. Assessing the domains of working memory, executive functioning, short-term memory and attention, and motor speed and accuracy, Luciana's group found several interesting differences between the depletion and the augmented groups. For instance, although both conditions lowered participants' scores of positive affect (as measured by the PANAS; Watson, Clark, & Tellegen, 1988), the depletion group exhibited faster response times on the Grooved Pegboard Test, more digits correct on the backward trial of the Digit Span test, and better ability at processing sad affective content compared to the augmented group. Conversely, although the augmentation group exhibited decreased motor coordination (as evidenced by the number of drops on the Grooved Pegboard Test), they made fewer errors of omission on a letter-cancellation test. No discernable differences were found between the two groups on the Digit Span forward trial, Spatial Span, verbal fluency, or spatial working memory. In both the depletion and augmentation conditions, total plasma tryptophan concentrations were significantly lowered and raised, respectively, compared to baseline levels. Overall, the authors concluded that augmentation results in transient reductions in affective and verbal working memory (as measured by the Digit Span Backward trial), as well as decreased motor-coordination while sparing (and even enhancing) immediate vigilant attention. Taken together, Luciana's group concluded that these findings are consistent with the notion that increased serotonin activity results in a reduction of the flow of information,

leading to declines in motor coordination, working memory, and short-term attention. Perhaps more importantly, results of this study demonstrate that just as depletion effects performance, so too does augmentation, highlighting the necessity of researchers including an augmented condition in future research to further our understanding of the effects of augmentation on neuropsychological performance.

Overall, it is clear that there is considerable controversy and contradiction within the literature on the effects of depletion and augmentation. This seems to be related to the following factors: a) the tests selected for use in the study, many of which do not always correspond with the instruments most widely used in clinical settings; b) the use of amino acids, which often result in adverse effects such as nausea or vomiting; c) gender ratios, which are rarely equal, especially as many studies only include males or provide no information on gender distributions; and d) high attrition rates and small sample sizes, making generalization of test results difficult. In an attempt to address some of these issues (e.g., nausea and small sample sizes), researchers have begun to look to other methods of altering tryptophan (and thus serotonin) levels, the most common of which is the administration of carbohydrate-rich, protein-poor meals.

Dietary Manipulations

The effect of diet on brain serotonin levels is also unclear as there are many factors influencing whether tryptophan will increase brain serotonin production or release. As discussed previously, merely ingesting tryptophan-rich foods does not necessarily alter brain- serotonin levels as it could get metabolized before it can cross the blood-brain barrier, or prevented from easily crossing the blood-brain barrier due to competition from the other LNAAs. This is because the volume of the LNAAs also

increase due to the ingestion of dietary proteins, thereby reducing the tryptophan:LNAA ratio. Ingesting carbohydrates in a protein-poor meal, however, appears to avoid both issues by increasing insulin, thereby sweeping the LNAAs into the surrounding tissues and allowing tryptophan to easily cross the blood-brain barrier to aid in the synthesis of serotonin.

Using the dietary method of altering tryptophan levels, Markus and colleagues (1998) studied the effects of carbohydrate-rich meals (designed to augment tryptophan levels) in comparison to protein-rich meals (designed to deplete tryptophan levels) on mood and memory in stress-prone participants during a stress-inducing task. Participants were divided into two groups, high-stress (n = 24) and low-stress (n = 24), based upon their scores on the Inadequacy Scale of the Dutch Personality Inventory (Luteijn, Starren, & van Dijk, 1975). All participants were randomly assigned to start with either the carbohydrate-rich/protein-poor meal, or the protein-rich/carbohydrate-poor meal. Each participant took part in both conditions, which were separated by a 4-week period. On each testing day, participants ate breakfast upon arriving at the testing site, followed by a snack 1.25 hours later. Lunch was served 45 minutes after consumption of the snack. The test battery, which was administered 1.5 hours after lunch, was preceded by the collection of a blood sample. The battery consisted of the Profile of Mood States, a stress induction task (a mental arithmetic task completed during noise interference), and a computerized memory scanning task measuring accuracy and response times. The first main effect Markus's group reported was that the ingestion of the carbohydrate-rich diet increased the tryptophan:LNAA ratio, rising from 0.074 on the protein-rich test day to 0.105 on the carbohydrate-rich day. They also reported that regardless of stress group,

the carbohydrate-rich meal resulted in slower reaction times on the computerized memory task. The authors concluded that the ingestion of carbohydrates increased brain tryptophan levels, and thus that the synthesis and activity of brain serotonin was also increased. They noted that this, in turn, resulted in slowed response times. Thus, their prediction that a carbohydrate-rich meal would protect stress-prone people from deteriorations in mood and cognitive performance in the face of a stressful task was met. Markus and colleagues assert that increased serotonin synthesis and activity likely mitigated the stress-response of the high stress group by increasing their sense of control in an otherwise uncontrollable situation (the stress-induction task). The general conclusion to be drawn from this study, however, is that carbohydrate-loading increased the tryptophan:LNAA ratio in a similar manner as the amino acid protocol, and neurocognitive effects were observed as a result. Thus, it seems that the dietary method can be used as an alternative to the amino acid protocol as a means of altering the rate of production and release of serotonin.

The utility of manipulating diet to measure the effects of varying macronutrients on performance and mood has been shown by many other researchers. For instance, to study the effects of tryptophan augmentation, Lieberman et al., (2002) provided 143 US Army soldiers with one of three carbohydrate drinks: 6% by volume, 12% by volume, or a placebo mixture, all of which looked and tasted identical. This was a between-subjects study, with each soldier assigned to only one condition. No blood samples were collected. The soldiers engaged in several physically demanding tasks throughout the day while wearing vigilance monitors (specialized watches). When soldiers heard the monitors beep, they had to respond as quickly as possible by pressing a button on the monitors.

Response times were utilized as an indication of vigilance. Profile of Mood States mood questionnaires were also completed at three separate times throughout the day. The authors found dose-related increases in sustaining vigilance and alertness, such that those in the 12% per volume group performed the best and those in the placebo group performed the worst, declining in vigilance at every designated test period. Dose-related effects were also found for confusion on the Profile of Mood States, wherein those in the placebo group scored as more confused than either of the carbohydrate groups, with the 12% group being the least confused. The authors conclude that during times of sustained physical activity, carbohydrates aid in the maintenance of vigilance and enable optimal cognitive functioning.

The finding that carbohydrates enhance attention and decision times, however, has not been replicated by all researchers. In a study by Fischer and colleagues (2002), participants were randomly assigned to one of three dietary groups: high-carbohydrate/low-protein (i.e., augmentation), low-carbohydrate/high-protein (i.e., depletion), or a balanced condition. All participants took part in each condition, with exactly 1 week separating each test condition. In each case, the cream-like meals were identical in taste, texture, and colour. Blood samples were collected and the cognitive domains of motor performance, information processing, short-term memory and peripheral attention were assessed. Overall, the authors found that cognitive performance, especially short-term memory, was greatest following the high-protein meal or the balanced meal, whereas the high-carbohydrate meal resulted in the poorest performance, producing only transient improvements in attention and decision times.

Neither the carbohydrate nor the protein meal affected peripheral attention, which the authors credited to a lack of sensitivity of the measure employed.

However, comparing protein (a depletion method) and two carbohydrate meals (one high in starch and one high in sugar, designed to augment tryptophan levels), Smith, Leekam, Ralph, and McNeill (1988) found that the carbohydrate meals resulted in slowed response times to peripheral targets whereas the protein meal resulted in greater distractibility on a centralized target-detection task. In addition, there were no overall effects of meal composition on attention or mood. Taken together, these findings suggest that protein increases vigilance and alertness compared to carbohydrates.

It has been found, however, that the effects of carbohydrates on performance differ depending on age and gender. For instance, Spring, Maller, Wurtman, Digman, and Cozolino (1983) examined the effects of protein and carbohydrates on a group of younger (aged 18-39 years) and older (aged 40-65 years) participants, each of whom were assigned to one of four groups: high-protein breakfast, high-protein lunch, high-carbohydrate breakfast, or high-carbohydrate lunch. Utilizing the Stanford Sleepiness Scale, the Profile of Mood States, visual analogue mood scales, an auditory response time task and a dichotic shadowing task measuring information processing of sustained and selective attention, they found significant effects for meal type, gender, and time of the meal. That is, those who consumed the high-protein meal performed with greater accuracy on the dichotic shadowing task, whereas there was no effect of meal on the auditory response time task. However, compared to the protein meals, the carbohydrate meals produced a greater feeling of sleepiness in women than in men, and a greater sense of calmness in men than in women. Furthermore, the protein meal resulted in those in the

older age group feeling more tense and less calm if it was ingested at breakfast time, whereas performance was worse on the dichotic shadowing task (i.e., sustained selective attention) following carbohydrates at lunchtime. The authors concluded that the consumption of a high-carbohydrate lunch affected concentration in older participants, likely resulting from faltering attention.

In an older sample of adults, however, Kaplan, Greenwood, Winocur, and Wolever (2001) found that carbohydrates, as well as protein and fat, improved several aspects of cognition in individuals 61-79 years of age. In this study, each participant took part in all four conditions, ingesting one drink (protein, carbohydrate, fat or placebo, 300 mL each) every 3-7 days. Within 15 minutes of consuming the drink, participants were administered three verbal memory tests: a list-learning task (testing immediate recall), and both an immediate and a delayed paragraph recall test. As fillers during the delay period, the Trail Making Test (Reitan, 1958) and an attention task were completed. All three drinks (save for the placebo) were found to improve delayed paragraph recall, with a trend towards improved immediate recall. More specifically, the carbohydrate drink was found to improve performance on the Trails task and delayed paragraph recall in men, whereas the protein drink resulted in greater immediate paragraph recall and the fat drink improved attention. Since all three drinks aided in increasing memory, the authors concluded that all three macronutrients likely exert different effects upon cognitive function.

Thus, it should be clear that the neuropsychological effects of tryptophan are varying and contradictory, especially when differing methodologies (i.e., amino acid protocol or dietary protocol) and manipulations (i.e., depletion or augmentation) are

considered. For instance, recall that Coull and colleagues (1995) found no effect of depletion on attention using the 52 g amino acid protocol, whereas Schmitt's group (2000) found that focused attention improved with the ingestion of a depletion 100 g protocol drink. Luciana and colleagues (2001) found that a 100 g augmentation mixture resulted in better attention than a 100 g depletion mixture. However, Hughes's team (2003) found that depletion using the 52 g protocol had no effect on any domain of cognitive functioning. Within the dietary manipulation literature, Markus's research group (1998) found that carbohydrates reduced response times, a finding in direct contradiction to that reported by Lieberman's group (2002). Despite such inconsistencies, however, there do appear to be several relatively reliable findings due to tryptophan manipulation. It appears that depletion, produced either via the amino acid protocol or the carbohydrate manipulation, is related to declines within the areas of LTM (Park et al., 1994; Riedel et al., 1999; Rogers et al., 1999) and mood (in susceptible individuals) (Luciana et al., 2001; Riedel et al., 1999; but see Hughes et al., 2003 and Shansis et al., 2000), with mixed results for effects on attention (Coull et al., 1995; Hughes et al., 2003; Lieberman et al., 2002; Schmitt et al., 2000; Shansis et al., 2000), response time (Lieberman et al., 2002; Markus et al., 1998) and verbal fluency (Schmitt et al., 2000). Conversely, augmentation seems to result in transient reductions in affective and verbal working memory, as well as motor coordination, while enhancing immediate vigilant attention (Luciana et al., 2001) (see Table 1 for summary of findings in the literature).

Table 1

Summary of Selected Findings Within the Literature

Research Group	Manipulation Method	Design	Conditions Employed	Effect of	Findings
Park et al., 1994 *n* = 12 M	amino acid	within	depletion & balanced	depletion	- no effect on mood - lower scores on LTM
Rogers et al., 1999 *n* = 31 (15 M)	amino acid	between	depletion & balanced	depletion	- reductions in visual discriminant - intact ability to shift attention
Riedel et al., 1999 *n* = 27 (12 M)	amino acid	mixed	depletion & balanced	depletion	- reductions in LTM scores - lower mood in those with family history of depression
Coull et al., 1995 *n* = 12[a]	amino acid	within	depletion & balanced	depletion	- no effect on attention
Schmitt et al., 2000 *n* = 20 (10 M)	amino acid	within	depletion & balanced	depletion	- no effect on planning, processing speed or divided attention - improvements in auditory and focused attention - delayed improvements in verbal fluency - reductions in LTM scores
Hughes et al., 2003 *n* = 20 M	amino acid	within	depletion & balanced	depletion	- no reductions on scores in any neuropsychological domains - no reductions in mood scores

Shansis et al., 2000 *n* = 12 M	amino acid	within	depletion & balanced	depletion	- no reductions in mood, attention or memory scores
Luciana et al., 2001 *n* = 19[a]	amino acid	within	depletion & augmentation	depletion vs. augment.	- faster response times - improvements in working memory - improvements in processing sad affect
				augment. vs. depletion	- reductions in affective processing - reductions in verbal working memory - decreased motor coordination - enhanced vigilant attention
Markus et al., 1998 *n* = 48 (13 M)	diet	mixed	depletion & augmentation	augment.	- slower response times
Lieberman et al., 2002 *n* = 143 M	diet	between	augmentation & balance	augment.	- improvements of vigilance & attention - lower scores on confusion ratings
Fischer et al., 2002 *n* = 15 M	diet	within	augmentation, depletion & balanced	depletion & balanced	- improvements in STM
				augment.	- transient improvements in attention and response times

Smith et al., 1988 *n* = 11 (5 M)	diet	within	augmentation & depletion	augment.	- slower response times
Spring et al., 1983 *n* = 184 (129 M)	diet	between	augmentation & depletion	augment.	- greater feeling of sleepiness in women - greater feeling of calmness in men - poor performance on dichotic shadowing task (at lunch only)
				depletion	- greater accuracy on dichotic shadowing task - greater feeling of tenseness and reduction in feelings of calmness in older adults (at breakfast only)
Kaplan et al., 2001 *n* = 22 (11 M)	diet	within	augmentation, depletion, balanced & fat	augment.	- improved delayed paragraph recall - improvements in Trail Making Test
				depletion	- improved delayed paragraph recall - greater immediate paragraph recall
				balanced	- no effect
				fat	- improved delayed paragraph recall improved attention

[a] = no gender information provided

Purpose and Hypotheses of the Present Study

The purpose of this study is to examine the effects of tryptophan on neuropsychological performance. Thus, the following hypotheses have been made:

Hypothesis 1: Depletion will result in lower scores on measures of LTM;

Hypothesis 2: Depletion will result in lower mood scores;

Hypothesis 3: Augmentation will lead to lower scores on measures of working memory;

Hypothesis 4: Augmentation will result in functional improvements within the areas of attention and vigilance;

Hypothesis 5: The balanced group will score higher than the depletion group on verbal LTM and on mood indices, and higher than the augmentation group on verbal working memory;

Hypothesis 6: The balanced group will score lower than those in the augmentation group on tests of attention and vigilance;

Exploratory analyses will also be conducted in order to examine any group differences that may arise on items comprising the composite attention and working memory scores, as well as any differences on the individual items of the PANAS.

The benefits of employing a dietary manipulation outweigh those of the amino acid protocol for several reasons. First, it is easy to attain the necessary materials – they are all readily available at any local grocery store. Second, it is affordable for most researchers and requires no special equipment or access to resources. With the amino acid protocol, each individual amino acid powder must be purchased separately and then mixed in the appropriate amounts. Using the dietary method, foods can be purchased

fresh, and possibly even in bulk, and stored in a refrigerator until needed. Third, it is better tolerated by participants, with lower chances of experiencing ill-effects (e.g., nausea, vomiting, etc.). Fourth, it is less aversive (Vered et al., 2001) given that participants are taking part in an activity (eating food) not typically associated with stress and anxiety. Lastly, it has potentially important implications for improving transient or occasional cognitive, physiological, or affective problems, including furthering our understanding of "emotional eating".

Unlike the present study, the majority of research within the literature does not include an augmented, a depleted *and* a balanced meal condition (e.g., Coull et al., 1995; Park et al., 1994; Rogers et al., 1999). Although Fischer and colleagues (2002) similarly employed all three conditions in the form of "cream-like" meals, all 15 of their participants were male. As the present study included both males and females, it will enable us to better tease apart the effects of tryptophan on neuropsychological performance for both males and females. What is more, many studies within the literature (e.g., Fischer et al., 2002; Rogers et al., 1999; Smith et al., 1988) do not include a comprehensive neuropsychological battery comprised of tests and measurements that are widely used within clinical settings; the present study does include such a battery, allowing for greater ecological validity. The present study also included the administration of commonly consumed foods, as opposed to the potent and potentially aversive amino acid mixtures used by some researchers (e.g., Riedel et al., 1999; Schmitt et al., 2000), allowing for further ecological validity. Lastly, although the majority of the research within the literature has employed within-subjects designs (e.g., Hughes et al., 2003), many have noted high attrition rates due to repeated testing sessions or reactions

to the amino acid protocol (e.g., 26% attrition rate in Riedel et al., 1999). Further, although a within-subjects design allows for comparison within the same participant across all three conditions, thereby eradicating such between-subject confounds as individual differences, it does introduce the possibility of such confounds as practice effects due to repeated testing, test expectancy, order effects (of test condition), fatigue and attrition, as mentioned previously. By employing a mixed design with between-subjects test variables, the present study avoided those pitfalls, thereby enabling the collection of data from a larger sample with a lower attrition rate and fewer confounds due to repeated testing.

The benefits and strengths of employing a dietary manipulation to investigate the effects of tryptophan manipulation on neuropsychological performance will help elucidate the role played by tryptophan on cognitive functioning.

Method

Participants

Participants were recruited in two ways. The primary method involved recruiting undergraduate students enrolled in a psychology course at the University of Windsor who had signed up for the online participant pool. These students received course credit in exchange for taking part in this study. A list of students who had signed up for the pool and who met our criteria for Body Mass Index (BMI) range (greater than 18.5, the lower cut-off for ‘underweight’, and less than 29.9, the upper cut-off for ‘overweight’) was emailed to the researcher. Those students were then sent an email advertisement for the study (see Appendix B for Study advertisement). This advertisement explained that in

order to take part in the testing session, participants must first complete an in-person screening interview, after which time they may elect to sign up for the testing session if they are eligible to do so. The screening session of the study was worth a total of 0.5 course credits and the testing session was worth a maximum of 5.5 bonus points, provided the participant completed the entire session. Those who were interested contacted the researcher and an appointment was set up to take part in the screener (see Appendix C for study screener). The secondary method of recruitment was comprised of posters which were placed throughout the campus community inviting students from other faculties to take part in the screener, with the option of signing up for the study should they be eligible (see Appendix D for poster advertisement). Those who were interested in taking part contacted the researcher and in return were sent the email advertisement of the study. If upon reading the email advertisement they were still interested in participating, an appointment was made to take part in the screening session. Of note, these participants were not eligible to earn bonus points towards their course(s). Thus, this was a two-part study consisting of a screening session (lasting approximately half an hour) and a testing session (lasting approximately 5.5 hours). All participants who signed up for and successfully completed the testing session, regardless of the recruitment method, were given the option of having their name entered into a draw to win 1 of 2 gift cards each valued at $50 CAD to either Devonshire Mall or Future Shop (the winners had the option of choosing their reward). Ethics approval was gained by the University of Windsor Research Ethics Board and informed consent was obtained from all participants before completing both the screener and the testing session (see Appendix E for the test screener consent form and Appendix F for the testing session consent form).

A total of 103 participants were screened for inclusion in this study, of which 82 were eligible to take part in the testing session. Of those deemed eligible, 73 participants successfully completed the testing session. Participants were split across conditions in the following manner: 25 in the Augmented group (17 female, 8 male), 25 in the Balanced group (17 female, 8 male), and 23 in the Depleted group (16 female, 7 male) (see Procedures for details on group assignment). All participants in this study regularly ate breakfast and lunch, were not colour blind, and had no motor problems. None of the females were pregnant or lactating. Complete demographic information for each group can been seen in Table 2.

Design

This study employed a double-blind, mixed design. Pre- and post-test measures of working memory (the Digit Span – backward trial) and affect (PANAS; Watson, Clark & Tellegen, 1988) served as within-subject variables to assess the success of the tryptophan manipulation (depletion, augmentation or balanced). All other test measures were between-subject variables. A maximum of 30 days separated the screening and testing sessions.

