The Effects of Serotonin versus Catecholamine Depletion on Emotional Processing: An Event-Related Potential Study on the International Affective Picture System

Clementine Thurgood, BA (Psychology & Psychophysiology)

Submitted in partial fulfillment of the requirements for the degree of Bachelor of Arts with Honours, Psychology Strand
Swinburne University of Technology
14th October, 2005

Supervisors: Associate Professors Rodney Croft and Pradeep Nathan, and Doctor Greg Murray
Student ID: 402835X
Word Count: 1140
Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td>(vi)</td>
</tr>
<tr>
<td>Acknowledgments</td>
<td>(vii)</td>
</tr>
<tr>
<td>Abstract</td>
<td>(viii)</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td></td>
</tr>
<tr>
<td>1.1 Overview</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Emotion</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Biases in emotional processing</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Emotional processing in depression</td>
<td>6</td>
</tr>
<tr>
<td>1.5 The role of neurotransmitters in emotional processing</td>
<td>8</td>
</tr>
<tr>
<td>1.5.1 Neurotransmitter precursor depletion techniques</td>
<td>9</td>
</tr>
<tr>
<td>1.5.2 The effects of serotonin manipulation on emotional processing</td>
<td>11</td>
</tr>
<tr>
<td>1.5.3 The effects of dopamine manipulation on emotional processing</td>
<td>15</td>
</tr>
<tr>
<td>1.5.4 The effects of simultaneous manipulation of serotonin and dopamine on emotional processing</td>
<td>16</td>
</tr>
<tr>
<td>1.6 Event-related potentials and attentional biases</td>
<td>17</td>
</tr>
<tr>
<td>1.7 Research aims</td>
<td>25</td>
</tr>
</tbody>
</table>
1.8 Hypotheses 25

Chapter 2: Method

2.1 Participants 27

2.2 Design 27

2.2.1 Amino acid mixture 28

2.3 Materials 29

2.3.1 Visual analogue mood scale 29

2.3.2 International affective picture system 30

2.4 Procedure 31

2.5 Data acquisition 33

2.6 Data analysis 34

Chapter 3: Results

3.1 Principal components analysis for the event-related potentials 35

3.2 Data screening for the event-related potentials and the visual analogue mood scale 36

3.3 Event-related potentials 37

3.4 The visual analogue mood scale 39

Chapter 4: Discussion

4.1 Overview of aims and findings 42
4.2 Early emotional processing 44
4.2.1 The effects of neurotransmitter depletion on early emotional processing 49
4.3 Mood 53
4.4 Limitations and directions for future research 55
4.5 Implications and conclusions 58

References 60

Appendix A  Copy of information and consent forms 76
Appendix B  Copy of the low protein diet 80
Appendix C  Copy of the international affective picture system stimulus codes 83
Appendix D  Copy of the visual analogue mood scale I 85
Appendix E  Copy of the visual analogue mood scale II 87
List of Tables

Table 1: Means and standard deviations for the ERP amplitudes to emotional stimuli in each drug condition  

Table 2: Means and standard deviations for quick witted-mentally slow, happy-sad, and amicable-antagonistic
Declaration

I declare that this report does not incorporate without acknowledgement any material previously submitted for a degree in any University, College of Advanced Education, or other educational institution, and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

I further declare that the ethical principles and procedures specified in the Faculty of Life and Social Sciences Human Research Ethics Committee document have been adhered to in the preparation of this report.

Name: Clementine Thurgood

Signed:
Acknowledgments

Firstly I would like to thank my supervisors Rodney Croft, Pradeep Nathan, and Greg Murray for their assistance with this project. Thank you all for being so approachable and for keeping me on track. Special thanks to Rodney for his assistance with the event-related potentials data analysis. I wouldn’t have known where to begin without his help!

Next I would like to acknowledge that this study was part of a larger amino acid depletion study. Thank you to Alan Dunne, Sumie Leung, Valerie Guille, and Kirsty Scholes for their contributions to the project.

To my parents, Ken and Dianne, and sister Madeleine for providing support and tolerating me this year!

To my friends who have been so supportive and encouraging. To the girls at Silver Moon for covering my shifts every time I left something to the last minute, and also for their genuine support and interest all along. I really appreciate it. And also thank you to John.
Abstract

This study examined the effects of serotonin, catecholamine, and combined serotonin and catecholamine depletion on attentional biases associated with emotional processing. The sample comprised ten healthy male participants aged between 22 and 42 years. Depletion, or reduction of neurotransmitter function, was achieved by administering an amino acid mixture that was lacking in the precursor necessary for the production of the corresponding neurotransmitter. Emotional processing was assessed by the early attention event-related potential component, the P100, to photographs from the International Affective Picture System. Mood was rated on the quick witted-mentally slow, happy-sad, and amicable-antagonistic scales of the Visual Analogue Mood Scale. Unexpectedly, the amplitude of the P100 was larger for positive stimuli than for negative or neutral stimuli. Also contrary to expectations, there were no differences among treatment conditions in terms of the P100 to any of the emotional stimuli. Consistent with expectations, serotonin depletion and catecholamine depletion did not have any effects on subjective ratings of mood. In contrast to expectations, however, combined serotonin and catecholamine depletion failed to have any effect on mood. These findings suggest that the neurotransmitters serotonin and dopamine do not affect attentional bias in the processing of emotional stimuli.
Chapter 1: Introduction

1.1 Overview

The affective disorder depression is now one of the most debilitating illnesses in Australia (Mathers, Vos, & Stevenson, 1999). Contributing to both the onset and maintenance of the depressed mood is the existence of a negativity bias in emotional processing (Beck, Rush, Shaw, & Emery, 1979). That is, depressed individuals are believed to selectively direct their information processing towards negative events (Beck et al., 1979). It is believed that disturbances in neurotransmitter function, in particular serotonin and dopamine, are responsible in part for the emotional processing deficits observed (Delgado et al., 1990).

The neurotransmitters serotonin and dopamine can be manipulated experimentally in order to increase or decrease their function. With a greater understanding of the neurotransmitters involved in emotional processing, we are able to come closer to identifying the mechanisms behind disorders such as depression.

In terms of the negative bias that is often observed in depression, psychological theories of depression contend that there is an attentional bias whereby attention is selectively directed towards unpleasant events (Beck et
al., 1979). The early event-related potential component, the P100 provides
the means by which processes such as early attention can be assessed
(Smith, Cacioppo, Larsen, & Chartrand, 2003). To date there have been no
studies that have examined early attentional biases in terms of the P100 in
depressed samples, or in samples undergoing neurotransmitter depletion.
Studies of the P100 in healthy controls have shown greater P100 amplitudes
to negative stimuli than positive stimuli (Smith et al., 2003, Delplanque,
Lavoie, Hot, Silvert, Sequeira, 2004).

This thesis will present a review of the literature surrounding the
negativity bias in emotional processing in healthy individuals and in
depressed patients. It will then describe the neurotransmitter abnormalities
associated with depression and their role in emotional processing. Next a
review of studies that have manipulated neurotransmitter levels in healthy
participants will be covered. Lastly, studies that have investigated the
negativity bias in terms of early attention will be reviewed.

1.2 Emotion

Because of the difficulty in defining an unobservable construct such
as emotion, much research has focused on emotional behaviours rather than
the actual experience of emotion itself. In fact, Lang (1995) proposed that
emotions are actually action dispositions that can be simplified into the two
dimensions of valence and arousal (Lang, 1995). Valence, or the degree of pleasantness versus unpleasantness, can be subdivided into appetitive and aversive systems that reflect the behavioural dispositions of approach and avoidance, respectively (Lang, 1995; Scherer & Peper, 2001). Arousal, the second dimension of the model, is reflected by the degree to which either system is activated, ranging from passive to active (Lane et al., 1999; Lang, 1995; Scherer & Peper, 2001).

As both resources and dangers are found unpredictably in the environment (Schupp, Junghofer, Weike, & Hamm 2003), individuals need to effectively extract clues that are of emotional significance. Hence, emotional processing, the perception and evaluation of emotional stimuli, is useful for ensuring the survival of the species by directing immediate and appropriate responses to emotionally salient stimuli (Sato, Kochiyama, Yoshikawa, & Matsumura, 2001).

1.3 Biases in emotional processing

Behavioural studies have provided evidence that emotional material receives prioritised processing over neutral material (Ohman, Flykt, & Esteves, 2001a; Purtois, Grandjean, Sander, & Vuilleumier, 2004). Furthermore, it seems that positive material and negative material also differ with respect to the degree of processing received. While the detection of
both resources and dangers is necessary for the benefits of both exploratory and self-preservative behaviour, respectively, the majority of research has emphasized the importance of detecting and responding to negative stimuli (Cacioppo, Larsen, Smith, & Berntson, 2004). It is proposed that negative events elicit stronger and more rapid responses than positive stimuli. That is, humans are said to possess a ‘negativity bias.’ This negativity bias is often measured in terms of speed of reaction time. Both photos and schematic representations of angry faces, for example, have been found to ‘pop-out’ of crowds of neutral or happy faces (Hansen & Hansen, 1988; Ohman, Lundqvist, & Esteves, 2001b). Likewise, pictures of snakes and spiders have been detected faster than pictures of flowers or mushrooms (Ohman et al., 2001a).

It is important to acknowledge that these studies have involved highly arousing, threat-related negative stimuli. This threat advantage is a likely result of the fact that fearful stimuli activate the amygdala to a greater extent even than highly arousing positive stimuli (LeDoux, 1995). The amygdala is a structure involved primarily in responding to aversive events and it has extensive connections with the visual cortex, an area involved in attention (LeDoux, 1995). In such instances the negativity bias observed may in fact be as a result of the arousing nature of the stimuli as opposed to the actual negative valence itself. In Hansen and Hansen’s (1988) study, for
instance, while angry faces popped out of crowds, sad faces did not. Perhaps the sad faces were not sufficiently arousing to activate the amygdala to the extent of the angry faces.

