The Pharmacology of the Loudness
Dependence of the Auditory Evoked
Potential (LDAEP)

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Abstract

The loudness dependence of the auditory evoked potential (LDAEP) has been proposed as a valid means of assessing brain serotonin (5-hydroxytryptamine, 5-HT) function. This electrophysiological biomarker may prove useful for diagnosis of psychiatric conditions and possible pharmacological intervention. While there is some evidence that supports the validity of this measure, there is also evidence suggesting that it may not be solely specific to the 5-HT system. The present thesis utilised a series of acute pharmacological challenges in healthy volunteers, in order to assess its sensitivity to other neurotransmitter systems and to clarify the relative specificity of the LDAEP for 5-HT.

Initially, the acute tryptophan depletion paradigm was used to assess the serotonergic sensitivity of the LDAEP. In addition, the acute tyrosine/phenylalanine depletion paradigm was employed to investigate whether depletion of central dopamine levels may affect the LDAEP. In an effort to elucidate possible synergistic interactions between the monoaminergic systems as regards the modulation of the LDAEP a recent novel approach, the combined tryptophan/tyrosine/phenylalanine depletion paradigm, was utilised. The selective and combined 5-HT and dopamine depletions had no effect on the LDAEP, suggesting the LDAEP may be insensitive to acute changes in serotonergic neurotransmission and possibly monoaminergic activity in general.

In the second study of this thesis, the dopaminergic sensitivity of the LDAEP was investigated further by augmenting dopamine neurotransmission utilising the dopamine agonists pergolide and bromocriptine. Despite previous evidence present in the literature, dopaminergic receptor stimulation resulted in no change in the LDAEP.

In addition to alterations induced by neuromodulators such as the monoamines, evoked potentials may be influenced by both inhibitory (i.e. γ-aminobutyric acid - GABA) and excitatory (i.e. glutamate) neurotransmitters. Hence the final experiment in this thesis examined the effects of acute administration of high-dose oral glycine (an excitatory NMDA receptor co-agonist/inhibitory glycine receptor agonist) on the LDAEP. Glycine administration attenuated the LDAEP though the mechanism by which this was achieved is yet to be ascertained.
Nevertheless this result demonstrates modulation of the LDAEP by neurochemical systems other than the serotonergic system.

In summary, acute dopaminergic stimulation, selective and combined monoaminergic depletion had no effect, while possible NMDA receptor stimulation resulted in a significant attenuation of the LDAEP in healthy humans. The implications of the findings of this thesis, when taken together with previous observations from pre-clinical and clinical literature, seem to indicate that the LDAEP may be insensitive to acute changes in monoaminergic neurotransmission as well as exhibiting a sensitivity to neurotransmitter systems other than 5-HT. However, before disregarding the LDAEP as a specific indicator of serotonergic function altogether, the chronic sensitivity and selectivity of this electrophysiological biomarker needs to be elucidated.
Acknowledgements

I would like to thank my supervisors for their help and guidance throughout the duration of this thesis. Firstly, I owe much thanks to Professor Pradeep Nathan for initially giving me the opportunity to study in Australia and for all his insights, ideas and encouragement along the way. The chance to study and live in Australia has been immeasurably rewarding and helped me grow as both a person and researcher and I am forever indebted to him. Secondly, I am extremely grateful to Professor Rodney Croft for his assistance with all things statistical and for his encouragement and invaluable advice at all times.

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To my Mum, Dad, brother Darragh and all my friends both at home in Ireland and here in Australia, thank you all so much for making these three years pass by so quickly. Had it not been for all your support and encouragement this thesis would not have been possible.

Finally, I would like to take this chance to both thank and remember my fellow PhD student, Alan Dunne. I had the pleasure of knowing Alan before his untimely passing and am grateful for having known such a fantastic and generous person.
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma except where due reference is made in the text of this thesis. To the best of my knowledge this thesis contains no material previously published or written by another person, except where due reference is made.

I declare that the ethical principles and procedures specified in the Swinburne University of Technology Human Research Ethics document on human research and experimentation have been adhered to in the presentation of this thesis.

Barry Vincent O’Neill

April 2008
“Serotonin is an enigma.

*It is at once implicated in virtually everything but

*responsible for nothing”*

B.L. Jacobs and C.A. Fornal
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Abbreviations

AEP    Auditory Evoked Potential
AMPT   α-methyl-paratyrosine
ANOVA  Analysis of Variance
ATD    Acute Tryptophan Depletion
ATPD   Acute Tyrosine/Phenylalanine depletion
ASF    Amplitude versus Stimulus intensity
function
BAL    Balanced/Control amino acid mixture
BBB    Blood Brain Barrier
cAMP   3’-5’ cyclic Adenosine MonoPhosphate
CMD    Combined Monoamine Depletion
CNS    Central Nervous System
COMT   Catechol-O-methyltransferase
CSF    CerebroSpinal Fluid
DA     Dopamine
DAT    Dopamine Transporter
dB     Decibel
DRD4 exon III Dopamine D4 Receptor Gene
DRN    Dorsal Raphé Nucleus
DSA    Dipole Source Analysis
EEG    Electroencephalography
EOG    Electro-OculoGram
ERP    Event Related Potential
g     Gram
GABA   γ-AminoButyric Acid
GAD    Generalised Anxiety Disorder
g/kg  Gram per kilogram
GlyR   Inhibitory Glycine Receptor
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
</tr>
<tr>
<td>GMS</td>
<td>Glycine Modulatory Site</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>IDAEP</td>
<td>Intensity Dependence of the auditory evoked potential</td>
</tr>
<tr>
<td>ILE</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>ISI</td>
<td>Inter-Stimulus Interval</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>LDAEP</td>
<td>Loudness Dependence of the Auditory Evoked Potential</td>
</tr>
<tr>
<td>LEU</td>
<td>Leucine</td>
</tr>
<tr>
<td>LNAA</td>
<td>Large Neutral Amino Acid</td>
</tr>
<tr>
<td>MDMA</td>
<td>MethyleneDioxyMethAmphetamine</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>MMN</td>
<td>MisMatch Negativity</td>
</tr>
<tr>
<td>MRN</td>
<td>Median Raphé Nucleus</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
<tr>
<td>NA</td>
<td>Noradrenaline</td>
</tr>
<tr>
<td>NMDAR</td>
<td>N-Methyl-$d$-aspartate Receptor</td>
</tr>
<tr>
<td>NRI</td>
<td>Noradrenaline Re-uptake Inhibitor</td>
</tr>
<tr>
<td>OCD</td>
<td>Obsessive Compulsive Disorder</td>
</tr>
<tr>
<td>PAC</td>
<td>Primary Auditory Cortex</td>
</tr>
<tr>
<td>PET</td>
<td>Positron Emission Tomography</td>
</tr>
<tr>
<td>PHE</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>PLC</td>
<td>PhosphoLipase C</td>
</tr>
<tr>
<td>p-r</td>
<td>Pseudo-random</td>
</tr>
<tr>
<td>r</td>
<td>Random</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin Transporter</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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xvii
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>SNP</td>
<td>Single Nucleotide Polymorphism</td>
</tr>
<tr>
<td>SOA</td>
<td>Stimulus Onset Asynchrony</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single Photon Emission Computerised Tomography</td>
</tr>
<tr>
<td>SPL</td>
<td>Sound Pressure Level</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SSRI</td>
<td>Selective Serotonin Re-uptake Inhibitor</td>
</tr>
<tr>
<td>TPH</td>
<td>Tryptophan Hydroxylase</td>
</tr>
<tr>
<td>TRP</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>TYR</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>VAL</td>
<td>Valine</td>
</tr>
<tr>
<td>VAMS</td>
<td>Visual Analogue Mood Scales</td>
</tr>
<tr>
<td>VMAT2</td>
<td>Vesicular Monoamine Transporter 2</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>5-HIA</td>
<td>5-HydroxyIndole Acetaldehyde</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>5-HydroxyIndoleAcetic Acid</td>
</tr>
<tr>
<td>5-HTP</td>
<td>5-HydroxyTryptoPhan</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-HydroxyTryptamine (Serotonin)</td>
</tr>
<tr>
<td>5-HTT</td>
<td>Serotonin transporter gene</td>
</tr>
<tr>
<td>5-HTTLPR</td>
<td>Polymorphism in the promoter region of the serotonin transporter gene</td>
</tr>
<tr>
<td>8-OH-DPAT</td>
<td>8-Hydroxy-DiPropylAminoTetralin</td>
</tr>
<tr>
<td>$[^{123}\text{I}]\text{ADAM}$</td>
<td>2-((2-((dimethylamino)methyl)phenyl)thio)-5-iodophenylamine</td>
</tr>
<tr>
<td>$[^{123}\text{I}]\beta\text{-CIT}$</td>
<td>2β-carbomethoxy-3β-(4-iodophenyl)tropane</td>
</tr>
<tr>
<td>μV</td>
<td>Microvolt</td>
</tr>
<tr>
<td>μV/dB</td>
<td>Microvolt per Decibel</td>
</tr>
<tr>
<td>-ve</td>
<td>negative</td>
</tr>
<tr>
<td>-----</td>
<td>----------</td>
</tr>
<tr>
<td>+ve</td>
<td>positive</td>
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1.1 Overview

In 2001 the World Health Organisation (WHO) estimated that 154 million people globally suffer from depression and 25 million people from schizophrenia (WHO, 2001). Monoaminergic neurotransmission (i.e. serotonin (5-hydroxytryptamine (5-HT)), dopamine (DA) and noradrenaline (NA)) plays a major role in the pathophysiology of these and other related psychiatric conditions (Carlsson, 1988, Charney et al., 1990, Ressler and Nemeroff, 2000). To date, despite progress in medical research, the inability to routinely and non-invasively determine the integrity of these monoaminergic systems, particularly the serotonergic system, has limited our understanding and treatment of neuropsychiatric disorders. Discovery of a valid means of assessing in vivo brain 5-HT function may provide for less problematic diagnosis of psychiatric conditions and aid in more accurate and effective pharmacological intervention.

Current methods for measuring central 5-HT activity are thought to be unreliable, inconvenient, and both time and resource consuming. In their place, an alternative neurophysiological approach has been proposed, the assessment of the loudness dependence of the auditory evoked potential (LDAEP). Converging preclinical and clinical evidence indicates that the LDAEP is related to serotonergic function in the primary auditory cortex, such that an increased LDAEP is reflective of decreased serotonergic function and vice versa (Hegerl and Juckel, 1993, Juckel et al., 1997, Hegerl et al., 1998, Juckel et al., 1999, Gallinat et al., 2000, Strobel et al., 2003 Juckel et al., 2003, Pogarell et al., 2004, Lee et al., 2005, Nathan et al., 2006, Hensch et al., 2006). Despite a relatively large body of research documenting an association between conditions thought to possess serotonergic disturbances and the influence of various serotonergic manipulations on the LDAEP, the exact nature of the relationship between this neuromodulator and the electrophysiological marker is yet to be elucidated. Throughout the extant literature there are inconsistent reports of the LDAEP remaining unaffected following serotonergic modulation.
in both patient populations and healthy controls, indeed there are also reports of other neuromodulators, other than 5-HT, influencing the LDAEP.

Accordingly, the aim of this thesis is to investigate the pharmacology of the loudness dependence of the auditory evoked potential (LDAEP). More specifically, it was to investigate the serotonergic sensitivity and specificity of the LDAEP by assessing the effects of modulating monoamine (i.e. 5-HT and dopamine) and neurotransmitter (i.e. glutamate) systems in healthy volunteers.

The first chapter of this thesis provides an introduction to 5-HT, electroencephalography (EEG), event related potentials (ERPs) and a review of the relevant LDAEP literature. In the first section of this chapter 5-HT, the human serotonergic system and its involvement in the auditory system are introduced. This is followed by a brief description of EEG and event related potentials (ERPs). Finally the rationale behind the LDAEP and a thorough review of the extant literature involving the LDAEP will be presented, with the chapter closing with specific aims and research questions to be addressed during the course of this thesis.

Chapter 2 will outline the general methodology used throughout the thesis. Chapters 3 to 5 are the experimental investigations and each of these chapters will outline the goals and results of each experiment and discuss their possible implications. Finally, the concluding chapter (Chapter 6) will discuss the findings of the thesis and outline the possible ramifications of these results for the literature as well as recommendations for future research.
1.2 Serotonin

As early as 1868 it was known that the blood contained a vasoconstrictive substance (Page, 1978). This substance, which was released in serum during platelet break-down, proved to be a problem to researchers investigating malignant hypertension. Some years later this compound was identified as serotonin (5-HT, 5-hydroxytryptamine), named in reference to its vasoconstrictive properties (Rapport, 1949). The key work on the isolation, chemical synthesis and the final identification of 5-HT took place between 1946 and 1953 after which it was distributed to interested scientists around the world (Green, 2006). From the laboratories of these scientists a research renaissance of sorts occurred, made possible by improving analytical techniques, delineation of pathways for synthesis and degradation of 5-HT, and further advances in knowledge of drugs involved in 5-HT related processes (Udenfriend, 1959, Page, 1968, Sjoerdsma et al., 1970, Pletscher, 1973).

Initially the focus of serotonergic research was on its actions in peripheral tissues (Gaddum and Hameed, 1954) but following research demonstrating the localised distribution of 5-HT neurons throughout the brain using fluorescence histochemistry (Dahlström and Fuxe, 1964), its role as a neurotransmitter became the focal point. Discoveries followed outlining the role of 5-HT in several affective disorders and as time passed its role in schizophrenia and several other neurological conditions came to light (for reviews see Jones and Blackburn, 2002, Green, 2006). Based on these discoveries, researchers were encouraged to engage in new research resulting in a wealth of literature which has proved to be the basis of our current knowledge on serotonergic neuropharmacology. Below is a brief outline of 5-HT synthesis and metabolism, 5-HT pathways and 5-HT receptors (for a more detailed account of the serotonergic system see Fuller, 1980, Tyce, 1990, Jacobs and Azmitia, 1992 and Barnes and Sharp, 1999).
1.2.1 Synthesis and metabolism of serotonin

5-HT is synthesised from the essential amino acid tryptophan (TRP) within neurons in the brain. Firstly, the rate-limiting enzyme tryptophan hydroxylase (TPH) catalyses the hydroxylation of TRP to 5-hydroxytryptophan (5-HTP), which is then rapidly converted to 5-hydroxytryptamine (5-HT, serotonin) by aromatic l-amino acid decarboxylase (Tyce, 1990) (Figure 1-1). Under normal conditions brain TPH is not saturated with its substrate (TRP); consequently the amount of TRP in the brain, influences the synthesis of 5-HT. In addition, the carrier system which actively transports TRP into the brain across the blood brain barrier (BBB) also transports other large neutral amino acids (LNAAs). Subsequently levels of TRP in the brain are not only influenced by its plasma concentrations but also by the plasma concentrations of other amino acids that compete for the same carrier system to transport them across the BBB (Oldendorf and Szabo 1976). These mechanisms may be manipulated in order to alter the concentration of 5-HT in the brain and such methods and their implications are discussed later (see Chapter 2 and Chapter 3).