Procedures

Screening Session. All participants who signed up for the screen came into the laboratory and, after signing an informed consent form, completed an initial screening interview with the author (see Appendix C). During this interview, participants were assessed for suitability for inclusion in the study according to the following criteria: absence of food allergies (especially peanuts); confirmation of a body mass index (BMI)

Table 2

Demographic Information for Each Condition

Variable		Augmented ($N = 25$)	Balanced ($N = 25$)	Depleted ($N = 23$)
Age *M* (*SD*)		21.6 (3.1)	22.6 (3.38)	21.3 (2.51)
Females		21.4 (2.92)	22.5 (3.13)	20.6 (2.00)
Males		22.1 (3.72)	22.9 (4.09)	23.0 (2.89)
Gender *N* (%)				
Female		17 (68)	17 (68)	16 (69.6)
Male		8 (32)	8 (32)	7 (30.4)
Screeners *N*	103			
Ineligible	17			
Declined Test Session	3			
No Show	1			
Completed/Eligible	82			
Testers *N*	82			
No Shows	3			
Cancellations	4			
Administration Errors	2			
Final Sample	73			
ESL *N* (%)				
Yes		7 (28)	9 (36)	8 (34.8)
No		18 (72)	16 (64)	15 (65.2)

Education *M* (*SD*)	15.0 (2.5)	15.4 (2.36)	15.11 (2.22)
Female	14.9 (2.64)	15.7 (2.40)	14.4 (1.89)
Male	15.3 (2.31)	14.69 (2.27)	16.6 (2.29)
BMI *M* (*SD*)	22.4 (2.57)	22.56 (2.88)	22.70 (3.02)
Female	22.0 (2.48)	22.0 (2.95)	21.8 (2.69)
Male	23.4 (2.64)	23.75 (2.48)	24.6 (2.98)
Weight (lbs) *M* (*SD*)			
Female	126.7 (13.9)	136.88 (17.5)	131.56 (14.00)
Male	167.4 (28.9)	165.88 (21.4)	167.29 (11.69)
Physical Activity Level (weekly) *N*			
Female			
Not at all	1	1	0
Minimal (walking to school)	12	8	1
Quite (3-5 times/week)	4	6	14
Very (6+ times/week)	0	2	1
Male			
Not at all	0	0	0
Minimal (walking to school)	2	5	1
Quite (3-5 times/week)	5	2	5
Very (6+ times/week)	1	1	1
Frequency of Food Consumption *N*			
Female			
Fast (0-2 hours)	5	2	4

Average (3-5 hours)	11	14	12
Slow (6+ hours)	1	1	0
Male			
Fast (0-2 hours)	2	0	1
Average (3-5 hours)	4	6	5
Slow (6+ hours)	2	2	1
Beck Depression Inventory – II			
(BDI - II) Score *M* (*SD*)			
Female	3.24 (4.06)	6.12 (5.12)	4.63 (3.48)
Male	5.38 (4.31)	5.00 (4.66)	8.14 (4.67)
History of Depression *N*			
Female	1	0	0
Male	0	0	0
History of Anxiety *N*			
Female	0	0	0
Male	0	0	0
Family History of Depression *N*			
Female	6	4	2
Male	2	1	0
Smoker *N*			
Female	1	1	0
Male	1	1	2
Birth control use (female) *N*	10	5	8

Hours of sleep/night *M* (*SD*)			
Female	6.88 (1.38)	7.79 (1.03)	7.50 (1.26)
Male	7.06 (1.05)	7.56 (1.08)	6.86 (1.11)
Amount of caffeine (daily) *N*			
Female			
Low (0-1 drinks/day)	13	14	14
Medium (2-3 drinks/day)	4	2	2
High (4 drinks/day)	0	1	0
Male			
Low (0-1 drinks/day)	5	5	4
Medium (2-3 drinks/day)	2	2	3
High (4 drinks/day)	1	1	0

between the values of 18.5 and 29.9; absence of chronic or current physical illness, including gastrointestinal disorders such as Crohn's disease, irritable bowel syndrome, or lactose intolerance as these conditions have been shown to impede absorption of nutrients (Murray, 1998); no neurological, endocrine or metabolic disorders; no current medication use (including SSRIs, MAOIs, narcotics, anti-psychotics, anxiolytics, or stimulants); no irregular diets (i.e., no 'fad' diets or skipping meals) or eating disorders; and no current episode of acute anxiety or depression. The Beck Depression Inventory – II (BDI-II; Beck, Steer, & Brown, 1996) was administered to measure current levels of depression. If a participant had a BDI score of 30 or greater (indicating severe depression), follow-up questions were asked as to the nature and severity of symptoms, including the likelihood of them hurting themselves or others. Any participant who scored in this range would have been provided with appropriate referrals to the Windsor Regional Hospital Mood and Anxiety Clinic, the Windsor Mood Disorders Self-Help Group, or the Student Counseling Centre on campus; this information was also listed on the consent forms, of which participants kept a copy. If anyone was currently suicidal or thinking about committing suicide, they would have been referred to the Community Crisis Centre (there is a telephone in the laboratory available for such use). Of note, no participant obtained a score of 30 or greater, and as a precaution, the author pointed out the referral information to any participant who obtained a score of 10 or greater. Furthermore, suicidal ideation was not endorsed by any participant. History of anxiety or depression, family history of mental illness, and diagnoses of diabetes were also documented, as was the typical amount of caffeine, alcohol and tobacco consumed daily, and the average number of hours of sleep per night. Information was also obtained about the participant's level of

physical activity, frequency of food consumption, type and frequency of food cravings, and use of oral birth control (for women).

At this point, if a participant was deemed eligible to continue after the screen, the participant was given the option of signing up for the testing session. To take part in the testing session, participants must have agreed to fast 12 hours prior to initiation of testing. They were informed that on the day of testing, they would be provided with breakfast, lunch and two snacks. They were also informed that the session would begin at 9 am and would last approximately 5 hours, although the actual testing would only take place during the last 1.5 hours of this 5 hour period. Participants were told that in the time between breakfast and the commencement of testing, they would be required to remain in the common waiting room (they were allowed to study, read, listen to music, and/or entertain themselves with their laptops; sleeping was not allowed). Lastly, participants were shown a list of foods they may be asked to eat when they come in to take part in the study (this allowed for the screening and confirmation of food allergies and/or sensitivities). If the participant agreed to these conditions, an appointment was made for the participant to come back another day to take part in the study. Each participant was instructed to abstain from alcohol consumption the day before testing, and to get a good night's sleep (ranging from 7-10 hours, or whatever is considered average for each particular person) the night prior to coming in. Every effort was made to accommodate the participant's school schedule (i.e., essay due dates and exams) when booking the testing session. Each participant thus came to the laboratory once for a screening session and once for the testing session. The author was responsible for conducting the screening sessions with participants, as well as for the quasi-randomization of participants into each

of the three meal conditions (augmented, balanced, or depleted tryptophan), matched for BMI and gender. In order to achieve quasi-randomization, BMI scores were split into lower (18.5-21.5), middle (22-25.5) and higher (26-29.9) scores for both males and females, with the aim of recruiting an equal number of males and females from each range of scores. In other words, the aim was to obtain an equal number of lower-male, lower-female, middle-male, middle -female, higher-male and higher-female participants for inclusion in this study. For example, if the first participant was a middle-male, he might randomly have been assigned to the Augmentation condition. Thus, the next middle-male would have been randomly assigned to either the Depletion or the Balanced condition. The third middle-male will thus be assigned to whichever condition is still empty for that gender and BMI range. The same process was repeated for female participants. Within each gender, an equal number of participants from each BMI range were assigned to each meal condition. As condition assignment was completed by the author and not by the research assistants who administered the neuropsychological test battery, the maintenance of a double-blind research design was thus ensured. A total of 103 participants were screened for this study, 82 of whom met eligibility criteria for inclusion in the testing session.

The meals for each participant were put together the night before each testing day by the author and labeled according to a master menu key (known only to the author), thereby ensuring that the research assistants administering the test battery were blind to the condition to which participants had been assigned. On the day of testing, the author provided participants with their meals and snacks, as well as with fresh copies of the PANAS at the designated times. The author was also responsible for ensuring that

participants completed their entire meal, and for removing all food trash from the waiting room (to ensure that the research assistants could not inadvertently deduce to which condition participants were assigned). The author informed participants that they were not to divulge any information to the research assistant with whom they were paired regarding the foods that were consumed. Participants were run either one or two at a time, depending on recruitment and availability of the research assistants. Although each participant worked with a separate research assistant once the testing started at 12:30 pm, participants were together in the common waiting room during the time between breakfast and the commencement of the testing (i.e., from 9 am until 12:30 pm). The author was responsible for checking in with participants during this wait period. A total of three research assistants were utilized in this study.

Testing Session. On the testing day, participants arrived at the laboratory by 8:45 am. Once they had signed the informed consent, they were quickly surveyed to ensure they had met with the previously discussed pre-conditions for taking part in the test session (namely: fasted for 12 hours, avoidance of alcohol and drugs the previous day, a minimum of 6 hours sleep and no caffeine since the previous day). This brief screen was conducted with each participant, one-on-one, by the author (on days when two participants were being run concurrently, a research assistant would conduct this brief screen with one of the participants). Upon confirmation that these conditions were met, the participant was administered a brief measure of attention (the Digit Span) and was then brought into the waiting room. Once in the waiting room, the participant filled out the first PANAS while their breakfast was prepared and served at 9 am. Immediately after breakfast and again after the first snack, which is provided at 10:15 am, each

participant filled out a PANAS. Lunch was provided at 11 am, after which the PANAS was completed for a fourth time. Given that research has shown that several hours are required for plasma and free tryptophan levels to reach peak depletion (e.g., Benkelfat et al., 1994), all participants completed the neuropsychological battery at 12:30 pm, 1.5 hours after the provision of lunch (3.5 hours after the first meal was consumed). This is in line with Markus's methodology. The fifth PANAS was completed just prior to the administration of the test battery. The test battery took less than 1.5 hours to complete, after which time participants completed the sixth and final PANAS, followed by the second and final administration of the Digit Span. Taken together, the PANAS and the Digit Span (backward condition) served as within-subject indicators of the effectiveness of the tryptophan manipulation, enabling pre- and post-test comparisons in affect and cognitive performance. At this point, all participants were provided with the option of a final snack to replete tryptophan levels and to help offset any possible reactions to tryptophan augmentation or depletion (see Table 3 for schedule of events). Water was available *ad libitum.* Of the 82 participants deemed eligible to take part in this study, 3 failed to show up on the day of testing, 4 cancelled their testing session, and 2 administration errors resulted in a total of 73 participants successfully completing the testing session. Participants were split across conditions in the following manner: 25 in the Augmented group (17 female, 8 male), 25 in the Balanced group (17 female, 8 male), and 23 in the Depleted group (16 female, 7 male).

Table 3

Schedule of Events

Time	Activity	Administered by
8:45 am	Arrive at lab, consent, pre-screen	Author
8:50 am	Complete pre-test	Author
8:55 am	Complete PANAS	Author
9:00 am	Breakfast is served	Author
9:20 am	PANAS is completed	Author
10:15 am	Snack is served	Author
10:30 am	PANAS is completed	Author
11:00 am	Lunch is served	Author
11:20 am	PANAS is completed	Author
12:25 pm	PANAS is completed	Author
12:30 pm	Neuropsychological test battery begins	RA
2:00 pm	Final PANAS is completed once neuropsychological test battery has finished	Author
2:05 pm	Final snack is served	Author

Dietary Manipulations

The meals created for each of the three conditions were modeled after the diets provided by Markus and colleagues (1998) (see Appendix G for composition of meals and snacks). As Markus's group did not provide the exact amount of each food provided to participants, instead citing only the total amount of carbohydrates, protein, fat and calorie intake in each meal, all meals for this study were created and analyzed by the author using the NutritionData website (NutritionData, 2009). This website enables the user to input the amount and type of food and creates a recipe label listing the total grams of carbohydrates, protein, fat, calories, and other nutritional facts about the foods contained therein. In this way, it was possible to replicate the menus created by Markus's group so as to obtain the same carbohydrate:protein ratios (see Table 4 for macronutrient content of each condition). Thus, the ratios employed in the present study are in keeping with those employed by Markus such that the augmentation meal in the present study contained 18.2 times more carbohydrates than proteins, and the depletion meal in the present study contained 1.47 times more carbohydrates than proteins. As Markus' group did not employ a balanced meal, the balanced meal in the present study was devised to have a carbohydrate:protein ratio between those used in the augmentation and depletion meals. Thus, the balanced meal has 8.37 times more carbohydrates than proteins. The final snack, composed of a pineapple cup and a yogurt cup, was chosen as these foods are known natural sources of tryptophan (Murray, 1998; Schmitt et al., 2000).

Table 4

Macronutrient Content of Each Condition

	Meal Type		
	Augmented	Depleted	Balanced
Carbohydrates			
Total grams	200	57	174
% of meal	67	19	58
% RDA	67	19	58
Protein			
Total grams	11	39	21
% of meal	15	45	2
% RDA	22	78	42
Fat			
Total grams	11	23	26
% of meal	18	36	40
% RDA	17	35	0.4
Calories			
Total grams	931	881	988

Note: Macronutrient content of these meals based on a 2,000 calorie diet. The Recommended Daily Allowance (RDA) for each macronutrient, based on a 2,000 calorie diet for males and females aged 4 and over as indicated by the Canada Food Inspection Agency ("Chapter 6", n.d.), is as follows: Carbohydrates = 300 g; Protein = 50 g; Fat = 65 g. All meals were modeled after those used by Markus and colleagues (1998) and were created and analyzed using the NutritionData website (NutritionData, 2009).

Neuropsychological tests administered

The following is a list of the neuropsychological test measures that were utilized in this study, organized by test domain. A brief description of each test is provided, along with the scoring criteria that were employed. The interested reader is directed to Lezak et al., (2004) for further detail regarding these measures.

Executive Functioning

Verbal Fluency ('F', 'A', 'S' + Animals; Benton, 1968)

The FAS is a measure of verbal fluency that assesses language generation and executive functions. Participants were instructed to list as many different words as possible in one minute that start with a specific letter while abiding by the following rules: no names or proper nouns (e.g., Brenda or Baltimore), no numbers, and no variants of a word (e.g., bank, banks, banked). For each of the three letters, participants were allotted one minute to respond. The total score was the total number of correct words (i.e., non-perseverative words that did not break any of the rules) for all three letters, combined into a single score. On the semantic fluency task, participants were instructed to name as many different animals as they could in one minute (e.g., mammals, reptiles, birds, insects, etc.). The total score on the semantic trial was the total number of real animals produced in one minute that were not perseverative. In both the phonemic and semantic conditions, rule breaks were coded as responses that were a number, a name or proper noun, or that constituted a variation of a previously stated word (e.g., paint, paints); perseverative responses were coded when a word was repeated more than once. Test-retest reliability for the FAS is quite high, over r = .70 for both the phonemic and the semantic trials (Basso, Bornstein, & Lang, 1999; Harrison, Buxton, Husain, & Wise,

2000). With regards to validity, the FAS is highly correlated with other measures of verbal fluency (e.g., the Controlled Oral Word Association Task, which uses the letters 'C', 'F', 'L'), ranging from .85 to .94 across varying populations and settings, including healthy controls (M. J. Cohen & Stanczak, 2000; Lacy, Gore, Pliskin & Henry, 1996).

Stroop Color-Word Test (SCWT; Golden, 1978; Stroop, 1935)

Although it is often utilized as a measure of selective attention, the Stroop is considered a measure of cognitive control as it requires the inhibition of pre-potent (i.e., automatic) responses, forcing participants to ignore task-irrelevant stimuli. The version of the Stroop employed in this study (Golden, 1978) consisted of three separate trials: word reading (black ink); colour naming (red, blue and green 'X's), and the colour-word reading interference task (e.g., the word "red" written in blue ink). On this latter task, participants were required to ignore the typed word and to name the colour of the ink in which the words were typed. Participants were allotted 45 seconds for each of the three trials. Scores were calculated for each individual trial, producing a Word Score, a Color Score and a Color-Word Score. An overall interference score was also derived by subtracting the participant's age and education predicted color-word score from the actual color-word score achieve by the participant. The interference score indicates the degree to which participants are able to inhibit pre-potent responses. Test-retest reliability has been reported at $r = .86$ for the Word trial, at $r = .82$ for the Color trial, and at $r = .73$ for the Color-Word trial (Golden, 1975). Chafetz and Matthew (2004) report moderate to high validity, and there is a moderate correlation between the Interference score and other measures of prepotent response inhibition (e.g., time of the stop-signal task, $r = .56$; May

& Hasher, 1998) and attention (r = .31 with errors of omission on continuous performance tasks; Weinstein, Silverstein, Nader & Turnbull, 1999).

Emotional Stroop (paper-and-pencil)

The emotional Stroop employed in this study followed the same principles as the Stroop Color Word Test, with the exception that the words listed in the latter two trials were emotionally salient. Modeled after the 1978 Golden version of the Stroop, four trials were administered: reading of neutral words (e.g., hat, swim, book); colour naming of neutral words; colour naming of negative words (e.g., disaster, failure, pathetic); and colour naming of positive words (e.g., happy, smile, clean). Each trial contained 100 words. For the latter three trials, participants were required to name the colour of the ink in which the words were printed. A total of 45 seconds was allotted for each trial. Scores were calculated for each individual trial, producing a Word Score, a Color Score and a Color-Positive Word Score, and a Color-Negative Word Score. An overall total interference score was also derived by subtracting the participant's combined score for the Positive and the Negative Word Scores from the combined Word and Color Score.

In creating the emotional Stroop test used in this study, a review of the literature provided information pertaining to the appropriate word parameters to employ. Larsen, Mercer, and Balota (2006) analyzed the lexical characteristics of words used in emotional stroop experiments. From the studies they analyzed that utilized negative, positive, and neutral conditions, we chose to use the neutral and positive word lists from Compton, Heller, Banich, Palmieri, and Miller (2000). Since these lists contained 12 words, we eliminated two words in order to fit our format (i.e., 10 positive, 10 negative and 10 neutral words). Since the negative word list from Compton's study included words of a

threatening nature, as opposed to a purely negative emotional nature, we created our own negative word list by utilizing the online MRC Psycholinguistic Database (http://www.psy.uwa.edu.au/mrcdatabase/uwa_mrc.htm) (Coltheart, 1981). We limited our word search to match the lists employed by Compton and Larsen's groups in terms of word length, number of syllables, concreteness, imageability, and familiarity. All words were one syllable and 3-6 letters long. The concreteness, imageability, and familiarity ratings for the positive word list used in Compton's study were 359, 435, and 609, respectively, whereas the ratings for the neutral word list were 373, 442, and 624, respectively. Limits in the Psycholinguistic database were set at 100 higher and lower than these ratings. The concreteness, imageability, and familiarity ratings for the negative word list created for this study were 358, 436, and 540, respectively. These ratings were similar to those of the neutral and positive lists used by Compton's group.

Attention/Concentration

Digit Span (DS; from the Wechsler Adult Intelligence Scale –Third Edition *(WAIS-III); Wechsler, 1997)*

The Digit Span is a measure of auditory attention span and working memory. In this task, participants were required to listen to and repeat an aurally presented string of numbers that increased in length with each successive trial. The task was discontinued following errors on both digit strings within a trial. The task was then completed in a backward condition wherein participants were required to repeat a string of aurally presented numbers backwards (i.e., in the reverse sequence as was presented). The backwards condition, which taps into the domain of working memory, is thus the harder of the two conditions. Discontinuation on the backward condition occurred when both

digit strings within a trial were incorrect. The total number of digits correctly recalled in both the forward and the backwards trials were calculated by simple summation of correct responses, resulting in a total forward score, a total backward score, and an overall total score. Scores range from 0-16 on the forward condition, and from 0-14 on the backward condition. Scores on the backward condition were employed as pre- and post-test measures of the tryptophan manipulation as there is evidence in the literature that this task is sensitive enough to differentiate between tryptophan augmentation and deletion (e.g., Luciana et al., 2001). The reliability of the Digit Span subset is high (r = .90). Digit Span correlates the best with the Letter-Number Sequencing subtest from the WAIS-III (r = .57), and demonstrates a moderate correlation with the Verbal Scale (r = .51) (Sattler, 2001).