To overcome limitations associated with the use of threatening stimuli, other, less arousing negative stimuli have also been studied in relation to the negativity bias. The Stroop task is a popular paradigm for examining biases in information processing (Pratto & John, 1991). In this task, participants are presented with a series of stimuli and are asked to name aloud the colour of the ink of the stimuli. Stroop studies involving emotional words have found longer colour-naming latencies for negative emotional words than for positive or neutral words, suggesting participants were automatically directing their attention the task-irrelevant negative word content (Pratto & John, 1991; Wentura, Rothermund, & Bak, 2000). Also, during person perception, when asked to form an impression of never-met-before person on the basis of a few descriptive sentences, participants have been found to look disproportionately longer at the negative statements than neutral or positive statements (Fiske, 1980). People are even believed to spend more time thinking spontaneously about negative events than positive events. When asked to list things commonly thought about, one study found participants listed primarily negative events such as a
threatened relationship, the challenge of a forthcoming event, and difficulties in pursuit of a goal (Klinger, Barda, & Maxeiner, 1980).

It is clear that there is evidence of a negativity bias in the emotional processing and behaviours of healthy individuals. The advantage of such a negativity bias is obvious. The consequences of ignoring or reacting slowly to an aversive stimulus are often more dramatic and dangerous than the consequences of ignoring or reacting slowly to appetitive or neutral stimuli (Cacioppo & Gardner, 1999; Carretie, Martin-Loeches, Hinojosa, & Mercado, 2001a; Carretie, Mercado, Tapia, & Hinojosa, 2001b; Crawford & Cacioppo, 2002; Pratto & John, 1991). Negative events typically involve greater time urgency than positive events and signal that the individual needs to change its current state or activity (Pratto & John, 1991). Hence it is proposed that humans possess an automatic vigilance, or readiness, to direct attentional resources towards aversive events (Pratto & John, 1991).

1.4 Emotional processing in depression

While the negativity bias appears to serve an adaptive advantage, it may be considered problematic when the bias makes a substantial contribution to depressed mood. Cognitive theories of depression suggest that depressed individuals are even more prone to direct their attention and information processing towards negative stimuli (Beck et al., 1979; Gotlib,
Krasnoperova, Yue, & Joormann, 2004). The affective disorder, depression involves a range of symptoms including disruptions to eating, sleeping, and concentration (DSM-IV-R, American Psychiatric Association, 1994). The disorder is also believed to be characterized by the two central themes of a pervasiveness of negativity of mood and emotion, together with a loss of interest or pleasure in virtually all activities (Dunn, Dalgleish, Lawrence, Cusack, & Ogilvie, 2004; Nevid, Rathus, & Greene, 2005).

Perhaps most emphasis has been placed on the sensitivity to negative information, or ‘negativity bias,’ as cognitive theories suggest this is involved in both the onset and maintenance of the disorder (Beck et al., 1979; Siegle, Ingram, & Matt, 2002). Behavioural studies of depression have found mood-congruent biases in reaction times and vigilance to negative emotional information (Bradley, Mogg, & Lee, 1997; Gotlib, Krasnoperova, Yue, & Joormann, 2004), biases towards negative words in emotional Stroop tasks (Gotlib & Cane, 1987; Segal, Gemar, Truchon, Guirguis, & Horowitz, 1995), and both implicit and explicit memory biases for negative material (Rinck & Becker, 2005). In addition, consistent with the idea of a reduced sensitivity to positive material, blunted responses to positive pictures together with increased sadness ratings to typically positive pictures have also been found (Dunn et al., 2004).
As mentioned, depression involves the persistence of negativity of mood and the above mentioned studies highlight a mood-congruent bias in emotional processing. Depression is also associated with decreased monoamine function (Coppen, Eccleston, & Peet, 1973; Delgado et al., 1990; Lambert, Johansson, Agren, & Friberg, 2000). Hence, it is assumed that neurotransmitters have a role in emotional processing.

1.5 The role of neurotransmitters in emotional processing

Serotonin and the catecholamines dopamine and noradrenaline have been implicated in the neurobiology of depression (Delgado et al., 1990). In support of a serotonin hypothesis, studies have shown that the brain serotonin receptors are decreased in depressed patients (Yatham et al., 2000). Also, tryptophan, the building block of serotonin, is reduced in depressed patients (Coppen et al., 1973; Cowen, Parry-Billings, & Newsholme, 1989). Alternatively, in support of a catecholamine hypothesis of depression, reduced levels of venoarterial norepinephrine and homovanillic acid, a dopamine metabolite, have been found (Lambert, Johansson, Agren, & Friberg, 2000).

Also, consistent with the view that monoamines are abnormally low in depression, pharmacological treatments are generally aimed at enhancing their function (Corrigan, Denahan, Wright, Ragual, & Evans, 2000; for
reviews see Kapur & Mann, 1992; Kasper & Heiden, 1995).

Antidepressants not only reduce the depressive symptoms such as sadness and anhedonia, but are also speculated to shift the emotional bias from negative to positive stimuli (Harmer, 2004). It is somewhat unclear, however, whether the effects of antidepressants are a direct result of the antidepressant treatment itself, or as an indirect result of symptom improvement (Harmer et al., 2004). By manipulating the levels of the neurotransmitters serotonin and dopamine in healthy controls, their direct roles in emotional processing can be exposed and not complicated by confounds in symptom or mood improvements. Manipulation of neurotransmitter levels can occur by several means. Two possible methods are the administration of agents to augment their function, or, in recent years by depletion techniques that reduce their functioning.

1.5.1 Neurotransmitter precursor depletion techniques

Depletion paradigms are based on the notion that the synthesis of monoamine neurotransmitters is dependent on the availability to the brain of the appropriate precursors located in the blood plasma (McLean et al., 2004; Williams, Shoaf, Hommer, Rawlings, & Linnoila, 1999; for a review see Reilly, McTavish, & Young, 1997). The synthesis of serotonin depends on the availability of the precursor, tryptophan, while the catecholamines dopamine and noradrenalin depend on the precursors tyrosine and
phenylalanine (for a review see Reilly, McTavish, & Young, 1997). Hence, the dietary administration of an otherwise nutritionally balanced amino acid mixture that is lacking in the relevant precursor should reduce the availability of the precursors to the brain (Young, Smith, Pihl, & Ervin, 1985). It does this by two possible means. Firstly the administration of the mixture induces synthesis of new protein. Tryptophan incorporated into protein comes from the blood and tissue stores which results in a decline in plasma and brain precursor levels (Moja, Lucini, Benedetti, & Lucca, 1996; Moja et al., 1988; for a review see Reilly et al., 1997). Secondly, the availability to the brain is reduced as the precursors are competing at a relative disadvantage with other large neutral amino acids that also share the same carrier system for transport across the blood-brain barrier (Fernstrom, 1977; Oldendorf & Szabo, 1976; Pardridge, 1977; for a review see Reilly et al., 1997). The end result of tryptophan depletion is reduced serotonin functioning. The end result of tyrosine/phenylalanine depletion is reduced catecholamine functioning, with dopamine being primarily affected (Harmer, McTavish, Clark, Goodwin, & Cowen, 2001; McTavish, Cowen, & Sharp, 1999; McTavish et al., 2001).

The effects of neurotransmitter depletion can be contrasted with those of neurotransmitter augmentation in order to ascertain the roles of the
neurotransmitters in emotional processing and ultimately lead to an understanding of the abnormalities underlying depression.

1.5.2 The effects of serotonin manipulation on emotional processing

Typical consequences of reduced serotonin are increased anxiety and depression, increased appetite, increased aggression, and impaired cognitive functioning (for a review see Booij et al., 2003). In contrast, research has shown that after one week of administration of the selective serotonin reuptake inhibitor, paroxetine, measures of hostility, assualtiveness, and negative affect decreased, while measures of social affiliation increased (Knutson et al., 1998). Also, chronic administration of the serotonin precursor, tryptophan, has been found to enhance social functioning by decreasing quarrelsome behaviour and simultaneously increasing dominant behaviour (Moskowitz, Pinard, Zuroff, Annable, & Young, 2001).

In terms of processing and responding to emotional stimuli, it seems that with increased serotonin there is an increase in the processing of positive stimuli (Harmer et al., 2004; Kemp et al., 2004a), while with decreased serotonin there is a decrease in the processing of positive stimuli (Murphy et al., 2004).
More specifically, with reduced serotonin function via tryptophan depletion, there seems to be reduced processing of positive stimuli together with an impairment in the ability to process fear. In terms of the reduction in positive information processing following tryptophan depletion, Murphy et al. (2004) reduced serotonin through tryptophan depletion and investigated the effects on an affective go/no-go task. In this paradigm, participants are required to respond to happy and sad target words, but withhold responses to non-target words. Following tryptophan depletion, although performance did not differ for sad targets, participants were significantly slower to respond to happy targets. In addition, the researchers found no changes in subjective ratings of mood following tryptophan depletion. The findings closely match those found in a study of depressed patients whereby patients were also slower to respond to happy targets but not sad targets (Murphy et al., 1999). While these results seem to show reduced responsiveness to positive stimuli rather than a negativity bias as such, Murphy et al. (2004) suggested that the slowing to positive targets could actually be due to distraction from the sad targets.

While Murphy et al. (2004) did not find any differences in response times to negative stimuli following tryptophan depletion, a study using fearful facial stimuli by Harmer et al. (2003b) did. Interestingly, female participants were significantly impaired in their ability to recognise fearful
faces following tryptophan depletion (Harmer et al., 2003b). Also, both females and males were slower in their reaction times to respond to fearful faces, suggesting that males too had at least some impairment in their ability to process fear following tryptophan depletion (Harmer et al., 2003b). In addition, these changes in emotional processing occurred in the absence of any changes in mood. The researchers highlighted the possibility that fear-related processing is compromised under conditions of reduced serotonin (Harmer et al., 2003b). In fact, it is proposed that serotonin has a dual role in the processing of fear stimuli (Graeff, Viana, & Mora, 1997). With acute increases in serotonin, fear related processing increases, but with prolonged increases in serotonin, fear-related processing decreases (Graeff et al., 1997).