Once synthesised 5-HT may be packaged in synaptic vesicles, via the action of vesicular monoamine transporter 2 (VMAT2) and then released into the extracellular fluid by calcium-dependent exocytosis (Tamir and Gershon, 1990). 5-HT that is not placed in vesicles for release may have two fates:

1. In most areas of the brain it is metabolised by monoamine oxidase B (located on the mitochondrial membrane) to 5-hydroxyindole acetaldehyde (5-HIA) and then by aldehyde dehydrogenase to the acid metabolite 5-hydroxyindoleacetic acid (5-HIAA), which is excreted from the cell by an energy-dependent clearance mechanism (Tyce, 1990, Boadle-Biber, 1993). 5-HIAA may then be actively transported out of the brain and excreted in urine (levels of 5-HIAA in cerebrospinal fluid (CSF), blood or urine are used in an attempt to assess central 5-HT function - see section 1.6)

2. In the pineal gland it is rapidly converted to melatonin through the actions of two enzymes, firstly 5-HT N-acetylase followed by 5-Hydroxyindole-O-methyltransferase (Tyce, 1990). Melatonin is a
hormone believed to be involved in circadian rhythms and skin pigmentation and has been used clinically to treat certain pigment disorders as well as a proposed treatment for jet lag (Lewy, 2007).

![Serotonin synthesis and metabolism](image)

**Figure 1-1:** Serotonin synthesis and metabolism. Adapted from Rang et al., 2003.

### 1.2.2 Serotonergic receptors

At present, it is known that 5-HT acts on 14 structurally and pharmacologically distinct mammalian 5-HT receptor subtypes (5-HT<sub>1A</sub>–7) (Figure 1-2) (for review, see Barnes & Sharp, 1999, Hoyer et al., 2002). With the exception of the 5-HT<sub>3</sub> receptors (a ligand-gated ion channel), 5-HT receptors belong to the G-protein-coupled receptor (GPCR) superfamily, making it one of the largest and most complex efferent systems in the central nervous system (CNS).

The 5-HT<sub>1</sub> receptor class is comprised of five receptor subtypes (5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub>) (Hoyer et al., 2002). 5-HT<sub>1E</sub> and 5-HT<sub>1F</sub> are given
the lower case appellation to denote that endogenous receptors with a physiological role have not yet been found whereas the other receptor types have been demonstrated functionally in a number of tissues in various species (Hoyer et al., 2002). The 5-HT\textsubscript{1C} designation has been left vacant as this receptor was reclassified to 5-HT\textsubscript{2C} due to similarities with the 5-HT\textsubscript{2} receptor subclass (Hoyer et al., 1994).

![Diagram of 5-HT receptors](image)

**Figure 1-2:** Graphical representation of the current classifications of 5-HT receptors. Receptor subtypes represented by shaded boxes and lower case designate receptors that have not been demonstrated to definitively function in native systems. (Abbreviations: 3'-5' cyclic adenosine monophosphate (cAMP); phospholipase C (PLC); negative (-ve); positive (+ve)) (Figure reproduced from Hoyer et al., 2002)

5-HT\textsubscript{1} receptors are located in both peripheral and central tissues and involved in numerous processes in both locations. A notable member of the 5-HT\textsubscript{1} family is the 5-HT\textsubscript{1A} receptor which is largely distributed throughout the CNS and acts as a critical mediator of 5-HT function. They are expressed on 5-HT neurons in the raphé nuclei and function as autoreceptors that negatively regulate 5-HT tone (Gozlan et al., 1983, Marcinkiewicz et al., 1984). Postsynaptically, they are located on structures thought to mediate cognition and emotion and affect several CNS structures including the cerebral cortex, hippocampus and
amygdala (for review, see Lanfumey and Hamon, 2000). In addition 5-HT_{1A} receptors are thought to play a role in several affective disorders including anxiety and depression (for review, see Hensler, 2003, Blier and Ward, 2003).

5-HT_{2} receptors comprise the 5-HT_{2A}, 5-HT_{2B} and the 5-HT_{2C} subtypes and like 5-HT_{1} receptors, this class of receptors are widely distributed in peripheral and central tissues and are involved in processes ranging from mediating contractile responses in vascular smooth muscle to playing a role in the antipsychotic efficacy of drugs such as clozapine and olanzapine (for review, see Leysen, 2004). 5-HT_{3} receptors are the only ligand-gated ion channel receptor within the serotonergic receptor superfamily and are located in several brain regions including the CA1 pyramidal cell layer of the hippocampus (Laporte et al., 1992) and peripheral tissues such as the gastrointestinal tract (De Ponti and Tonini, 2001). 5-HT_{3} receptors are used clinically for the treatment of chemotherapy and radiotherapy-induced nausea and vomiting. Animal studies involving 5-HT_{3} antagonists have suggested a possible role for these agents in the treatment of anxiety and schizophrenia as well as reported cognition enhancing properties in rats (for review, see Costall and Naylor, 2004), though these results are yet to be replicated in human trials.

5-HT_{4} receptors possess several isoforms and are thought to be expressed in intestinal vascular smooth muscle and several areas throughout the CNS (Hoyer et al., 2002). Functionally, these receptors are involved in gastrointestinal motility, secretory responses of the intestinal mucosa in the periphery and modulation of neurotransmitter release as well as enhancement of synaptic transmission in the CNS. 5-HT_{4} receptors have also been implicated in memory enhancement, but further affirmative results in clinical trials are still needed (for review, see Bockaert et al., 2004). Of the remaining receptors the 5-HT_{5} receptor is the least characterised. These receptors have been identified in the human CNS but no functional role has been outlined as yet (for review, see Nelson, 2004). The 5-HT_{6} receptor is expressed in the CNS and targeted as a possible area of treatment for cholinergic deficits in cognitive dysfunction such as Alzheimer’s disease and several antipsychotic and antidepressant agents have displayed a high affinity and antagonistic properties at the 5-HT_{6} receptor.
suggesting a possible role for this receptor in psychiatric disorders (for review, see Branchek and Blackburn 2000; Woolley et al., 2004).

Finally, the 5-HT$_7$ receptor is found throughout the periphery and is involved in the relaxation of smooth muscle (both in the intestinal mucosa and cardiovascular areas). The recent advent of 5-HT$_7$ receptor specific antagonists has shed some light on the possible roles of this receptor in the CNS, with studies utilising these compounds suggesting that it is involved in the modulation of neuronal function in several areas including the hippocampus and thalamus. These receptors are also implicated in the control of circadian rhythms and sleep, as well as neurological disorders such as anxiety, cognitive disorders and migraine, though further studies are needed to confirm the promising therapeutic potential of this receptor type (for review, see Thomas and Hagan, 2004).

1.2.3 Serotonin pathways

It has been estimated that several hundred thousand serotonergic neurons reside in the human brain. When one considers that the CNS consists of billions of neurons this seems like a nominal amount, however despite being few in number, serotonergic neurons exert a strong modulatory influence in almost all parts of the CNS. These neurons chiefly originate in the midline of the upper brainstem, where the cell bodies aggregate into clusters called the raphé nuclei (Figure 1-3). The raphé 5-HT system innervates practically all the central nervous system (CNS) and is the first neurotransmitter system to develop in the embryo (Wilson and Molliver, 1991, Jacobs and Azmitia, 1992). The most caudal clusters innervate the medulla and spinal cord, the dorsal raphé (DRN) and the median raphé (MRN) (also known as the rostral nuclei) innervate much of the rest of the CNS by means of numerous and sometimes diffuse projection pathways (see Jacobs and Azmitia, 1992 for review). Although the DRN and MRN have overlapping terminal fields, the DRN preferentially innervates the cerebral cortex (including the primary auditory cortex), thalamus and striatal regions, whereas the MRN innervates the hippocampus, septum and other limbic regions (Figure 1-3) (Wilson and Molliver, 1991, Jacobs and Azmitia,
1992). Of relevance for this thesis is the serotonergic innervation of the auditory pathways in the CNS.

The auditory pathways begin in the organ of corti in the cochlear region and travel through the brainstem areas including the cochlear nuclei, superior olivary complexes and inferior colliculi and are innervated by serotonergic fibres throughout (Thompson et al., 1994). They then follow the auditory radiation to terminate in the auditory cortex located on the temporal lobe in the posterior half of the superior temporal gyrus and overlaps into the lateral sulcus as the transverse temporal gyri (also called Heschl's gyri) (Figure 1-4). The auditory cortex is divided into three separate parts, the primary, secondary and tertiary auditory cortex. These structures are formed concentrically around one another, with the primary auditory cortex (PAC) in the middle and the tertiary auditory cortex on the outside. The PAC is divided into 6 distinct cortical layers numbered I through VI, each with its own distinct cellular constituents (Table 1-1). The highest concentrations of cortical 5-HT and synthesis rates have repeatedly been found in the primary sensory cortices, especially in the PAC (Brown et al., 1979, Azmitia and Gannon 1986, Lewis et al., 1986, Campbell et al., 1987). Within the PAC, it is thought that cells of layer IV of the cortex, receiving specific thalamic sensory input (Winer, 1984) and projecting to pyramidal cell layers III and V, receive the greatest amount of serotonergic innervation (Morrison and Foote, 1982, 1986, Lewis et al., 1986, Berger et al., 1988).

The serotonergic innervation of the auditory pathways and of the cortical layers of the PAC, places the 5-HT system in an ideal position to modulate auditory processing in the CNS. A non-invasive investigative tool for observing neurochemical modulation of such processes is the electroencephalogram (EEG). The origins and basis of EEG are outlined in the following sections leading onto a description of the LDAEP itself.
Figure 1.3: The major serotonin pathways in the human brain (Sourced from www.CNSforum.com)

Figure 1.4: Ascending auditory pathway (Sourced from www.keck.ucla.edu)
<table>
<thead>
<tr>
<th>Layer</th>
<th>Description of cellular content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer I</td>
<td>Contains few scattered neurons and consists mainly of extensions of apical dendrites and horizontally oriented axons, and some Cajal-Retzius and spiny stellate neurons can be found.</td>
</tr>
<tr>
<td>Layer II</td>
<td>Contains small pyramidal neurons and numerous stellate neurons.</td>
</tr>
<tr>
<td>Layer III</td>
<td>Contains predominantly small and medium sized pyramidal neurons, as well as non-pyramidal neurons with vertically-oriented intracortical axons. Layers I through III are the main target of interhemispheric corticocortical afferents, and layer III is the principal source of corticocortical efferents.</td>
</tr>
<tr>
<td>Layer IV</td>
<td>Contains different types of stellate and pyramidal neurons, and is the main target of thalamocortical afferents as well as intra-hemispheric corticocortical afferents.</td>
</tr>
<tr>
<td>Layer V</td>
<td>Contains large pyramidal neurons as well as interneurons, and it has two sub-layers - a superficial cell-sparse layer and a deeper cell rich layer with large pyramidal neurons.</td>
</tr>
<tr>
<td>Layer VI</td>
<td>Contains few large pyramidal neurons and many small spindle-like pyramidal and multiform neurons. This layer sends efferent fibres to the thalamus establishing a very precise reciprocal interconnection between the cortex and the thalamus.</td>
</tr>
</tbody>
</table>

**Table 1-1: Summary of Laminar organisation of the Cerebral Cortex**

1.3 The Electroencephalogram

The EEG was first described more than a century ago in rabbits (Caton, 1875) with the first report concerning human EEG appearing more than 75 years ago (Berger, 1929). Since this time much has been learned about the nature and origin of EEG, making it an important research tool in both academic and clinical settings. EEG is the non-invasive measurement of the electrical activity of large numbers of neurons in the living brain, recorded with electrodes placed on the scalp. The electrodes are used to measure voltage fluctuations caused by variations in electrical potential between different areas of the CNS. As the EEG
is directly coupled to neuronal electrical activity it provides millisecond precision and allows the greatest temporal resolution compared to other brain imaging techniques (Debener et al., 2006). EEG is particularly valuable for measurement of an individual’s behavioural state (i.e. sleep, wakefulness, anaesthetised), in addition to its use as a clinical tool for observing and diagnosing neurological conditions such as epilepsy, sleep disorders and brain trauma.

1.4 Event related potentials

The term event related potential was first attributed to Herb Vaughan, who in 1969 wrote,

_The term “event related potentials” (ERPs) is proposed to designate the general class of potentials that display stable time relationships to a definable reference event (Vaughan, 1969)_

(Most electrophysiological research now utilises this nomenclature though exceptions are present, but for the purpose of this thesis ERP will suffice).

ERPs represent a series of positive and negative voltage deflections within an EEG that are time-locked to a particular neural event. This event may be a sensory stimulus (visual, auditory, somatosensory), omission of a stimulus (e.g. increased latency between presented stimuli) or a higher level cognitive event. Despite ERPs possessing a significantly lower voltage relative to the high background electrical noise of the spontaneous EEG, these small signals can be separated from the EEG by averaging a number of responses to a particular stimulus. ERP components may be interpreted in a number of ways but the principle factors are amplitude and latency, with amplitude of the ERP an indication of the intensity of neuronal activity and latency providing a measure of the speed of cognitive processing. The focus of this thesis is on the sensory stimuli, particularly the auditory evoked potential (AEP).
1.5 The loudness dependence of the auditory evoked potential (LDAEP)

Following presentation of an auditory stimulus there are notable components visible within the ERP (Figure 1-5):

- Early latency AEPs occur in the first 10-12 milliseconds (ms).
- Middle latency AEPs occurring between 12-50 ms.
- Long latency AEPs occur after 50 ms.

Only the components relevant to this thesis will be discussed, namely those occurring after 50 ms, the long latency AEPs. These potentials can be subdivided into exogenous (N100 or N1, P100 or P1, and P200 or P2) and endogenous components (P300). These exogenous components are primarily dependent upon characteristics of the external stimulus (i.e. loudness (intensity) or latency of the stimulus), and the endogenous components like P300 or the mismatch negativity (MMN), which are more dependent upon internal cognitive processes. The exogenous components of the AEP are the principle focus of this thesis.