Letter-Number Sequencing (from WAIS-III; Wechsler, 1997)

Letter-number Sequencing is a measure of working memory and attention that involves the aural presentation of complex auditory material (letters and numbers) that the participant must mentally manipulate and reproduce according to specific criteria (i.e., letters in alphabetical order, followed by numbers in numerical order). There is no time limit on this task, and discontinuation occurred when all three trials of an item are incorrect. The total number of correct trials was calculated. The reliability of the Letter-Number Sequencing subtest is high (r = .82), demonstrating a moderate correlation with the Verbal Scale of the WAIS-III (r = .62). Of the other subtests comprising the WAIS-III, Letter-Number Sequencing best correlates with Digit Span (r = .57) (Sattler, 2001).

Digit Symbol – Coding (from WAIS-III; Wechsler, 1997)

Digit Symbol – Coding is a task of visual attention and working memory. In this task, participants were required to re-code a series of symbols using the key-code provided at the top of the page. This is a timed task, and discontinuation occurred after two minutes had lapsed. The total number of correctly recoded symbols served as the score for this task. The Digit Symbol – Coding subtest of the WAIS-III is reliable (r = .84) and demonstrates a moderate correlation with the Performance Scale (r = .50) (Sattler, 2001).

Ruff 2 & 7 Selective Attention Test (Ruff & Allen, 1996)

The Ruff 2 and 7 Selective Attention Test is a selective visual cancellation task that assesses the difference between automatic and controlled visual search (i.e., processing speed). In this task, participants were presented with 20 blocks of stimuli, each of which consisted of either three rows of letters (automatic search) or three rows of numbers (controlled search). Participants were instructed to cross off (with a pen) all of the 2's and 7's that they could within a 15 second block of time. At the end of each 15 second period of time, participants were instructed to stop and to continue with the next block of stimuli. This task takes a total of 5 minutes to complete and is scored according to both speed and accuracy of automatic and controlled search. Thus, for the letter trials, the following scores were derived: Automatic Detection Speed (i.e., total number of correct hits), Automatic Detection Errors (i.e., the total number of errors) and Automatic Detection Accuracy scores (calculated in the following manner: Automatic Detection Speed divided by the sum of the Automatic Detection Speed score plus the Automatic Detection Errors score, multiplied by one hundred). For the digit trials, scores were

derived for Controlled Search Speed (i.e., total number of correct hits), Controlled Search Errors (i.e., the total number of errors), and Controlled Search Accuracy (i.e., Controlled Speed score divided by the sum of Controlled Search Speed score plus Controlled Search Errors, multiplied by one hundred). Total Speed and Total Accuracy scores, as well as Speed Differences, Accuracy Differences, and Total Difference were also calculated by adding or subtracting speed and accuracy scores, respectively. Ruff and Allen (1996) report that Speed scores yield higher test-retest reliability than do Accuracy scores; Lemay, Bedard, Rouleau, and Tremblay (2004) found a .85 correlation between Speed scores. Although content validity is mainly based on theoretical grounds (Strauss, Sherman, and Spreen, 2006), it has been reported that Digit Symbol, which also assesses processing speed, is the test most highly correlated with the Ruff 2 & 7 (r = .35-.40) and in normal samples it is not correlated with other tests designed to measure attention (e.g., Digit Span, Stroop; Ruff & Allen, 1996).

Trail Making Test (Trails A & B; Reitan, 1958)

The Trail Making Test is a speeded visual attention task involving simple sequencing of numbers. On the first trial (Trails A), participants were instructed to connect the numbers on the page as quickly as they could without taking their pen off of the page. The second trial (Trails B) was essentially the same task save for the fact that it entails alternating between letters and numbers, making it the more complex of the two tasks. This is a timed test requiring participants to connect the circles of letters (or letters and numbers) as quickly as possible. The total time required for each condition served as the score on each trial. Dikmen, Heaton, Grant, and Temkin (1999) report reliability for both Trails A (.79) and Trails B (.89) as adequate to high, respectively. There is a modest

correlation between Trails A and B (r = .31-6), a range that is thought to reflect the greater cognitive demand of the latter as opposed to the former trial (Heilbronner, Henry, Buck, Adams, & Fogle, 1991).

Memory

California Verbal Learning Test - II (CVLT-II; Delis, Kramer, Kaplan, & Ober, 2000)

The CVLT-II is an aurally presented list learning task requiring the participant to learn and recall verbal information immediately after presentation. There are five learning trials, in which the list is repeated each time after which the participant must reproduce as many words from the list as possible. An interference trial was administered, followed by Short-Delay Free Recall and Short-Delay Cued Recall trials. After a 20 minute delay, Long-Delay Free Recall, Long-Delay Cued Recall, and Long-Delay Recognition trials were administered. Thus, scores were calculated for: Trials 1-5 Recall Total Correct; List B Free Recall Correct; Short-Delay Free Recall; Short-Delay Cued Recall; Long-Delay Free Recall; Long-Delay Cued Recall; Free Recall Intrusions; Cued-Recall Intrusions; Total Intrusions; Total Repetitions; Long-Delay Recognition; Long-Delay Recognition False Positives; and Long-Delay Forced Choice Recognition Accuracy. According to the test manual, reliability is high (r = .80-.89) for Trials 1-5 Recall Total Correct, Short- and Long-Delayed Free Recall, and Total Recognition. The test manual also reports validity scores ranging from .60-.69 for Free-Recall Intrusions to .80-.89 for Long-Delayed Recognition False Positives.

Rey-Osterrieth Complex Figure Test (R-OCFT; Rey, 1941; Osterrieth, 1944)

The R-OCFT measures a participant's ability to accurately copy a complex figure onto a sheet of paper. Successful completion requires that participants be able to accurately perceive and synthesize the numerous components of the figure. After the copy trial, immediate (3 minute delay) and delayed (25-35 minute delay) recall trials were administered to assess the integration, memory and synthesis of the figure. Participants also completed a recognition trial to help determine whether performance was affected by difficulties with perception, synthesis, or retrieval of complex visual information. Thus, scores were calculated for Immediate Recall, Delayed Recall, Recognition Total Correct, Copy, and Time to Copy, as well as for Recognition True Positives, Recognition False Positives, Recognition True Negatives and Recognition False Negatives. Meyers and Meyers (1995) report reliability scores of $r = .76$ for Immediate Recall, of $r = .89$ for Delayed Recall, and of $r = .87$ for Recognition Total Correct. According to Strauss and colleagues (2006), the *R-OCFT* is a valid measure of visual-construction (via the copy trial) and memory (recall and recognition trials).

Mood/Affect

Positive and Negative Affect Scale (PANAS; Watson, Clark, & Tellegen, 1988)

The PANAS is a self-report inventory comprised of both a negative affect and a positive affect subscale. Each scale consists of 10 items, and participants were required to rate (on a 5-point scale) how well each descriptor described them at that particular moment. Examples of negative descriptors are irritable, distressed, guilty, or scared; positive descriptors include such items as determined, strong, enthusiastic, or proud. Watson (1988) reported correlations of between -. 12 to -.26 for the PANAS scales,

indicating that the negative affect and the positive affect scales are independent of one another. With regards to validity, Watson (1988) stated that "[a]ll of the scales are reasonably convergent with other measures of the same factor, and one must conclude [that the NA and PA] scales have comparable correlates and lead to similar conclusions" (p. 138). Discriminant validity between the NA and PA scales has also been reported, with correlations in the range of r = -.48 (Warr, et al., 1983) to r = .54 (Watson, 1988).

Beck Depression Inventory-II (BDI-II; Beck, Steer, & Brown, 1996)

The BDI-II is a self-report inventory consisting of 21 items, each of which is scored on a four-point scale. Indecisiveness, Mood, and Sense of Failure are examples of items on the scale, each of which pertains to a particular symptom of depression. The scores for each item were summed together to arrive at a total score; lower scores translate into lesser degrees of depression. A score of 30 or greater indicates severe depression. If a participant scored in this range, follow up questions were asked by the researcher as to the nature and severity of symptoms, including the likelihood of the participant hurting him or herself or others. No participants scored in this range. As a precaution, all participants who scored 10 or above were provided with referrals to the appropriate services, all of which have their contact information listed on the consent form signed by the participant. If anyone had of endorsed the suicide item, they would have been walked over to the Student Counseling Centre at the CAW by the researcher. No one endorsed this item. The BDI-II was only administered as part of the screening procedures in order to ensure no current depression; it was not administered during the testing session.

Results

Prior to analyses, all variables were examined for accuracy of data, missing values, normality and outliers. Of the 73 participants who completed this study, five participants failed to complete one of the six PANAS forms and thus these participants with missing data were excluded from analyses relevant to the PANAS; all other analyses contained data from all 73 participants. Data was screened for multivariate outliers using Mahalanobis's distances, and none were found. All assumptions of analyses of variance (ANOVA), multivariate analyses of variance (MANOVA) and repeated measures were met (or corrected for). Raw test scores were used in all analyses, and data was analyzed using SPSS for Windows Version 17 (SPSS Inc., Chicago, IL, USA).

A correlation matrix was conducted in order to examine the relationship between the background variables (e.g., age, education, physical activity level, BDI score, etc.) and all cognitive and affective measures. Results of this analysis can be seen in Table 5.

As can be seen from Table 5, several key measures did not correlate with any background variables (e.g., CVLT Long Delay Recall, Rey-O Delayed Recall); every background variable correlated with at least one test measure.

As we were unable to recruit equivalent numbers of males and females, it was unclear whether there were any gender differences on the background variables of English as a Second Language (ESL), eating frequency[1], level of physical activity[2] or BMI. This was an important consideration as many researchers within the field have collected data only

[1] 'Eating frequency' is the term used in this document to refer to the average number of hours between eating each meal or snack as reported by the participant during the screening session.

[2] Levels of physical activity was measured via self-report by each participant during the screener. A 4-point Likert scale was utilized, enabling participants to report their levels of physical activity during a typical week as either "Not at all" (i.e., no exercise of any kind), "Minimal" (e.g., walking to school), "Quite" (physical activity 3-5 times per week), or "Very" (physical activity 6 or more times during the week). See Appendix C for more detail.

Table 5

Correlations Between Background Variables and Tests Comprising Predictions and Composite Scores[a].

Test	Age	Educ[b].	BMI[c]	Weight (lbs)	Height (in.)	Eating Freq.[d]	Sleep (hrs)	BDI[e]	Physical Activity[f]
1. CVLT-II[g] Long Delay Recall	.22	.28	.18	.12	-.03	-.10	-.09	-.17	-.04
2. Rey-O Delayed Recall	.12	.20	.21	.10	-.07	.18	-.16	-.03	-.22
3. Stroop Interference	.09	.09	-.14	-.11	.01	-.06	.10	.00	-.20
4. Trails A	-.25*	-.20	-.28*	-.33**	-.19	.16	.25*	-.05	.16
5. Trails B	-.13	-.06	-.10	-.20	-.16	.07	.14	.07	.13
6. Ruff 2 & 7 Total Speed	.25*	.29*	.22	.31**	.23	-.05	-.02	.02	-.20
7. Ruff 2 & 7 Total Accuracy	.12	.15	-.24	-.05	.14	-.12	-.08	-.11	-.40
8. Digit Symbol Coding	.01	.09	-.07	-.08	-.00	-.32**	.15	-.15	-.14
9. Letter Number Sequencing	.01	-.04	-.02	.09	.14	-.23	.00	-.09	-.25
10. EStroop Positive Interference	-.08	-.10	-.08	-.02	.07	-.24*	.15	.08	-.10
11. EStroop Negative Interference	-.06	.04	-.08	-.22	-.21	-.35**	-.10	-.21	-.12
12. Digit Span 2 Total	.30*	.24*	.05	.13	.11	-.02	.07	-.26*	-.34

Test	Age	Educ[b].	BMI[c]	Weight (lbs)	Height (in.)	Eating Freq.[d]	Sleep (hrs)	BDI[e]	Physical Activity[f]
13. FAS[h] Total	.33**	.20	.17	.05	-.05	.05	.05	-.17	-.16
14. Working Memory	.27*	.17	.08	.17	.13	-.13	.05	-.22	-.32
15. Attention	.11	.16	-.18	-.06	.09	-.17	.16	-.17	-.34
16. Positive PANAS[i] 1	.20	.13	.03	.13	.16	.20	-.22	.04	.13
17. Positive PANAS 2	.08	.04	.03	.10	.11	.13	-.24	.05	.07
18. Positive PANAS 3	-.02	-.04	.01	.15	.20	.07	-.21	.10	.11
19. Positive PANAS 4	-.01	.00	-.11	.04	.15	.14	-.30*	.11	.13
20. Positive PANAS 5	.04	.04	.01	.12	.16	.06	-.34**	.16	.13
21. Positive PANAS 6	.04	.50	-.05	.08	.18	.01	-.24	.11	.17
22. Negative PANAS 1	-.14	-.18	.07	.12	.14	.02	-.13	.58**	.20
23. Negative PANAS 2	-.09	-.12	.08	.06	.02	.09	-.09	.51**	.10
24. Negative PANAS 3	-.15	-.17	.16	.08	-.04	.04	-.18	.58**	.20
25. Negative PANAS 4	-.12	-.16	.07	.09	.07	-.03	.03	.58**	.13
26. Negative PANAS 5	-.12	-.19	.11	.08	.02	-.04	-.05	.54**	.15

Test	Age	Educ[b].	BMI[c]	Weight (lbs)	Height (in.)	Eating Freq.[d]	Sleep (hrs)	BDI[e]	Physical Activity[f]
27. Negative PANAS 6	-.08	-.11	.09	.08	.05	.04	-.01	.49**	.06

** $p < .01$, * $p < .05$

[a] Collapsed across gender; [b] Education (years); [c] Body Mass Index; [d] Eating Frequency = average time (hrs) between each meal and/or snack; [e] Beck Depression Inventory - II score; [f] biserial correlation conducted on dichotomized (i.e., 'low' and 'high') variable; [g] California Verbal Learning Test-II; [h] 'F', 'A', 'S' verbal fluency test; [i] Positive And Negative Affect Scale. *Note*. All PANAS correlations excluded participants with missing data ($n = 68$).

on males (e.g., Fischer et al., 2002; Hughes et al. 2003; Lieberman et al., 2002; Park et al., 1994) or females (e.g., Nabb & Benton, 2006). Thus, examining each gender separately allowed us to better compare our findings to those in the literature.

Looking at each gender separately, ESL proportions did not differ for females [$\chi^2(2, N = 50) = .01, p = .994$] or males [$\chi^2(2, N = 23) = 1.10, p = .577$]. With regards to eating frequency, it had been suggested by Craig (1986) that the amount of time between each meal is likely to influence the effect of the manipulation on performance in that those with a faster metabolism may have a different peak level of augmentation or depletion than those with a slower metabolism. For this reason, a chi-square analysis was also conducted on eating frequency, demonstrating no significant group differences for either females [$\chi^2(4, N = 50) = 2.60, p = .627$] or males [$\chi^2(4, N = 23) = 2.63, p = .622$]. The chi-square analysis examining the proportions of physical activity levels across meal types for females, however, violated the assumption of expected frequencies, with more than 20% of the cells having an expected cell count of less than 5, thus rendering this analysis uninterpretable. Examination of the chi-square table illustrated that the lowest (i.e., 'Not at all') and highest (i.e., 'Very') categories of physical activity for each meal condition were the cells with expected frequency counts of less than 5.

This analysis was repeated for the males, with the same result being obtained. The assumption of expected frequencies was violated, as all nine cells had an expected cell count of less than 5, again rendering the analysis uninterpretable. Examination of cell counts in the chi-square table, it is evident that the lowest category (i.e., 'Not at all'), which was not endorsed by any males, and the highest category (i.e., 'Very') of physical

activity for each meal condition were the cells that violated the assumption of expected frequencies most gravely.

Since the values in the 'Not at all' and 'Very' categories for both the females and the males rendered the analyses uninterpretable due to their low levels of endorsement, we collapsed across these more extreme activity levels to instead create two categories of physical activity. The 'low' category was generated by collapsing across the 'Not at all' and the 'Minimal' levels of activity, whereas the 'high' category was created by collapsing across the 'Quite' and the 'Very' levels of activity. By doing so, the chi-square analysis of physical activity levels no longer violated the assumption of expected frequencies for females. Levels of physical activity (using these dichotomized 'low' and 'high' physical activity levels) were significantly disproportionate across meal conditions for females [$\chi^2(2, N = 50) = 16.86, p = .000$]. The Augmented group had a greater proportion of participants within the 'low' level of physical activity ($n = 13$, 76.5%), whereas the Depleted group had a greater proportion of participants within the 'high' category of physical activity ($n = 15$, 93.8%) (see Table 6).

A chi-square analysis examining levels of physical activity (using the dichotomized 'low' and 'high' physical activity levels) was also conducted for the males and demonstrated a violation of the assumption of expected frequencies. Since more than 20% of the cells had an expected cell count of less than 5, the analysis was uninterpretable (see Table 7).

Table 6

Chi-square Results for Proportion and Percent Total of Dichotomized Physical Activity Levels by Meal Type for Females

	Physical Activity Levels	
	Low	High
Meal	Count (%)	Count (%)
Augmented	13 (76.5)	4 (23.5)
Depleted	1 (6.3)	15 (93.8)
Balanced	9 (52.9)	8 (47.1)

Table 7

Chi-square Results for Proportion and Percent Total of Dichotomized Physical Activity Levels by Meal Type for Males

	Physical Activity Levels	
	Low	High
Meal	Count (%)	Count (%)
Augmented	2 (25.0)*	6 (75.0)
Depleted	1 (14.3)*	6 (87.5)*
Balanced	5 (62.5)*	3 (37.5)

*expected cell count of less than 5.

When BMI was analyzed separately for each gender, no significant difference emerged between meal types for either the females [$F(2, 47) = .02$, $p = .985$, partial $\eta^2 = .00$] or the males [$F(2, 20) = .43$, $p = .656$, partial $\eta^2 = .04$] (see Table 8 for group means and SDs).

Overall, no gender differences were found for ESL or eating frequency. Levels of physical activity, although significant for the females when dichotomized into 'low' and 'high' levels of activity, was not significantly correlated with any of the variables on which the predictions were based. Thus, despite there being an unequal proportion of levels of physical activity among female participants in Augmented and Depleted conditions (respectively), it does not appear that this difference was great enough to influence test scores. For this reason, level of physical activity is excluded from all further analyses. For exploratory purposes, however, all female analyses were repeated, examining low- and high-activity level participants separately on each test measure. The results were largely the same. The only significant difference was on the EStroop Positive Interference for the high-activity females [$F(2, 24) = 3.84$, $p = .036$, partial $\eta^2 = .24$]. Post-hoc comparisons showed a significant difference between those in the Augmented group ($M = 12.00$, $SD = 4.24$; $n = 4$) and those in the Depleted group ($M = 2.80$, $SD = 7.04$; $n = 15$). With regards to BMI, when taken together with the results of the correlation matrix and above mentioned ANOVA, results of these analyses indicate that the BMI distribution is equal between meal groups and the difference is thus not large enough to influence test scores. For this reason, BMI was excluded from further analyses. This is in keeping with the norm within the literature in that researchers may

Table 8

Mean and Standard Deviation of BMI Scores by Gender and Meal Type

	BMI Score			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
Augmented	21.97	2.48	23.38	2.64
Depleted	21.84	2.69	24.64	2.98
Balanced	22.00	2.95	23.75	2.48

control for BMI when recruiting participants (e.g., Markus et al., 1998; Nabb & Benton, 2006), but they do not include it in subsequent analyses.

Effects of the Tryptophan Manipulation

Working memory and self-report affect indices have been found to be sensitive measures in previous studies examining the effects of tryptophan manipulation on neuropsychological performance (e.g., Kanarek & Swinney, 1990; Lieberman et al., 2002; Luciana et al., 2001). For this reason, the Digit Span Backwards scores and the PANAS NA and PA scores over time were employed to serve as indicators of the effectiveness of the tryptophan manipulation. In order to assess whether the dietary method of tryptophan manipulation successfully altered levels of circulating tryptophan in females, a one-way ANCOVA of meal type (augmentation, depletion, or balanced) was conducted on the Digit Span Backward Time 2 raw scores, with the Digit Span Backward Time 1 raw score entered as a covariate. There was no main effect of meal type [$F(2, 46) = .61$, $p = .549$, partial $\eta^2 = .03$]. As expected, however, Digit Span Backward Time 1 score was a significant covariate [$F(1, 46) = 24.54$, $p = .000$, partial $\eta^2 = .35$]. An identical analysis was conducted for the male participants. As with the females, there was no main effect of meal type [$F(2, 19) = .34$, $p = .713$, partial $\eta^2 = .04$], but Digit Span Backward Time 1 score was a significant covariate [$F(1, 19) = 15.04$, $p = .001$, partial $\eta^2 = .44$]. These results indicate that there were no significant between group differences for either females or males on the Digit Span Backward Time 2 score, one of the two pre- post-test measures selected to examine the effectiveness of the manipulation (see Table 9 for mean scores on Digit Span Backward 2 scores split by gender and meal type).