The dual role of serotonin in fear processing perhaps explains the contradictory findings regarding acute administration of serotonin enhancing agents and chronic administration. With acute administration of the serotonin precursor, tryptophan (Attenburrow et al., 2003) and the selective serotonin reuptake inhibitor, citalopram (Harmer et al., 2003a), studies have found the superior detection of both happy and fearful faces with no changes in mood. While this finding does not seem compatible with the idea of antidepressants reducing responsiveness to threatening stimuli, Harmer et al. (2004) explained these findings in terms of an initial increase
in anxiety that often occurs before therapeutic effects are seen. With repeated administration of citalopram, however, decreased processing of fearful stimuli has been found (Harmer et al., 2004).

In fact, with repeated administration of citalopram, the superior processing of positive stimuli together with reduced processing of negative stimuli has been found (Harmer et al., 2004). In a study involving facial stimuli, Harmer et al. (2004) found that repeated administration of citalopram decreased recognition of the facial expressions of fear, disgust, and surprise, and it also increased the likelihood of participants misclassifying negative emotions as happy. In terms of memory, citalopram resulted in the recall of a higher percentage of positive words than those receiving the placebo. Lastly, citalopram abolished the potentiation of the startle response that is normally observed in response to negative images. Similar results were found in Kemp et al. ’s (2004a) steady-state probe topography study involving emotional photographs. Following acute administration of citalopram, electrophysiological activation to unpleasant images within frontal and occipital regions was attenuated, while activation to pleasant stimuli within parieto-occipital regions was enhanced. In addition, the findings of both Harmer et al. (2004) and Kemp et al. (2004a) occurred in the absence of any changes in mood.
Taken together, the findings of Harmer et al. (2004) and Kemp et al. (2004a) suggest that antidepressants work by shifting the emotional bias from negative to positive stimuli. It is unlikely, however, that any single neurotransmitter alone can fully explain emotional processing or account for the emotional processing deficits observed in depression (for reviews see Kalia, 2005; Mayberg, 1997). Hence, manipulation of other neurotransmitters such as dopamine is also useful for understanding biases in emotional processing.

1.5.3 The effects of dopamine manipulation on emotional processing

Generally serotonin has been primarily investigated in relation to depression and emotional processing. In recent times, however, the role of dopamine has also gained considerable interest, especially given the involvement of dopamine in motivated behaviour (Kapur & Mann, 1992; Willner, 1995). With reduced dopamine, proneness to addiction, decreased vigour, and impaired emotional and working memory are common consequences (for a review see Booij et al., 2003).

In terms of the role of dopamine in emotional processing, it appears that individuals display a tendency to direct processing towards negative events, and are also less sensitive to reward (McLean et al., 2004). Following the administration of a tyrosine and phenylalanine deficient
mixture, subjects have been shown to display a bias towards sad targets in affective go/no-go tasks while the subjects receiving a balanced mixture displayed a happy bias (McLean et al., 2004). A study involving participants recovered from depression also showed a small negativity bias in a similar task that was close to approaching significance (Roiser et al., 2005).

Studies have also found more conservative bets in decision-making tasks following tyrosine/phenylalanine depletion in both healthy participants (McLean et al., 2004) and in patients recovered from depression (Roiser et al., 2005). This supports the idea that tyrosine/phenylalanine depletion disrupts the ability to evaluate and act effectively. This finding also complements the fact that depressed individuals experience difficulties in making decisions (APA, 1994) and that dopamine has an important role in affect and reward-based processing (McLean et al., 2004). It is likely that as a result of the depletion, participants were betting less due to a reduction in reward sensitivity (Roiser et al., 2005).

1.5.4 The effects of simultaneous manipulation of serotonin and dopamine on emotional processing

It is clear that the neurotransmitters serotonin and dopamine are involved in affective processes. As mentioned, however, it is unlikely that any single neurotransmitter alone would account for emotional processing.
It is more than likely that there are complex interactions between monoaminergic systems (for a review see Mongeau, Blier, & de Montigny, 1997) especially given that serotonin and dopamine act on common brain circuitry (Bremner et al., 2003). In line with this, a newly developed technique, combined monoamine depletion, was developed to deplete the three precursors tryptophan, tyrosine, and phenylalanine simultaneously. This technique, like tryptophan and tyrosine/phenylalanine depletion, can achieve significant plasma monoamine depletion in the range expected to affect brain monoamine function (Hughes et al., 2004; Leyton, Pun, Benkelfat, & Young, 2003; Nathan, Hughes, McInerney, & Harrison, 2004).

As combined monoamine depletion is a relatively newly developed technique, there is very little in the way of past research in this area. A study by Hughes et al. (2004) examined the effects of combined monoamine depletion on emotional processing, but failed to find any changes in performance on an Emotional Stroop task, or in response to emotionally evocative photographs from the International Affective Picture System (IAPS; Lang, Bradley, & Cuthbert, 1999). The study did find, however, that following combined monoamine depletion participants became significantly sadder, more antagonistic, and mentally slower (Hughes et al., 2004). Participants have also been found to become significantly more bored and
irritated following combined monoamine depletion (Leyton, Pun, Benkelfat, & Young, 2003).

The fact that mood-lowering has been found following combined-monoamine depletion (Hughes et al., 2004; Leyton et al., 2003) but has not consistently been found with either serotonin or dopamine manipulation techniques alone, suggests that substantial changes in mood may only occur when two or more monoamines are altered. In fact, in terms of tryptophan depletion, it seems that those individuals that have displayed any mood-lowering effects, are either belonging to the subgroups of patients recovered from depression (for a review, see Van der Does, 2001), or healthy individuals with a genetic vulnerability to depression (Booij, Van der Does, & Riedel, 2003). In one rare instance, mood-lowering was found for both tryptophan depletion and tyrosine/phenylalanine depletion paradigms, but this was only found to be significant after the induction of a psychological challenge (Leyton et al., 2000).

While mood seems to be relatively unaffected by neurotransmitter depletion, the previously mentioned depletion and antidepressant studies did, however, affect emotional processing. This suggests that monoamines may directly modulate the neural processing of emotional material independently, or at a lower threshold than changes in mood. Hence,
emotional processing appears to be a more sensitive measure of monoamine function than mood. As it can be seen that neurotransmitters do have a role in emotional processing, it would be useful to know when exactly this differentiation between positive and negative takes place. The study by Harmer et al. (2004) showed that there was an increase in positive information processing, together with a decrease in negative information processing following administration antidepressants. This suggests that antidepressants shift biases in emotional processing from negative to positive stimuli. It could be that neurotransmitters affect emotional processing by influencing attentional biases.

1.6 Event-related potentials and attentional biases

It has already been suggested that humans possess a negativity bias in emotional processing. Furthermore, it is apparent that this bias is particularly prominent in depression, possibly due in part to aberrant neurotransmitter transmission. In understanding the superior processing of negative material relative to positive, it seems that the mechanism behind the negativity bias is attention. That is, negative stimuli possess attributes that capture attention. Although behavioural studies have pointed to an attentional bias in emotional processing, these studies are limited in that they do not specify where exactly in the information processing stream more attention is allocated towards negative stimuli. It could be that negative
events receive more attention very early on in stimulus processing (Smith et al., 2003). Alternatively, rather than initially receiving more attention than positive events, it could be that negative events receive greater processing downstream, hence resulting in stronger response dispositions (Smith et al., 2003).

Event-related potentials (ERPs) are to be used in this study to provide the means by which moment-by-moment attention allocation can be assessed. ERPs are measures of the electrical activity of the brain in response to the processing of a stimulus (Andreassi, 2000; Smith et al., 2003). They are time-locked to stimulus events and are advantageous in that they provide an account of brain responses to a stimulus even in the absence of any overt behavioural response (Andreassi, 2000). When describing ERPs, the labels “P” or “N” are used to indicate positive or negative deflections, together with a number to indicate the timing or latency of the peak (Smith et al., 2003). These deflections, or components, each correspond to a different information-processing function (Andreassi, 2000; Smith et al., 2003).

Early ERP components occurring prior to 300 ms, such as the P100, are of particular relevance to the current study as they are sensitive to early attention allocation (Luck et al., 2000; Smith et al., 2003). As the name
suggests, the P100 is a positive-going component that peaks at approximately 100-150 ms post-stimulus onset (Smith et al., 2003). It is maximally recorded over the occipital lobe (Luck et al., 2000; Smith et al., 2003), and is the result of activation of neurons in the extrastriate area of the visual cortex (Fu, Caggiano, Greenwood, & Parasuraman, 2005; Purtois et al., 2004; Smith et al., 2003). With increasing amounts of attention allocation towards a stimulus, more neurons are recruited and this is reflected by an increase in P100 amplitude (Smith et al., 2003).

The majority of electrophysiological studies on emotional processing have investigated later ERP components, with only relatively few studies that have investigated earlier, attention-related components. The few studies that have been conducted in relation to the P100 have been successful, however, in showing emotional modulation of early attention (Delplanque, Lavoie, Hot, Silvert, & Sequeira, 2004; Smith et al., 2003).

Smith et al. (2003) provided convincing evidence of a negativity bias as early as the P100. In their design participants were presented with a background picture that was either negative or positive and then a target picture that was either negative or positive was presented after 4, 6, or 8 s. By presenting a contrasting background image, target pictures tend to be judged as more extreme. Such a design enables strong valence
manipulation; hence differences in amplitude can be attributed to valence. As expected, the researchers found that the P100 was larger for negative stimuli than for positive stimuli.

Providing further support for the negativity bias, a follow-up study by Smith et al. (2003) presented participants with predominantly neutral pictures with the occasional positive and negative picture interspersed. The frequently presented neutral stimuli actually elicited larger P100 amplitudes than the rare, valenced targets. This was explained in terms of stimulus probability effects, whereby expected stimuli attract large amounts of attention, as the individual is primed to detect these stimuli. The negative stimuli did, however, elicit larger P100 amplitudes than the positive stimuli, suggesting again that more attention is allocated to negative material than positive material (Smith et al., 2003).