At approximately 100 ms after a stimulus tone there is a noticeable increase in negative electrical activity of the ERP termed the “N1” followed by an increase in positive activity at 200 ms termed the “P2”. This pattern is called the N1/P2 complex of AEP and is thought to represent the processing of the tone in the auditory cortex. The intensity of the stimulus (i.e. loudness of the tone), affects the amplitude of the N1/P2 complex, such that louder tones evoke a greater increase in the amplitude of the N1/P2. This increase in amplitude in response to increasing stimulus loudness describes the loudness dependence of the auditory evoked potential (LDAEP). The LDAEP is based on the concept of augmenting/reducing, a phenomenon noted several decades ago whereby, individuals who show an increase in ERP amplitudes with increasing stimulus intensity are classed as augmenters and individuals who display a decrease in ERP amplitudes with increasing intensities are categorised as reducers (Buchsbaum and Silverman, 1968). It is believed that this concept represents
individual differences in the modulation of sensory input and reflects a central mechanism regulating neuronal sensitivity and protecting the organism from sensory overstimulation (Buchsbaum and Silverman, 1968, Hegerl and Juckel, 1993). However, the concept of augmenting/reducing was criticised on the grounds that it may be too dependent on methodological aspects i.e. electrode recording site, intensity range and stimulus modality (auditory vs. visual) (Connolly and Gruzelier, 1986, Connolly, 1987, Carrillo-de-la-Peña, 1992) and in its place the loudness dependence paradigm was hypothesised (Hegerl and Juckel, 1993).

Figure 1-5: Breakdown of an auditory evoked potential into its basic components (sourced from http://neurocog.psy.tufts.edu/images/erp.htm).

A measure of loudness dependence is achieved when the difference between the N1 and P2 of the N1/P2 complex are graphed against the loudness of the stimulus tone, and a single slope is calculated from these points. There are two accepted ways to calculate the slope with the preferred method being the use of a regression equation to calculate the line of best fit, that is, the amplitude versus stimulus intensity function, or LDAEP slope (also known as ASF slope (Hegerl
(and Juckel, 1993)). The second is to obtain the slope from the median of all possible lines between any two points on the graph. Although these methods produce slightly different slopes, for both the steepness reflects the degree of loudness dependency; that is, steeper slopes represent a larger LDAEP, or greater increase in N1/P2 amplitude with increasing loudness. Flatter slopes indicate lesser changes in amplitude with incrementing intensity levels (Figure 1-6). Differing terminology still exists in the extant literature to describe the loudness dependence of the AEP. Some authors use the possibly more adequate term “IDAEP” (intensity dependence of the auditory evoked potential) (Linka et al., 2004, 2005, 2007, Hensch et al., 2006) to describe the amplitude versus stimulus intensity function however, for this thesis I will use the term LDAEP as utilised in my previous publications and by several other research groups (Gallinat et al., 2000, Juckel et al., 2004, Lee et al., 2005, Daumann et al., 2006, Norra et al., 2008).

Figure 1-6: In this figure subject 1 possesses a steep LDAEP (a large increase in N1/P2 amplitude with increasing loudness) whereas subject 2 shows a shallow LDAEP (a small increase in N1/P2 amplitude with increasing loudness). Adapted from Senkowski et al. (2003)
1.6 Serotonin and the LDAEP – rationale and pre-clinical evidence

Biochemical parameters such as cerebrospinal fluid (CSF) or plasma/serum levels of 5-HT and its major metabolite, 5-hydroxyindole acetic acid (5-HIAA), have been studied as possible indicators of central 5-HT activity (Murphy, 1990, Pletscher, 1988). Also, neuroendocrine, psychological and thermoregulatory responses to challenge tests using a number of serotonergic drugs have been suggested to provide “probes” of central 5-HT function (for review see Murphy, 1990). However, the reliability of these methods and their relevance as indicators of central serotonergic function have been questioned (Auerbach et al., 1989, Murphy 1990). For example, CSF 5-HIAA has been examined as a possible indicator of 5-HT turnover in the CNS but many separate metabolic steps are involved in the synthesis, storage and release of 5-HT and 5-HIAA (Tyce, 1990, Boadle-Biber, 1993). Accordingly it is possible that altered 5-HIAA in CSF may reflect a dysfunction in 5-HT metabolism (synthesis, storage and release) or a change in the total number of 5-HT neurons, thus functional consequences cannot be easily deciphered from CSF 5-HIAA levels alone. In addition, changes in hormonal plasma concentrations and the use of serotonergic drug challenges may be unreliable due to interactions with other monoaminergic systems, namely the dopaminergic and noradrenergic systems.

A more recent technological advance to gauge 5-HT function in the living brain involves the use of molecular imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). Over the last decade the number of studies utilising molecular imaging procedures such as PET and SPECT has risen considerably. For example, the serotonin transporter (SERT) is involved in the termination of serotonergic action at the synapse by removing 5-HT from the synaptic cleft, thus it has an integral role in serotonergic function (Cooper et al., 1996). Using SERT specific radioligands and PET, several researchers have demonstrated significant global reduction in SERT binding in methylenedioxymethamphetamine (MDMA) users (MDMA has been suggested to cause 5-HT nerve terminal loss in both animals and humans (Ricaurte et al., 1992; 2000 and McCann et al., 1998; 2000, Green et al., 2003)) (McCann et al., 1998, Buchert et al., 2004, McCann et al., 2005).
In addition, increasingly selective SERT ligands used with SPECT (i.e. \(^{123}I\) ADAM) have been used to assess central serotonergic function in disease states and healthy controls (Newberg et al., 2005, Herold et al., 2006, Koch et al., 2007). However, despite the neurochemical specificity and sensitivity of PET and SPECT and their ability to assess the integrity of the serotonergic system, they possess limitations. Firstly, they are more adept at examining molecular rather than “functional” aspects of the 5-HT system. Secondly, they are invasive procedures, requiring the use of radioactive materials which themselves carry inherent risks. As such, using these radionucleotides limits the ability for repeated testing. Finally, they are also time and resource consuming, requiring lengthy visits as well as specific costly equipment.

In place of these existing methods the LDAEP has been proposed as a direct, reliable, non-invasive marker of \textit{in vivo} central 5-HT function. Though the specific anatomic structures and processes generating scalp-recorded ERPs are still not wholly identified (Hegerl and Juckel, 1993), it is thought that the amplitude of cortical evoked potentials is related to the phasic release of glutamate or GABA (Zemon et al. 1986, Simpson and Knight, 1993). It has also been postulated that the loudness dependence of the auditory evoked potential (that is, the more global activity of cortical pyramidal cells of the auditory cortex) to various stimulus intensities may be influenced more readily by neuromodulatory systems located both in the cortex and subcortically (Connolly, 1987). It has been proposed that pyramidal cells in layer IV, projecting to layers III and V are the initial stage of all major intracortical pathways including those generating the late-evoked potentials such as the N1/P2 (Barth and Di, 1990). As innervation of layer IV in the primary sensory cortices contains mostly projections from serotonergic neurons in the DRN (Wilson and Molliver, 1991) and as the DRN receives a high level of specific thalamic sensory input, it is conceivable that 5-HT, either at cortical or subcortical locations could regulate the loudness dependence of the N1/P2 component (Figure 1-7). This proposition is supported by the gross physiological properties of the serotonergic system. Serotonergic neurons, especially of the DRN, are characterised by a regular, stable discharge (Aghajanian and VanderMaelen, 1982) leading to the suggestion that the serotonergic system may possess a general activating
function in the mammalian CNS in order to “set the tone” of cortical networks (Bloom, 1988, Jacobs et al., 1990).

In support, Juckel et al (1999) demonstrated an increased loudness dependence of the P12 component of the cat AEP (the first positive component of the cat AEP with the highest functional similarity to that of humans (Juckel et al 1997, 1999)), following local application of the 5-HT$_{1A}$ agonist 8-OH-DPAT in the dorsal raphé nucleus (DRN) (i.e. which decreases 5-HT release through activation of the pre-synaptic 5-HT$_{1A}$ autoreceptor) and the 5-HT$_{2}$ antagonist ketanserin (i.e. via post-synaptic 5-HT$_{2}$ receptor antagonism). In contrast a decreased loudness dependence of the P12 component was reported following administration of the 5-HT$_{1A}$ antagonist spiperone in the DRN (i.e. which increases 5-HT release via antagonism of pre-synaptic 5-HT$_{1A}$ autoreceptor) (Juckel et al., 1999) and stimulation of postsynaptic 5-HT$_{1A}$ receptors with 8-OH-DPAT (Juckel et al., 1997) (Figure 1-7). It is interesting to note that these studies reported an inverse relationship between 5-HT function and the loudness dependence of the cat AEP. The preceding anatomical evidence suggests that the serotonergic system may be well suited for regulating cortical processing by providing a stable and tonic preactivation level, but why would a low level of preactivation (i.e. reduced 5-HT activity) result in increased loudness dependence of ERPs and vice versa, as in these animal studies. This inverse relationship is thought to be a feature of competitive neuronal networks comparable to those proposed by Grossberg (Grossberg, 1984, Grossberg and Gutowski, 1987). In these networks, unspecific preactivation does not lead to an output because of lateral inhibition, but after an additional specific sensory input, activity resulting in an evoked response (i.e. N1/P2) will be observed (Hegerl and Juckel, 1993). Accordingly, the loudness dependence of the N1/P2 component may depend on the preactivation level, such that, if the baseline serotonergic activity is low, an increase of specific sensory input, caused by an increase in stimulus loudness, is followed by a relatively greater activity increase and vice versa (Hegerl and Juckel, 1993) (Figure 1-7). This assertion is in agreement with the results of the aforementioned preliminary animal studies however, in both studies the investigators noted that their results needed to be regarded with some caution. Firstly, the small number of animals used, displayed a wide variability of baseline values of loudness dependence.
Figure 1-7: The left hand side of this figure illustrates a global view of the cortex. It also depicts the high or low serotonergic activity of neurons in the DRN and their consequent effects in the primary auditory cortex (PAC) which results in a decreased or increased LDAEP respectively (adapted from Hegerl et al., (2001)). The right hand side is a microscopic view of the cortical generators of the LDAEP in the PAC (i.e. pyramidal cells) and their hypothesised serotonergic modulation (Juckel et al., 1997, 1999).

(Abbreviations: Pyr – cortical pyramidal cell, DRN – Dorsal Raphé Nucleus, 5-HT$_2$ – 5-HT$_2$ receptor, 5-HT$_{1A}$ – 5-HT$_{1A}$ receptor).
In addition the loudness dependence of the cat AEP component (which has the highest functional similarity to that of the N1/P2 component in humans) was used in these studies and the parallels between human and animal data are difficult to make due to species differences. Despite the relative lack of pre-clinical evidence that 5-HT function is directly related to the LDAEP and emerging evidence that other neuromodulators may play a role, this particular topic has been extensively studied in clinical settings examining the relationship between the LDAEP, 5-HT and other constructs or behaviours thought to relate to 5-HT dysfunction, such as in clinical disorders.

1.7 LDAEP and Indirect Human Studies from Clinical Groups

As outlined above, the theory of an inverse relationship between 5-HT and the LDAEP is largely based on pharmacological studies in animals (Juckel et al., 1997, 1999). Despite this relative shortcoming a significant body of research has been undertaken based on the premises outlined in these studies, which are; (a) the LDAEP is a sensitive marker of 5-HT function, and (b) the LDAEP is unlikely to be modulated by other neuromodulatory systems. Discussed below are the studies in humans using the LDAEP in clinical populations consisting of patients with conditions involving a presumed serotonin abnormality.

Given the role of 5-HT in obsessive compulsive disorder (OCD) (for review, see Baumgarten and Grozdanovic, 1998, Stein, 2002) the LDAEP has been used to examine serotonergic dysfunction in OCD. An initial study found no difference in the LDAEP of 22 medication-free OCD patients when compared to 22 age- and gender-matched healthy subjects (Carrillo-de-la-Peña et al., 2000). While this study provided no evidence for 5-HT dysfunction in this group of OCD patients using the LDAEP, a more recent study using single photon emission computed tomography (SPECT) found a link between the LDAEP, serotonin (SERT) and dopamine (DAT) transporter availabilities in patients with OCD (Pogarell et al., 2004). That study used SPECT with the radiotracer \([^{123}\text{I}]\text{2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^{123}\text{I}]β-CIT)}\) and reported increased \([^{123}\text{I}]β\text{-CIT binding in the midbrain and pons regions (indicating elevated SERT availability and indirectly suggesting reduced 5-HT)}\) and in the striatum.
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(indicating elevated DAT availability and indirectly suggesting reduced dopamine) was correlated with an increased LDAEP. Taken together the results of these studies involving the LDAEP and OCD indicate that serotonergic dysfunction may be involved in OCD, the nature of which remains to be elucidated due to the possibility of interactions with the dopaminergic system. Additionally, the correlation between the LDAEP, striatal DAT binding and SERT binding may point towards a synergistic relationship between these monoamine systems in the modulation of the LDAEP. The possibility of such a synergistic relationship becomes plausible if one considers the well documented interactions between the serotonergic and dopaminergic/noradrenergic systems respectively, as regards the pathophysiology of depression and anxiety disorders and their pharmacological treatment (for review, see Ressler and Nemoroff, 2000, Esposito, 2006). Conversely the results of the above studies may lead one to believe that there is no complete specificity of the LDAEP for the serotonergic system.

Dysfunction in the serotonergic system has been reported in generalised anxiety disorder (GAD) (for reviews, see Connor and Davidson 1998, Davidson, 2001). In an attempt to further elucidate serotonergic involvement in GAD, Senkowski and colleagues (2003) investigated the LDAEP in medication-free patients with GAD and reported a significant decrease in the LDAEP in patients compared to controls. While the exact role of 5-HT in GAD is unknown, with reports of both increases and decreases in 5-HT function linked with GAD (Connor and Davidson, 1998), the aforementioned study may indicate a serotonergic hyperfunction in GAD (i.e. based on a reduced LDAEP compared to controls). However, these findings are contrary to what would be predicted based on the efficacy of selective serotonin re-uptake inhibitors (SSRIs) in the treatment of GAD. Senkowski and colleagues (2003) propose that the effectiveness of SSRIs may involve the enhancement of inhibitory serotonergic afferents that inhibit other neuromodulators that are linked to the aetiology of anxiety, such as noradrenaline (Connor and Davidson, 1998, Sullivan et al., 1999, Ressler and Nemerooff, 2000). However, as acknowledged by the authors, this study cannot determine whether the increased serotonergic activity is the cause or consequence of anxiety-related behaviour. Nevertheless, this study would seem
to support a serotonergic role in the aetiology of GAD, but due to the possible involvement of the noradrenergic system and as the effects of noradrenergic modulation of the LDAEP are unknown, it adds further uncertainty as to the serotonergic specificity of the LDAEP.