Table 9

Mean and Standard Deviation of Test Scores on Digit Span Backward 2 Scores by Gender and Meal Type

	Digit Span Backward 2 Scores			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
Augmented	8.47	2.27	8.63	2.62
Depleted	8.19	2.51	8.71	2.56
Balanced	7.29	2.42	7.50	2.73

In order to determine whether mood scores changed over time as a function of the manipulation, a repeated measures ANOVA of meal type on the NA scale of the PANAS (administrations 1 through 6) was conducted for each gender separately. For the females, Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of NA, $\chi^2(14) = 60.28$, $p = .000$. Thus, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .65$ for the main effect of NA). The main effect of NA was not significant [$F(3.24, 149.23) = 1.23$, $p = .301$, partial $\eta^2 = .03$], nor was the main effect of meal type [$F(2, 46) = .08$, $p = .923$, partial $\eta^2 = .00$]. The interaction between NA and meal type was also not significant [$F(10, 230) = .42$, $p = .936$, partial $\eta^2 = .02$].

These analyses were repeated for the males. As with the females, Mauchly's test indicated that the assumption of sphericity had been violated for the main effect of NA, $\chi^2(14) = 54.57$, $p = .000$. Thus, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon = .50$ for the main effect of NA). The main effect of NA was not significant [$F(2.52, 40.24) = 4.00$, $p = .019$, partial $\eta^2 = .20$], nor was the main effect of meal type [$F(2, 16) = .51$, $p = .611$, partial $\eta^2 = .06$]. The interaction between NA and meal type was also not significant [$F(10, 80) = 1.14$, $p = .343$, partial $\eta^2 = .12$] (see Table 10 for mean NA scale scores split by gender and condition).

An identical set of repeated measures ANOVAs were conducted examining the effect of meal type on the PA scale of the PANAS (administrations 1 through 6) for each gender. For the females, Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the main effect of PA, $\chi^2(14) = 34.48$, $p = .002$. Thus, degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity ($\varepsilon =$

Table 10

Mean and Standard Deviation of Test Scores on Negative Affect (NA) Scale by Gender and Meal Type

	NA Scale Scores			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
		NA Scale 1		
Augmented	12.69	4.25	14.17	5.91
Depleted	12.56	3.48	12.83	3.66
Balanced	12.47	4.35	15.57	4.04
		NA Scale 2		
Augmented	11.50	2.76	12.17	2.48
Depleted	12.44	4.46	12.17	3.25
Balanced	11.76	4.76	12.86	2.48
		NA Scale 3		
Augmented	12.38	3.14	12.67	3.08
Depleted	12.44	3.03	12.17	3.37
Balanced	11.71	2.95	12.86	1.95
		NA Scale 4		
Augmented	11.88	3.69	12.17	1.94
Depleted	12.13	2.47	11.83	2.56
Balanced	12.18	3.19	12.29	1.80

	NA Scale Scores			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
		NA Scale 5		
Augmented	12.06	2.79	12.67	2.66
Depleted	12.00	2.16	11.50	1.52
Balanced	12.00	2.96	13.00	2.31
		NA Scale 6		
Augmented	12.19	4.02	12.33	2.34
Depleted	13.31	5.21	13.67	3.61
Balanced	12.47	2.18	16.57	6.90

.76 for the main effect of PA). There was no significant main effect for PA [F(3.80, 174.93) = 1.69, p=.157, partial η^2= .04]. The main effect of meal type was also not significant [F(2, 46) = .59, p =.599, partial η^2= .03]. There were no significant interactions between PA and meal type [F(10, 230) = .17, p =.998, partial η^2=.01].

These analyses were repeated for the males. As with the females, there was no significant main effect for PA [F(5, 75) = .95, p =.455, partial η^2= .06]. The main effect of meal type was also not significant [F(2, 15) = .66, p =.530, partial η^2= .08]. There was, however, a significant interaction between PA and meal type [F(10, 75) = 2.19, p =.028, partial η^2= .23]. Post-hoc comparisons indicate that there was a near-significant difference between meal types on the fourth administration of the PA, t(15) = -2.13, r = .48, with those in the Augmented group scoring lower than those in the Depleted group. As this value is equal to the established p = .05 cut-point for a significant finding and is only present on one of the six PANAS administrations, it is likely that this result is spurious and not meaningful (see Table 11 for mean PA scale scores split by gender and condition).

Overall, these analyses indicate that there were no significant between group differences for males or for females on pre- and post-test affective scores as measured by the PANAS NA and PA scales.

Hypothesis 1: Depletion will result in lower scores on measures of LTM.

An ANOVA was conducted examining the effect of meal type on the CVLT Long-delay Free Recall scores for females. There was no significant main effect of meal type on the total number of words recalled during the long-delay free recall [F(2, 47) = .13, p =.882, partial η^2=.01]. An identical analysis was conducted for the male

Table 11

Mean and Standard Deviation of Test Scores on Positive Affect (PA) Scale by Gender and Meal Type

	PA Scale Scores			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
		PA Scale 1		
Augmented	26.69	5.75	29.50	6.57
Depleted	23.75	6.95	31.00	6.60
Balanced	23.76	7.01	32.14	9.19
		PA Scale 2		
Augmented	26.38	2.27	26.17	8.89
Depleted	23.38	9.16	30.80	6.14
Balanced	23.53	7.15	31.14	9.23
		PA Scale 3		
Augmented	26.56	7.66	28.83	10.76
Depleted	24.56	7.62	34.80	4.09
Balanced	24.35	7.37	31.00	12.52
		PA Scale 4		
Augmented	26.63	8.50	24.00*	8.67
Depleted	24.25	6.66	35.00	4.90
Balanced	25.12	7.21	31.29	10.13

	PA Scale Scores			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
		PA Scale 5		
Augmented	25.88	8.45	28.50	7.79
Depleted	24.69	8.30	36.80	5.50
Balanced	24.41	9.10	30.00	7.57
		PA Scale 6		
Augmented	28.38	8.29	31.33	6.53
Depleted	25.75	8.42	32.60	3.78
Balanced	25.76	10.65	29.00	12.82

*p =.05

participants. The assumption of homogeneity of variance was violated, however, necessitating the use of the Brown-Forsythe *F* correction for unequal group sizes; the Brown-Forsythe correction is a robust test of the equality of means. Using this correction, the main effect of meal type on total words recalled was not significant [$F(2, 12.07) = .73$, $p = .493$, partial $\eta^2 = .07$].

An identical ANOVA was also conducted to examine the effect of meal type on Rey-O Delayed Recall scores for females. The main effect of meal type on the number of elements correctly recalled during the long-delay recall trial was not significant [$F(2, 47) = .22$, $p = .806$, partial $\eta^2 = .01$]. This same analysis was conducted for the male participants, wherein a significant main effect of meal type was found [$F(2, 20) = 5.21$, $p = .015$, partial $\eta^2 = .34$]. Post-hoc comparisons indicate that males in the Depleted condition scored significantly higher on the Rey-O Long Delay subtest than did those in the Balanced condition (see Table 12 for mean scores on the CVLT Long Delay Free and the Rey-O Long Delay split by gender and meal type).

Taken together, the prediction that depletion would result in lower scores on measures of LTM was not met. In fact, males in the Depleted group scored significantly higher than did males in the Balanced condition and higher (though not significantly) than those in the Augmented condition on Rey-O Long Delay Recall, a measure of visuospatial LTM.

Hypothesis 2: Depletion will result in lower mood scores.

This prediction was tested by conducting a repeated measures ANOVA with the NA and PA scores from all six administrations of the PANAS. Although this measure was used to assess the efficacy of the tryptophan manipulation, it was also employed in

Table 12

Mean and Standard Deviation of Test Scores on the CVLT Long Delay Free Recall and the Rey-O Long Delay by Gender and Meal Type

	Test			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
	CVLT-Long Delay			
Augmented	13.65	2.18	11.63	4.14
Depleted	13.38	2.34	13.14	2.12
Balanced	13.24	7.05	13.13	1.55
	Rey-O Long Delay			
Augmented	21.88	7.15	21.06 [c]	4.28
Depleted	20.50	5.68	25.21 [a]	5.71
Balanced	20.65	7.05	16.63 [b]	5.44

[a] $p < .05$ Significantly different from [b] but not significantly different from [c]; [b] is not significantly different from [c].

examining the prediction that tryptophan depletion will lower mood scores as it directly measures changes in self-reported affect throughout the testing session. The prediction was also assessed by conducting an ANOVA with the Positive and Negative Interference scores for the Emotional Stroop.

As detailed in the repeated measures ANOVA described in the *Effects of the Tryptophan Manipulation* section, four analyses were conducted looking at the NA and PA scales of the PANAS for each gender separately. There were no significant main effects for NA or for meal type, nor were there any significant interactions between NA and meal type for either the females or the males. For the PA analysis, no significant main effects were found for PA or for meal type for either gender, nor were there significant interactions between PA and meal type for females. There was, however, a significant interaction between PA and meal type for the males on the 4th administration of the PANAS. This interaction, although significant, is not interpretively meaningful.

An ANOVA of meal type on Emotional Stroop Negative Interference scores was conducted for each gender. No significant main effect was found for meal type on negative interference scores for females [$F(2, 47) = .11$, $p = .899$, partial $\eta^2 = .01$], or for males [$F(2, 20) = .14$, $p = .874$, partial $\eta^2 = .01$].

An identical set of analyses were conducted using the Positive Interference score of the Emotional Stroop. The main effect of meal type on positive interference scores was not significant for females [$F(2, 47) = .79$, $p = .459$, partial $\eta^2 = .03$], or for males [$F(2, 20) = .02$, $p = .985$, partial $\eta^2 = .00$] (see Table 13 for mean scores on the Positive and Negative EStroop test split by gender and meal type).

Table 13

Mean and Standard Deviation of Test Scores on the Negative and Positive EStroop by Gender and Meal Type

	Test			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
	EStroop Negative Interference			
Augmented	10.06	6.18	5.38	5.55
Depleted	9.31	6.67	4.43	5.38
Balanced	8.88	9.24	3.88	6.45
	EStroop Positive Interference			
Augmented	5.35	6.97	1.50	6.55
Depleted	3.06	6.88	1.86	4.10
Balanced	6.29	8.64	2.00	6.37

Taken together, these analyses indicate that the prediction that those in the Depletion group would score lower on measures of mood was not met.

Hypothesis 3: Augmentation will lead to lower scores on measures of working memory.

To assess this prediction, participants' total raw scores for Letter-number Sequencing, verbal fluency and Digit Span (total score on the second administration) were converted into z-scores which were then averaged to arrive at a composite working memory score for each participant. An ANOVA was conducted examining the effect of meal type on the working memory composite scores for females. There was no significant main effect of meal type on working memory scores [$F(2, 47) = 1.29$, $p = .286$, partial $\eta^2 = .05$]. The same analysis was conducted for males, indicating that the main effect of meal type on working memory scores was not significant [$F(2, 20) = .18$, $p = .837$, partial $\eta^2 = .02$] (see Table 14 for mean scores on the working memory composite split by gender and meal type). Thus, the prediction that those in the Augmented condition would obtain lower scores on measures of working memory was not met.

Hypothesis 4: Augmentation will result in functional improvements within the areas of attention and vigilance.

To test this prediction, a composite attention score was created for each participant. This was done by converting raw scores for the measures of attention into z-scores and averaging them to arrive at a composite attention score for each participant. The following tests were included in this composite score: Digit Span total score (from the second administration); Letter-number Sequencing total score; Digit-symbol Coding total score; Ruff 2 & 7 Total Speed and Total Accuracy scores; Trails A time; and Trails

Table 14

Mean and Standard Deviation of Test Scores on the Working Memory Composite by Gender and Meal Type

	Working Memory Composite Score			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
Augmented	.13	.64	.03	.92
Depleted	-.01	.89	.29	.86
Balanced	-.27	.63	.04	1.04

B time. An ANOVA of meal type on composite attention scores was conducted for the females. Results of this analysis revealed no significant main effect of meal type on attention composite scores [$F(2, 46) = .10$, $p = .904$, partial $\eta^2 = .00$]. The same analysis was conducted for the males, again revealing no significant main effect of meal type on attention composite score [$F(2, 19) = .80$, $p = .464$, partial $\eta^2 = .08$] (see Table 15 for mean attention composite scores split by gender and meal type). Thus, the prediction that Augmentation would result in functional improvements in attention and vigilance was not met.

Hypothesis 5: The balanced group will score higher than the depletion group on LTM and on mood indices, and higher than the augmentation group on working memory.

These hypotheses, which were tested via the analyses for Hypotheses 1 through 3 (detailed above), were not met as neither females nor males in the Balanced group obtained statistically significant higher scores than those in the Depleted or Augmented groups on these measures. Females in the Balanced group did not score higher than the females in the Depletion group on the CVLT Long-delay Free Recall [$F(2, 47) = .13$, $p = .882$, partial $\eta^2 = .01$]. Similarly, there were no significant group differences between females on the Rey-O Delayed Recall [$F(2, 47) = .22$, $p = .806$, partial $\eta^2 = .07$]. Scores on the CVLT Long-delay Free Recall were also not significantly different between males in the Balanced and Depleted groups [$F(2, 12.07) = .73$, $p = .493$, partial $\eta^2 = .01$]. Scores on the Rey-O Delayed Recall, however, were significantly different between males in the Balanced and Depleted groups [$F(2, 20) = 5.21$, $p = .015$, partial $\eta^2 = .34$], with those in the Depleted group obtaining higher scores than those in the Balanced group (see Table 12 for mean scores). Overall, the prediction that the Balanced group would score higher

Table 15

Mean and Standard Deviation of Test Scores on the Attention Composite by Gender and Meal Type

	Attention Composite Score			
	Female		Male	
Meal Type	*M*	*SD*	*M*	*SD*
Augmented	-.00	.54	.10	.39
Depleted	.03	.48	-.02	.35
Balanced	.06	.37	-.17	.49

than the Depleted group on measures of LTM was not met. In fact, males in the Balanced group scored significantly lower than those in the Depleted group on the Rey-O Delay Recall, a measure of visuospatial LTM (see Hypothesis 1 for further details).

The hypothesis that those in the Balanced group would obtain higher scores in the mood indices was also not met. Neither the females [$F(2, 46) = .08$, $p = .923$, partial $\eta^2 = .00$] nor the males [$F(2, 16) = .51$, $p = .611$, partial $\eta^2 = .06$] in the Balanced group scored higher than those in the Depletion group on the NA scale of the PANAS. Similarly, there were no group differences on the PA scale for the females [$F(2, 46) = .59$, $p = .599$, partial $\eta^2 = .03$] or for the males [$F(2, 15) = .66$, $p = .530$, partial $\eta^2 = .08$]. Females in the Balanced group also failed to score higher than the Depletion group on the Emotional Stroop Negative [$F(2, 47) = .11$, $p = .899$, partial $\eta^2 = .11$] or Positive [$F(2, 47) = .79$, $p = .459$, partial $\eta^2 = .03$] Interference trials. Similarly, males in the Balanced group failed to score higher than those in the Depletion group on either of the Negative [$F(2, 20) = .14$, $p = .874$, partial $\eta^2 = .01$] or Positive [$F(2, 20) = .02$, $p = .985$, partial $\eta^2 = .00$] Interference trials of the Emotional Stroop (see Table 13 for mean scores). Taken together, these analyses demonstrated that the Balanced group did not score higher than the Depletion group on mood indices (see Hypothesis 2 for further details).

The hypothesis that those in the Balanced group would score higher than those in the Augmented group on measures of working memory was also not met for either the females $F(2, 47) = 1.29$, $p = .286$, partial $\eta^2 = .05$] or the males [$F(2, 20) = .18$, $p = .837$, partial $\eta^2 = .02$] (see Table 14 for mean scores; see Hypothesis 3 for further details).

Overall, hypotheses made in Hypothesis 4 were not met as neither the females nor the males in the Balanced group obtained statistically significantly higher scores than

those in the Depleted group on measures of LTM and mood, and they did not obtain statistically significant higher working memory scores than those in the Augmented groups.

Hypothesis 6: The balanced group will score lower than those in the augmentation group on tests of attention and vigilance.

As discussed in the ANOVA of meal type on attention composite scores detailed in Hypothesis 4, neither the females [$F(2, 46) = .10$, $p = .904$, partial $\eta^2 = .00$] nor the males [$F(2, 19) = .80$, $p = .464$, partial $\eta^2 = .08$] in the Balanced group obtained a lower score on tests of attention and vigilance than did those in the Augmented group (see Table 15 for mean scores; see Hypothesis 4 for greater details). Thus, this hypothesis was not met as there were no statistically significant between group differences on the attention composite.

Exploratory Analyses

Exploratory analyses were also conducted in order to assess whether there were any group differences on the other cognitive measures comprising the composite scores or on the individual items of the PANAS. As the nature of exploratory analyses is to examine whether any findings emerge that would warrant further exploration in future research, no corrections for multiple comparisons were conducted; we were more concerned with Type II error (i.e., sensitivity) than with Type I error (i.e., specificity) (S. Miller, personal communication, March 17, 2010). In keeping with previous analyses, females and males were analyzed separately.

In an exploratory way, the individual items of the PANAS were analyzed in order to examine the individual affects (e.g., upset, interested, enthusiastic) captured by the PANAS. As initial exploratory analyses, a MANOVA was conducted for each gender examining the effect of meal type on the difference scores for each item. The difference scores were created by subtracting the score on PANAS Time 6 from the score on PANAS Time 1 for each individual item, resulting in 20 difference scores, one for each item on the PANAS. No significant between group differences emerged for females for any of the items (see Table 16).

This analysis was repeated for the male participants. A significant between group difference was found for males on the Excited difference scores [$F(2, 16) = 3.81$, $p = .044$, partial $\eta^2 = .32$] (see Table 17). Post-hoc comparisons indicate that the Excited item was significantly different between the Balanced and Depleted groups, $t(16) = 2.51$, $p = .023$, $r = .47$.

In order to determine whether or not the individual items on the PANAS were in fact stable and reliable across time, test-retest reliability analyses were conducted on the NA scale as prior research has found the NA scale to be the most sensitive to tryptophan manipulation. These analyses, which were conducted on the Balanced group, demonstrated that the NA is in fact stable over time, $r = .78$, $p < .01$. Further examination revealed that the Excited item is also reliable over time, with an average correlation of $r = .72$ (when collapsed across gender) or of $r = .80$ (when only males are included). As difference scores have an inherently greater degree of error in them compared to repeated measures analyses, it was unclear whether there was, in fact, a change in excitement levels over time. For this reason, a repeated measures ANOVA was conducted on meal

Table 16

MANOVA for Each PANAS Item for Females

PANAS Item	*df*	*F*	partial η^2	*p*
Interested	2	.22	.01	.807
Distressed	2	2.29	.09	.113
Excited	2	.08	.00	.921
Upset	2	.14	.01	.870
Strong	2	.27	.01	.767
Guilty	2	.24	.01	.791
Scared	2	.04	.00	.965
Hostile	2	.97	.04	.386
Enthusiastic	2	.22	.01	.801
Proud	2	.19	.01	.827
Irritable	2	1.44	.06	.248
Alert	2	.40	.02	.674
Ashamed	2	.18	.01	.839
Inspired	2	.03	.00	.966
Nervous	2	.76	.03	.474
Determined	2	.25	.01	.784
Attentive	2	.54	.02	.588
Jittery	2	.02	.00	.982
Active	2	.85	.04	.432
Afraid	2	.05	.00	.951
Error	46			

Table 17

MANOVA for Each PANAS Item for Males

PANAS Item	*df*	*F*	partial η^2	*p*
Interested	2	.23	.03	.794
Distressed	2	.95	.12	.408
Excited	2	3.81	.32	.044
Upset	2	1.75	.18	.206
Strong	2	1.44	.15	.266
Guilty	2	.84	.10	.449
Scared	2	.61	.07	.556
Hostile	2	2.02	.20	.165
Enthusiastic	2	1.30	.14	.301
Proud	2	2.58	.24	.107
Irritable	2	1.01	.11	.385
Alert	2	.31	.04	.739
Ashamed	2	.26	.03	.777
Inspired	2	.68	.08	.521
Nervous	2	1.16	.13	.338
Determined	2	1.30	.14	.300
Attentive	2	.22	.03	.809
Jittery	2	1.22	.13	.321
Active	2	1.16	.13	.340
Afraid	2	1.10	.12	.358
Error	16			

type on Excited scores for all 6 times (male participants only). Mauchly's test of sphericity indicated that the assumption of sphericity had been violated for the main effect of Excited, $\chi^2(14) = 27.72$, $p = .017$. Thus, degrees of freedom had been corrected for using Greenhouse-Geisser estimate of sphericity ($\varepsilon = .65$ for the main effect of Excited). There was no significant main effect for Excited [$F(3.24, 51.88) = .53$, $p = .677$, partial $\eta^2 = .03$]. The main effect of meal type was also not significant [$F(2, 16) = 2.64$, $p = .102$, partial $\eta^2 = .25$], nor was the interaction between Excited and meal type [$F(10, 80) = 1.49$, $p = .158$, partial $\eta^2 = .16$] (see Table 18 for mean Excited scores by meal type over time).