Similar results have also been found in response to other types of emotional photographs (Carretie, Hinojosa, Martin-Loeches, Mercado, & Tapia, 2004) and in response to facial stimuli (Purtois et al., 2004). Carretie et al. (2004) presented participants with mainly neutral pictures and then the occasional highly arousing positive or negative picture. As expected, negative pictures elicited the greatest P100 amplitude. Likewise, Purtois et al. (2004) found that photographs of fearful faces elicited greater amplitudes
of the occipital P100 than neutral or happy faces. In addition, this physiological data was complemented by the behavioural finding that participants detected the orientation of bars replacing emotional faces faster than those replacing neutral faces (Purtois et al., 2004).

It must be recognised that the ERP studies of Carretie et al. (2001b), Purtois et al. (2004) and Smith et al. (2003) used stimuli that were high on arousal. While Smith et al. (2003) attempted valence manipulation by the presentation of a contrasting background image, it is still possible that the negativity bias exhibited was actually as a result of the arousal level of the target pictures. As previously mentioned, stimuli that are high in arousal activate the amygdala to a greater extent than low arousal stimuli (LeDoux, 1995). As the amygdala has extensive connections to attention-related areas of the cortex, it may be that it is the dimension of arousal that is eliciting the bias, rather then the negative valence dimension.

To overcome limitations associated with highly arousing stimuli, Delplanque et al. (2004) attempted to show the existence of valence-modulation of the P100 to low-arousal IAPS. In their design, participants were presented with checkerboards and were instructed to press a button upon presentation of rare, low-arousal IAPS targets. Higher parieto-occipital P100 amplitudes were found for negative images than for positive images.
This finding was interpreted as evidence of a negativity bias in attention allocation. As the bias occurred even on presentation of low-arousal images, the effect was confidently attributed to the negative valence of the stimuli, rather than arousal influences.

The results from the aforementioned studies provided evidence for the superior processing of negative stimuli relative to positive stimuli. The fact that this discrimination occurs very early on in stimulus processing is consistent with the adaptive explanation that the faster negative and positive stimuli can be differentiated, the faster the individual can engage in an appropriate response behaviour, and the more successful it will be in interacting with the environment (Balconi & Pozzoli, 2003; Purtois et al., 2004; Smith et al., 2003).

To date very little research has been conducted on early attentional biases in emotional processing. As the P100 provides an index of early attention, the use of this measure should hopefully prove advantageous in elucidating the relative contributions of the neurotransmitters, serotonin and dopamine, in early attentional biases to negative stimuli.
1.7 Research aims

The current research aimed to elicit the negativity bias to emotional stimuli as early as the P100 in healthy male volunteers. Participants were each examined under a control condition, a serotonin depletion condition, a catecholamine depletion condition, and a combined monoamine depletion condition in order to assess the effects of the neurotransmitters on attentional biases associated with emotional processing. More specifically, it was intended to use event-related potentials (ERPs) to ascertain whether the three different neurotransmitter depletion techniques would differentially affect the P100 in response to stimuli of different emotional valences. The effects of the various depletion techniques on mood were also assessed.

1.8 Hypotheses

1. There will be an increase in P100 amplitude to unpleasant stimuli relative to pleasant and neutral stimuli in all treatment conditions.

2. Depletion of serotonin (tryptophan depletion), dopamine (tyrosine/phenylalanine depletion) and monoamines (tryptophan and tyrosine/phenylalanine depletion) will increase the P100 amplitude to unpleasant stimuli relative to the control condition.
3. There will be no mood changes associated with serotonin and dopamine depletion but a reduction in mood with monoamine depletion.
2.1 Participants

Ten healthy males aged between 22 and 42 years (mean, 28.40 yr; S.D., 6.77 yr) were recruited via university advertisements and friends of the researchers. Seven other participants were originally involved but were excluded after experiencing adverse side effects. Participants underwent a physical examination and semi-structured clinical interview conducted by a medical physician to ensure suitability for participation in the study. The exclusion criteria included: smokers, current medication or recreational drugs, history of recreational drugs, personal or family history of neurological or psychiatric illness, or a head injury. Females were excluded due to time constraints involved in testing during the follicular stage of the menstrual cycle (Harrison et al., 2004) and also because the rate of serotonin synthesis is considerably lower in females (Nishizawa et al., 1997). Participants gave written informed consent for their participation and the study was granted approval by the Swinburne University Human Research Ethics Committee.

2.2 Design

The study was a randomized, double-blind, placebo-controlled design. All participants were tested under four treatment conditions each
separated by a washout period of at least seven days; the balanced control/placebo condition (BAL), the tryptophan depletion condition (TRP), the tyrosine/phenylalanine depletion condition (TYR), and the combined monoamine depletion condition (CMD).

2.2.1 Amino acid mixture

The amino acid (AA) mixture in the current study was based on the 100 g nutritionally balanced mixture developed by Young et al. (1985). The BAL condition consisted of the following AAs: L-Alanine, 5.5 g; L-Arginine, 4.9 g; L-Cysteine, 2.7 g; Glycine, 3.2 g; L-Histidine, 3.2 g; L-Isoleucine, 8.0 g; L-Leucine, 13.5 g; L-Lysine monohydrochloride, 11.0 g; L-Methionine, 3.0 g; L-Phenylalanine, 5.7 g; L-Proline, 12.2 g; L-Serine, 6.9 g; L-Threonine, 6.5 g; L-Tryptophan, 2.3 g; L-Tyrosine, 6.9 g; and L-Valine, 8.9 g. As the balanced mixture is not lacking in the precursors necessary for serotonin or dopamine, this serves as the placebo or control condition. In the three experimental conditions, the amino acid mixtures were of identical composition to the BAL mixture with the exception that tryptophan was excluded from both the TRP and CMD conditions, and tyrosine and phenylalanine were excluded from both the TYR and CMD conditions. Cysteine, arginine, and methionine were given in capsule form due to the unpleasant taste. Drinks were prepared by mixing the powdered
acids with 180 ml of orange juice within a few minutes of oral ingestion following the administration of the capsules.

2.3 Materials

2.3.1 Visual analogue mood scale

The Visual Analogue Mood Scale (VAMS; Bond and Lader, 1974) consists of 16 100 mm horizontal lines each representing a pair of bipolar adjectives describing a mood state. The scales of interest to the current study are “Quick Witted-Mentally Slow,” “Happy-Sad,” and “Amicable-Antagonistic” as ratings on these have been shown to change following monoamine depletion (Hughes et al., 2004). Participants were required to mark, with a vertical line, the extent to which they felt a particular mood at the current moment in time. Scores for each scale were summed by measuring in mm the distance from the end of the line to the subject’s mark. Therefore possible scores for each scale ranged from 0-100 with high scores indicating that the subject reported to be experiencing a greater intensity of the given mood-state.

Although the VAMS in general has been shown through factor analysis to successfully reflect the three underlying factors of alertness, contentedness, and calmness (Bond & Lader, 1974), reliability and validity statistics are not available. Inter-item reliability is not possible as each scale
is designed to measure a different trait. Measures of test-retest are not practical given that the scale does not measure a stable trait, and reliability from parallel forms is also impossible because of the difficulties involved in creating a comparable scale.

A copy of the VAMS for pre- and post-treatment conditions is included as Appendices D and E, respectively.

2.3.1 International affective picture system

Participants viewed 200 pictures from the International Affective Picture System (IAPS; Lang Bradley, & Cuthbert, 1999) to assess changes in emotional processing. The IAPS is a set of emotionally evocative photographs of varying arousal and valence levels. The current study presented 50 pleasant images such as family scenes, 50 unpleasant images such as scenes of pollution, and 100 neutral pictures including common household objects. All images were matched on normative arousal ratings with a mean of 4.47, however, images from the three categories differed from each other on normative valence ratings with mean ratings for pleasant, unpleasant, and neutral contents reported at 7.17, 2.83, and 5, respectively (Lang Bradley, & Cuthbert, 1999). Low-arousal images were assessed so that any differences in amplitude to the various stimuli can be attributed to differences in valence. The presentation of pictures was random
with the exception that there were no more than four images in a row of the same valence category.

The IAPS have been used extensively in past research and are proposed to be successful in eliciting a range of emotions similar to those experienced outside of the laboratory (Lang et al., 1998). To establish normative ratings of valence and arousal, a series of studies were carried out on populations of both children and adults (Lang et al., 1999). The split-half coefficients for the valence and arousal dimensions were highly reliable \( (p < .001) \) for both pencil and paper administration \( (r = .94 \text{ and } r = .94, \text{ respectively}) \) and computer administration \( (r = .94 \text{ and } r = .93, \text{ respectively}) \).

A complete description of valence and arousal ratings, together with stimulus codes is included as Appendix C.

2.4 Procedure

Initially, all interested participants were contacted for a telephone interview to assess their suitability for the study. During this time they were informed about the study purpose and procedure and were free to ask any questions about the research. They were also assured of the confidentiality of their results and that they were free to withdraw from participation at any time during the study.
On each of the four testing days subjects arrived at the Brain Sciences Institute (BSI) after having followed a low-protein diet and fasting from 19:00 hours on the previous day. A copy of the low protein diet is included as Appendix B. A low protein diet was undertaken as dietary restriction of tryptophan has been shown to reduce plasma tryptophan levels (Delgado, Charney, Price, Landis, & Heninger, 1989; Fernstrom, 1977). Hence, the combination of a low-protein diet together with acute tryptophan depletion should result in a substantial decline in serotonin function.

Participants were provided with a list of food types appropriate for their consumption. This process of undertaking a low-protein diet has been conducted in past research with no adverse effects (Carpenter et al., 1998; Williams et al., 1999). Upon arrival, the mood questionnaire (the VAMS) was completed. Following this, subjects ingested one of the amino acid treatment mixtures and capsules. Chewing gum and water were provided to counteract the unpleasant taste of the amino acids.

Throughout the day subjects were asked to remain in the BSI but were permitted to do non-physical activities of their choice (such as reading and watching TV) provided that the content was emotionally neutral. The mood ratings were obtained again at 5:00 h post amino acid administration. At 5:30 h post amino acid administration, the IAPS task and EEG recording
took place. Each testing day ended at approximately 7:15 h post amino acid administration, at which time the subjects were free to go home.