The drug of abuse methylenedioxymethamphetamine (MDMA or “ecstasy”) has been suggested to cause 5-HT nerve terminal loss in both animals and humans (Ricaurte et al., 1992; 2000 and McCann et al., 1998; 2000, Green et al., 2003). This loss of 5-HT has been linked with learning and memory impairments, elevated impulsivity and neuroendocrine abnormalities in MDMA users (Morgan, 1998, Gerra et al., 1998, Parrott and Lasky, 1998, Morgan, 1999, Rodgers, 2000, Gouzoulis-Mayfrank et al., 2003). Consequently, the LDAEP has been employed in several studies to examine the functional status of the 5-HT system. Consistent with the link between 5-HT and the LDAEP, Tuchtenhagen and colleagues (2000) demonstrated that abstinent recreational MDMA users exhibited an increased LDAEP (suggesting reduced 5-HT function) when compared with two control groups (one consisting of drug-naïve volunteers - matched for age, sex and education with the MDMA group and the other of cannabis users - matched for age, sex, education and cannabis use with the MDMA group). An additional investigation reported similar results with an increased LDAEP noted in long-term MDMA users relative to separate control groups consisting of drug-naïve subjects and long-term-cannabis users (Croft et al., 2001). In a more recent study Daumann and co-workers (2006) performed an 18 month longitudinal investigation in polydrug MDMA users and discovered an association between the LDAEP and aspects of MDMA use, such as frequency of use, cumulative lifetime dose and abstinence. Unexpectedly, they failed to demonstrate any difference in LDAEP between those that continued and those that abstained from drug use after 18 months follow-up. One may expect abstinence from MDMA to lead to a restorative effect on 5-HT neurons and a subsequent change in the LDAEP however, this result may have been due to the nature of the MDMA-related 5-HT toxicity, that is, regular MDMA consumption may have toxic effects on serotonergic neurons which even after 18 months of abstinence would still (Hatzidimitriou et al., 1999). Furthermore not all participants in the study reported complete abstinence during the follow-up
period and the authors speculated that the amount of MDMA ingested during the follow-up period may not have been enough to worsen pre-existing serotonergic deficits. Other forms of amphetamines (e.g. d-methamphetamine) have been shown to possess neurotoxic potential with reported depletion of dopamine and 5-HT and damage of dopamine and 5-HT nerve terminals (Kita et al., 2003). As the population studied were polydrug users (i.e. in addition to MDMA, consumption of cannabis and amphetamine-like derivatives), taken together with the reported correlation of the dopamine transporter and the LDAEP (discussed previously) (Pogarell et al., 2004), the effects of dopamine on the LDAEP in this population cannot be ruled out. Further studies are needed in this area to determine whether the serotonergic alterations in ecstasy users are related to the well documented neurotoxic potential of MDMA on the serotonergic system, or alternatively, are connected with pre-existing traits and the influence of other illicit drugs (i.e. affecting the dopamine and opioid systems). Nevertheless, these studies provide evidence for the sensitivity of the LDAEP to chronic or long term changes in central 5-HT.

1.8 LDAEP and Changes in Serotonergic Neurotransmission in Humans: Genetic Polymorphism Studies of Serotonergic Function

The serotonin transporter (SERT) is involved in the termination of serotonergic action at the synapse by removing 5-HT from the synaptic cleft, thereby playing an integral role in serotonergic function (Cooper et al., 1996). Polymorphisms of the serotonin transporter gene (5-HTT) appear to be associated not only with susceptibility to suffer affective disorders (Ogilvie et al., 1996; Bellivier et al., 1998; Furlong et al., 1998), but also with treatment response to selective serotonin re-uptake inhibitors (SSRIs) (Kim et al., 2000; Zanardi et al., 2000; Serretti et al., 2001; Rausch et al., 2002). Consequently a particular polymorphism in the promoter region of the serotonin transporter gene (SHTTLPR) has become a focus of psychiatric research. Specifically, this polymorphism is composed of either 14 or 16 repeat elements containing 20-23 base-pairs that appear to be of functional relevance: The shorter s-allele (14 repeats) is thought to be associated with lower transcription and efficiency of the
5-HTT gene (and presumably with increased synaptic 5-HT levels) while the longer \(l\)-allele (16 repeats) is associated with higher transcription and efficiency (and presumably with decreased synaptic 5-HT levels) (Lesch et al., 1996). The shorter of the two alleles, the \(s\)-allele, is associated with conditions associated with serotonergic dysfunction, including affective disorders (Lasky-Su et al., 2005) and alcohol dependence (Nilsson et al., 2005), while the \(l\)-allele is associated with a diminished amygdala response during emotional processing tasks, a process which is thought to be 5-HT dependent (Harari et al., 2002, 2005, Pezewas et al., 2005).

However, it is generally accepted that the influence of a single polymorphism on complex behaviour such as affective disorders and alcohol dependence, involving multiple genes and environmental factors, would be small and difficult to detect (Reif and Lesch 2003). Accordingly, Hariri and Weinberger (2003) proposed the examination of the effects of genes on more “direct” indices of serotonergic function. To date, the 5HTTLPR \(s\)-allele has been correlated with lower CSF 5-HT metabolites (Williams et al., 2003), an indirect index of central serotonergic function. Recently the LDAEP has been investigated in an attempt to elucidate the influence of genetic variations on this purported “direct” serotonergic marker. Results to date have been mixed with studies reporting an association between the \(l/l\) genotype (linked with reduced synaptic 5-HT) and both a reduced (Gallinat et al., 2003) and increased LDAEP (Strobel et al., 2003). While these studies seem to support some association between the LDAEP and the 5HTTLPR, the inconsistencies in predicting directional changes in 5-HT neurotransmission may question the reliability of the LDAEP. In an attempt to clarify the issue, Hensch and co-workers (2006) investigated the LDAEP and the 5HTTLPR while controlling for confounding variables that are known to affect either serotonergic neurotransmission, the 5HTTLPR or the LDAEP (i.e. gender, smoking, age, psychiatric or neurological disorders, centrally acting medication, recreational drugs and LDAEP methodology). The study replicated the findings of Strobel et al., (2003), with \(l/l\) individuals (with presumably lower synaptic 5-HT) having an increased LDAEP when compared to individuals with the \(s/s\) or \(s/l\) genotype (Hensch et al., 2006).
Hensch and colleagues acknowledge that the results of their study need to be interpreted with caution due to methodological limitations pertinent to the LDAEP, in particular stimulus intensity (Hensch et al., 2006). However, in addition to LDAEP methodologies there are numerous other influences which may have an effect on allelic variations of SERT function, such as gender, age and environmental factors. Indeed, Juckel and colleagues (2007c) noted that the aforementioned studies may have been biased in their recruitment strategies. In an effort to rectify this they randomly selected a community-based sample of healthy volunteers from the general population, analysed the association between the LDAEP and the 5HTTLPR and extended the analysis to include four further variations in the SERT gene (Juckel et al., 2007c). Unexpectedly, they found no association between the LDAEP and any of the variables tested. The authors suggest that methodological limitations may have contributed to their result (i.e. LDAEP methodology, sample size) but also speculate that the LDAEP may be more closely related to genetic variants controlling 5-HT release (5-HT autoreceptors) and synthesis (tryptophan hydroxylase), rather than re-uptake (Juckel et al., 2007c). In line with this suggestion, the same research group recently investigated a single nucleotide polymorphism (SNP) in the gene coding for the 5-HT\textsubscript{1B} receptor and its relationship to the LDAEP (Juckel et al., 2007b). The 5-HT\textsubscript{1B} receptor is located presynaptically on serotonergic axons in terminal areas (such as the primary auditory cortex) and in the brainstem raphé nuclei (Moret and Briley, 2000). As well as controlling 5-HT release in terminal areas, this receptor inhibits the release and synthesis of 5-HT and also reduces the firing rate of serotonergic neurons via a negative feedback loop in the raphé nuclei (Moret and Briley, 2000, Sari, 2004). Accordingly the 5-HT\textsubscript{1B} receptor may play an important role in the modulation of the LDAEP. In support, Juckel and co-workers reported that 5-HT\textsubscript{1B} alleles were related to an increased LDAEP (Juckel et al., 2007b). But the interpretation of this result is tempered by the fact that the 5-HT\textsubscript{1B} receptor can function as a heteroreceptor at non-serotonergic neurons controlling the release of neurotransmitters other than 5-HT (such as dopamine, acetylcholine and glutamate) (Moret and Briley, 2000). Although these studies provide some evidence for the sensitivity of the LDAEP to aspects of central serotonergic function in humans, they also raise questions as to the reliability of the LDAEP.
1.9 LDAEP and Global Changes in Serotonin Neurotransmission: Effects of Pharmacological and Biochemical Manipulations in Humans.

The LDAEP would be expected to change under conditions of varying 5-HT function (i.e. changes in synaptic 5-HT release), such that increasing 5-HT levels in the brain would be expected to decrease the LDAEP and vice versa. However, as discussed below, inconsistent results have been reported when overall 5-HT levels are acutely altered in the brain.

1.9.1 LDAEP and Central Serotonin Depletion

Acute tryptophan depletion (ATD) is a dietary intervention that rapidly lowers plasma tryptophan and alters 5-HT synthesis; metabolism and release (Young et al., 1985). It involves the administration of an amino acid mixture which lacks tryptophan, the precursor of 5-HT. Extensive reviews of ATD methodology conclude that ATD is a valid procedure to induce a reversible reduction of central 5-HT function in humans (for review, see Reilly et al., 1997 and Moore et al., 2000). Consequently this paradigm has been investigated in conjunction with the LDAEP to assess the sensitivity of the LDAEP to acute changes in central serotonergic neurotransmission. To date results have been supportive of ATD, and accordingly 5-HT reduction, having no effects on the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Norra et al., 2008). However, one study reported a paradoxical decrease in the LDAEP following ATD (Kähkönen et al., 2002). One possible reason for this result may be the use of dipole sources analysis (DSA) by Kähkönen and colleagues, a technique which is thought to allow for the separation of the auditory evoked N1/P2 component into subcomponents generated by the primary and secondary auditory cortices. It has been suggested that the LDAEP generated from the primary auditory cortex, which is highly innervated by serotonergic fibres (Lewis et al., 1986), is more sensitive to 5-HT function and may be isolated and analysed using DSA (Hegerl and Juckel 1993, Gallinat and Hegerl, 1994). However, the advantage of DSA in comparison to scalp measurement has not
been directly shown to date, with comparable results reported using both methods. For example, in the aforementioned studies, ATD did not have the predicted effect on the LDAEP as measured by either scalp analysis (Debener et al., 2002, Massey et al., 2004), or DSA (Dierks et al., 1999, Norra et al., 2008). Another possibility for the paradoxical result of Kähkönen and colleagues may be methodological differences with respect to the ATD paradigm such as time of testing, amino acid mixture concentrations and gender. However, as the majority of studies have reported no change in the LDAEP following ATD, this suggests that methodological differences are not the reason for the lack of effect. Taken together these studies involving the LDAEP and ATD in healthy humans seem to question the sensitivity of the LDAEP to acute changes in serotonergic neurotransmission.

1.9.2 LDAEP and Central Serotonin Augmentation

Acutely increasing central 5-HT in humans has also resulted in variable results. Researchers have investigated the LDAEP utilising pharmacological agents that possess serotonergic effects in conditions with serotonergic abnormalities and in healthy controls. One such investigation reported a marked decrease in the LDAEP in alcoholic patients and healthy controls following ethanol administration (which among other mechanisms also increases central 5-HT neurotransmission (McBride et al., 1990, Yoshimoto et al., 1992)) (Hegerl et al., 1996). Lithium is thought to be effective in the treatment of affective disorders in part due to minor serotonergic agonistic properties (Price et al., 1989, Hegerl et al., 1992, Juckel et al., 2004) but Hegerl and co-workers reported no change in the LDAEP following lithium administration in healthy controls (Hegerl et al., 1990). However, other studies in clinical populations have reported an increased LDAEP in responders to lithium treatment when compared to non-responders (Hegerl et al., 1992, Juckel et al., 2004). This would seem to suggest that an increased pre-treatment LDAEP (implying low 5-HT) may be a useful indicator of a favourable response to lithium treatment. However, the strength of these conclusions is weakened by the actions of the pharmacological agents utilised. Alcohol’s primary site of action in the CNS is at the GABA<sub>A</sub> receptor as well as its influence on other systems (i.e. dopamine and glutamate) (for
An alternative method to acutely modulate central 5-HT function is to increase synaptic 5-HT levels using increasingly specific serotonergic agents such as SSRIs (e.g. citalopram). SSRIs increase synaptic 5-HT by inhibiting the re-uptake of synaptically released 5-HT from the synaptic cleft through the binding to and inhibiting of the SERT, and are especially effective in the treatment of depression and other affective disorders (Cooper et al., 1996). However, despite the use of increasingly specific serotonergic probes in the form of SSRIs, results of acute studies involving the LDAEP are varied. In depressed patients studies have reported no change in the LDAEP following 4-week treatment with SSRIs (citalopram, paroxetine, sertraline, fluoxetine) (Gallinat et al., 2000, Lee et al., 2005). Although the slope remained unchanged over the treatment period in these studies, similar with earlier studies involving lithium, patients possessing a steeper pre-treatment slope (indicating reduced 5-HT) exhibited a greater reduction in depressive symptoms over the course of SSRI treatment. This result suggests that an increased pre-treatment LDAEP (implying low 5-HT) may be a useful indicator of a favourable response to SSRI treatment (Gallinat et al., 2000, Juckel et al., 2004). However, these results do not explain why there was no change in the LDAEP over the treatment period of these respective studies when SSRI administration would have presumably enhanced serotonergic neurotransmission.

In an effort to further examine the relationship between 5-HT and the LDAEP without the confounding effects of disease, several studies have examined the effects of acute SSRI administration on the LDAEP in healthy subjects. A study by our group (Nathan et al., 2006) reported a reduced LDAEP after acute enhancement of 5-HT levels following citalopram administration, directly supporting the inverse relationship between 5-HT and the LDAEP in humans for the first time. However, subsequent studies in healthy participants have not replicated these findings, with no change in the LDAEP observed following citalopram (Uhl et al., 2006), citalopram, sertraline or escitalopram administration (Guille et al., 2008). Overall, the findings suggest that acute...
augmentation of 5-HT neurotransmission with SSRIs has no effect on the LDAEP in healthy subjects. However, we did observe a decrease in the LDAEP in our earlier study with citalopram (Nathan et al., 2006) using the same methodology to that used in our subsequent study which found no effects of citalopram, escitalopram and sertraline (Guille et al., 2008). This suggests that the sensitivity of the LDAEP to acute changes in 5-HT may be influenced by other factors. One likely and significant factor may be genetic polymorphisms within the 5-HT system. As discussed previously the effect of genetic variants on the LDAEP is yet to be elucidated with associations reported between an increased or decreased LDAEP and components of 5-HT release and re-uptake (5-HT1B receptor alleles and 5HTTLPR). Additionally gender may play a role as it has been shown to influence serotonergic sensitivity, evoked potential research (Bruneau et al., 1986, Camposano and Lolas, 1992, Nishizawa et al., 1997, Walpurger et al., 2004) as well as response to pharmacological agents such as SSRIs (Berlanga and Flores-Ramos 2006). Alternatively, the more consistent findings in conditions characterised by longer term serotonergic dysfunction (i.e. predicting treatment response in depression (Buchsbaum et al., 1971, Gallinat et al., 2000, Juckel et al., 2004, Lee et al., 2005) and serotonergic impairments in MDMA users (Croft et al., 2001; Tuchtenhagen et al., 2000, Daumann et al., 2006)), suggest that the LDAEP may not be reliably sensitive to acute changes in 5-HT neurotransmission but may be a more effective marker of chronic changes.