Although the overarching omnibus analysis was not significant, post-hoc comparisons were examined for exploratory purposes to see if any trends existed within the data. Post-hoc comparisons indicate that the Excited item was significantly different between the Augmented and Depleted groups on Time 4, $t(16) = -2.74$, $p = .015$, $r = 0.75$, Time 5, $t(16) = -2.14$, $p = .048$, $r = 0.47$, and on Time 6, $t(16) = -2.12$, $p = .050$, $r = 0.47$. These post-hoc comparisons indicate a trend wherein those in the Depleted group experienced a fairly modest, but linear, increase in excitement level over time, whereas excitement was lowered between administration two through four before increasing drastically by the sixth administration for those in the Augmented group. Participants in the Balanced condition demonstrated a subtle decrease in excitement levels over time (see Figure 2 for mean Excited scores by meal type over time).

Taken together, these analyses suggest that there is a trend between meal type and level of excitement, indicating that those in the Depleted group experienced an increase in level of excitement as the study progressed, whereas those in the Augmented group

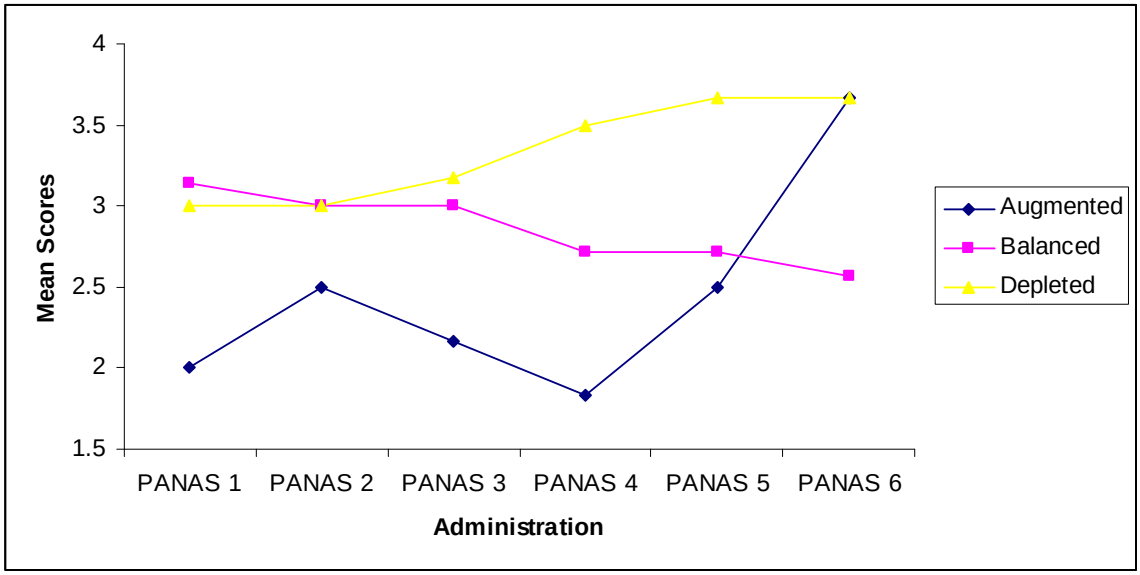
Table 18

Mean and Standard Deviation of Excited Item Over Time for Males

	Administration	
Meal Type	*M*	*SD*
	Excited 1	
Augmented	2.00	.63
Depleted	3.00	.89
Balanced	3.14	1.07
	Excited 2	
Augmented	2.50	.84
Depleted	3.00	1.27
Balanced	3.00	1.16
	Excited 3	
Augmented	2.17	1.33
Depleted	3.17	.41
Balanced	3.00	1.29
	Excited 4	
Augmented	1.83	.98
Depleted	3.50	.55
Balanced	2.71	1.38

	Administration	
Meal Type	*M*	*SD*
	Excited 5	
Augmented	2.50	.84
Depleted	3.67	.82
Balanced	2.71	1.11
	Excited 6	
Augmented	2.50	.84
Depleted	3.67	.52
Balanced	2.57	1.27

Figure 2. Mean Excited scores by meal type over time.



experienced a dip in excitement levels followed by a steady increase in excitement towards the latter half of the testing session. The subtle decrease in excitement experienced by the Balanced group over time was not statistically significant. These findings may provide some limited evidence for the effect of the manipulation.

Oneway ANOVAs were conducted on all other tests and measures employed in this study in order to examine the effect of meal type on neuropsychological and emotional performance; females and males were analyzed separately. As can be seen in Table 19, the only significant result was found for male participants on the Ruff 2 & 7 Total Speed subtest [$F(2, 20) = 4.68$, $p = .022$, partial $\eta^2 = .32$]; all other analyses failed to reject the null hypothesis. Post-hoc Bonferroni comparisons on the Ruff 2 & 7 Total Speed subtest revealed significant differences in mean time scores between the Augmented and Balanced groups but not between the Augmented and Depleted groups, or between the Balanced and Depleted groups. The Augmented group obtained a mean speed score of 112.50 ($SD = 16.44$), the Balanced group obtained the fastest speed score, with a mean score of 88.38 ($SD = 17.44$), and the Depleted group obtained a mean speed score of 104.86 ($SD = 13.91$). There was no significant difference in time scores between the Augmented and the Depleted group, or on any other measures for either females or males.

Discussion

In this study, the effects of tryptophan manipulation on cognitive functioning and affective state were examined. More specifically, a dietary method of tryptophan manipulation was employed in order to assess any differences in performance produced by tryptophan augmentation as compared to tryptophan depletion or balanced tryptophan

Table 19

ANOVAs on the Effect of Meal Type on All Test Measures Examined in Exploratory Analyses, Separated by Gender

Test	*df*	*F*	partial η^2	*p*
Digit Symbol Coding				
Female	2, 47	.13	.01	.878
Male	2, 20	1.26	.11	.305
FAS				
Female	2, 47	.71	.03	.495
Male	2, 20	1.16	.10	.334
Letter Number Sequencing				
Female	2, 47	.87	.04	.425
Male	2, 20	.22	.02	.808
Stroop Interference				
Female	2, 44	.25	.01	.783
Male	2, 18	.15	.02	.865
Ruff 2 & 7 Total Accuracy				
Female	2, 46	.86	.04	.428
Male	2, 19	2.00	.17	.163
Ruff 2 & 7 Total Speed				
Female	2, 46	.12	.01	.890
Male	2, 20	4.68	.32	.022
Trails A Time (sec)				
Female	2, 47	3.16	.12	.052
Male	2, 20	2.01	.17	.160
Trails B Time (sec)				
Female	2, 47	.66	.03	.521
Male	2, 20	1.03	.09	.374
Digit Span 2 Total				
Female	2, 47	.98	.04	.383
Male	2, 20	.24	.02	.787

levels. Although the cognitive measure of pre-test/post-test differences (Digit Span Backwards) failed to detect any significant between group differences for either males or females, there were marginally significant group differences in positive affect between males in the Augmented and Depleted group. These differences in PA levels seem to be primarily driven by the trend in excitement levels between males in the Augmented and Depleted conditions over time. Group differences were also found for the males on a visuospatial measure of long-term memory, as well as on a measure of attention and concentration. Results of this study support the hypothesis that dietary manipulations aimed at altering tryptophan levels had an effect on some cognitive tests and positive affect, at least with regards to males.

Did the manipulation affect affect?

Of all the measures employed in this study, it was believed that the PANAS would be the most sensitive to tryptophan manipulation and it can thus be viewed as a check on the efficacy of the manipulation. Although no significant results emerged on the NA scale for either the males or the females, indicating that participants reported no change in negative affect, a marginally significant finding emerged for males on the PA scale, but not for females. More specifically, it was found that on the fourth administration of the PANAS (which was administered immediately after lunch), males in the Depleted group scored higher on the PA scale than did males in the Augmented group. As only one of the six administrations demonstrated a near-significant effect of meal type, with all others being not significant, it is likely that this finding is spurious and not interpretively meaningful. Thus, despite this trend, no meaningful results emerged on either the NA or the PA scale for either the females or the males, indicating that

participants reported no increase or decrease in overall negative or positive affect. This finding was somewhat unexpected as it was predicted that negative affect (i.e. NA scale scores) would be affected, whereas a subtle change in positive affect was actually found.

The results of the study by Luciana and colleagues (2001), however, are the opposite as they found that tryptophan depletion resulted in transient decreases in negative affect, and a lowering of positive affect, whereas tryptophan augmentation had no effect on negative affect, but lowered positive affect. Their findings seem to indicate that positive affect can be decreased by either augmentation or depletion of tryptophan, whereas the effect on negative affect is transient at best. This is in contrast to the finding by several researchers that negative affect is most affected by augmentation of serotonergic transmission (and thus, presumably, tryptophan; e.g., Bodkin et al., 1997; Shelton & Brown, 2000; Shelton & Tomarken, 2001), which would indicate that increased serotonin likely plays a role in reducing feelings of general distress (Dichter et al., 2005). Reviewing the literature on the effects of tryptophan manipulation on mood and affect, however, illustrates that there is no consensus within the field. For instance, many researchers have found no effect of tryptophan manipulation on mood (e.g., Hughes et al., 2003; Park et al., 1994; Shansis et al., 2000; Smith et al., 1988), whereas many others have found that tryptophan depletion lowers mood (e.g., Benkelfat et al., 1994; Luciana et al., 2001; Riedel et al., 1999; Schmitt et al., 2000; Spring et al., 1983; Young et al., 1985).

One possible reason for the lack of an effect of tryptophan manipulation on NA scores in the present study is that perhaps the manipulation was not as strong as expected. Another possibility is that the inclusion of a macronutrient-manipulated breakfast, lunch

and snack for each participant lessened (or even perhaps eradicated) the effects of tryptophan manipulation on negative affect. For instance, many of the studies within the literature include only a macronutrient-manipulated breakfast or lunch and few include snacks (e.g., Fischer et al., 2002; Riedel et al., 1999; Spring et al., 1983; Wurtman et al., 2003). This makes comparison between studies difficult as each study will thus report differing amounts of carbohydrates, proteins, fats and calories in their meals. For instance, Wurtman and colleagues employed breakfasts in their study and reported a total of 175.70 calories for the high carbohydrate meal, and 34.29 calories for the high protein meal. In the present study, those in the Augmented condition (i.e., high carbohydrate condition) ingested 931 calories in total, whereas those in the Depleted condition (i.e., the high protein condition) consumed a total of 881 calories. Since the present study included two meals and a snack, the calorie intake was thus much higher, making it difficult to determine whether the manipulation was too weak, or whether there were too many competing macronutrients for subtle differences in affect to emerge.

An alternative possibility for the lack of an effect of tryptophan manipulation on negative affect is that perhaps macronutrient content does not exert a strong influence in the first place, which would consequently make detecting changes difficult. In line with this notion, Teff, Young and Blundell (1989) reported that a carbohydrate meal containing as little as 4% protein will counteract the rise in availability of tryptophan to the brain that would otherwise occur. However, in a study that sampled lumbar cerebrospinal fluid from 3 normal pressure hydrocephalus patients, Teff, Young, Marchand, and Botez (1989) found that neither carbohydrates nor protein, ingested 2.5 hours prior to lumbar puncture, resulted in significantly altered levels of central nervous

system tryptophan or serotonin. While it is possible that this conclusion was the result of either too little time elapsing between treatment and measurement of the effect, by the small sample size, or by the sample characteristics (i.e., normal pressure hydrocephalus patients), it does raise the question of how much of a given macronutrient is required to influence tryptophan or serotonin levels in the brain and spinal cord, and over what time is this change detectable? In a more recent study by Lindseth, Petros, Jensen, Lindseth and Fossum (2006) that examined the effects of macronutrients on the flight performance of pilots, it was found that a high-fat diet resulted in significantly better performance than the high-carbohydrate, high-protein or control diets. Furthermore, they concluded that when on the high-protein diet, pilots performed the worst. These contradictory findings suggest that the effects of protein (or carbohydrates) on tryptophan availability are not straightforward. In the present study, the Augmented group had 15% of its total caloric intake comprised of protein, whereas the Balanced meal only had 2% of it's calories coming from protein. In consideration of the findings by Teff, Young and Blundell, the Balanced group should have displayed some effect of tryptophan manipulation given that there was not enough protein to quash the emergence of tryptophan-driven effects. Regarding the statement by Teff, Young, Marchand, and Botez, however, both groups should have demonstrated some effect of tryptophan based solely on the premise that protein content does not appear to matter. Fischer and colleagues (2002) had concluded that their lack of tryptophan-related findings was due to a protein content in the meals that was still too high, based on the idea that even 4% protein would abolish effects; the same could possibly be said for the present study regarding the Augmented group. However, we are not convinced that this is the case in the present study as there appears

to be no clear consensus on how much protein is required, if any at all, to erase tryptophan-mediated effects on affect (or cognition). Further, S. N. Young (1993) states that, "[s]ince few meals eaten by humans will contain less than 4% protein, serotonin-mediated changes in behaviour after carbohydrate meals are unlikely to be a normal physiological phenomenon" (p. 240).

A final possible reason for the lack of findings of an effect of tryptophan manipulation on NA scores pertains to screening criteria as it seems likely that the exclusionary criteria used during recruitment would play a critical role in the outcome. For instance, previous studies have found differing effects of tryptophan manipulation on mood when studying those with a family history of depression (e.g., Riedel et al., 1999), personal history of mood disorder (e.g., Price, Charney, Delgado & Heninger, 1991; S. N. Young, 1993), stress level (e.g., Markus et al., 1998), and even age or gender (Spring et al., 1983). The present study employed very rigorous exclusionary criteria which makes it possible that it over-controlled for potential confounding factors, thereby decreasing the likelihood that any effect would emerge. While this certainly reduces the likelihood of making a Type I error, it begs the question as to whether the over-stringent controls made it difficult for more subtle effects to be detected (i.e., Type II error). Reviewing the literature, it is clear that there is no standard set of exclusionary criteria for this line of research. For example, the requirements for many studies were merely being 'healthy' and free from medication/drug use (e.g., Blum et al., 1992; Coull et al., 1995; Park et al., 1994). Some studies did not list exclusion criteria at all, only stating that participants were interviewed about their medical and/or psychiatric history (e.g. Lyons & Truswell, 1988; Wurtman et al., 2003). On the other hand, some studies employed very strict

controls similar to those used in the present study (e.g., Luciana et al., 2001; Riedel et al., 1999; Schmitt et al., 2000) and still found effects of the tryptophan manipulation on affect (and cognition). All of these studies, however, employed within-subjects designs and all (with the exception of Fischer and colleagues, 2002) utilized an amino acid tryptophan depletion protocol. The present study utilized a mixed design with a dietary manipulation of tryptophan. Thus, it is possible that the use of strict exclusionary criteria, in conjunction with the mixed design and dietary tryptophan manipulation employed in the present study, prevented the emergence of affective differences. The mechanism behind such action, however, is still unclear as there are others within the literature who found effects of tryptophan using a dietary manipulation, including those who employed between-subject designs but who did not utilize strict exclusionary criteria (e.g., Spring et al., 1983). Thus, there is some degree of interplay between these three factors (controls, design and mode of manipulation) that influences whether or not effects on mood are detected. As more research is conducted within this field, a consensus should begin to emerge that will indicate which methods and controls provide the most accurate and replicable results as to the effect of tryptophan on positive and negative affect.

With regards to specific affects comprising the NA and the PA scales, exploratory repeated measures analyses of individual affects on the PANAS detected a trend for males, indicating group differences in levels of excitement on the fourth, fifth and almost the sixth administrations; no significant group differences were found for either gender on any of the individual affects. More specifically, it was found that those in the Depleted group demonstrated a fairly linear (albeit modest) increase in excitement over

time, whereas those in the Augmented group demonstrated a dip in excitement from administration two through four before experiencing a drastic increase in excitement by time six. A limitation of exploratory analyses is that the risk of committing a Type I error (i.e., of finding a result that is not really there by rejecting the null when it is actually true) is increased. It is possible that this is the case in the present study as it is difficult to interpret, from a theoretical perspective, the reason for increased excitement levels in males in the Augmented and Depleted conditions, the two conditions in which participants received opposite carbohydrate:protein proportions in their meals, whereas those in the Balanced condition demonstrated a minor decrease in excitement levels. Since this finding was not predicted from the outset and does not have a theoretical basis to support it, any interpretation must be handled with caution. Regardless, one possible explanation is that perhaps it is not the macronutrient content per se, but rather the ingestion of greater than normal amounts of dietary components (i.e., carbohydrates or proteins) that is driving this finding, which could also explain why those who received a balanced diet demonstrated a minor decrease in level of excitement. This decrease would not be surprising when one considers that participants were in the lab from 8:45am until roughly 2pm, a span of approximately 5 hours, during which time it would seem plausible that activity levels and the degree of alertness would decrease. It is difficult to maintain a high level of arousal over an extended period of time, so the body naturally begins to lower its degree of arousal in response. In the current sample, however, the average score (collapsed across gender and meal type) on the Alert item at time 6 was 3.43 (out of 5), indicating a 'Moderate' degree of alertness, which is actually slightly higher than the average rating of 2.75 (out of 5) that indicated only a 'Slight' degree of

alertness at the start of the study. In a similar vein, scores on the Active item did not decline over time, with an average score of 2.24 (out of 5) on the sixth administration, compared to an average score of 2.68 (out of 5) on the first administration. These results show that in fact, a minor increase in levels of alertness and activity were found over time, not the expected decrease.

With regards to why the Excited item would be most sensitive to the manipulation, as opposed to the PA scale in general, it has been reported by Watson, Clark and Carey (1988), that "[p]ositive affect is a dimension reflecting one's level of pleasurable engagement with the environment" (p. 347). Excitement can also be seen as a pleasurable state of being – we get excited about events to come, or about things we are presently experiencing. As to why there would be differing levels of excitement over time for the different meal types, however, is somewhat more difficult to understand. Recall that in the present study significant differences between the Augmented and the Depleted group were found for level of excitement at Time 4, Time 5 and almost at Time 6 (for males only), with those in the Depleted group displaying the greatest levels of excitement at all times. More specifically, Time 4 represents the fourth administration of the PANAS, which was completed immediately after lunch, and Time 5 (i.e., the fifth administration) was immediately before the test battery began. Immediately after lunch, those in the Augmented group experienced the lowest levels of excitement which might be explained by the finding that carbohydrate-rich foods result in decreased feelings of dysphoria, alertness and general distress, and increased feelings of calmness (Christensen, 1993; Christensen, Krietsch, & White, 1989; Pühringer, Wirz-Justice, Graw, LaCoste & Gastpar, 1976; Spring et al., 2008). Recall that carbohydrate consumption

leads to increased insulin secretion which acts to draw the large neutral amino acids (LNAAs), with the exception of tryptophan, into the surrounding tissue. This action enables tryptophan to pass through the blood brain barrier with little competition, where 5-HTP synthesis occurs, which is immediately converted into serotonin. This process results in increased serotonin synthesis and activity within the limbic system, including the amygdala and the prefrontal cortex, which in turn produces a feeling of calmness and even euphoria (Pühringer, et al., 1976). It is also known that 5-HTP raises endorphin levels in the brain and endorphins act as the body's natural defense against pain and stress of all kinds (physical, psychological, and emotional) (Murray, 1998). Thus, greater levels of 5-HTP in the brain result in more endorphins being released, which in turn decreases feelings of stress (Murray, 1998). This decrease in stress could in turn result in decreased levels of excitement.