The timing of the emotional processing task was chosen to coincide with the timeframe suitable for inducing maximum levels of precursor depletion as found in previous research. For instance, plasma levels of tryptophan have been reduced by 70-90% approximately 6 hours post administration (Young et al., 1985). In addition, continuous cerebrospinal fluid (CSF) sampling amino acid depletion techniques have shown plasma levels and CSF levels of tryptophan to fall to approximately 86% and 92% of baseline, respectively, and this effect was greatest 6-8 hours after consuming the mixture (Carpenter et al., 1998; Williams et al., 1999). Furthermore, levels of CSF 5-HIAA, a principal metabolite of serotonin, have been found to decrease by approximately 32% (Carpenter et al., 1998; Williams et al., 1999). Likewise, tyrosine/phenylalanine depletion techniques have been found to reduce plasma phenylalanine and tyrosine by up to 87.3% and 70.2% of baseline, respectively, and this was greatest at 6 hours post-treatment (Moja et al., 1996).

2.5 Data acquisition

EEG data were recorded and processed from 64 scalp sites based on an extended version of the International 10/20 system using tin electrodes.
Each of the sites were on-line referenced to the centre the top of the scalp. Horizontal and vertical electro-oculograms were detected by four facial bipolar electrodes placed inferior to the right eye and both inferior and superior to the left eye. Impedance was below 5 kΩ before beginning the recording session. Data was continuously digitized at a rate of 500 Hz, with a low-pass filter set at 100 Hz, and a high-pass filter set at .05 Hz.

2.6 Data Analysis

A visual inspection of the data was performed to manually check for bad epochs. Then, in order to eliminate extraneous noise, several off-line steps were carried out. The data were re-referenced to linked mastoids. To remove contamination caused by eye-blinks, an artefact-aligned average procedure was used for EOG correction (Croft & Barry, 2000). A low-pass zero phase shift filter at 30 Hz (12 dB roll-off) was applied. The data were sorted into valences (neutral, pleasant, and unpleasant) and were epoched from 200 ms prior to stimulus onset and 600 ms post-stimulus onset. To remove baseline differences, the average amplitude of each electrode for each trial was set to zero. In order to remove electrical activity that was not time-locked to the stimuli, epochs were averaged separately for each stimulus type (low-arousal neutral, low-arousal pleasant, low-arousal unpleasant) in each treatment condition so that 12 averages were generated for each participant.
3.1 Principal components analysis for the event-related potentials

Before the ERP hypotheses could be tested, the P100 component was first quantified. A Principal Components Analysis (PCA) was used to separate overlapping components from the background noise. This technique involves examining a set of averaged ERP waveforms for covariations in amplitude across time points. From this, PCA reduces the total variance of the waveforms into a smaller number of underlying components whereby each component represents a selection of the overall variance. Each component consists of a component loading for each time point. This indicates the degree to which that component is influencing the particular time point, whereby higher loadings specify time points where the component is strongly active. A set of component scores is also created and these explain the degree to which a particular component is present in a waveform (Smith et al., 2003).

The output of the PCA was subjected to a varimax rotation and three components were extracted. A matrix of component loadings and a matrix of component scores were produced by the PCA. To investigate the P100, the component in the appropriate time region was analysed. To qualify as a P100, the component was required to peak between 100-150 ms post-
stimulus onset, have a maximum amplitude at occipital sites, and be positive-going at those scalp sites (Smith et al., 2003). Consistent with these criterions, component three was found to peak at approximately 148 ms, was maximal at occipital sites, and was positive-going at those sites. Hence, component three was labelled the P100.

To determine the extent to which the component is present in any given experimental condition, the component scores of the P100 at all scalp electrode sites are then subjected to further examination by repeated measures analysis of variance.

3.2 Data screening for the event-related potentials and the visual analogue mood scale

Both the emotional processing and the mood data were analysed using the Statistical Package for Social Sciences (SPSS) version 12.0. Preliminary data analyses were performed on the data to ensure suitability for further analysis. The screening revealed that there were no out-of-range or missing values. To examine any deviations from normality, skewness statistics and histograms were examined. All the variables were normally distributed with the exception of “Tryptophan Pleasant Stimulus” and “Post-Tyrosine Sad” from the ERP and VAMS data, respectively. The skewness statistics revealed these variables to be positively skewed at the .05 level. To
test if there were any outliers that may be affecting the distribution of variables, boxplots then were created. While an outlier was found for each of the two variables, these were both within three standard deviations of the mean. A square root transformation was applied to both the variables. The transformed “Tryptophan Pleasant stimulus” variable was not significantly improved. Also, while the histogram, boxplot, and skewness statistic for the transformed “Post Tyrosine Sad” variable successfully reduced the skewness to that of a normally distributed variable, the mean was substantially reduced and did not seem like a realistic value when compared with the means of the other variables. Hence, given the small sample size, all cases were retained and the data for both the ERPs and VAMS were analysed in the original form.

3.3 Event-related potentials

The means and standard deviations for the ERP data are presented in Table 1. Firstly, to assess whether the negativity bias would be replicated in this sample under normal conditions, a 1 (placebo treatment) x 3 (stimulus valence) repeated measures ANOVA was conducted for the placebo condition. Secondly, to compare the effects of the different treatment conditions of the P100 to the different emotional stimuli, a 4 (treatment condition) x 3 (stimulus valence) repeated measures ANOVA was performed.
Table 1

*Means and Standard Deviations of the ERP Amplitudes in Response to the Different Emotional Valences for each Treatment Condition*

<table>
<thead>
<tr>
<th>Treatment Condition</th>
<th>Stimuli Valence</th>
<th>M</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>Neutral</td>
<td>191.03</td>
<td>157.62</td>
</tr>
<tr>
<td></td>
<td>Pleasant</td>
<td>232.69</td>
<td>155.40</td>
</tr>
<tr>
<td></td>
<td>Unpleasant</td>
<td>199.41</td>
<td>141.64</td>
</tr>
<tr>
<td>CMD</td>
<td>Neutral</td>
<td>195.11</td>
<td>158.87</td>
</tr>
<tr>
<td></td>
<td>Pleasant</td>
<td>204.09</td>
<td>152.55</td>
</tr>
<tr>
<td></td>
<td>Unpleasant</td>
<td>211.06</td>
<td>174.09</td>
</tr>
<tr>
<td>TRP</td>
<td>Neutral</td>
<td>202.79</td>
<td>151.94</td>
</tr>
<tr>
<td></td>
<td>Pleasant</td>
<td>223.84</td>
<td>146.86</td>
</tr>
<tr>
<td></td>
<td>Unpleasant</td>
<td>200.31</td>
<td>152.81</td>
</tr>
<tr>
<td>TYR</td>
<td>Neutral</td>
<td>214.52</td>
<td>158.62</td>
</tr>
<tr>
<td></td>
<td>Pleasant</td>
<td>232.58</td>
<td>148.46</td>
</tr>
<tr>
<td></td>
<td>Unpleasant</td>
<td>235.60</td>
<td>144.66</td>
</tr>
</tbody>
</table>

\(N=10\)

Note: BAL= placebo condition, CMD= combined monoamine depletion condition, TRP= tryptophan depletion condition, and TYR= tyrosine depletion condition.

For the results regarding the control condition analysis, Mauchly’s test was not violated so sphericity was assumed. The results revealed a significant effect of valence \(F(2,18)=9.90, p<.01, \text{partial } \eta^2=.52\).
Furthermore, Post-Hoc tests with Bonferroni correction ($p = .017$) revealed that the amplitude of the P100 was significantly larger for pleasant images than for neutral images ($F(1,9) = 30.04, p < .001$, partial $\eta^2 = .77$) and significantly larger for pleasant images than for unpleasant images ($F(1,9) = 9.35, p = .01$, partial $\eta^2 = .51$). There were no significant differences in amplitude, however, between neutral and unpleasant images ($F(1,9) = .60, p = .46$, observed power = .11).

For the analysis involving all treatment conditions, Mauchly’s test was again not violated so sphericity was assumed. The results indicated that there was no significant interaction between stimulus valence and treatment condition ($F(6, 54) = 1.05, p = .40$, observed power = .38).

### 3.4 The visual analogue mood scale

In order to assess the effects of treatment condition over time on mood, the next section outlines the results for the VAMS. The means and standard deviations for the ‘quick witted-mentally slow,’ ‘happy-sad,’ and ‘amicable-antagonistic’ items are presented in Table 2. The table will then be followed by a $2 \times 4$ (time x treatment condition) repeated measures analysis of variance (ANOVA) for each scale.
Table 1

*Means and Standard Deviations for ‘Quick Witted-Mentally Slow,’ ‘Happy-Sad,’ and ‘Amicable-Antagonistic’*

<table>
<thead>
<tr>
<th></th>
<th>BAL</th>
<th>CMD</th>
<th>TRP</th>
<th>TYR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Q-M</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>35.40(20.21)</td>
<td>31.40(14.79)</td>
<td>32.80(21.56)</td>
<td>40.30(21.56)</td>
</tr>
<tr>
<td>Post</td>
<td>41.40(23.16)</td>
<td>49.50(26.00)</td>
<td>42.70(25.84)</td>
<td>45.60(29.25)</td>
</tr>
<tr>
<td><strong>H-S</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>24.75(20.50)</td>
<td>25.90(18.43)</td>
<td>28.80(16.77)</td>
<td>29.20(19.58)</td>
</tr>
<tr>
<td>Post</td>
<td>27.10(15.85)</td>
<td>28.20(17.94)</td>
<td>26.40(14.71)</td>
<td>31.80(24.75)</td>
</tr>
<tr>
<td><strong>A-A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>26.60(17.65)</td>
<td>25.60(13.76)</td>
<td>29.30(18.30)</td>
<td>30.10(18.47)</td>
</tr>
<tr>
<td>Post</td>
<td>32.70(18.00)</td>
<td>29.90(15.41)</td>
<td>32.00(20.69)</td>
<td>35.30(26.38)</td>
</tr>
</tbody>
</table>

*N= 10*

Note: BAL= placebo condition, CMD= combined monoamine depletion condition, TRP= tryptophan depletion condition, TYR= tyrosine depletion condition, Q-M= ‘Quick Witted-Mentally Slow,’ H-S= ‘Happy-Sad,’ and A-A= ‘Amicable-Antagonistic.’