1.10 Beyond serotonin: the LDAEP and its Sensitivity to Multiple Neurotransmitter Systems in Humans

It has been suggested that neuromodulators other than 5-HT may be in a position to modulate the LDAEP. This suggestion has arisen from evidence obtained in previous investigations, 5-HT’s ubiquitous nature in the CNS, and multiple possible modulatory sites within cortical and subcortical areas (Bruneau et al., 1986, Paige et al., 1994, Juckel et al., 1997, Strobel et al., 2003, Pogarell et al., 2004). Early evidence for dopaminergic modulation arose from studies on event related potentials (ERPs) which reported that a high intensity dependence of
auditory and visual evoked potentials were related to low levels of dopamine metabolites (i.e. homovanillic acid) in CSF and urine (Von Knorring and Perris, 1981, Bruneau et al., 1986). More recently, the dopamine receptor agonist apomorphine was shown to decrease the loudness dependence of auditory evoked potentials in animals; though the effect was delayed indicating it may have been mediated through dopaminergic effects on 5-HT neurons in the DRN (Juckel et al., 1997). Indeed it has been reported that 5-HT output as well as the firing rate of serotonergic neurons in the dorsal raphé nucleus is enhanced following administration of D₂ receptor agonists (Ferre and Artigas 1993; Martin-Ruiz et al., 2001). This preliminary evidence in animals is supported by studies in humans including a SPECT study using \[^{123}\text{I}]\beta\text{-CIT}\) which found that increased \[^{123}\text{I}]\beta\text{-CIT}\ binding in the striatum (indicating elevated DAT availability and reduced dopamine) was correlated with an increased LDAEP (Pogarell et al., 2004), as well as an investigation of the 5-HTTLPR that noted that when the dopamine D₄ receptor gene (DRD4exon III) was considered in the analysis, individuals with the l/l genotype displayed a more pronounced loudness dependence when compared to individuals with the l/s genotype (Strobel et al., 2003). Additionally, Paige and colleagues (1995) noted that depressive patients, who exhibited a positive therapeutic response following treatment with the dopaminergic/noradrenergic antidepressant bupropion, had an increased pre-treatment loudness dependency similar to patients treated with an SSRI (Gallinat et al., 2000, Lee et al., 2005).

In addition to dopaminergic modulation of the LDAEP the potential influence of other neuromodulators has been noted. Juckel et al., (1997) reported that atropine; a cholinergic muscarinic receptor antagonist, increased the intensity dependence of the cat AEP component, leading them to postulate that the cholinergic system may be in a position to modulate the LDAEP. The possibility of a cholinergic influence is credible given that in the middle layers of the auditory cortex, muscarinic receptors are abundant and located at pyramidal cells and interneurons in a ratio of 4:1 (Zilles et al., 1989). To date, investigations in humans involving the LDAEP and noradrenergic modulation are scarce but one such investigation reported a significant correlation between lower N1 amplitude and favourable response to the antidepressant reboxetine (as discussed earlier), but without a change in N1 slope over the course of
reboxetine treatment (Linka et al., 2005). While this study provides some indirect evidence for a link between the noradrenergic system and modulation of components of the LDAEP (i.e. N1), more direct studies are required to elucidate a possible role of noradrenaline in the modulation of the LDAEP itself.

The majority of previous research involving the LDAEP has predominantly focused on studies investigating monoaminergic systems and the LDAEP (particularly 5-HT), but very little is known about other neurotransmitter systems. It is thought that the amplitude of cortical evoked potentials, in addition to being modulated by 5-HT and other monoamines, may be related to the phasic release of excitatory (i.e. glutamate) or inhibitory (i.e. GABA) neurotransmitters in the cortex (Zemon et al. 1986, Simpson and Knight, 1993). To date this has not yet been investigated in relation to the LDAEP but given the roles of glutamate and GABA in excitatory and inhibitory neurotransmission respectively, such studies may be warranted.

1.11 Summary of LDAEP pharmacology

The major evidence for a direct relationship between the LDAEP and 5-HT function is derived from early animal studies (Juckel et al., 1997, 1999) with subsequent clinical studies in conditions possessing purported serotonergic dysfunction providing indirect support (Tuchtenhagen et al., 2000, Croft et al., 2001, Senkowski et al., 2003, Pogarell et al., 2004, Daumann et al., 2006). To date, direct evidence linking the LDAEP and 5-HT function in healthy humans, firstly by investigating genetic polymorphisms in the 5-HT system (Gallinat et al., 2003, Strobel et al., 2003, Hensch et al., 2006, Juckel et al., 2007b, c) and secondly using direct acute pharmacological manipulations (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Nathan et al., 2006, Uhl et al., 2006, Norra et al., 2008, Guille et al., 2008) has largely failed to identify a link between changes in 5-HT function and the LDAEP. Further there have also been a number of inconsistent reports of other neuromodulators and neurotransmitter systems influencing the LDAEP, casting doubt on its specificity as a marker of 5-HT function (Strobel et al., 2003, Pogarell et al., 2004).
1.12 Aims and outline of the current thesis

The general aim of this thesis is to investigate the pharmacology of the loudness dependence of the auditory evoked potential (LDAEP). More specifically, it was to investigate the sensitivity of the LDAEP to neurotransmitter systems other than 5-HT and to elucidate the specificity of the LDAEP for serotonergic neurotransmission. This was achieved by acutely modulating monoamine (i.e. 5-HT and dopamine) and neurotransmitter (i.e. glutamate) systems in healthy volunteers. The first of these pharmacological manipulations (Chapter 3) investigates the effects of global changes in 5-HT and dopamine neurotransmission on the LDAEP. This study examines for the first time, the effects of the acute depletion of the amino acid precursors for 5-HT (i.e. tryptophan) and dopamine (i.e. tyrosine/phenylalanine) alone, and simultaneously (to examine the effects of synergistic interactions) on the LDAEP in healthy subjects. This experiment has been published in the peer reviewed journal *Human Psychopharmacology: Clinical and Experimental* (see Appendix 4 for reprint).

The second investigation involves the administration of the dopaminergic receptor agonists, bromocriptine (D2/D3 receptor agonist) and pergolide (D1/D2/D3 agonist). This experiment, outlined in Chapter 4, aimed to examine the effects of directly modulating the dopaminergic receptor system on the LDAEP. This experiment has been published in the peer reviewed journal *Psychopharmacology* (see Appendix 8 for reprint). The final experiment of this thesis (Chapter 5) is also a novel study investigating, for the first time, the effects of acute administration of high dose glycine, thereby possibly stimulating excitatory glutamate receptors (N-Methyl-d-aspartate receptors), on the LDAEP. This experiment was also published in the peer reviewed journal *Psychopharmacology* (see Appendix 7 for a reprint).

Within each experimental chapter the results and possible ramifications for the literature are discussed. This thesis concludes with a final chapter (Chapter 6) including a general discussion on each of the experimental chapters and a more
specific discussion on the results of this thesis and their implications for the literature as well as recommendations for future research.
Chapter 2: General Methods

This chapter outlines the methodology used in this thesis. It outlines methodologies common to all experimental chapters including participant recruitment, data acquisition, stimuli presentation, pharmacological techniques utilised, data analysis and statistical analysis. Methodologies specific to each experiment are described within each experimental chapter.

2.1 Participants

Participants in all experiments of this thesis were male. Participants for all studies were recruited through university advertisements. Participants were considered for selection if they were aged between 18 and 45 years, reported to have normal or corrected-to-normal vision and no hearing impairments, were reported to be drug-free, non-smokers, with no personal or family history of neurological or psychiatric illness as determined by telephone screening using the Prime-MD (Spitzer et al., 1994) and a subsequent semi-structured examination conducted by a medical physician (see Appendix 1a and 1b). All subjects gave written informed consent to take part in the study, which was approved by the Swinburne Human Research Ethics Committee (see Appendix 2).

2.2 Data Acquisition

Participants were seated upright in a comfortable chair with their eyes open; approximately 60 cm from a computer screen, in a sound attenuated and electrically shielded room. They were instructed to relax throughout the recording and to avoid excessive facial muscle movements throughout stimulus presentation. EEG was recorded with tin electrodes inserted in an elasticised cap (Quik-Caps, Neuro Scan Inc, Sterling, VA, USA) from 64 scalp sites based on the international 10/20 system, as illustrated in Figure 2-1.
Before application of any facial electrodes the participant’s skin was cleaned at each site using alcohol wipes (WEBCOL®️, Kendal Healthcare, Mansfield, MA, USA) and with an abrasive gel (Nuprep™️ gel, D.O Weaver & Co, Aurora, CO, USA). This procedure was used to help reduce impedance of facial electrodes to below 5 kΩms. Five tin 9 mm diameter facial electrodes were employed: a bipolar montage below the right eye field to record electromyographic activity, an electrode above and below the left eye to record eye movement activity (electro-oculogram, EOG), and an electrode on the nose (Figure 2-2). To assist in the reduction of cap electrode impedances to below 5 kΩms, a small amount of water-based gel (Electro-cap International Inc., Eaton, OH, USA) was inserted into each electrode. The data were recorded using NeuroScan equipment with Synamps2™️ amplifiers (Neuroscan Inc. Sterling, Va, USA) and collected with a sampling rate of 500 Hz (Chapter 3 and 5) and 1000 Hz (Chapter 4) and a band-pass filter of 0.15 to 200 Hz.

Figure 2-1: The international 10-20 system seen from (A) left and (B) above the head. A = Ear lobe, C = central, Pg = nasopharyngeal, P = parietal, F = frontal, Fp = frontal polar, O = occipital. 
Source: http://butler.cc.tut.fi/~malmivuo/bem/bembook/13/13x/1302ax.htm
2.3 Stimuli

Fifty stimulus tones (1000 Hz, 100 ms duration including 10 ms rise and 10 ms fall time, stimulus onset asynchrony (SOA) randomised between 1,600 ms and 2,100 ms) at each of five intensities (60, 70, 80, 90, 100 dB, sound pressure level (SPL) generated by the STIM II sound generator and STIM software (Neuroscan Inc. Sterling, Va, USA)) were presented binaurally in a pseudo-randomised fashion through single-use foam EAR inserts (EARlink 3A, Aero company auditory system, Indianapolis, IN, USA). During the presentation of auditory stimuli, participants were presented with a series of faces and asked to respond with a button press if the face had a nose (they were asked to ignore faces without a nose).

Figure 2-2: Facial electrode set-up (printed with permission).

The purpose of this visual task was to distract attention away from the auditory stimuli as attention has been shown to modulate the intensity dependence of ERPs in humans (Baribeau and Laurent 1987). The visual task was counterbalanced across conditions and delivered in a pseudo-randomised form in each of the studies, with visual stimuli temporally distinct from the auditory stimuli, with a separation from the LDAEP stimuli of approx 500-1,800 ms.
2.4 EEG Analysis

EEG data were re-referenced to linked mastoids, lowpass filtered at 30 Hz (12 dB/oct) and the Croft and Barry (2000) method employed to correct for ocular artefact. Data were then visually inspected using Neuroscan Edit 4.3 (Neuroscan Inc. Sterling, Va, USA) and rejected where artifactual, and epoched -100 to 500 ms post auditory stimulus (discarding the first 5 stimuli to reduce novelty effects). Averages were then created for each of the five intensities separately, with a mean of 47.81 ± 2.23 sweeps per intensity. N1-peaks (80-120 ms) and P2-peaks (150 – 230 ms) were determined automatically at the C\textsubscript{Z} electrode (C\textsubscript{Z} was chosen due to its reliability for the recording of auditory evoked potentials (Orlebekele et al., 1989, Carrillo-de-la-Peña, 1999, Beauducel et al., 2000)) and subsequently inspected by the investigator to ensure they were accurate. The N1/P2 amplitude was calculated as the difference in amplitude between P2 and N1. For each experiment, participant and condition, the slope of the N1/P2 was calculated as the least-squares linear regression slope, with stimulus intensity the independent variable and N1/P2 amplitude (at the C\textsubscript{Z} electrode) the dependant variable. The investigator analyzing the AEP data was blind to the coding of all treatment conditions throughout the studies.

2.5 Statistical Analysis

For all studies, analyses of the ERP data were performed using the Statistical Package for the Social Sciences (SPSS) 12.0 - 14.0 for Windows (SPSS Inc. Chicago, USA). Statistical analyses relevant to each study are described within the methods section of each experimental chapter. ERP data were analysed with parametric statistics if the normality assumptions were met. If the assumption of normal distribution was not met, the data was first transformed. Non-parametric analyses were used if, even after transformation, data could not be normalised. All statistical tests were two-tailed with a significance level of $p<0.05$ used to indicate a significant difference between means, unless otherwise stated. As these tests were to one degree of freedom, no adjustments for sphericity were made (Tabachnick and Fidell, 2001).
2.6 Pharmacological and Biochemical Manipulation of Neurochemical Systems

2.6.1 Acute tryptophan depletion.

The primary biochemical method to modulate the serotonergic system in this thesis was acute tryptophan depletion (ATD), a dietary intervention that rapidly lowers plasma tryptophan (TRP) and alters 5-HT synthesis, metabolism and release (Young et al., 1985). As outlined in Chapter 1 the synthesis of 5-HT is dependent on the availability of its amino acid precursor, tryptophan (TRP). Accordingly, ATD is based on the premise that decreasing TRP, the precursor for 5-HT, leads to depletion of brain 5-HT (for review see Reilly et al., 1997). It involves the combination of a low protein diet and administration of an amino acid mixture containing 15 amino acids in the same proportion as human milk (Hood et al., 2005). This mixture includes large amounts of large neutral amino acids (LNAAs) (i.e. isoleucine, leucine, valine, tyrosine and phenylalanine) and lacks TRP, aspartic acid and glutamic acid (the latter two because of concerns about toxicity) (Table 2-1) (Young et al., 1985). Often the amino acids that contain sulphur (i.e. methionine, cysteine and arginine) are omitted from the mixture and given in an encapsulated form due to their unpleasant taste, as is the case in Chapter 3 of this thesis.