Conversely, the (male) Depleted group in the present study experienced higher levels of excitement over time than did the Augmented group, which seems indicative of greater sympathetic nervous system arousal. In the present study, the depleted meal was composed of the lowest level of carbohydrates and the highest level of protein (57 g of carbohydrates and 39 g of protein, compared to 200 g of carbohydrates and 11 g of protein in the Augmented condition); thus, it should be expected that the effects of the depleted meal would be opposite to those of the augmented meal. The finding that those in the Depleted group experienced the highest levels of excitement over time could be interpreted to indicate that protein increases one's level of excitement. Ingestion of protein has also been linked with self-reported ratings of happiness. For instance, Verger, Lagarde, Batejat and Maitre (1998) stated that participants reported feeling happier after

consuming a protein meal than after a ingesting a protein-free meal. However, a study by DeCastro (1987) reported the opposite result from protein consumption. In his study he had participants keep a detailed food diary for 9 consecutive days and found that over the 9-day period those who reported a protein-rich diet also reported greater overall levels of depression. Reconciling these divergent findings is difficult and likely stems from differing samples, designs and methodologies.

As should be clear, the findings pertaining to the relationship between protein, carbohydrates and mood are not entirely straightforward as several researchers have noted that although ingestion of simple carbohydrates leads to increased feelings of energy in the short-term (e.g., Blouin et al., 1991; Thayer, 1987), in the long-term they result in increased feelings of fatigue and lower energy levels (e.g., D. P. Wyons & I. Wyons, unpublished data). Further, as discussed earlier, protein has been found to influence feelings of happiness and depression (DeCastro, 1987; Verger et al., 1998). In the present study, participants in the Augmented condition consumed carbohydrates for an extended period of time (breakfast, lunch and a snack), resulting in decreased excitement levels following the morning snack. The Depleted group, however, exhibited a subtle increase in excitement levels over time. By contrast, those in the Balanced group, whose meals contained carbohydrate:protein ratios between those ingested by the other two groups, demonstrated a minor decrease in excitement over time. While it is not clear from the literature exactly what effect specific macronutrients have on excitement levels, it has generally been found that increases in negative mood typically follow the consumption of lunch (Craig, 1986; Kanarek, 1997). This conclusion was supported by males in the Augmented condition of the present study. Nonetheless, these

interpretations must be made with caution as there are many factors that can influence these findings, including the size of the meal, it's caloric and macronutrient content, whether the participant is a habitual breakfast-eater, and whether the foods ingested were similar to those normally consumed by participants at home (Kanarek, 1997; Lloyd, Rogers, Hedderley, & Walker, 1996). It is evident that further research within the area of the effects of specific macronutrients on individual affects is needed before a clearer picture can begin to emerge.

Overall, the effect of macronutrients on specific affects or scales is not clear within the literature. For instance, Lieberman and colleagues (1983) found that tryptophan resulted in reduced alertness, as measured by the Visual Analogue Mood Scale (VAMS), whereas scores on the fatigue-inertia scale of the POMS were increased and scores on the vigor-activity scale of the POMS were decreased. In a later study conducted by Lieberman (Lieberman et al., 2002), carbohydrates were found to increase alertness and decrease scores on the confusion scale of the POMS. These results seem contradictory in light of the fact that carbohydrate consumption has been shown to increase tryptophan levels in the blood (e.g., Fernstrom & Wurtman, 1971) and has been found to increase alertness by some researchers (Lieberman et al., 2002) and to decrease alertness by others (Lieberman et al., 1983). Thus, the results of Lieberman's respective studies seem to indicate that tryptophan both increases and decreases levels of alertness. Taken together, the results of the present study and those in the literature highlight the need for more research examining not just positive and negative affect on the whole, but specific affects as well, so that a greater understanding of the influence of tryptophan on mood and affect can begin to emerge.

Did the manipulation affect cognitive functioning?

In an attempt to determine the effect of tryptophan manipulation on cognitive functioning, the present study employed measures aimed at testing the domains of memory (specifically long-term memory), attention/concentration, and executive functioning. The Digit Span Backwards was also utilized as a measure of the effectiveness of the manipulation as there is some evidence within the literature that it is sensitive enough to detect the effects of tryptophan manipulation (Kanarek & Swinney, 1990; Luciana et al., 2001). The present study, however, failed to replicate these findings. This result in and of itself does not necessarily indicate that the tryptophan manipulation did not work, but rather it may indicate that Digit Span Backwards may not be sensitive enough to detect these effects. This issue of test sensitivity has also been raised with regards to difficult to measure constructs such as attention. It has been suggested that paper-and-pencil tests such as those employed in the present study may not be sensitive enough to detect subtle changes in cognitive functioning produced by altered levels of brain tryptophan (e.g., Hoyland, Lawton & Dye, 2008; Shansis et al., 2000). This argument does not hold in light of the fact that the CANTAB battery, which is known to be sensitive to subtle neuropsychological changes and which was employed by Hughes's group (2003) and Park and colleagues (1994), has also failed to reliably detect tryptophan-induced changes in cognitive performance. Park's group found that low levels of tryptophan resulted in poor consolidation of information, which in turn resulted in lower scores on measures of long-term memory and learning. Conversely, despite utilizing the exact same depletion methodology as Park, employing many of the same CANTAB tests, and recruiting a larger sample size, Hughes's group failed to find

any significant effects of tryptophan depletion on neuropsychological performance, including long-term memory. Thus, it appears that even tests deemed to be highly sensitive to subtle changes in cognitive function, such as those produced by tryptophan manipulation, fail to produce replicable results. Hoyland and colleagues (2008) go one step further by stating that "[i]t is feasible that commonly employed tasks may be inadequate measures of performance that mask real effects" (p. 74). In their review of the macronutrient effects of food on cognition, they concluded that task difficulty seems to be the best indicator of task sensitivity in that tasks that require the greatest cognitive load are most sensitive to macronutrient manipulations.

Regardless, significant group differences were found on a visuospatial measure of LTM. It was predicted that those in the Depletion group would score lower on measures of LTM. This prediction was in part supported by the finding that males in the Depleted group scored differently than the other two groups on LTM measures. The prediction that they would score lower, however, was not met as males in the Depleted group scored significantly higher than did males in the Balanced group on the Rey-O long delay recall; no effect was found for the males on the verbal LTM test, or for females on either the verbal or the visuospatial measure of LTM. The finding for the males on the Rey-O was somewhat unexpected for the simple reason that most researchers only measure verbal LTM (e.g., Kaplan et al., 2001; Riedel et al., 1999; Schmitt et al., 2000), whereas those that do employ visuospatial measures of LTM (e.g., Hughes et al., 2003; Shansis et al., 2000) find no effect of meal type. Both Hughes and Shansis, however, employed a within-subject design and used the amino acid protocol for altering tryptophan levels. The present study employed a mixed-design, and used a dietary manipulation for

tryptophan. Thus, it is possible that the difference in findings could be related to methodology. In particular, it seems to bolster the notion broached earlier that extreme amounts of specific macronutrients could influence cognitive functioning, as the Depleted group, who ingested low levels of carbohydrates but high levels of protein, performed significantly better than those in the Balanced group, who did not receive greater-than-normal amounts of protein (or carbohydrates). Indeed, Kaplan and colleagues (2001) state that "the ingestion of energy, regardless of source, appears to improve memory" (p. 691). They found that carbohydrates, protein and fat intake all improved delayed paragraph recall in their sample of elderly adults (aged 61-79), although protein in particular enabled participants to recall more details during the delay recall than on the immediate recall trial. Why this would be so is somewhat unclear. It is known, however, that protein-containing foods increase phenylalanine, from which tyrosine is synthesized (Curzon, 1985). Tyrosine, in turn, is the precursor to dopamine, norepinephrine and epinephrine, the latter two of which are known for their excitatory effect in readying the body for activity and for increasing alertness (Kolb & Whishaw, 1996; Spring, 1986). According to Lieberman and colleagues (1983), however, "tyrosine appears to have little or no effect on rested, unstressed volunteers" (p. 246). Dopamine, on the other hand, plays a role in memory by way of dopaminergic connections between the basal ganglia and substantia nigra (Kolb & Whishaw, 1996). Thus, it could be that the findings by Kaplan's group are mediated by dopaminergic processes. Memory impairments for nonverbal stimuli, such as objects or drawings, however, are known to result from damage to the right temporal lobe and surrounding areas, including the amygdala, hippocampus, and prefrontal cortex (Kolb & Whishaw, 1996). As discussed

earlier, the hippocampus in particular plays a role in spatial memory as the memory and perceptual systems are closely linked (Zola & Squire, 2000). The thalamus also plays a role in memory in that connections between the temporal and prefrontal cortices pass through the thalamus. Neocortical and brainstem systems, including acetylcholine, serotonin and norepinephrine systems, all provide input to the temporal lobe and other regions involved in memory processes (Kolb & Whishaw, 1996). Together, these processes can lead to greater LTM for visuospatial information.

During exploratory analyses, a significant between-group difference was also found for males on the Ruff 2 & 7 Selective Attention Test, with those in Augmented group obtaining a faster speed score than those in the Balanced group; no difference was found for females. A possible explanation for the findings with males could be due to cerebellar function, as this area of the brain, although primarily thought of for its motor control, is involved in speed of information processing (Botez et al., 1985). It does this through its non-motor pathways, connecting to the thalamus and receiving input from the frontal, parietal and superior temporal cortices (Schmahmann & Sherman, 1998). These pathways also influence working memory (Desmond et al., 1997), as well as general memory and learning (Nyberg, 1998). Together, these functions are all required in order to successfully complete the Ruff 2 & 7, as the speed with which the task is completed also requires being able to keep the rules of the task in working memory during completion. The majority of the serotonergic innervation to all layers of the cerebral cortex, including temporal, parietal, frontal and cerebellar cortices, comes from the dorsal raphe nuclei (Graeff, 1997; Molliver, 1987). Thus, a reduction to the amount of serotonin available to the structures would likely result from lesions to these cerebellar

pathways. Although it has been reported in the literature that the speed of information processing slows as a result of such lesions (e.g., Botez et al., 1985), it is not yet clear whether this outcome is due to decreased tryptophan or due to some other process influenced by such lesions. For instance, Smith and colleagues (1988) found that response times to peripheral stimuli on a visual search task were slowed following a high carbohydrate lunch (i.e., increased tryptophan), whereas those in the high-protein condition (i.e., reduced tryptophan) experienced greater distractibility to non-relevant stimuli. These results do not concur with those of the present study, which found faster response times for those in the Augmented (i.e., increased tryptophan) condition compared to those in the Balanced and Depleted conditions. An important difference between these studies, however, is that although both employed a dietary method of manipulating tryptophan, Smith's group utilized a within-subject design, whereas the present study used a mixed-design. Furthermore, the present study analyzed each gender separately, whereas the study by Smith did not, resulting in a total sample size of 23 males in the present study, versus a mixed sample of 11 participants (5 males, 6 females) in the latter study. Regardless, the effect of tryptophan manipulation on visual search tasks is not unanimous within the literature. For instance, Fischer and colleagues (2002) reported transiently improved attention following carbohydrate consumption (although they concluded that the balanced and protein conditions resulted in better overall cognitive performance), whereas several groups have failed to find any effect of augmented or depleted tryptophan (Hughes et al., 2003; Luciana et al., 2001; Shansis et al., 2000). As with Smith's group, the studies that failed to detect any between-group differences all employed a within-subjects design. As has been discussed previously,

reconciling the results of the present study with the findings in the literature is difficult due to the utilization of differing methods of manipulation and different research designs.

Thus, there are several possible reasons for such discrepancies between the present study and those reported in the literature. For instance, as has been discussed earlier, there appears to be no real consensus within the field as to the best way to manipulate tryptophan levels. There is ample evidence, as presented above, to suggest that at least within those studies that found significant results, the amino acid protocol is equally effective as the dietary (carbohydrate-loading) method at altering tryptophan levels. However, even among researchers using the same method, there is little consistency with regard to dose, with researchers employing macronutrient protocols ranging from 25g to 100g (e.g., Ford, Scholey, Ayre, & Wesnes, 2000; Green, Taylor, Elliman, & Rhodes, 2001; Hughes et al., 2003; Park et al., 1994; S. N. Young et al., 1985). Within the dietary literature, there is even less consistency with regards to dose or type of manipulation, with some studies employing carbohydrate or macronutrient drinks (e.g., Kaplan et al., 2001; Lieberman et al., 2002), some utilizing full meals or foods (e.g. Markus et al., 1998; Smith et al., 1988; Spring et al., 1983), and still others using cream-like pastes or puddings (e.g., Fischer et al., 2002; Teff, Young & Blundell, 1989). To further complicate matters, there is evidence that the time of day in which the protocol is administered, in conjunction with the age and gender of participants, also influences results (e.g., Craig, 1986; Spring et al., 1983). Researchers have also employed different delay periods between administration of the depletion method and testing session, which may also influence results. For instance, although Benkelfat and colleagues (1994) found that 5 hours are needed for tryptophan levels to reach their peak level of depletion

following the amino acid protocol, some researchers have tested participants 2 to 24 hours after the manipulation (e.g., Coull et al., 1995; Markus et al., 1998; Reidel et al., 1999; Schmitt et al., 2000), whereas others have tested participants only 15-30 minutes after ingestion of the macronutrient drinks (e.g., Kanarek & Swinney, 1990; Kaplan et al., 2001). Furthermore, some researchers have employed the use of maintenance doses throughout the testing session (e.g., Schmitt et al., 2000), and others still have made use of practice sessions in order to obtain baselines measures of performance (e.g., Fischer et al., 2002; Luciana et al., 2001; Riedel et al., 1999). It has been postulated by Hoyland and colleagues (2008) that the inability of researchers to successfully and consistently replicate the findings reported in the literature is likely due to these issues of varying methodologies. In other words, the paucity of research that employs the same tests and means of manipulation could be the very thing that is hindering progress within this field of research.

Bellisle (2001) and others have pointed out that there are a myriad of other factors that can influence test results. For instance, motivation, arousal, previous learning, time of day, fatigue, and individual characteristics and abilities could all lead to discrepant findings. Craig (1986) posits that both the quantity and the quality (i.e., the carbohydrate:protein ratio) of the meal is also important, as is the "nature and disposition" (p. 163) of the person consuming the meal, such that a person accustomed to eating large meals will be less affected by the macronutrient components of a large meal than would a be person who usually eats smaller meals. Further, he states that the amount of time between each meal is also likely to influence the outcome as someone who is hungry is likely to be more affected than is someone who is not hungry. This

reasoning could be extended to include metabolic rate, as someone with a fast metabolism, who is used to eating more frequently, would likely react differently than would someone with a slower metabolism. Nonetheless, metabolism is a variable not often accounted for within the literature (but see Smith et al., 1988) and is typically excluded from analyses. Although the present study failed to detect any group differences for females or for males with regards to the average amount of time between each meal or snack (i.e., the eating frequency variable), it cannot be concluded that this variable is unimportant. In the present study, participants were asked during the screening session how many hours typically pass between each meal or snack, which is admittedly an inaccurate measurement of metabolic rate, especially as participants reported guessing how many hours passed as they could not pin down the exact amount of time (on average) between each meal. This could be a promising area of investigation as metabolism would influence the digestion speed of the meals consumed such that someone with a faster metabolism may have a different window of opportunity in which to measure cognitive or affective changes than would someone with a slower metabolic rate.

Individual differences in neurotransmitter synthesis and activity would also presumably influence the effectiveness of tryptophan manipulation in that higher or lower baseline levels would result in greater or lesser amounts of depletion or augmentation. Along these lines, Steckler and Sahgal (1995) suggest that different and overlapping neurotransmitter systems, such as the cholinergic and serotonergic systems, exert different effects on cognitive processes and that manipulating availability of neurotransmitters in one system could influence the other. In other words, a

compensatory process could emerge with one system taking over the function(s) of the incapacitated system until normal functioning is reestablished. This could become even more apparent when individual differences in these systems get taken into account.

Lastly, results of the present study also suggest that demographic variables may influence results to some degree and on some tests, a finding echoing that of Spring and colleagues (1983). Unlike the finding by Sambeth and colleagues (2007), who stated that females carried the effect for results of tryptophan manipulation on (verbal) LTM, the present study only found results for males (on visuospatial LTM). With regards to other demographic variables, it seems that the present study is one of the first to include demographic variables in the analyses. Certain variables, such as ESL status, BMI, and eating frequency, were found to exert no influence on test results. However, eating frequency, as discussed earlier with regards to metabolic rate, was not a well-designed measure so the results from this analysis are not entirely conclusive. Level of physical activity, however, did demonstrate some influence on cognitive performance for the females. It is possible that if data had been collected on a larger sample of males, and an interval level scale used instead of an ordinal scale, more significant findings would have emerged. Future research should be sure to employ continuous measurements of physical activity, as well as metabolic rate and other key variables, in order to ensure more accurate analyses of factors potentially influencing the effect of tryptophan manipulation on cognitive and affective measures.

Strengths of the Current Study

This study has several notable strengths, the first of which is the inclusion of an augmented condition. The majority of the studies in this field of research employ only a

depletion condition and a control (or, balanced) condition (e.g., Coull et al., 1995; Park et al., 1994), whereas the present study included both a depleted and an augmented condition, in addition to a balanced condition, in order to facilitate greater understanding of any between-groups difference that could emerge. What is more, our stratification of participants allowed us to ensure that all three groups were comparable in terms of the age, education level, BMI, eating frequency, ESL status, and gender ratios of the participants. Had we failed to do so, we may have missed finding important differences between the Augmented and Balanced conditions on the Ruff 2 & 7 Speed task and between the Depleted and Balanced conditions on the Rey-O delay recall task. Thus, had other studies employed all three levels of manipulation, perhaps more findings would be present within the literature.

Another strength to the present study is that our test battery was a fairly comprehensive assessment of cognitive functioning, consisting of reliable and well-validated measures that are commonly used clinically. Our inclusion of a visuospatial measure of LTM, a task often omitted from the test battery of other researchers, enabled the detection of group differences between males in the Depleted and Balanced conditions.

A final strength to the present study, compared to many studies in the literature, pertains to the sample collected. We obtained roughly the same female to male ratio in each of the three meal conditions (approximately 2:1, respectively), whereas many past studies have included only male (e.g., Hughes et al., 2003; Park et al., 1994) or only female participants (e.g., Nabb & Benton, 2006). While it might be argued that the sample employed in the present study was too small, there are several findings within the

literature that support the sample size and gender split utilized. A mega-analysis by Sambeth and colleagues (2007) was conducted on nine studies employing acute tryptophan depletion in order to investigate the effects of tryptophan depletion on declarative LTM and the mediating variables of age, gender and serotonergic dysregulation. Their results indicate that tryptophan depletion results in lower scores on measures of delayed (and, to some degree, immediate) recall, but that the effect is larger for women. Further, they noted that gender was the only variable found to be a factor of serotonergic vulnerability. The difference in tryptophan levels between the depletion and control conditions was 9.90 for females (effect size = .60), but only 4.13 for males (effect size = .29). They calculated that the sample size required for a power of .8 (deemed a large effect size, according to Cohen, 1992) is 19 for females, but 74 for males. This female sample size is not altogether dissimilar from that collected in the present study (Augmented = 17 females; Balanced = 17 females; Depleted = 16 females; total female *n* = 50). Cohen (1992) states that for a three group ANOVA with a large effect size (.8) using alpha at .05, a total of 21 participants per group are required; this number is less than that collected in the present study, provided we collapse across gender (Augmented, *n* = 25; Balanced, *n* = 25; Depleted, *n* = 23) . If gender is considered (i.e., a six group ANOVA), a total of 14 participants per meal and gender group would be needed (Cohen, 1992). The present study has more than 14 participants in each meal condition (collapsed across gender), but admittedly fewer than 14 males per meal condition when gender is taken into account. There are, however, more than 14 females in each condition. Further, as discussed by Sambeth's group (2007), the effect of tryptophan manipulation is carried by females, not males, which suggests that our current female sample size is

likely adequate. This point is particularly relevant as the mega-analysis by Sambeth and colleagues (2007) only included studies that employed verbal measures of LTM. As the present study found effects for males on a measure of visuospatial LTM, our smaller male sample size is likely not an issue. Lastly, examination of the sample sizes of other between-subject studies within the literature that employed a dietary manipulation of tryptophan reveals that most contain cell sizes that are either comparable to, or much smaller than, those in the present study (for both males and females). For example, Vered (2001) reported cell sizes of between 4 and 6, Rogers and colleagues (1999) report cell sizes of between 7 and 8, and Markus's group (1998) reports cell sizes ranging from 6 to 18. Other, larger studies, such as Spring and colleagues (1982) do not explicitly state their cell sizes, only informing the reader that their 184 participants were split into 4 groups, that 129 were male, 55 were female, and that 81 were aged 18-36, whereas 103 were aged 40-65. Similarly, Lieberman's group (2002) collected a total sample size of 143 male participants randomly assigned to three groups, but does not indicate the exact number of participants per condition, leaving the reader to speculate that approximately 47 participants were in each condition. Lastly, Nabb and Benton (2006) report that they collected data on 189 female participants who were then split into 8 groups with between 23 and 25 participants each. Overall, these studies indicate that the sample size of the present study is at least comparable to, if not greater than, the standard reported within the literature which, when taken together with the findings of Sambeth's group and the established power analyses of Cohen (1992), indicate the adequacy of the sample in the present study. Thus, the sample size in this study may be considered a relative strength in comparison to many studies (particularly those employing a between-subjects design),

but also a weakness in terms of Cohen's established levels of adequate power (but only with regard to the males).