For ‘quick witted-mentally slow,’ the assumption of covariance was violated for the interaction of time and treatment condition (*p*<.001). Hence, Lower-bound correction was used. The results indicated a significant difference in levels of ‘quick witted-mentally slow’ over time (*F*(1,9)= 7.43, *p*=.02, *partial η²* = .45) whereby participants were significantly mentally slower at the second time of VAMS administration compared to the first. There was no significant effect of treatment condition (*F*(3, 27)= .56, *p*=.47,
observed power= .10) nor was there a significant interaction between
treatment condition and time \((F(3, 27)=.95, p=.36, \text{observed power}= .14)\).

As Mauchly’s test for ‘happy-sad’ was not violated, sphericity was
assumed. The results indicated no significant difference in levels of ‘happy-
sad’ over time \((F(1,9)= .40, p=.54, \text{observed power}= .09)\) or across
treatment conditions \((F(3, 27)= .40, p=.76, \text{observed power}= .12)\). There
was also no significant interaction between treatment condition and time
\((F(3, 27)=.45, p=.72, \text{observed power}= .13)\).

There was a moderate violation to Mauchly’s test of sphericity for
the interaction term of ‘amicable-antagonistic’ \((p=.02)\). Hence, Huynh-Feldt
correction was used. The results indicated a significant difference in levels
of ‘amicable-antagonistic’ over time \((F(1,9)= 7.93, p= .02, \text{partial } \eta^2= .47)\)
whereby participants were significantly more antagonistic at the second
administration of the VAMS compared to the first. There was no significant
effect of treatment condition \((F(3, 27)= .61, p= .57, \text{observed power}= .14)\)
and no significant interaction between treatment condition and time \((F(3,
27)=.14, p=.91, \text{observed power}= .07)\).
Chapter 4: Discussion

4.1 Overview of aims and findings

The current research aimed to examine attentional bias to emotional stimuli, measured with the P100 and how this was modulated by depletion of serotonin (with tryptophan depletion) and dopamine (tyrosine/phenylalanine depletion) and simultaneous monoamine depletion (with combined tryptophan/tyrosine/phenylalanine depletion). The findings suggested; (1) a greater P100 amplitude to positive relative to negative or neutral stimuli and (2) that the P100 amplitudes were not modulated by depletion of serotonin, dopamine or monoamines. Furthermore no subjective changes in mood were found.

The prediction that negative stimuli would elicit the largest P100 amplitudes was not supported. In fact it positive stimuli actually elicited larger P100 amplitudes than negative or neutral stimuli. This is inconsistent with studies that have found greater P100 amplitudes in response to negative stimuli than to positive or neutral (Carretie et al., 2004; Delplanque et al., 2004; Smith et al., 2003). The greater processing of positive stimuli relative to neutral or negative was, however, similar to a recent study that found faster reaction times to low-arousal positive images than low-arousal negative images (Robinson, Storbeck, Meier, & Kirkeby, 2004).
The hypothesis that the depletion conditions (tryptophan depletion, tyrosine/phenylalanine depletion, and combined monoamine depletion) would increase the P100 to negative stimuli relative to the control condition was also not supported.

The hypothesis that ratings on the VAMS would not be affected by either tryptophan or tyrosine/phenylalanine depletion conditions alone was supported. None of the depletion conditions affected the ‘Quick Witted-Mentally Slow,’ ‘Happy-Sad,’ or ‘Amicable-Antagonistic’ scales of the VAMS. These findings are consistent with a growing body of research suggesting that serotonin depletion with tryptophan depletion and dopamine depletion via tryrosine depletion does not modulate mood in healthy subjects (Harmer et al., 2003a, Harmer et al., 2004; Kemp et al., 2004a). However contrary to expectations, combined depletion of all monoamines (tryptophan/tyrosine/phenylalanine depletion) did not affect mood. These findings are not consistent with recent studies that showed a mood lowering effect of the combined depletion in healthy subjects (Hughes et al., 2004, Leyton et al., 2003).
4.2 Early emotional processing

The results of the current study regarding emotional processing of the IAPS failed to show evidence of a negativity bias. This is inconsistent with past research that successfully showed larger P100 amplitudes to negative stimuli than positive stimuli (Carretie et al., 2004; Delplanque et al., 2004; Purtois et al., 2004; Smith et al., 2003). The current study actually found the reverse effect, with positive stimuli eliciting larger P100 amplitudes in the control condition than negative stimuli. In addition, negative and neutral stimuli did not elicit significantly different P100 amplitudes. An examination of the means, however, shows that they were in the expected direction, with the average P100 amplitude peak being slightly higher for negative stimuli than for neutral stimuli. In addition to these findings regarding the control condition, the findings regarding all the drug conditions revealed that there were no significant differences between drug conditions in P100 amplitudes to the IAPS. Hence, it was established that the negativity bias failed to manifest in any of the treatment conditions.

There were several methodological differences between the current study and the past research that may help to explain the discrepant results. The major difference between the current study and those of Carretie et al. (2004), Purtois et al. (2004) and Smith et al. (2003) was that the past studies used stimuli that were high in arousal. As mentioned, highly arousing
negative images activate the amygdala to a greater extent than even highly arousing positive pictures (LeDoux, 1995). Hence, the greater attention allocated towards the negative stimuli may not be due to the stimulus valence, but instead the arousing content of the stimulus. In contrast to Carretie et al. (2004), Purtois et al. (2004) and Smith et al. (2003), the current study used only low-arousal images. This was a deliberate attempt to see whether the negativity bias can be attributed to valence. However as the negative pictures were not high in arousal, they were not able to activate the amygdala to a greater extent than the positive pictures. Hence, the negativity bias was not observed.

While the existence of a true negativity bias may now seem questionable, the study by Delplanque et al. (2004) was successful in finding a negativity bias even with the use of low-arousal images. Delplanque et al.’s (2004) study involved a very different paradigm, however, to the current study. The current study required that the participants passively view randomly presented pictures from the IAPS. Delplanque et al. (2004) presented participants with checkerboard images and then occasional stimuli from the IAPS. It could be that, compared to the checkerboard images, the low-arousal negative pictures were perceived as more arousing than if they had have been presented directly following other stimuli from the IAPS.
The failure of the current study to find a negativity bias with the use of low-arousal stimuli is not, however, a detrimental finding. In fact, a promising alternative argument suggests that valence and arousal interact in evaluative processing. As a result, participants have been found to respond faster to stimuli that are either both highly arousing and negative, or low in arousal and positive than, their valence-matched equivalents (Robinson, Storbeck, Meier, & Kirkeby, 2004). Likewise, low-arousal positive IAPS and word stimuli have been found to be judged as more intense than low-arousal negative IAPS and word stimuli, while high-arousal negative IAPS and words were rated more intense than high-arousal positive IAPS and words (Ito, Cacioppo, & Lang, 1998a). These findings can be directly related to the behavioural dispositions of approach and avoidance. It is suggested that with moderate levels of intensity, or arousal, approach behaviours are facilitated. In contrast, at higher levels of intensity or arousal, avoidance behaviours are facilitated (Robinson et al., 2004).

In understanding the superior responsiveness to highly arousing negative stimuli, it is suggested that throughout evolution, high intensity stimulation has been associated with danger, therefore individuals are tuned to respond to such stimuli (Robinson et al., 2004). As already mentioned, the consequences of reacting slowly to negative stimuli are often more dangerous than those of positive stimuli (Cacioppo & Gardner, 1999;
Carretie et al., 2001a; Carretie et al., 2001b; Crawford & Cacioppo, 2002; Pratto & John, 1991) and negative events signal that the individual needs to change its current state or activity (Pratto & John, 1991). Hence, it follows that individuals possess a mechanism that enables the rapid detection of negative stimuli. This effect may not be as strong for low-arousal negative images as such images often result in a conflicted state divided between approach and avoidance behaviours (Lang, 1995).

In terms of highly arousing positive stimuli, processing tends to be slowed until the arousing nature of the stimuli is understood. With arousing stimuli, whether they are negative or positive, there tends to be an initial evaluation of the stimulus as potentially dangerous (Robinson et al., 2004). Although this conflict is eventually resolved, it may take some time. In contrast, positive stimuli that are low on arousal bring pleasure but no immediate harm or uncertainty. Hence, evaluations of these stimuli are immediate, and not slowed by evaluations of arousal (Robinson et al., 2004). This effect is likely to have occurred in the current study given that only low arousal images were used. In the current study, this was evident as a positivity bias, or offset. As an alternative argument to the negativity bias, it is sometimes argued that it is positive material that has the advantage in information processing. There is some speculation that healthy individuals tend to see the world through ‘rose-coloured’ glasses and are unrealistically
optimistic about the likelihood of negative events happening to themselves relative to others (Deldin et al., 2001a; Deldin et al., 2001b). Such a positivity offset is evident when the motivation to approach exceeds that to withdraw (Ito & Cacioppo, 2005; Ito et al., 1998) and without such a bias there would be little motivation to approach new situations and therefore little opportunity to learn about their potential value or threat (Cacioppo & Gardner, 1999).

In summary, there is an interaction between valence and arousal in emotional processing so that a positivity offset is observed under conditions of low motivation, and a negativity bias under conditions of high motivation. The presence of both a positivity offset and negativity bias enables the individual to engage in exploratory behaviour but also maintain self-preservation (Cacioppo, Larsen, Smith, & Berntson, 2004).

In addition to differences in stimulus arousal, Carretie et al. (2004), Delplanque et al. (2004) and Smith et al. (2003), all involved either mixed gender samples, or exclusively female samples. The current study involved an exclusively male sample. While males were specifically chosen due to time constraints involved with testing females during the follicular stage of the menstrual cycle (Harrison et al., 2002), it has been established that males and females differ in their processing of emotional stimuli. In a SSPT study
by Kemp, Silberstein, Armstrong, and Nathan (2004b), females were more responsive than males to negatively-valenced IAPS. Additionally, in Kemp et al.’s (2004a) SSPT study, following repeated administration of the serotonin enhancing agent citalopram, responses to pleasant images were predominantly influenced by males, whereas responses to unpleasant images were predominantly influenced by females. Hence had females been included in the sample, it is possible that they would have displayed greater early attention to the negative stimuli than the positive stimuli.