Before administration of the depletion mixture, participants were requested to adhere to a low protein diet for the 24 hours preceding testing and this technique has been reported to reduce cerebrospinal fluid (CSF) TRP levels by 15-20% and is used to standardize dietary intake before and during test days (Reilly et al., 1997). The effect of ATD on serotonin synthesis and release is twofold (Young et al. 1985). Firstly, the amino acid mixture deficient in TRP promotes synthesis of new proteins resulting in a decline in plasma and brain TRP as any TRP utilised comes from blood and tissue pools (Gessa et al., 1974). Secondly, as all LNAAs compete for the same carrier system to transport them across the blood brain barrier (BBB) (Figure 2-3) (Oldendorf and Szabo 1976), administration of a mixture loaded with LNAAs and deficient in TRP increases
such competition at the BBB resulting in a decline in brain TRP (Gessa et al., 1974). Following ingestion of the mixture, peak effects are usually seen at 5-7 hours which is the point of maximal plasma TRP depletion (Reilly et al., 1997).

<table>
<thead>
<tr>
<th>AMINO ACID</th>
<th>STANDARD WEIGHT (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-alanine</td>
<td>5.5</td>
</tr>
<tr>
<td>L-arginine</td>
<td>4.9</td>
</tr>
<tr>
<td>L-cysteine</td>
<td>2.7</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.2</td>
</tr>
<tr>
<td>L-histidine</td>
<td>3.2</td>
</tr>
<tr>
<td>L-lysine</td>
<td>11.0</td>
</tr>
<tr>
<td>L-leucine</td>
<td>13.5</td>
</tr>
<tr>
<td>L-isoleucine</td>
<td>8.0</td>
</tr>
<tr>
<td>L-methionine</td>
<td>3.0</td>
</tr>
<tr>
<td>L-phenylalanine</td>
<td>5.7</td>
</tr>
<tr>
<td>L-proline</td>
<td>12.2</td>
</tr>
<tr>
<td>L-serine</td>
<td>6.9</td>
</tr>
<tr>
<td>L-threonine</td>
<td>6.5</td>
</tr>
<tr>
<td>L-tyrosine</td>
<td>6.9</td>
</tr>
<tr>
<td>L-valine</td>
<td>8.9</td>
</tr>
<tr>
<td>L-tryptophan</td>
<td>2.3</td>
</tr>
<tr>
<td>Total (minus L-tryptophan)</td>
<td>102.1</td>
</tr>
<tr>
<td>Total (plus L-tryptophan)</td>
<td>104.4</td>
</tr>
</tbody>
</table>

**Table 2-1:** Composition of the amino acid mixture (based on Young et al., 1985)

Ingestion of TRP-free mixtures in laboratory animals has been shown to produce marked decreases in plasma TRP and brain 5-HT content (Biggio et al., 1974, Gessa et al., 1974, Moja et al., 1989) and to alter behavioural indices of 5-HT function such as acoustic startle (Walters et al., 1979), effects which have been reversed once central TRP is restored. In humans, ATD has been reported to cause an 80-90 % reduction in CSF TRP in healthy volunteers (Carpenter et al., 1998, Williams et al., 1999). In addition a study examining central 5-HT synthesis using positron emission tomography (PET) discovered a more
dramatic effect, with reductions in the rate of synthesis of a factor of about 9.5 in men and 40 in women (Nishizawa et al., 1997). Further to this, a study in healthy women found a reduction in 5-HT\textsubscript{2} receptor binding following ATD (Yatham et al., 2001) and in a neuroendocrine study involving healthy volunteers, ATD has been shown to attenuate the prolactin response to a D-fenfluaramine challenge indicating that ATD reduces levels in the releasable pool of 5-HT in the pre-synaptic neuron (Coccaro et al., 1998).

In addition to modulating facets of 5-HT synthesis and release, ATD has been shown to impair serotonin-dependant cognitive processes such as memory consolidation and learning (Harrison et al., 2004; Sambeth et al., 2007). ATD has also been used to investigate the role of 5-HT in emotional processing (Harmer et al., 2003, Fusar-Poli et al., 2007), mood (for review see Van der Does, 2001) and various psychiatric conditions (for reviews see, Moore et al., 2000, Bell et al., 2005, Neumeister, 2004, Fusar-Poli et al., 2006). The aforementioned studies are part of a large body of both preclinical and clinical data that support the notion that depletion of plasma TRP using ATD induces a safe and reversible reduction of brain 5-HT content and function.

\textit{2.6.2 Acute tyrosine/phenylalanine depletion}

One method used to modulate the dopamine system in this thesis was acute tyrosine/phenylalanine depletion (ATPD), which is based on the same principles as ATD. Synthesis of dopamine (and noradrenaline) requires the presence of its amino acid precursor tyrosine (TYR) (and the tyrosine precursor phenylalanine (PHE)). As for ATD, the depletion of these amino acids leads to an acute reversible reduction in central dopamine and noradrenaline levels. The mechanism of depletion is comparable to ATD, with the amino acid mixture deficient in TYR and PHE promoting synthesis of new proteins resulting in a decline in plasma and brain TYR/PHE and an increase in competition with other LNAAs for the same carrier system to transport them across the blood brain barrier.
Figure 2-3: Tryptophan entry into the brain and 5-HT synthesis is regulated by the relative availability of tryptophan versus large neutral amino acids that compete with it for transport across the blood brain barrier. Abbreviations: 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT)

Similar to ATD, it was first discovered that administration of an amino acid mixture deficient in TYR and PHE resulted in a reduction of TYR/PHE levels in serum and whole brain of the rat (Biggio et al., 1976). More recently these findings have been supported by further studies in laboratory animals (McTavish et al., 1999a, b, c) and more importantly McTavish and colleagues (1999a, b, c) demonstrated that ATPD appears to be dopamine specific with minimal or no effect on noradrenaline function. This finding confers ATPD with a dopamine specificity not enjoyed by other methods of dopamine depletion, such as the catecholamine synthesis inhibitor, α-methyl-paratyrosine (AMPT -
an inhibitor of the rate limiting enzyme tyrosine hydroxylase involved in dopamine synthesis) which has been shown to deplete both dopamine and noradrenaline (Verhoeff et al., 2003). The preliminary findings in animals were further supported by results in humans (Moja et al., 1996, Sheehan et al., 1996). In addition, the dopaminergic specificity of ATPD has since been replicated in humans in a study by Harmer and colleagues (2001) where it was noted that ATPD caused changes in prolactin levels and impaired dopamine dependent processes such as working memory in healthy volunteers. Since these initial studies in humans the relationship between ATPD, plasma levels of TYR/PHE and dopamine dependent processes has been consistently reproduced in several studies (Montgomery et al., 2003, Harrison et al., 2004, Leyton et al., 2004, Lythe et al., 2005, Mehta et al., 2005). Thus, it seems that ATPD is a safe and reliable method for inducing an acute reversible reduction in central dopamine synthesis and function.

2.6.3 Combined tryptophan/tyrosine/phenylalanine depletion

Monoamines levels were simultaneously modulated by combined tryptophan/tyrosine/phenylalanine depletion or combined monoamine depletion (CMD). Pharmacological intervention in some psychiatric conditions consists of compounds possessing primary effects at 5-HT, dopamine or noradrenaline receptors and/or transporters with resultant secondary effects modulating all monoaminergic systems (Spoont, 1992, Mongeau et al., 1997, Delgado, 2000). Accordingly, it may be that simultaneous depletion of both 5-HT and catecholamines may serve as a more accurate probe of monoamine function. Indeed this technique has been explored using combined ATD and AMPT administration in an effort to investigate their effects on mood and clinical symptoms (Salomon et al., 1997, Berman et al., 2002). However, the combination of these depletion techniques required 2-4 days of sequential testing accompanied by an overnight stay, making it inconvenient and time-consuming.

More recently a modified version of the amino acid mixture used by Young and colleagues (1985) has been proposed, which involves omitting TRP, TYR and
PHE from the balanced amino acid mixture (Table 2-1) (Leyton et al., 2003, Nathan et al., 2004). The plasma depletion levels and the ratio of LNAAs to amino acid precursors achieved by Nathan and colleagues (2004) are comparable to those in previous research utilizing ATD and ATPD separately. This result was further supported by the use of CMD methodology in healthy volunteers and the observation of mood (Hughes et al., 2004) and cognitive effects (Matrenza et al., 2004, Mann et al., 2007). Evidence from these studies suggests that the CMD methodology is an appropriate method to simultaneously deplete both 5-HT and DA synthesis and function.

2.6.4 Dopamine receptor stimulation

Dopamine receptors are modulated by dopamine receptor agonists. There are at least five distinct receptors for dopamine (D_1-D_5), but because of a lack of selective agonists for each receptor they are usually classified as D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) (Vallone et al., 2000). For this thesis two dopamine receptor agonists were chosen based on their availability for human use and their differential selectivity for dopamine receptors. Firstly, pergolide was selected due to its affinity for the D_1/D_2/D_3 receptor sites (Perachon et al., 1999), previous evidence of its sensory effects, cognitive effects and tolerability in healthy populations (Muller et al., 1998, Bartholomeusz et al., 2003). Bromocriptine was also chosen because of its affinity for the D_2/D_3 (and not D_1) receptors and evidence previously documented of its cognitive and sensory effects and tolerability in healthy populations (Luciana et al., 1992, Abduljawad et al., 1998, Oranje et al., 2004). In addition, both pergolide and bromocriptine readily cross the BBB (Deleu et al., 2002) providing for easy oral administration to participants. Following oral administration both drugs are rapidly absorbed and reach peak plasma concentration (T_{max}) between 1-3 hours for pergolide (Markham and Banfield 1997, Bartholomeusz et al., 2003) and 0.5-3.5 hours for bromocriptine (Friis et al., 1979, Luciana et al., 1992). The dose of each drug pergolide (0.1 mg) and bromocriptine (2.5 mg)) was chosen based on previous research that found significant behavioural drug effects (Luciana et al., 1992, Muller et al., 1998) and minimizing the possibility of side-effects, such as nausea, which could confound the study results.
2.6.5 Glycine administration

Glycine was used to modulate the glutamate system and particularly the N-methyl-d-aspartate receptors (NMDAR). Glycine is an amino acid that is used primarily in the synthesis of proteins in mammals with only a small fraction of the cellular pool of glycine retained for release as a neurotransmitter. Originally it was postulated that glycine was the major inhibitory neurotransmitter in the mammalian spinal cord (Aprison and Werman 1965b) and brainstem, however since this discovery it has come to light that glycine has a more ubiquitous role in inhibitory transmission throughout the entire mammalian CNS. With the advent of increasingly sensitive immunohistochemical techniques, glycine-fibre and receptor immunoreactivity has been reported in upper brainstem areas such as the DRN (Rampon et al., 1996a, Sato et al., 1991; Aoki et al., 1988). Other areas displaying glycinergic fibers and receptors include the forebrain (Rampon et al., 1996a), the central auditory system, including the cochlear nuclei and inferior colliculus (Wenthold and Hunter, 1990) and throughout the cerebral cortex (Naas et al., 1991). However, the majority of early immunohistochemical studies were performed in rats and other rodents, with few conducted in the human brain. Accordingly a collection of recent studies utilizing an extensively expressed protein, gephyrin, which generates a protein scaffold to anchor inhibitory neurotransmitter receptors in the postsynaptic membrane of the human CNS, mirrors the results of the aforementioned investigations with glycine receptors located throughout the entire CNS (Waldvogel et al., 2003; Baer et al., 2003). Inhibitory glycine receptors (GlyR) are implicated in the control of motor rhythm generation, coordination of reflex responses and processing of sensory signals.

In addition to its inhibitory role, glycine possesses a major excitatory function in the CNS through its glycine modulatory site (GMS) located on the NMDAR. Glutamate is a major excitatory neurotransmitter in the mammalian CNS and there are several types of glutamate receptors, including the NMDA receptor. The normal activation of NMDA receptors requires the presence of the essential co-factor, glycine, in moderate concentrations (Parsons et al., 1998). The subsequent non-competitive binding of glycine to its GMS causes allosteric conformation changes of the receptor to promote the binding of glutamate to
enhance NMDA receptor function (Millan 2005; D'Souza et al., 1995). Within
the glutamate system, NMDA receptors play a critical role in modulating
pyramidal cell activity and are involved in brain development, learning and
memory and general neural processing of information (Danysz and Parsons
1998, Frick et al., 2001, Lewis and Moghaddam 2006). NMDA receptors are
widely distributed throughout the CNS (Coyle and Tsai 2004; Dansyz and
Parsons 1998), including the cortex, where these receptors are found on various
cortical layers and primarily located on the cell bodies and apical dendrites of
pyramidal cells (Bowen et al 1993).

As NMDA receptor hypo-function is implicated in the pathology of
schizophrenia (Tuominen et al., 2006; Millan 2005; Coyle and Tsai 2004; Javitt
et al., 2001; Evins et al., 2000; Danysz and Parsons 1998), previous psychiatric
research involving glycine has primarily focused on the adjuvant treatment of
schizophrenia (Heresco-Levy et al., 1999, Javitt et al., 2001). Accordingly the
doze utilised in Chapter 5 of this thesis (0.8g/kg of body weight) is based on
these studies which displayed beneficial clinical effects and minimum adverse
effects (Heresco-Levy et al., 1999, Javitt et al., 2001). Following oral
administration glycine is rapidly absorbed reaching $T_{\text{max}}$ after 30-45 minutes
(Truong and Fahn 1988, Gannon et al., 2002).
3.1 Introduction

Upon review of the literature involving the LDAEP it is clear that studies attempting to directly assess the relationship between 5-HT and the LDAEP in humans have been inconsistent. For example, while a stronger LDAEP has been linked with low cerebrospinal fluid (CSF) levels of the serotonin metabolite, 5-hydroxyindole acetic acid (5-HIAA) in humans (von Knorring and Perris 1981), increasing 5-HT with the selective serotonin reuptake inhibitor (SSRI), citalopram, has been shown to decrease (Nathan et al., 2006) and have no effects (Uhl et al., 2006, Guille et al., 2008) on the LDAEP. Studies examining 5-HTTLPR have shown a stronger LDAEP in subjects homozygous for the l allele (Strobel et al., 2003; Hensch et al., 2006) as well as in subjects homozygous for the s allele (Gallinat et al., 2003).