Regardless, what is of interest is that unlike Sambeth and colleagues' (2007) findings that females carry the effect for tryptophan depletion lowering verbal LTM, the present study found that males carried the effect for tryptophan depletion improving visuospatial LTM. This finding is perhaps due to gender effects showing that males are better at visuospatial tasks than are females (Burnett et al., 1982; Kaploun & Abeare, 2007; Lewis & Harris, 1990; Linn & Petersen, 1985; Peters et al., 1995) (although these findings largely disappear when degree of hemispheric lateralization is taken into account; e.g., Burnett, Lane & Dratt, 1982; Kaploun & Abeare, 2007; McGlone & Kertesz, 1973; McGlone, 1980). It is also possible that the smaller sample of males (compared to females in this study) made individual differences stand out more, allowing them to influence results more than would be the case with a larger sample of males. Lastly, it could be that these findings are the result of some peculiarity of the sample that cannot be accounted for and that may not likely be replicated by other researchers. Whatever the reason, future research should be sure to include equal numbers of men and women in their sample as this appears to be an important factor in influencing results.

Overall, these factors indicate that our investigation was a strong test of the hypothesis that dietary manipulation of tryptophan levels affects cognitive functioning and affective processes.

Limitations of the Current Study

There are, however, some limitations to this study. First, in deference to the within-subject design commonly found within the literature, the present study employed a

mixed design. Although such a design does introduce inter-individual variability that would otherwise be absent in a within-subject study, it does reduce the chances of test expectancy and repeated testing influencing results, as well as enabling the recruitment of larger sample sizes with lower attrition rates. This latter point is key as differences in the findings of previous studies and the present one could be due to sample size of the males. Thus, it could be that individual differences get disproportionately represented in small within-subject studies due to the lack of variability within the sample itself. This could be the reason for the results found for the PANAS PA and the trend in Excitement levels for males in the present study. This problem is avoided by mixed designs employing between-subject variables with larger samples.

Another limitation was that we were unable to draw blood assays in order to assess for alterations in free and total plasma levels of tryptophan, resulting in the inability to definitively discern whether or not the dietary manipulation successfully altered circulating tryptophan levels. As several other studies reported in the literature, however, have similarly failed to draw blood samples from participants (e.g., Kanarek & Swinney, 1990; Lieberman et al., 2002; Smith et al., 1988; Spring et al., 1983), it was not felt that this was a strong limitation. There are, in addition, several theoretical and practical reasons for rightfully excluding the use of blood assays. For instance, although sampling blood would enable us to determine whether the dietary manipulation successfully altered circulating tryptophan levels, it does not allow us to definitively determine whether the levels of tryptophan in the brain have been similarly altered. Based on this data, we can infer that a change has occurred, but we have no way of directly measuring brain tryptophan levels or the levels of its metabolites, including

serotonin. One means of measuring tryptophan metabolism is to measure levels of 5-HIAA (the end product of metabolized serotonin) in cerebral spinal fluid (CSF). It has been assumed that CSF levels of 5-HIAA indicate how much serotonin has been produced and released in that higher levels of CSF 5-HIAA would correlate with greater serotonergic activity. For example, in a study by Carpenter and colleagues (1998), a sample of 5 healthy participants (3 male, 2 female) underwent a tryptophan depletion protocol (amino acid drink) and then had their lumbar CSF sampled every 15 minutes over a 13.5 hour time span. The researchers utilized 5-HIAA as a measure of central serotonin levels, and collected blood samples to measure plasma levels. Despite the counterintuitive findings that a 92% drop in CSF 5-HIAA levels and an 85% decrease in blood tryptophan levels occurred, no behavioural or mood changes were found following tryptophan depletion. From these results, there can be no doubt that tryptophan levels changed (at least in free-circulating blood levels), but whether these changes lead to decreases in brain tryptophan or its metabolites, such as serotonin, is unclear. Further, it is unclear as to whether such changes in blood serum tryptophan levels are even related to CSF tryptophan levels. It has been found by several researchers (e.g., Perez-Cruet, Chase & Murphy, 1974; S. N. Young et al., 1976), however, that it is not CSF tryptophan levels, but rather free tryptophan blood serum levels, that correlate with CSF 5-HIAA. Together, these findings suggest that CSF 5-HIAA levels can only be used to indirectly estimate the degree of change in central nervous system tryptophan and serotonin levels (Carpenter et al., 1998; Degrell & Nagy, 1990; Nishizawa et al., 1997). What is more, Anderson and colleagues (1990) assert that since serotonin concentrations in CSF are extremely low and the current methods available for detecting subtle changes in central

(i.e., brain) CSF are imprecise, it is not clinically useful to assess central serotonin activity via CSF analysis. Furthermore, Garelis, Young, Lal and Sourket (1974) assert that lumbar CSF may include 5-HIAA from brain or spinal catabolism of serotonin, highlighting the fact that lumbar CSF may not be directly related to brain (i.e., ventricular) CSF. Urinanalysis of 5-HIAA levels has also been utilized to detect serotonergic production and release throughout the body. As discussed earlier, an adult male human body contains approximately 10 mg of serotonin; roughly 1-2% of that 10 mg is found within the brain (Sirek & Sirek, 1970). Thus, 5-HIAA in urine could be from tryptophan metabolism anywhere in the body, not just in the brain. Furthermore, 5-HIAA is usually only found in very small quantities in urine as larger amounts are indicative of carcinoid tumors (Feldman, 1986). Thus, it is clear that urinalysis of 5-HIAA is an inadequate means of measuring brain tryptophan or serotonin as there is no way to determine if the 5-HIAA is a result of central or peripheral tryptophan metabolism.

Overall, it should be clear that although blood assays would enable one to ensure whether or not a tryptophan manipulation actually altered serum levels of tryptophan within the circulating blood, it is an inadequate measure of brain tryptophan levels. Furthermore, there is no direct way to measure tryptophan or serotonin levels in the brain as neither lumbar nor ventricular tryptophan or serotonin levels can directly assess the availability of these substances in the brain. Taken together, these findings suggest that employing blood assays as a measure of tryptophan levels in the brain is not necessarily a requirement in this field of research as it is at best an indirect method of inference.

Is Dietary Manipulation An Effective Means of Measuring Tryptophan Manipulation?

Although the present study did not detect as many effects of tryptophan manipulation as would have been hoped, there are several reasons why it is felt that the dietary method of tryptophan manipulation is worth pursuing. As discussed earlier, it is a relatively inexpensive and a much more feasible means of manipulating tryptophan levels (compared to the amino acid protocol), enabling more researchers who do not have large research grants to implement this method. Purchasing fresh foods and bulk items is relatively economical, even for those with small research budgets. This fact alone should make it a much more appealing alternative to researchers looking to work within this field of study. Using different samples and designs, several researchers have shown that dietary methods of tryptophan manipulation do produce cognitive and affective changes in participants (e.g., Fischer et al., 2002; Lieberman et al., 2002; Markus et al., 1998; Smith et al., 1988). As more researchers employ this method, our knowledge about the effects of dietary manipulations will continue to increase which will help bring about some consensus within the field as to the appropriate amounts of each food, and other methodological and research issues concerning the effects of tryptophan upon cognition and affect.

Furthermore, it must be kept in mind that this line of research is still in its infancy. While it is true that the backbone of this field of study arguably started in 1948 with the isolation of serotonin, it has hopefully been made clear that no consensus has been reached as to the appropriate design and method of this type of research. At present, the literature is a veritable cornucopia of differing sample sizes and demographics, methodologies (dietary or amino acid mixture), designs (within-, between-, and mixed-

designs), and other possibly confounding variables that make it extraordinarily difficult to arrive at consistent, replicable, and generalizeable results. As more researchers pursue this line of study greater consensus will begin to emerge within the literature, which should also help bring us closer to a unified theory.

It must also be kept in mind that this line of research is incredibly complex. All research must take into account such variables as individual differences and other confounding factors, but within this field of study there are a myriad of other factors that could bear weight upon the outcome, such as inherent differences in macronutrient metabolism and catabolism, as well as the production and activity of resulting neurotransmitters. The interplay between different amino acids and macronutrients, as well as between different neurotransmitters, may also influence whether or not effects of the tryptophan manipulation are detected. In other words, the same manipulation may be much stronger for one participant than for another, even though all other variables are similar. For this reason, this field of study may be better suited to the examination of manipulation responders versus non-responders. By investigating these two groups separately, we may begin to get a better picture of what makes someone a responder versus as non-responder, as well as the ways in which they differ cognitively and affectively following tryptophan manipulation.

The importance of this line of research cannot be overstated. It is not just students or patients that would benefit from a greater understanding of the effects of tryptophan on neuropsychological performance. If consumption of everyday foods was found to result in significant effects on cognition or affect, then it is possible that in our daily lives we are inadvertently affecting our baseline level of functioning. What is more, we could

unknowingly be exacerbating existing conditions or conditions to which we are predisposed (e.g., depression). For instance, patients taking monoamine oxidase inhibitors (MAOIs) for the treatment of depression must limit foods high in tyramine, such as cheese, alcohol, and chocolate to prevent spikes in blood pressure that could lead to stroke (McCabe, 1986). While it is not being argued that tryptophan, found in the levels present in everyday food, exert the same effects as major antidepressant medications, it could be that even trace amounts of tryptophan could interact with medications taken for various conditions. It is possible that even small amounts of tryptophan or other macronutrients could be enough to produce, without our even being aware of it, variations in our day-to-day behaviour (including mood, cognition, and physiological or metabolic states). Maier and Watkins (2000) have proposed a similar idea regarding our immune systems, suggesting that since we are continuously coming into contact with unknown pathogens, variations in our spontaneous "normal" behaviour are caused by immune system responses. Further, they state that in response to immune system activation, serotonin is released in the hippocampus, highlighting the complexities of human neuropsychological function and the many factors that can influence them.

Overall, it should be clear that the complexities of this field of research make it difficult to boil down results to one simple set of findings. The ease and accessibility of employing a dietary manipulation of tryptophan in order to investigate the effects of tryptophan manipulation on cognitive and affective outcomes should make this field of study more appealing to researchers, and through increased research the complexities of this area of research will begin to unravel.

Summary and Future Directions

In conclusion, although no significant results were found for females, significant effects were found for males on the Rey-O delay recall, and the Ruff 2 & 7 Total Speed measure, as well as minor differences in PA and excitement levels over time. These findings are relatively unique within the literature as few studies have found significant results for PA (as opposed to NA), or for visuospatial LTM, especially for males. Although some caution must be exercised in comparing the results of the present study to those of within-subject studies, it is apparent that the current study found effects of tryptophan manipulation that, to the best our knowledge, have not yet been reported within the literature. Unlike Lieberman and colleagues (2002), the present study did not find that carbohydrates resulted in increased vigilance in males, nor did it result in greater sleepiness in women, or greater calmness in men (Spring et al., 1983). Furthermore, no between-subject studies employing a dietary method of tryptophan manipulation found any effect of tryptophan on visuospatial LTM or on tests of selected visual attention. Hoyland and colleagues (2008) bolster the belief of the author that the lack of consensus within the literature pertaining to the effects of tryptophan manipulation could at least in part be due to the variability of research designs and methods, with too few studies employing the same means of manipulating tryptophan or of measuring its effect. They also suggest that different foods likely produce cognitive effects along differing timelines, and thus it might be best if performance (on cognitive measures) was assessed on multiple occasions following the manipulation in order to assess differences in performance across time. Furthermore, there is a lack of consensus within the literature as to whether manipulating tryptophan levels in healthy participants, such as in the

present study and many others (e.g., Lieberman et al., 2002; Luciana et al., 2001; Riedel et al., 1999), leads to findings as robust as those seen in psychiatric populations (e.g., Benkelfat et al., 1994; LeMarquand et al., 1998). Thus, a combination of participant characteristics, sample size, method of manipulation and test sensitivity could all be responsible for the lack of consistent findings within this field of research.

Future directions for this field of study should include the analysis of tryptophan manipulation responsiveness (as suggested earlier), coding participants as either responders or non-responders. This idea had occurred to the author of the present study after completing data collection. However, as this was not an *a priori* analysis, it was discovered that the data from the present study do not lend themselves to coding participants as responders or not. For this type of analysis to be done, a greater range of scores must be available on the pre- and post-test measures used to assess the effectiveness of the manipulation. The PANAS and Digit Span Backwards scores did not provide the ranges required to discern not only whether someone responded to the manipulation, but also whether or not they responded in the predicted direction based on their meal condition. Responder analyses were attempted, but they were deemed meaningless as the categories for 'responder' or 'non-responder' were far too narrow, making it impossible to accurately determine whether a participant did in fact respond to the manipulation (in the manner predicted or not). Future studies should consider this analysis from the start of their design conception and should detail exactly how they will define and code for responsiveness in order to ensure an adequate range of scores is attainable.

Other, more technologically complex designs should also be considered for future research. For instance, PET studies employing the injection of radio-labelled tryptophan would also provide a wealth of information concerning whether exogenous doses of tryptophan actually increase brain tryptophan (and therefore serotonin production and release), and whether such changes are detectable via behavioural or affective output. In a study by Perreau-Linck and colleagues (2007) using PET, professional actors were instructed to self-induce a particular mood state (happy, sad or neutral, counterbalanced across 3 testing days) and were then injected with radio-labelled tryptophan (^{11}C-labelled α-methy-L-tryptophan, a synthetic version of L-tryptophan that crosses the blood-brain barrier and is used to estimate serotonin synthesis within the brain). Although no changes were detected in free or total venous tryptophan levels, the researchers found that serotonergic activity in the right anterior cingulate cortex was positively correlated with self-induced happiness, whereas a negative correlation was found between self-induced sadness and the subcallosal region of the right anterior cingulate cortex. These results indicate that even though measurable levels of free circulating tryptophan did not appear to change, changes to serotonin activity within the brain were detected. In a similar vein, Praschak-Reider and colleagues (2004) conducted a tryptophan depletion study using PET with 8 currently-remitted depressed patients. Although they measured a drop of 86% in total tryptophan plasma following tryptophan depletion, they failed to detect any changes in brain serotonin levels or activity. The tryptophan depletion did, however, induce a transient relapse of depressive symptoms in 6 of their 8 participants, indicating that small changes in brain serotonin levels are sufficient to induce short-term relapse in depressed patients normally being treated with

SSRIs. Taken together, these studies seem to suggest that even small changes in brain tryptophan or serotonin levels can lead to changes in activity in neuroanatomical structures known to be implicated in mood. Future studies should include a more comprehensive assessment of other neuropsychological functions, such as memory or attention. Hoyland and colleagues (2008) suggests that the following tests seem most consistently sensitive to macronutrient manipulation: the serial sevens task (a measure of working memory); free word recall with delay trials (verbal LTM); and cued word recall (verbal LTM). Thus, the inclusion of measures similar to these should be employed in PET studies.

As a follow-up to the present study, a mixed-design study should be employed with both between- and within-subject participants being recruited. Such a design will allow the direct comparison of these two methods to help determine which is the stronger design. This finding would guide researchers in deciding which design to utilize in future research. A larger sample size for both between- and within-subject samples, with equal gender ratios in each condition, should be obtained to further strengthen the findings of the present study and to prevent possible power issues from confounding results. The inclusion of additional sensitive measures, such as those suggested by Hoyland and colleagues (2008), should also be employed to provide the widest net for catching any between-group or between-condition effects of tryptophan manipulation on neuropsychological performance.

Overall, through future research that employs the above-discussed methods of measuring and assessing tryptophan and serotonin levels in the brain, a better

understanding of the effects of tryptophan manipulation on neuroanatomical, cognitive and affective functioning should begin to emerge.

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Appendix A

Glossary of Terms

CNS	Central Nervous System: the part of the nervous system comprised of the brain and spinal cord that coordinates activities of the body.
LNAAs	Large Neutral Amino Acids: a term used to describe amino acids that compete with each other for uptake into the brain and subsequent neurotransmitter synthesis.
5-HT	The chemical name for the neurotransmitter 5-hydroxytryptamine (aka: serotonin), the final end product of tryptophan metabolism.
5-HTP	The chemical name for 5-hydroxytryptophan, the amino acid metabolite of tryptophan and the precursor to serotonin (5-HT).
5-HIAA	The chemical name of 5-hydroxyindoleacetic acid, the primary metabolite of serotonin (5-HT) that is excreted in the urine.
Depletion	The term used to denote acute tryptophan depletion produced either via the amino acid protocol drink, excluding the amino acid tryptophan, or via dietary manipulations (i.e., protein-loading).
Augmentation	The term used to denote acute tryptophan augmentation produced either via the amino acid protocol drink, with the inclusion of increased amounts of the amino acid tryptopahn, or via dietary manipulations (i.e., carbohydrate-loading).
Balanced	The term used to denote the balanced condition between acute tryptophan depletion and acute tryptophan augmentation, produced

	either via the amino acid protocol drink, wherein the proportion of LNAAs produces an equal effect as the amount of tryptophan on plasma levels, or via dietary manipulations (i.e., the carbohydrate:protein ratio produces an even balance between LNAAs and tryptophan).
BMI	Body Mass Index: a measure of body fat based on weight and height.

Appendix B

Study Advertisement (Participant Pool)

Participant Pool Recruitment Statement – Effects of Food on Cognition

Hello,

My name is Kristen Kaploun and I am a PhD Candidate in the Clinical Neuropsychology program here at that University of Windsor. I am contacting you in the hopes that you are interested in taking part in my research study examining the effect of food on cognition. This is a two-part study, requiring participants to first take part in a screening session; it is this session for which I am now recruiting participants.

The purpose of this screen is to determine your eligibility for inclusion in the study entitled, "The Effects of Food on Cognition". This screening is completely non-invasive. If you volunteer to participate, you will be asked to come into the laboratory for approximately 30 minutes in order to complete a screening interview. During the interview, you will be asked to provide information about your medical history and general health, as well as other demographic criteria (e.g., age, gender, program of study). The screen is worth 0.5 credits. If you are deemed eligible to continue, and you wish to do so, a separate appointment will be set up for you to come in and take part in the testing session. The testing session takes approximately 5.5 hours, during which time you receive breakfast, lunch and two snacks. The testing session is worth 5.5 credits and the chance to win one of two $50 gift cards (more information will be provided about the study during the screening session).

If you are interested in taking part, please email me at: hodgesk@uwindsor.ca and we can set up an appointment. The screening will take place in the basement of Chrysler Hall South, Room 73.

I thank you in advance and I look forward to hearing from you.

Appendix C

SCREENER

Effects of Food on Cognition

***Are you allergic to peanuts?** ☐ No ☐ Yes

If Yes, please leave now _____

Date: _____

Age: _____

Sex: _____ ESL?: _____

Weight: _____

Height: _____

BMI: _____ (BMI category: _____)

- Underweight = <18.5 (less than 17.5 = anorexia)
- **Normal weight = 18.5 - 24.9**
- Overweight = 25 - 29.9
- Obesity = BMI of 30 or greater

Do you have any food allergies? ☐ No ☐ Yes

If Yes: _____

Chronic illnesses? (incl. Crohn's disease, celiac disease, IBS, lactose intolerance)

- _____
- _____
- _____
- _____
- Do you have diabetes? ☐ No ☐ Yes If 'Yes', Type I or II ?

Current illnesses?