4.2.1 The effects of neurotransmitter depletion on emotional processing

Contrary to expectations, there were no differences in emotional processing between any of the treatment conditions. That is, reduced serotonin (via tryptophan depletion), reduced dopamine (via tyrosine/phenylalanine depletion) and combined monoamine depletion (via tryptophan and tyrosine/phenylalanine depletion) did not increase the P100 to negative stimuli relative to the control. They also did not differ in P100 to each other. In terms of reduced serotonin, an examination of the means revealed that the average amplitude for the P100 to negative stimuli was greater than that for the control condition, the interaction between stimulus valence and treatment condition failed, however to reach significance.
Likewise, with reduced dopamine, the means revealed that the average P100 amplitude peak was higher, although not significantly, towards negative stimuli in the catecholamine depletion condition compared with the control condition. Additionally, after combined monoamine depletion, the means revealed that the amplitude of the P100 was higher to negative IAPS, and lower to positive IAPS, as compared with the control condition. While this seems to be in the direction of what would be expected, again, this failed to reach significance.

As there have been no prior studies that have examined neurotransmitter manipulation and early emotional processing in terms of attention, the results of the current study cannot be directly compared to past research. While other studies have been successful in showing neurotransmitter modulation of emotion, they have generally measured emotional processing in terms of reaction time, recognition, memory and so on. The fact that differences in emotional processing following depletion have been found in the past, together with the fact that the current study failed to find any effects of depletion on the P100 suggests that the neurotransmitters perhaps do not actually modulate emotion as early as attention.
With serotonin manipulation, for instance, past research has shown participants to be significantly slower to respond to happy targets (Murphy et al., 2004) and to be impaired in their ability to recognise fearful faces (Harmer et al., 2003) following depletion. Murphy et al. (2004) suggested that it was actually distraction from negative stimuli that resulted in the poor performance for the positive stimuli, hence providing indirect evidence of a negativity bias. The current study on the other hand did not find either reduced responsiveness to positive stimuli, or increased responsiveness to negative stimuli following serotonin depletion. The results regarding impaired recognition of fearful faces following tryptophan depletion are also difficult to reconcile with the current research, given that fearful stimuli are highly arousing and the current study only involved low-arousal stimuli.

With administration of serotonin past research has found increased processing of positive information together with decreased processing of negative information (Harmer et al., 2004; Kemp et al., 2004a). In Harmer et al.’s study, a range of findings supported the idea of a shift in emotional processing from negative to positive stimuli following increased serotonin. In their study, for instance, participants had a decreased recognition of negative facial expressions, together with increased likelihood of perceiving negative emotions as positive. Likewise, following the administration of the antidepressant, citalopram, Kemp et al. (2004a) found attenuation of cortical
responses to negative stimuli together with enhanced responses to positive stimuli. Clearly then, manipulation of serotonin does have an effect on emotional processing. The fact that the current study did not find neurotransmitter modulation of the P100 suggests, however, that these drugs are unlikely to be influencing emotional processing by affecting attentional biases.

Likewise, past research involving catecholamine depletion has shown modulation of emotional processing. McLean et al. (2004) found participants to display negativity biases towards sad words (McLean et al., 2004) and conservative bets in decision-making tasks (McLean et al., 2004). This suggest firstly, that with reduced dopamine there is increased processing of negative material, and secondly there is a reduction in the processing of positive material. Again, caution must be advised when comparing the results of the current study to those of past research as the past research involved measures far less sensitive than the P100. Again it can be assumed that while manipulation of dopamine does affect emotional processing, it is unlikely to be affecting early attentional biases.

In line with the results of serotonin and dopamine modulation of emotion, it is difficult to compare the current P100 findings for combined monoamine depletion with past research as no other studies have as yet been
conducted in this area. A behavioural study of emotional processing did not find any changes relative to placebo following combined on an emotional Stroop task, or in response to IAPS (Hughes et al., 2004). Like event-related potentials, the Stroop is also sensitive to attention. Given that the Stroop findings of Hughes et al. (2004) and the current P100 findings failed to find neurotransmitter modulation of attention, it can again be assumed that the neurotransmitters, serotonin and dopamine are not affecting early attention.

4.3 Mood

Participants did not become mentally slower, sadder, and more antagonistic over time as a function of treatment condition. They did, however, become mentally slower and more antagonistic over time independent of the treatment condition. It is likely that the mood effects observed occurred due to the long nature of the testing days. The failure to find any mood effects as a result of treatment condition are somewhat unsurprising given the inconsistency of mood findings in tryptophan and tyrosine/phenylalanine depletion studies. As previously mentioned, tryptophan depletion is thought to only result in lowered ratings of mood when the subjects involved are belonging to subgroups recovering from depression or healthy individuals with a genetic susceptibility to depression (for reviews see Booij et al., 2003; Van der Does, 2001). Likewise tyrosine/phenylalanine depletion is also not a reliable manipulator of mood. It seems that both tryptophan
depletion and tyrosine/phenylalanine depletion are more likely to result in mood changes in healthy individuals upon subjection to psychological stress (Leyton et al., 2000). The participants in the current study underwent a physical and psychological examination by a qualified medical practitioner and were excluded from participation if they had a personal or family history of depression. Also, the current study did not involve any psychological challenges. Hence, it is understandable that drug-related effects of mood failed to occur under either tryptophan or tyrosine depletion.

The combined monoamine depletion did not produce any changes in mood. This finding was inconsistent with Hughes et al. (2004) who found participants became mentally slower, more antagonistic, and sadder following administration of the combined monoamine depletion mixture. Still, it must be noted that the study by Hughes et al. (2004) used only female participants whereas the current study involved only males. As already mentioned, the rate of serotonin synthesis in females is considerably lower than that in males (Nishizawa et al., 1997). Hence, following tryptophan depletion, there is a larger decrease in the rate of serotonin synthesis in females than in males (Nishizawa et al., 1997) explaining why females are more susceptible than males to a lowering of mood following
tryptophan depletion (Ellenbogen, Young, Dean, Palmour, & Benkelfat, 1996).

4.4 Limitations and directions for future research

This study did have a few drawbacks that may have jeopardised the results. As mentioned, the current study only involved male participants. Males were chosen on purpose due to time constraints involved with testing females during the follicular stage of the menstrual cycle (Harrison et al., 2004). Upcoming studies would benefit, however, from a mixed gender sample. This would not only increase the meaningfulness of the findings to the population at large, but would also be more likely to highlight any effects of the depletion techniques. It has already been established that with tryptophan depletion females are more susceptible than males to mood-lowering (Ellenbogen et al., 1996). In addition, gender differences have been found in the processing of emotional stimuli with (Kemp et al., 2004a) and without the administration of the serotonin-enhancing agent, citalopram (Kemp et al., 2004b).

The sample was also very small in number, due to the extensive testing procedure involved in testing one subject. A power analysis revealed, however, that to be 95% confident of finding even a very small effect size (.12) with a power of .80, the sample would need to include at
least 1122 participants. Given the extremely large sample size required, it is unlikely that neurotransmitter depletion is actually having a biological effect on attentional processes.

One important limitation of the current study concerns the neurotransmitter depletion techniques themselves. While tryptophan depletion techniques have been found to lower plasma levels by up to 70-90% (Young et al., 1985) and tyrosine/phenylalanine depletion techniques have been found to lower plasma tyrosine and phenylalanine by up 70.2% and 87.3% and 70.2%, respectively (Moja et al., 1996), these are not direct measures of the levels of depletion achieved in the brain. Hence, it is not yet possible to tell exactly how much central serotonin and dopamine depletion has been achieved.

Despite the drawbacks, this study leads the way to several ideas for future research. Firstly, future research would benefit from comparing attentional biases to low and high arousal stimuli. The current study deliberately chose all low arousal stimuli to see whether the negativity bias would operate even under low arousal. Given that it is suggested both low-arousal positive stimuli, and high-arousal negative stimuli involve greater processing than their valence-matched counterparts, it would be interesting,
to compare early emotional processing in a paradigm involving both low and high arousal positive and negative stimuli.

It would also be advantageous to compare early emotional processing of healthy controls with depressed individuals. Emotional processing of the controls could be assessed under normal conditions, and also under neurotransmitter depletion. As depression involves chronic dysfunction in the neurotransmitters serotonin and dopamine, it would be interesting to compare these effects with the acute effects of neurotransmitter depletion.

Furthermore, the effects on emotional processing of antidepressants could be compared to the effects of neurotransmitter depletion. The depletion and antidepressant studies discussed have used a range of behavioural measures to assess emotional processing, making it relatively difficult to directly compare the effects of increased versus decreased neurotransmitter function. By applying an early attention paradigm such as the one used in the current study under conditions of decreased and increased neurotransmitter function, the roles of the neurotransmitters in emotional processing could be better established.
4.5 Implications and conclusions

The results of the current study were successful in showing valence-modulation of the P100 to emotional stimuli even when the stimuli were low in arousal. While the discrimination between negative and positive occurred very early on in processing, attention allocation was not in the expected direction. The fact that positive stimuli engaged attentional resources to a greater degree than negative stimuli points to the possible existence of a positivity bias, or offset. It seems that with low levels of arousal, approach behaviours are facilitated and this resulted in a greater response to the low-arousal positive images. Such results have implications for how we perceive and react to emotional situations at large.

Understanding the interactions of arousal and valence may help to interpret social interactions. For instance, most people would have encountered a situation whereby a highly dominant person evoked stronger, more negative reactions than a quiet, softly spoken person.