Like the serotonergic system, there have been inconsistencies in studies examining the effects of dopaminergic modulation of the LDAEP. Early studies on ERPs reported that a high loudness dependence of auditory and visual evoked potentials was related to low levels of dopamine metabolites (i.e. homovanillic acid) in CSF and urine (Von Knorring and Perris 1981, Bruneau et al., 1986) while findings in animal studies have demonstrated a decrease in the loudness dependence of auditory evoked potentials following administration of the dopamine agonist, apomorphine (Juckel et al., 1997). Also, Pogarell and colleagues (2004) found a correlation between the LDAEP and both serotonin transporter (SERT) binding and striatal dopamine transporter (DAT) binding in patients with obsessive compulsive disorder (Pogarell et al., 2004). Additionally, an investigation on the 5-HTTLPR found that individuals with the l/l genotype displayed a stronger intensity dependence when the dopamine D_4 receptor gene (DRD4exon III) was considered in the analysis (Strobel et al., 2003), suggesting a possible synergistic interaction between the 5-HT and dopamine systems with regard to the modulation of the LDAEP.
together, these findings suggest that while there is evidence for both selective and synergistic serotonergic and dopaminergic modulation of the LDAEP, findings have been inconsistent and require further investigation.

Consequently, in this experiment we aim to examine the effects of an alternative method, namely, the acute depletion of the amino acid precursors for 5-HT (i.e. tryptophan) and dopamine (i.e. tyrosine/phenylalanine) alone, and simultaneously (to examine the effects of synergistic interactions) on the LDAEP in healthy subjects. Acute tryptophan (i.e. 5-HT) depletion (ATD) has been shown to be a reliable procedure to induce a reversible reduction of central serotonin function (for reviews see Reilly et al., 1997, Moore et al., 2000). In humans, acute tryptophan depletion (ATD) has been shown to acutely decrease 5-HT and its metabolites (Moja et al., 1989, Nishizawa et al., 1997, Carpenter et al., 1998, Williams et al., 1999), and impair 5-HT-dependant cognitive processes such as memory consolidation and learning (Harrison et al., 2004; Sambeth et al., 2007). Using this procedure, a number of studies have examined the effects of ATD on the LDAEP, showing no effect (Dierks et al., 1999; Debener et al., 2002; Massey et al., 2004, Norra et al., 2008), or a paradoxical decrease in the LDAEP (Kähkönen et al., 2002).

Acute tyrosine/phenylalanine depletion (ATPD) has been used as a method to reduce dopamine function. ATPD has been shown to decrease dopamine synthesis and release, (Leyton et al., 1999, McTavish et al., 1999a, Montgomery et al., 2003) and impair dopamine dependent cognitive processes such as working memory (Harmer et al., 2001; Harrison et al., 2004). Similarly, simultaneous tryptophan, tyrosine and phenylalanine depletion (combined monoamine depletion; CMD) has been used to simultaneously deplete monamines (5-HT and dopamine) (Nathan et al., 2004) and provides the means to examine the possible synergistic effects of monoamines in the human brain. CMD has also been shown to deplete plasma monoamine precursors by greater than 80% (Nathan et al., 2004). While the effects of ATD on the LDAEP have been previously examined (with mixed findings), the effects of ATPD and CMD on the LDAEP are yet to be examined. Based on clinical findings suggesting an inverse relationship between 5-HT neurotransmission and the
LDAEP, we hypothesized that both ATD and CMD would increase and ATPD would have no effects on the LDAEP.

3.2 Methods

3.2.1 Participants

Fourteen healthy male participants aged 21-42 years ($M=27.09$ years, $SD=6.92$ years) were included in the study.

3.2.2 Study Design

The study utilised a double-blind, placebo-controlled, cross-over design, in which each participant was tested under four acute treatment conditions; (a) 100 g nutritionally balanced control treatment (BAL), (b) acute tryptophan depletion treatment (ATD) (i.e. 5-HT depletion), (c) acute tyrosine and phenylalanine depletion treatment (ATPD) (i.e. dopamine depletion), and (d) acute tyrosine, tryptophan and phenylalanine depletion treatment (CMD) (i.e. combined monoamine depletion). Individual assignment to order of treatment condition was randomized and counterbalanced to ensure that equal numbers of subjects were tested under each condition. Completion of each condition was separated by a minimum 7-day washout period.

3.2.3 Amino Acid Composition

The amino acid composition for the depletion treatments were based on the 100 g balanced mixture developed by Young et al., (1985) and Nathan et al., (2004) and as described in Chapter 2. In the current study, the balanced control mixture consisted of 5.5 g of L-alanine, 3.2 g of glycine, 3.2 g of L-histidine, 8.0 g of L-isoleucine, 13.5 g of leucine, 11.0 g of L-lysine monohydrochloride, 5.7 g of L-phenylalanine, 12.2 g of L-proline, 6.9 g of L-serine, 6.5 g of L-threonine, 2.3 g of L-tryptophan, 6.9 g of L-tyrosine and 8.9 g of L-valine. L-Arginine (4.9 g), L-cysteine (2.7 g) and L-methionine (3.0 g) were encapsulated in 22 gelatin capsules and were administered separately due to their unpleasant
taste. All treatment mixtures were identical in composition to the BAL mixture, however in the ATD condition the mixture was deficient of L-tryptophan; in the ATPD condition the mixture was deficient of L-tyrosine and L-phenylalanine; and in the CMD condition the mixture was deficient of L-tryptophan, L-tyrosine and L-phenylalanine.

3.2.4 Procedure

On the day before each testing session, participants were required to adhere to a low protein diet, with their total protein consumption to be less than 20 g (Young et al., 1985). In addition, participants were also required to fast from 19:00 h that evening (with the exception of the consumption of water). This procedure has been employed in many previous studies as it has been suggested that it may enhance the effect of monoamine depletion and lessen the variability in baseline monoamine levels (Bel & Artigas 1996, Reilly et al., 1997, Harrison et al., 2004, Hood et al., 2005). On arrival for testing, participants were required to complete the Visual Analogue Mood Scales (VAMS) (Bond and Lader 1974; for example see Appendix 4) and then they had a small sample of blood taken (12 ml) to establish baseline mood and amino acid levels respectively (Table 3-1). Participants were then administered the amino acid drink and capsules. The powdered amino acids were mixed with 180 ml of orange juice a few minutes prior to oral administration. Participants consumed the twenty-two capsules and then swallowed the amino acid suspension immediately after. The participants were advised to drink this as quickly as possible given the unfamiliar and unpleasant taste. Upon completion of ingestion of the amino acids, participants were provided with sugar free chewing gum and a glass of water to cleanse the mouth. The process of amino acid administration took approximately ten minutes. To enable the depletion effects to occur, participants rested for the following four hours. During this time, they were allowed to consume water freely, but were restricted from any physical activity. At two hours post amino acid administration, participants were provided with a low protein snack, consisting of one carrot and an apple. At four and a half hours post ingestion participants were prepared for electrophysiological recording.
Preparation lasted approximately one hour and at 5.5 h post amino acid ingestion participants underwent electrophysiological recording. Immediately prior to recording participants were again administered the VAMS to examine mood changes following treatment, and were required to give another sample of blood (12 ml) in order to establish the levels of amino acid depletion achieved. This latency period for testing was chosen to coincide with the timing of maximal monoamine depletion determined in previous research detailing the time course of monoamine depletion in both animals and humans (for reviews see Reilly et al., 1997; Moore et al., 2000). In addition, this time period has frequently been employed in previous studies investigating the effects of ATD on the LDAEP (Debener et al., 2002; Kähkönen et al., 2002; Massey et al., 2004). Upon conclusion of the testing procedure, participants were provided with high protein snacks in order to replenish their amino acid levels. Participants resumed their normal diet between each of the testing sessions.

3.2.5 Subjective mood assessment

Possible mood changes were evaluated with a modified Visual Analogue Mood Scale (VAMS) (Bond and Lader 1974; see Appendix 4). The VAMS consists of sixteen, 100 mm horizontal scales such as Happy-Sad, Sociable-Withdrawn and Relaxed-Tense. Participants were asked to place a mark on each line that described their current mood state.

3.2.6 Data acquisition

As described in Chapter 2. In addition, data were collected with a sampling rate of 500 Hz, and a bandpass filter of 0.5 to 100 Hz.

3.2.7 Data Analysis

As described in Chapter 2. However in this particular study, data were epoched -100 to 400 ms post auditory stimulus.
3.2.8 Stimuli

As described in Chapter 2.

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 am</td>
<td>Participant arrives, completion of baseline mood questionnaire (VAMS) + 1st blood sample</td>
</tr>
<tr>
<td>10:30 am</td>
<td>Amino acid mixture administration</td>
</tr>
<tr>
<td>2:30-3 pm</td>
<td>Completion of mood questionnaires + 2nd blood sample</td>
</tr>
<tr>
<td>3:00-3:55 pm</td>
<td>EEG set-up</td>
</tr>
<tr>
<td>4:00 pm</td>
<td>EEG recording starting</td>
</tr>
<tr>
<td>5:5:30 pm</td>
<td>EEG recording completed</td>
</tr>
</tbody>
</table>

Table 3-1: Timetable of each testing session

3.2.9 Biochemical Analysis

Plasma samples were thawed at room temperature. 100 μL of plasma was sampled and diluted 1:1 with internal standard solution and deproteinized by ultrafiltration through a membrane with a 10 KDa nominal molecular weight cut-off (Ultrafree MC with PL-10 membrane, Millipore, MA, USA). The resulting filtrate (100 μL) was used to determine the concentrations of free amino acids tryptophan (TRP), tyrosine (TYR), phenylalanine (PHE), valine (VAL), leucine (LEU), and isoleucine (ILE) in plasma. The free amino acid concentration was determined in the filtrates using precolumn derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate and quantified by reversed phase high performance liquid chromatography using the Waters AccTag amino acid analysis system (Waters Corporation, MA, USA) (Cohen 2000). Amino acids were detected by fluorescence, except for TRP, which
required UV detection. VAL, LEU and ILE levels were analysed to calculate the ratio of plasma TRP, TYR, or PHE, to other large neutral amino acids (LNAAs).

3.3 Statistical Analysis

3.3.1 Subjective mood assessment

Repeated-measures analysis of variance (ANOVA) was performed to determine whether there were pre-existing differences in participants’ moods prior to treatment administration and to determine whether there was a treatment-related change in mood. The VAMS subscales were collapsed to form 3 factors (alertness, contentedness and calmness) (Bond and Lader, 1974) and the average of the VAMS sub-scale scores for each Factor was the dependent variable. The independent variables were treatment (BAL, ATD, ATPD and CMD) and time (before and after treatment).

3.3.2 Amino Acid Concentrations

As a result of a number of outliers and skewed variables within the biochemical data (which were not corrected with transformation), the biochemical data were analyzed using non-parametric Friedman’s tests. Significant results ($p<0.05$) were followed up with the Wilcoxon Signed Ranks Test.

3.3.3 LDAEP

A repeated measures linear contrast was employed to determine whether N1/P2 amplitude increased linearly with stimulus intensity, in the placebo (BAL) condition. Repeated measures ANOVAs were employed predicting; (1) a significant increase in the slope of the LDAEP in the ATD condition relative to the BAL condition (2) no change in the slope of the LDAEP in the ATPD condition relative to the BAL condition and (3) a significant increase in the slope of the LDAEP in the CMD condition compared to BAL condition. All of the above were determined for the least squares linear regression slopes across the 60-100 dB range. Slope means for the treatment conditions were expressed
as mean ± standard deviation (Significant results = $p<0.05$). All statistical tests were performed using the SPSS 14.0 package for Windows (SPSS Inc. Chicago, USA)

3.4 Results

3.4.1 Subjective mood

There was no significant treatment x time interaction ($F_{(3,39)}=0.59$, $p=0.63$) for the VAMS, indicating no pre-existing differences in participant’s mood prior to treatment administration or difference in participant mood after treatment administration. Furthermore, there was no treatment x VAMS factor ($F_{(6,78)}=1.35$, $p=0.09$) interaction, indicating that neither ATD, ATPD or CMD had any effects on the alertness, contentedness or calmness subscales.

3.4.2 Amino Acid Concentrations

A Friedman’s test demonstrated a significant effect of treatment on plasma levels of the amino acid precursors (tryptophan, tyrosine and phenylalanine) ($\chi^2_{(23)}=148.41$, $p<0.001$). Wilcoxon Signed Ranks tests (significance set at $p<0.05$) showed that, when compared to baseline concentrations of amino acids, there were significant increases in all amino acids after the BAL treatment (141.5 % TRP, 98.6 % TYR and 126.3 % PHE), and significant decreases in all after the CMD depletion treatment (68.1 % TRYP, 77.6 % TYR and 81.7 % PHE) (Table 3-2). Following ATD there was a significant decrease in concentrations of TRP (84.3 %), and increase in PHE (127.3 %) and no significant change in TYR compared to baseline levels (Table 3-2). After ATPD there was a significant decrease in TYR (72.3 %) and PHE levels (81.4 %) and an increase in TRP levels (256.2 %), when compared to baseline (Table 3-2).

In addition, a separate Friedman’s test revealed a significant effect of treatment on amino acid ratios ($\chi^2_{(23)}=138.06$, $p<0.001$). Wilcoxon Signed Ranks Tests (significance set at $p<0.05$) indicated that after BAL treatment there was a significant decrease in the ratio of TYR:ΣLNAAs (32.7 %), but no significant
change in the ratios of TRP:∑LNAAs or PHE:∑LNAAs, compared to baseline precursor ratios (Table 3-2). Following CMD treatment there was a significant reduction in all amino acid ratios, when compared to baseline ratios (86.7 % TRP, 90.7 % TYR, 92.6 % PHE) (Table 3-2). After ATD treatment there was a significant reduction in the ratio of TRP:∑LNAAs (93.3 %) but no significant decreases in the ratio of TYR:∑LNAAs or PHE:∑LNAAs, when compared to baseline ratios (Table 3-2). Finally, following ATPD there were significant reduction in the ratio of TYR:∑LNAAs (89.9 %) and PHE:∑LNAAs (93.3 %), however there was no significant change in the ratio of TRP:∑LNAAs, when compared to baseline ratios (Table 3-2).

In an attempt to investigate whether levels of amino acid depletion achieved after the CMD treatment were comparable to those achieved in the ATD and ATPD conditions, percentage change of the amino acid ratio (((post-pre)/pre)*100) was compared across treatment conditions. An additional Wilcoxon Signed Ranks test (significance set at \(p<0.05\)) revealed no significant difference in percentage change in the ratios of TYR:∑LNAAs or PHE:∑LNAAs between ATPD and CMD, similarly there was no significant difference in the percentage change of the ratio of TRP: ∑LNAAs, between ATD and CMD, suggesting that similar levels of depletion of TYR, PHE and TRP were achieved under selective and combined depletion methods.