- _____
- _____
- _____

- _____

Current medications? (incl. birth control, vitamins, herbal supplements, allergy or asthma meds)

- _____
- _____
- _____
- _____

Exclusionary: SSRIs, MAOIs, narcotics, anti-psychotics, anti-depressants, anxiolytics (anti-anxiety)

Do you use any recreational drugs?

☐ No ☐ Yes If Yes: _____

Hallucinogens: PCP, angel dust, ketamine, Special K, LSD, acid, MDMA/methamphetamine**,** ecstasy, mescaline, peyote, psilocybin (mushrooms)

Frequency**:** _____ Amount: _____

Pain Killers: Codeine, opium, heroin, morphine, smack, Vicodin, methadone, Dilaudid, Oxycodone/Oxycontin, Percodan

Frequency**:** _____ Amount: _____

Tranquilizers: Benzodiazepines, Valium, Ativan, Diazepam, pentobarbital, amobarbital, Seconal, roofies, Nembutal, barbituates, inhalants, alcohol

Frequency**:** _____ Amount: _____

Stimulants: Cocaine, coke, crack, amphetamines, methamphetamines, speed, crystal meth, ice, crank, Ritalin, tobacco, caffeine

Frequency**:** _____ Amount: _____

Marijuana: Marijuana, pot, weed, hashish, hashish oil

Frequency**:** _____ Amount: _____

Steroids: Any kind: _____

Frequency**:** _____ Amount: _____

Any prescription? ☐ No ☐ Yes Yes: _____

Do you smoke? ☐ No ☐ Yes

If Yes: How many per day?_____ How long have you smoked? _____

Is your diet irregular? ☐ No

☐ Yes: _____

Does your weight tend to fluctuate? ☐ No ☐ Yes Range: _____

Have you ever / do you ever binge eat? ☐ No ☐ Yes When last?_____

Have you ever been diagnosed with: Anorexia Nervosa ☐ No ☐ Yes
Bulimia ☐ No ☐ Yes

Do you usually eat breakfast? ☐ No ☐ Yes

Do you usually eat lunch? ☐ No ☐ Yes

Athleticism: ☐ Very (daily) ☐ Quite (3-5 time/wk) ☐ Minimal (e.g., walk to school) ☐ Not at all (e.g., drive to school)

Metabolic Rate: How often do you eat during a normal day (every X hours): _____

Do you eat more than most people at each meal? ☐ No ☐ Yes

What types of foods do you crave the most? _____

Current Depression? ☐ No

☐ Yes:_____

Current Anxiety? ☐ No

☐ Yes:_____

Family history of mental illness? (include who has what disorder)

- _____
- _____
- _____
- _____

History of stroke, seizures or other neurological impairments/injuries? (incl. age of injury and most recent event)

- _____
- _____
- _____
- _____

How many hours of sleep do you get in a typical night? _____

Do you feel rested upon waking? _____

How much caffeine do you typically consume in a day?

☐ Pop: _____ ☐ Coffee: _____ ☐ Decaf Coffee: _____

☐ Decaf Pop: _____ ☐ Tea: _____ ☐ Decaf Tea: _____

☐ Chocolate: _____ ☐ Hot Chocolate: _____

☐ Energy Drinks: _____ ☐ Other: _____

Do you ever take caffeine pills? _____

Other:

Are you pregnant? ☐ No ☐ Yes

Stage of menstrual cycle (females only): Last day: _____

First day: _____

General

How did you find out about this study? _____

- Recruitment method (circle one): Participant Pool Poster

What motivated you to take part in this study?

_____

Are you a student? ☐ No ☐ Yes

If 'Yes', what is your Major? _____

- **What year are you currently in? _____**

If 'No', what kind of work do you do? _____

Appendix D

Study Advertisement (Community Sample)

INTERESTED IN THE EFFECTS OF FOOD ON COGNITION?

Women and men 17 years or older are invited to participate in a study exploring the effects of food on cognitive performance.

Participation is voluntary and confidential.
Participants will receive breakfast, lunch and two snacks, as well as the chance to win 1 of 2 gift cards valued at $50 for a total of 6 hours of their time (30 minutes for the pre-screening, and 5.5 hours for the testing session).

This study is being conducted by Kristen Kaploun, M.A., a clinical neuropsychology doctoral student at the University of Windsor, and her research advisor, Dr. Chris Abeare, Ph.D. The research study has received ethics clearance from the University of Windsor Research Ethics Board.

For further information about this study, please contact Kristen at hodgesk@uwindsor.ca.

Contact Kristen at: hodgesk@uwindsor.ca	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**	**Contact Kristen at: hodgesk@uwindsor.ca**

Appendix E – Screener Consent Form

CONSENT TO PARTICIPATE IN SCREEN

To determine eligibility for participation in the study entitled, "The Effects of Food on Cognition"

Title of Study: The Effects of Food on Cognition

You are asked to participate in a research screen for a study conducted by Kristen Kaploun, MA (student researcher) and Dr. Chris Abeare, PhD (faculty supervisor), from the Psychology Department at the University of Windsor. This screen comprises a part of Kristen Kaploun's PhD Dissertation.

If you have any questions or concerns about the research, please feel free to contact Kristen Kaploun at hodgesk@uwindsor.ca, or Dr. Chris Abeare at 519-253-3000, ext. 2231.

PURPOSE OF THE STUDY

The purpose of this screen is to determine your eligibility for participation in the study, "The Effects of Food on Cognition".

PROCEDURES

If you volunteer to participate in this screen, we would ask you to do the following things:

Screening Procedures:

Upon signing up for the study, you will be asked to come into the laboratory. Upon providing informed consent, you will be asked to complete an initial screening interview. You will be asked you will be asked to provide information about your medical history and general health, as well as other demographic criteria (e.g., age, gender, program of study). If you are deemed eligible to participate in the study and you wish to do so, a separate appointment will be made for you to return to the lab to take part in the study. The screening session should last approximately 30 minutes and will take place one-on-one with the examiner in the basement of Chrysler Hall South, Room 73. It is worth 0.5 credits.

Exclusionary Criteria:

Participants will be excluded if they meet criteria for any of the following:

- Obesity, Anorexia, or Bulimia (other eating disorders)
- Diabetes
- Food allergies
- Lactose intolerance
- Irritable Bowel Syndrome (IBS)

- Crohn's disease
- Celiac disease
- Other chronic illnesses (incl. Endocrine and metabolic disorders)
- Current depression
- Current anxiety
- Illicit drug use (e.g., marijuana, narcotics)
- Stimulants (incl. more than the equivalent of 5 cups of coffee/day, or 1 package of cigarettes/day)
- Current medication use (incl. SSRIs, MAOIs, psychotropic medications, cold medications such as Nyquil)
- History of neurological disorders/impairments

POTENTIAL RISKS AND DISCOMFORTS

All information provided is completely confidential. However, it is possible that by participating in this screen, you may experience discomfort discussing medical and health matters. If so, you may elect to skip certain questions and still remain in the screen.

If by taking part in this screener you experience certain negative feelings, such as anxiety, sadness or worry, and you would like to talk to someone about these feelings, here is a list of local resources that we encourage you to contact:

The Student Counseling Centre	**CAW Student Centre Room 293 2nd Floor (519) 253-3000 Ext. 46160 Email: scc@uwindsor.ca**
Windsor Regional Hospital Mood and Anxiety Clinic	**(519) 257-5125**
Windsor Mood Disorders Self-Help Group	**(519) 979-5089**
Community Crisis Centre	**(519) 973-4435**

POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

By participating in this screen, you will learn about the different variables that may influence the effects of meal composition on cognitive performance. If you are deemed eligible to take part in the study upon completion of the screen, you will learn more specific information regarding the impact of food choices on cognition. The scientific community, society and those involved in helping patients who experience transient or occasional cognitive, affective, or physiological problems could benefit from a greater understanding of role played by various food components on mood and cognition.

PAYMENT FOR PARTICIPATION

As a participant, you will earn 0.5 credits for participating in this screen. If you are deemed eligible for taking part in the testing session and you wish to do so, you will be able to earn up to an additional (and maximum of) 5.5 credits for completing the study (please note that credits cannot be "banked" from one semester to another). If you are deemed eligible to take part in the testing session, more information will be provided about the procedures, at which point you may decide if you want to take part. Even if you chose to **not** take part in the testing session (i.e., upon hearing the details it is not of interest to you), you will still earn the 0.5 credits for taking part in the screening session.

CONFIDENTIALITY

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your written permission. Your identity will be known to the researcher for the purposes of scheduling and compensation only and any information collected will be kept in file cabinets and locked up in the laboratory. Upon entering the lab for the study screen, you will be assigned an ID number. This number is kept on a master key alongside your name and contact information. All data that is subsequently collected will be identifiable by ID number only. All data will be stored in cabinets and locked up in the laboratory; the master key, which will be stored separately from all data, will also remain locked in the laboratory. Data will be retained, locked away in this fashion, for at least two (2) years until the study is completed and accepted for publication.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this screen or not. If you volunteer to be in this screen, you may withdraw at any time. However, please note that if you decide to withdraw from the screen, you will only be compensated for the time in which you participated (i.e., a maximum of 0.5 credits). The investigator may withdraw you from this research if circumstances arise which warrant doing so (for example, if you fail to follow test instructions, or if it is deemed unsafe for you to continue to participate). As a participant in this study, you will have the option of removing your screening data from the study.

FEEDBACK OF THE RESULTS OF THIS STUDY TO THE SUBJECTS

Participants will have the option of learning the results of the study (not the screen) simply by contacting the researcher. Those who are ineligible for participating in the study will be notified and provided with the reason for their exclusion. Participants who complete the study may also log onto the REB website and read the results of this study by selecting it from the available list.

Web address: http://www.uwindsor.ca/reb
Date when results are available: April 30, 2011

SUBSEQUENT USE OF DATA

This data will be used in subsequent studies.

RIGHTS OF RESEARCH SUBJECTS

You may withdraw your consent at any time and discontinue participation without penalty. If you have questions regarding your rights as a research subject, contact: Research Ethics Coordinator, University of Windsor, Windsor, Ontario, N9B 3P4; Telephone: 519-253-3000, ext. 3948; e-mail: ethics@uwindsor.ca

SIGNATURE OF RESEARCH SUBJECT/LEGAL REPRESENTATIVE

I understand the information provided for the screen for the study “The Effects of Food on Cognition” as described herein. My questions have been answered to my satisfaction, and I agree to participate in this screen. I have been given a copy of this form.

_____
Name of Subject

_____ _____
Signature of Subject Date

SIGNATURE OF INVESTIGATOR

These are the terms under which I will conduct research.

_____ _____
Signature of Investigator Date

Appendix F – Testing Session Consent Form

CONSENT TO PARTICIPATE IN RESEARCH – TESTING SESSION

Title of Study: The Effects of Food on Cognition

You are asked to participate in a research study conducted by Kristen Kaploun, MA (student researcher) and Dr. Chris Abeare, PhD (faculty supervisor), from the Psychology Department at the University of Windsor. This study comprises Kristen Kaploun's PhD Dissertation.

If you have any questions or concerns about the research, please feel free to contact Kristen Kaploun at hodgesk@uwindsor.ca, or Dr. Chris Abeare at 519-253-3000, ext. 2231.

PURPOSE OF THE STUDY

The purpose of this study is to determine the effects of different foods on cognitive performance.

PROCEDURES

If you volunteer to participate in this study, we would ask you to do the following things:

Testing Procedures:

On the day of testing, informed consent will be obtained along with a short intake survey. You will have been randomly assigned to one of three conditions, each of which receive a different set of meals. Upon completing the intake survey you will be provided with breakfast; lunch, and two snacks will also be provided before the end of the testing session. Please note that you will **not** have the option of refusing to eat some or all of the food provided during this study. If you do not wish to eat some or all of the food that is provided, you are free to withdraw at any time. If you chose to exercise this right, please note that you will only be compensated for the time in which you participated (i.e., you will not receive the full 5.5 credits; please see 'Participation and Withdrawal' section below for further details). One and a half hours following the provision of lunch, you will complete the test battery, comprised of several paper-and-pencil measures aimed at assessing various cognitive functions (e.g., attention). Indices of mood and affect will also be administered upon arrival at the laboratory on the day of testing, after each meal, and upon completion of the test battery.

The total length of time for participation will be approximately 5.5 hrs done in one sitting (the actual testing session will last approximately 1.5 hours). During the waiting period, you must remain in the waiting room (save for washroom breaks). You will be allowed to study, read, watch a movie on the computer or play a board game; you will not, however, be permitted to eat or sleep. Water will be provided upon request. This study will take place one-on-one with the examiner in the basement of Chrysler Hall South, Room 73 (the same room as the screening interview).

Exclusionary Criteria:

Participants will be excluded if they meet criteria for any of the following:

- Obesity, Anorexia, or Bulimia (other eating disorders)
- Diabetes
- Food allergies
- Lactose intolerance
- Irritable Bowel Syndrome (IBS)
- Crohn's disease
- Celiac disease
- Other chronic illnesses (incl. Endocrine and metabolic disorders)
- Current depression
- Current anxiety
- Illicit drug use (e.g., marijuana, narcotics)
- Stimulants (incl. more than the equivalent of 5 cups of coffee/day, or 1 package of cigarettes/day)
- Current medication use (incl. SSRIs, MAOIs, psychotropic medications, cold medications such as Nyquil)
- History of neurological disorders/impairments

POTENTIAL RISKS AND DISCOMFORTS

It is possible that by participating in this study, you may experience a mild alteration of your mood (e.g., mild irritation or lowering of mood). Any such effects, if experienced at all, will be mild and transient, consistent with what might occur had you skipped breakfast or lunch. The snack provided upon completing the testing will help return your mood to baseline levels should any deviation occur.

If by taking part in this study you experience certain negative feelings, such as anxiety, sadness or worry, and you would like to talk to someone about these feelings, here is a list of local resources that we encourage you to contact:

The Student Counseling Centre	**CAW Student Centre Room 293 2nd Floor (519) 253-3000 Ext. 46160 Email: scc@uwindsor.ca**
Windsor Regional Hospital Mood and Anxiety Clinic	**(519) 257-5125**
Windsor Mood Disorders Self-Help Group	**(519) 979-5089**
Community Crisis Centre	**(519) 973-4435**

POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

You will learn about the effects of meal composition on mood and cognitive performance. The scientific community, society and those involved in helping patients who experience transient or occasional cognitive, affective, or physiological problems could benefit from a greater understanding of role played by various food components on mood and cognition.

PAYMENT FOR PARTICIPATION

Participants will earn up to a maximum 5.5 course credits for participating in, and completing, this study (please note that credits cannot be "banked" from one semester to another). The screening session (completed previously) is worth 0.5 credits. Thus, by fully completing this study, participants can earn up to a total of 6 credits (0.5 for the screening session, and 5.5 for the testing session). Completion of the study will also deem the participant eligible for inclusion (if s/he so wishes) in a draw to win 1 of 2 gift cards valued at $50 CAD to either Devonshire Mall or Future Shop. Please refer below ("Participation and Withdrawal") for information regarding withdrawing from this study.

CONFIDENTIALITY

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your written permission. Your identity will be known to the researcher for the purposes of scheduling and compensation only and any information collected will be kept in file cabinets and locked up in the laboratory. Upon entering the lab for the study screen, you were assigned an ID number. This number is kept on a master key alongside your name and contact information. All data that is subsequently collected will be identifiable by ID number only. All data will be stored in cabinets and locked up in the laboratory; the master key, which will be stored separately from all data, will also remain locked in the laboratory. Data will be retained, locked away in this fashion, for at least two (2) years until the study is completed and accepted for publication.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time. However, please note that if you decide to withdraw from the study, you will only be compensated for the time in which you participated. In other words, if you completed 2 hours of the study, you will receive 2 credits; if you completed 4 hours, you will receive 4 credits. Also, choosing not to complete the testing session will deem you ineligible for inclusion in the draw to win 1 of 2 gift cards valued at $50 CAD to either Devonshire Mall or Future Shop. However, as a participant in this study, you may refuse to answer any questions you do not want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so (for example, if you fail to follow test instructions, or if it is deemed unsafe for you to continue to participate). Lastly, in the event that you choose to withdraw from the study, *you will still receive the credits you earned for participating in the screen* (i.e., you will not be docked previously earned points). As an example, if you withdrew from the study after 2 hours, you would have earned a total of 2.5 credits – 2 for the study, and 0.5 for the screener completed previously. As a participant in this study, you will have the option of removing your data from the study.

FEEDBACK OF THE RESULTS OF THIS STUDY TO THE SUBJECTS

Participants will have the option of learning the results of the study simply by contacting the researcher. They may also log onto the REB website and read the results of this study by selecting it from the available list.

Web address: http://www.uwindsor.ca/reb
Date when results are available: April 30, 2011

SUBSEQUENT USE OF DATA

This data will be used in subsequent studies.

RIGHTS OF RESEARCH SUBJECTS

You may withdraw your consent at any time and discontinue participation without penalty. If you have questions regarding your rights as a research subject, contact: Research Ethics Coordinator, University of Windsor, Windsor, Ontario, N9B 3P4; Telephone: 519-253-3000, ext. 3948; e-mail: ethics@uwindsor.ca

SIGNATURE OF RESEARCH SUBJECT/LEGAL REPRESENTATIVE

I understand the information provided for the study "The Effects of Food on Cognition" as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____
Name of Subject

_____ _____
Signature of Subject Date

SIGNATURE OF INVESTIGATOR

These are the terms under which I will conduct research.

_____ _____
Signature of Investigator Date

Appendix G

Composition of tryptophan augmented (ATA), tryptophan depleted (ATD), and balanced (B) meals and snacks.

Augmentation Meal

Breakfast

Bread – 1 slice (26 g)
Low-fat margarine – 1 tbsp (14 g)
Grape jam – 1 oz (28 g)
Tea – black decaf – 1 cup (8 fl oz) (237 g)
Sugar (in tea) – 2 tsp (4 g)
Grape juice – 1 cup (253 g)

Snack

Black coffee (decaf) – 1 cup (8 fl oz) (237 g)
Sugar (in coffee) – 2 tsp (4 g)
3 Musketeer candy bar – 1 2.13 oz bar (60 g)

Lunch

Bread – 1 slice (26 g)
Low-fat margarine – 1 tbsp (14 g)
Grape jam – 1 oz (28 g)
Grape juice – 1 cup (253 g)

Depletion Meal

Breakfast

Bread – 2 slices (26 g each)
Low-fat margarine – 1 tbsp (14 g)
Cheese – low-fat mozzarella – 1 oz (28 g)
Tea – black decaf – 1 cup (8 fl oz) (237 g)
Milk (in tea) – whole milk – 1 oz (28 g)

Snack

Coffee – decaf – 1 cup (8 fl oz) (237 g)
Milk (in coffee) – 1% milk – 1 oz (28 g)
Peanuts – roasted with salt – 1 oz (28 g)

Lunch

Bread – 2 slices (26 g each)
Low-fat margarine – 1 tbsp (14 g)
Roast beef – lean – 1 oz (28 g)

Balanced Meal

Breakfast

Bread – 1 slices (26 g each)
Butter – salted – 1 pat (5 g)
Grape jam – 2 oz (28 g each)
Tea – black decaf – 1 cup (8 fl oz) (237 g)
Sugar (in tea) – 2 tsp (4 g each)

Snack

Black coffee (decaf) – 1 cup (8 fl oz) (237 g)
Sugar (in coffee) – 2 tsp (4 g)
Milk – 1% milk – 1 oz (28 g)
2 fun-size "3 Musketeers" candy bars - (28 g)

Lunch

Bread – 2 slices (26 g each)
Peanut Butter (unsalted) – 1 oz (28 g)
Grape jam – 1 oz (28 g)
Grape juice – 1 cup (253 g)

Final Snack for all groups:

Yogurt cup
Pineapple fruit cup

All meals were modeled after those used by Markus and colleagues (1998). They were created and analyzed using the NutritionData website (NutritionData, 2009). RDA values based on a 2,000 calorie diet for males and females aged 4 and over as indicated by the Canada Food Inspection Agency (http://www.inspection.gc.ca/english/fssa/labeti/guide/ch6e.shtml#a6_3).

VITA AUCTORIS

NAME:	Kristen A. Kaploun
PLACE OF BIRTH	Toronto, Ontario
YEAR OF BIRTH	1982
EDUCATION	Lawrence Park Collegiate Institute, Toronto 1996-2001 University of Guelph, Guelph, Ontario 2001-2005 B.A. Honours University of Windsor, Windsor, Ontario 2005-2007 M.A. University of Windsor, Windsor, Ontario 2007-2010 Ph. D.