The current study failed to find any effects on early emotional processing as a consequence of serotonin, dopamine, and combined monoamine depletion. Yet neurotransmitters clearly have been shown to influence emotional processing. Likewise, antidepressants are successful in correcting the negative bias observed in depression. Thus it seems that
neurotransmitters do not influence emotional processing in terms of early attentional biases to positive and negative stimuli.
References


Delgado, P.L., Charney, D.S., Price, L.H., Aghajanian, G.K., Landis, H.,
Heninger, G.R. (1990). Serotonin function and the mechanism of
antidepressant action. *Archives of General Psychiatry, 47*, 411-418.
(1989). Neuroendocrine and behavioural effects of dietary
tryptophan restriction in healthy subjects. *Life Sciences, 45*, 2323-
2332.
Delgado, P.L., Price, L.H., Miller, H.L., Saloman, R.M., Aghajanian, G.K.,
neurobiology of depression. *Archives of General Psychiatry, 51*,
865-874.
face processing anomaly in depression. *Journal of Abnormal
Psychology, 109*, 116-121.
and emotion in neuropsychological models of depression. *Cognition
and Emotion, 15*, 787-802.
Modulation of cognitive processing by emotional valence studied
through event-related potentials in humans. *Neuroscience Letters,
356*, 1-4.


Harmer, C.J., Shelley, N.C., Cowen, P.J., & Goodwin, G.M. (2004). Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and


Roiser, J.P., McLean, A., Oglivie, A.D., Blackwell, A.D., Bamber, D.J.,
and cognitive effects of acute phenylalanine and tyrosine depletion
in patients recovered from depression. *Neuropsychopharmacology*,
1-11.

Emotional expression boosts early visual processing of the face:
ERP recording and its decomposition by independent component
analysis. *Cognitive Neuroscience and Neuropsychology, 12*, 709-
714.

and neuropsychological research. In F. Boller & J. Grafman (Eds.).
Handbook of Neuropsychology. Vol. 5 (Emotional behavior and its

and emotion: and ERP analysis of facilitated emotional stimulus
processing. *Cognitive Neuroscience and Neuropsychology, 14*, 1107-
1110.

priming methodology for studying self-representation in major


Appendix A

Copy of information and consent forms
Project Title:

The Effects of Dopamine Depletion and Serotonin Depletion on Emotional Processing and Cognition

Principal Investigators:

Ms Valerie Guille
Ms Sumie Leung
Mr Alan Dunne

Senior and Associated Investigators:

A/Prof Pradeep Nathan, A/Prof Rodney Croft, Dr. Susan Ilic, Ms Kirsty Scholes, Ms Hayley Lawrence and Ms Clementine Thurgood
Participant’s Name:________________________________________ Participant ID Code:____________

Only the Primary Investigators will have knowledge of the names and code numbers used. It is the responsibility of the Primary Investigators to destroy this information at the end of the study. If confidentiality is required to be broken, this may only be done by the Primary Investigators after consultation with the Participant in writing.

I, ……………………………………………………………………………………

(Name of participant) agree to participate in a research project entitled: The Effects of Dopamine Depletion and Serotonin Depletion on Emotional Processing and Cognition. conducted by Ms Valerie Guille, Ms Sumie Leung, Mr Alan Dunne, A/Prof. Pradeep Nathan, A/Prof Rodney Croft, Ms Kirsty Scholes, Ms Hayley Lawrence and Ms Clementine Thurgood. I have read and understood the information given to me regarding this project and any questions I have asked have been answered to my satisfaction.

My agreement is based on the understanding that:

• I agree to participate in this activity, realising that my identity will remain confidential, and that I may withdraw at any time.

• I do not have epilepsy, or a personal history of epilepsy

• I do not have any physical or psychiatric disorders

• I have been given a full explanation and a copy of the information sheet outlining the purpose of this study, the procedures involved, and what I will be expected to do.

• I am not on any medication

• I do not smoke
• I have been given an explanation of how the amino acid depletion procedure work and have been informed about the possible side effects.

• I understand that participation in this study involves the donation of 10 ml of blood, twice during each testing session

• My consent to participate in this project is given freely.

• I understand the time involved in the medical screening session and each of the four recording sessions.

• I agree that research data collected for the study may be published or provided to other researchers on the condition that anonymity is preserved and that I cannot be identified.

• I agree to follow the diet recommended by the investigators on the day prior to testing.

SIGNED……………………………………………………………………DATE……..
………………………….
(Participant)

SIGNED……………………………………………………………………DATE……..
………………………….
(Researcher)

1 Privacy Act, 1988 Commonwealth of Australia
1 The December 1991 Guidelines for Good Clinical Research Practice in Australia, published by the Therapeutic Goods Administration of the Commonwealth Department of Health and Family Services, recommends retention of data for at least 15 years.
1 Uniform Requirement for Manuscripts Submitted to Biomedical Journals as presented in JAMA 1993: 269:2282-6
Appendix B

Copy of the low protein diet
Thank you for volunteering to participate in our research and we hope it will turn out to be a learning experience for all involved. As we will be attempting to modify your amino acid concentrations with a dietary intervention, it is essential for the success of the study that you follow the suggested diet on the day before testing (attached). This diet has been carefully selected to be nutritionally balanced and healthy. You do not have to eat everything on the list, nor do you have to be strict with each category. E.g. You could have a salad for lunch with lettuce, carrot, celery, tomato and cucumber. You may substitute/swap items on the list, but it is important that you do not consume any foods high in protein. Also, it is very important that you do not eat after 7pm the day before the test. If you have any further questions feel free to contact any of the investigators and we will be happy to assist you. Thank you again for your assistance and we look forward to working with you.
# Low Protein Diet

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

Copy of the international affective picture system stimulus codes
Normative Valence and Arousal Ratings for the International Affective Picture System

<table>
<thead>
<tr>
<th>Description</th>
<th>Slide No.</th>
<th>Valence</th>
<th>Arousal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutilation</td>
<td>3064</td>
<td>1.78</td>
<td>5.44</td>
</tr>
<tr>
<td>Cemetery</td>
<td>9220</td>
<td>2.27</td>
<td>3.83</td>
</tr>
<tr>
<td>BatteredFem</td>
<td>3180</td>
<td>2.27</td>
<td>5.17</td>
</tr>
<tr>
<td>DyingMan</td>
<td>3230</td>
<td>2.44</td>
<td>5</td>
</tr>
<tr>
<td>Soldier</td>
<td>9421</td>
<td>2.47</td>
<td>4.86</td>
</tr>
<tr>
<td>Cat</td>
<td>9571</td>
<td>2.65</td>
<td>4.68</td>
</tr>
<tr>
<td>Cigarettes</td>
<td>9830</td>
<td>2.65</td>
<td>4.8</td>
</tr>
<tr>
<td>Baby</td>
<td>2053</td>
<td>2.78</td>
<td>4.65</td>
</tr>
<tr>
<td>BatteredFem</td>
<td>3181</td>
<td>2.79</td>
<td>4.9</td>
</tr>
<tr>
<td>Police</td>
<td>6831</td>
<td>2.98</td>
<td>5</td>
</tr>
<tr>
<td>Garbage</td>
<td>9330</td>
<td>3</td>
<td>4.26</td>
</tr>
<tr>
<td>Girl</td>
<td>2276</td>
<td>3.17</td>
<td>4.02</td>
</tr>
<tr>
<td>Sick kitty</td>
<td>9561</td>
<td>3.2</td>
<td>4.18</td>
</tr>
<tr>
<td>Speeding</td>
<td>9417</td>
<td>3.4</td>
<td>4.37</td>
</tr>
<tr>
<td>ScaredChild</td>
<td>9041</td>
<td>3.43</td>
<td>4.3</td>
</tr>
<tr>
<td>Family</td>
<td>9046</td>
<td>3.87</td>
<td>3.85</td>
</tr>
</tbody>
</table>

(Lang, Bradley, & Cuthbert, 1999)
Appendix D

Copy of the visual analogue mood scale I
**Visual Analogue Mood Scale**

**I**

**Instructions:**
*Please rate the way you feel in terms of the dimensions given below*
*Regard the line as representing the full range of each dimension*
*Rate your feelings as they are at the moment*
*Mark clearly and perpendicularly across each line*

e.g. .................................................................

<table>
<thead>
<tr>
<th>Alert</th>
<th>Drowsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calm</td>
<td>Excited</td>
</tr>
<tr>
<td>Strong</td>
<td>Feeble</td>
</tr>
<tr>
<td>Muzzy</td>
<td>Clear-headed</td>
</tr>
<tr>
<td>Well-coordinated</td>
<td>Clumsy</td>
</tr>
<tr>
<td>Lethargic</td>
<td>Energetic</td>
</tr>
<tr>
<td>Contented</td>
<td>Discontented</td>
</tr>
<tr>
<td>Troubled</td>
<td>Tranquil</td>
</tr>
<tr>
<td>Mentally slow</td>
<td>Quick-witted</td>
</tr>
<tr>
<td>Tense</td>
<td>Relaxed</td>
</tr>
<tr>
<td>Attentive</td>
<td>Dreamy</td>
</tr>
<tr>
<td>Incompetent</td>
<td>Proficient</td>
</tr>
<tr>
<td>Happy</td>
<td>Sad</td>
</tr>
<tr>
<td>Antagonistic</td>
<td>Amicable</td>
</tr>
<tr>
<td>Interested</td>
<td>Bored</td>
</tr>
<tr>
<td>Withdrawn</td>
<td>Sociable</td>
</tr>
</tbody>
</table>
Appendix E

Copy of the visual analogue mood scale II
Visual Analogue Mood Scale

II

Instructions:
*Please rate the way you feel in terms of the dimensions given below*
*Regard the line as representing the full range of each dimension*
*Rate your feelings as they are at the moment*
*Mark clearly and perpendicularly across each line*

e.g. ...................................................

Alert ...................................................... Drowsy
Calm ...................................................... Excited
Strong ..................................................... Feeble
Muzzy ...................................................... Clear-headed
Well-coordinated ..................................... Clumsy
Lethargic ................................................ Energetic
Contented .............................................. Discontented
Troubled ............................................... Tranquil
Mentally slow ......................................... Quick-witted
Tense ..................................................... Relaxed
Attentive ............................................... Dreamy
Incompetent .......................................... Proficient
Happy ................................................... Sad
Antagonistic .......................................... Amicable
Interested ............................................. Bored
Withdrawn ............................................. Sociable