**3.4.3 LDAEP**

A repeated measures ANOVA found a linear increase in N1/P2 amplitude with increasing stimulus intensity in the placebo condition (\(F_{(1,13)}=66.33, \ p<0.001\)). No significant difference in the slope of the LDAEP with increasing tone loudness was observed under the ATD condition (\(F_{(1,13)}=1.08, \ p=0.318\)) (ATD: Mean: 0.29+0.03; range 0.05-0.47), under ATPD treatment (\(F_{(1,13)}=4.21, \ p=0.061\)) (ATPD: Mean: 0.26+.03; range 0.04-0.41) or under CMD treatment (\(F_{(1,13)}=3.05, \ p=0.104\)) (CMD: Mean: 0.26+.03; range 0.04-0.47), relative to BAL (BAL: Mean: 0.32+0.04; range 0.13-0.61) (Figure 3.1 (a) and (b) and Figure 3.2).
Figure 3-1: (A) Mean Slope Values (Mean ± Standard deviation) (n=14) of LDAEP (µV/10 dB) at C2 for balanced (BAL), combined monoamine depletion (CMD), selective tryptophan depletion (ATD) and selective tyrosine/phenylalanine depletion (ATPD) conditions. (B) Mean N1/P2 amplitude (µV) plotted against stimulus intensity (loudness (dB)) (n=14), for BAL, CMD, ATD and ATPD conditions separately. No significant differences in slope values between treatment conditions are reported.
<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Treatment Condition</th>
<th>Baseline</th>
<th>4.5 h Post Treatment</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma free TRP</td>
<td>BAL</td>
<td>4.53 (1.88)</td>
<td>10.94 (3.42)</td>
<td>141.50**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>4.30 (1.68)</td>
<td>1.37 (0.52)</td>
<td>-68.14**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>5.10 (1.61)</td>
<td>0.80 (0.23)</td>
<td>-84.31**</td>
</tr>
<tr>
<td></td>
<td>ATPD</td>
<td>4.23 (1.92)</td>
<td>15.07 (7.78)</td>
<td>256.26**</td>
</tr>
<tr>
<td>Plasma TYR</td>
<td>BAL</td>
<td>49.06 (11.35)</td>
<td>97.41 (27.26)</td>
<td>98.55**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>47.33 (10.83)</td>
<td>10.60 (3.33)</td>
<td>-77.60**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>65.49 (46.78)</td>
<td>96.74 (36.59)</td>
<td>47.72</td>
</tr>
<tr>
<td></td>
<td>ATPD</td>
<td>46.73 (14.02)</td>
<td>12.93 (4.42)</td>
<td>-72.33**</td>
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<tr>
<td>Plasma PHE</td>
<td>BAL</td>
<td>44.96 (9.31)</td>
<td>101.74 (43.86)</td>
<td>126.29**</td>
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<tr>
<td></td>
<td>CMD</td>
<td>43.90 (8.77)</td>
<td>8.04 (3.12)</td>
<td>-81.69**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>50.31 (14.51)</td>
<td>114.37 (52.31)</td>
<td>127.33*</td>
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<tr>
<td></td>
<td>ATPD</td>
<td>41.76 (9.22)</td>
<td>7.77 (1.76)</td>
<td>-81.39**</td>
</tr>
<tr>
<td>TRP/ΣLNAAs</td>
<td>BAL</td>
<td>0.015 (0.006)</td>
<td>0.012 (0.003)</td>
<td>-20.00</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.015 (0.005)</td>
<td>0.002 (0.001)</td>
<td>-86.67**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>0.015 (0.005)</td>
<td>0.001 (0.001)</td>
<td>-93.33**</td>
</tr>
<tr>
<td></td>
<td>ATPD</td>
<td>0.014 (0.008)</td>
<td>0.016 (0.006)</td>
<td>14.29</td>
</tr>
<tr>
<td>TYR/ΣLNAAs</td>
<td>BAL</td>
<td>0.162 (0.031)</td>
<td>0.109 (0.024)</td>
<td>-32.72**</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.161 (0.033)</td>
<td>0.015 (0.005)</td>
<td>-90.68**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>0.162 (0.030)</td>
<td>0.131 (0.060)</td>
<td>-19.14</td>
</tr>
<tr>
<td></td>
<td>ATPD</td>
<td>0.149 (0.059)</td>
<td>0.015 (0.005)</td>
<td>-89.93**</td>
</tr>
<tr>
<td>PHE/ΣLNAAs</td>
<td>BAL</td>
<td>0.149 (0.022)</td>
<td>0.111 (0.033)</td>
<td>-25.50</td>
</tr>
<tr>
<td></td>
<td>CMD</td>
<td>0.149 (0.023)</td>
<td>0.011 (0.004)</td>
<td>-92.62**</td>
</tr>
<tr>
<td></td>
<td>ATD</td>
<td>0.142 (0.034)</td>
<td>0.140 (0.034)</td>
<td>-1.41</td>
</tr>
<tr>
<td></td>
<td>ATPD</td>
<td>0.134 (0.027)</td>
<td>0.009 (0.003)</td>
<td>-93.28**</td>
</tr>
</tbody>
</table>

**Table 3-2:** Plasma Concentrations of amino acids (μmol/l) (mean, SD) (n=7) (*p<0.05, **p<0.02). BAL = Balanced Condition, CMD= Combined Monoamine Depletion, ATD= Acute Tryptophan Depletion, ATPD= Acute Tyrosine/Phenylalanine Depletion.
3.5 Discussion

The current experiment utilised acute monoamine precursor depletion to investigate the effects of selective 5-HT and dopamine as well as combined 5-HT and dopamine depletion on the LDAEP in healthy male subjects. Contrary to predictions from the clinical literature linking 5-HT and the LDAEP (Hegerl et al., 1998, Gallinat et al., 2000, Tuchtenhagen et al., 2000, Croft et al., 2001, Senkowski et al., 2003, Juckel et al., 2003), selective 5-HT depletion using ATD had no significant effect on the LDAEP. In addition, acute depletion of dopamine using ATPD and simultaneous depletion of both 5-HT and dopamine using CMD had no effect on the LDAEP. These findings add to the growing literature outlining minimal effects of acute changes in serotonergic neurotransmission on the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Lee et al., 2005, Uhl et al., 2006, Norra et al., 2008, Guille et al., 2008).

Acute tryptophan depletion (ATD) resulted in a 93 % decrease in the ratio of tryptophan to other large neutral amino acids (LNAAs). This ratio of amino acid precursor to large neutral amino acid is thought to be a more accurate reflection of the amount of central depletion of the precursor (Fernstrom, 1979, Van der Does, 2001). This level of depletion is comparable to, if not exceeding that obtained in other studies involving the LDAEP (Dierks et al., 1999, Debener et al., 2002 and Kähkönen et al., 2002). In addition, these levels of depletion have been shown to significantly affect measures of central serotonergic function such as brain 5-HT synthesis (Moja et al., 1989; Nishizawa et al., 1997) and CSF concentrations of 5-HT metabolites (Carpenter et al., 1998; Williams et al., 1999). However, despite such pronounced levels of tryptophan depletion in the current study, no significant effect was noted on the LDAEP. This result is consistent with the majority of previous studies using ATD which similarly found no effects on the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004), although a paradoxical decrease in the LDAEP has been noted following ATD in one study (Kähkönen et al., 2002).
Figure 3-2: Grand mean auditory evoked potentials at Cz (n=14) for the balanced control treatment (BAL), acute tryptophan depletion treatment (ATD), acute tyrosine and phenylalanine depletion treatment (ATPD) and the acute tyrosine, tryptophan and phenylalanine depletion treatment (CMD) separately, at 60 dB, 80 dB and 100 dB intensities of auditory stimuli.
These variable results using ATD methodology are analogous to other investigations reporting inconsistent outcomes following acute 5-HT augmentation using selective serotonin reuptake inhibitors (SSRIs). In these investigations both a lack of modulation of the LDAEP (Uhl et al., 2006, Guille et al., 2008) and a noted decrease in the LDAEP (Nathan et al., 2006) have been reported.

While the reasons for these inconsistencies are as yet unknown a number of possible factors may be responsible. Firstly, it is possible that the LDAEP is not reliably sensitive to acute changes in 5-HT function. Indeed the strongest link between 5-HT and the LDAEP is derived from animal studies (Juckel et al., 1997, 1999) and investigations involving possible chronic serotonergic dysfunction (Buchsbaum and Pfeferbaum 1971, Hegerl et al., 1998, Gallinat et al., 2000, Senkowski et al., 2003, Juckel et al., 2003, Lee et al., 2005, Daumann et al., 2006). Second, inconsistencies in acute studies involving the LDAEP may relate to genetic variations in the serotonin transporter and in particular, a polymorphism in the promoter region of the serotonin transporter gene (5HTTLPR). Gallinat and colleagues (2003) reported a decreased LDAEP in participants with the l/l genotype of this polymorphism (longer l-allele associated with higher transcriptional efficiency, thereby leading to increased serotonin transporter levels and serotonin reuptake when compared to the shorter s-allele (Lesch et al., 1996)) and unexpectedly, in other studies involving individuals with the l/l genotype, an increased loudness dependence was reported (Strobel et al., 2003, Hensch et al., 2006). These studies seem to support an association between the LDAEP and serotonergic function and it is possible that the inconsistent results achieved to date in acute studies involving the LDAEP may be partly explained by these genetic variations that influence serotonergic neurotransmission.

Third, ATD findings may be confounded by methodological differences. For example, the physiological effects of ATD may occur at later than five hours post-administration. In previous investigations involving ATD and the LDAEP, a five hour period has been applied (Dierks et al., 1999; Kähkönen et al., 2002) as plasma tryptophan concentrations reach their nadir after this time. However, apparently more specific measures of central serotonergic function (i.e. CSF
tryptophan and CSF 5-HIAA) have been shown to reach maximal depletion from seven to ten hours after ATD with decreases in CSF 5-HIAA observed up to twelve to fourteen hours following ATD (Carpenter et al., 1998, Williams et al., 1999). Accordingly a later time window may be more sensitive to ATD effects on the LDAEP. Debener and colleagues (2002) investigated the possibility of greater effects of ATD at a later time window by performing recordings at five and six hours post-depletion. The authors noticed an increased LDAEP at six hours post-ingestion that was not statistically significant but this observation suggests that in future studies an even longer time window (i.e. six to twelve hours) may be more sensitive to reveal ATD effects on the LDAEP.

Finally, several acute studies involving the LDAEP included a mixed group of men and women (Dierks et al., 1999, Kähkönen et al., 2002, Nathan et al., 2006), yet men and women have been shown to differ greatly in markers of serotonin function, including serotonin synthesis rates (Nishizawa et al., 1997). Similar to the current study, some studies included males only (Massey et al., 2004), while the effects of the ATD paradigm is purported to have much more significant effects on central 5-HT synthesis in women (Nishizawa et al., 1997). A previous investigation involving ATD and LDAEP on a population entirely composed of women utilised the low dose 50 g amino acid mixture (Debener et al., 2002). Evidence from animal research points to a dose-response relationship between the amount of amino acids administered and the decline in brain tryptophan, free plasma tryptophan, brain 5-HT and brain 5-HIAA (Moja et al., 1989). Accordingly it may prove beneficial to include a study population of women only, with the 100g amino acid mixture and possibly a longer time window. However, a recent study in an entirely female population utilising a 75 g mixture (based on the 100g mixture of Young et al. 1985, with weights adjusted for lower body weight of females) and testing after 5 hours, found no effect of ATD on the LDAEP (Norra et al., 2008). Taken together these studies involving the LDAEP and ATD in healthy humans seem to question the sensitivity of the LDAEP to acute changes in serotonergic neurotransmission.

Acute tyrosine/phenylalanine depletion (ATPD) resulted in 90 % reduction in the ratio of tyrosine and a 93 % reduction in the ratio of phenylalanine to other LNAAs. These levels of depletion are comparable to those achieved in other
studies involving ATPD and healthy volunteers (Leyton et al., 1999, Harmer et al., 2001). Furthermore, ATPD studies in animals with depletion levels of this magnitude have noted significant reductions in catecholamine metabolites (Palmour et al., 1998) and catecholamine synthesis (McTavish et al., 1999a, b) and significant increases in plasma prolactin levels (an indirect measure of dopamine function) (Harmer et al., 2001). Despite significant depletion of plasma precursors likely to influence dopamine function, no significant effect was observed on the LDAEP in the current study. However, our findings are inconsistent with animal studies (Juckel et al., 1997) and more recent brain imaging and genetic studies linking dopamine markers and the LDAEP (Strobel et al., 2003, Pogarell et al., 2004).

The differences between our findings and previous research may be related to methodological discrepancies between studies. The methodological differences are discussed extensively in the following chapter (Chapter 4) but in summary, the study by Juckel et al., (1997) was performed in cats and should be interpreted with caution due to interspecies differences and the large variability in the small numbers of animals used. The studies in humans reporting a dopaminergic influence on the LDAEP were confounded by possible interactions with the 5-HT system. Namely, a relationship between the D4 receptor gene and the LDAEP was only observed when it was investigated in association with the l/l genotype of 5-HTTLPR (Strobel et al., 2003). Additionally, the radioligand used by Pogarell and colleagues (2004) ([123]β-CIT) lacked specificity for the dopamine transporter, as it also binds to the 5-HT transporter and the study was undertaken in patients with obsessive compulsive disorder adding the possibility of underlying pathophysiology (including a serotonergic dysfunction) confounding the reported result.

In an attempt to examine possible synergistic interactions between the 5-HT and dopamine systems based on previous studies (Strobel et al., 2003, Pogarell et al., 2004), we examined the effects of CMD on the LDAEP. CMD (i.e. acute tryptophan, tyrosine and phenylalanine depletion) resulted in an 86 % decrease in the ratio of tryptophan, a 90 % decrease in the ratio of tyrosine and a 92 % decrease in the ratio of phenylalanine to other LNAAs. These plasma depletion levels are comparable to those achieved in previous research utilizing CMD.
methodology in healthy volunteers and displaying cognitive effects (Matrenza et al., 2004, Mann et al., 2007). Despite significant depletion of plasma monoamine precursors (which were similar in magnitude to the selective depletions of each monoamine), CMD did not modulate the LDAEP. In accordance with the suggested relationship between 5-HT and the LDAEP (Hegerl and Juckel 1993, Gallinat et al., 2000, Hegerl et al., 2001), we expected CMD to modulate the LDAEP through concurrently depleting serotonin (i.e. via tryptophan depletion). However, as with ATD, there was no effect of CMD on the LDAEP. Interestingly the additional tyrosine (i.e. dopamine) depletion in the CMD did not result in modulation of the LDAEP.

These findings suggest that using the CMD method, we could not demonstrate any evidence for a synergistic interaction between 5-HT and dopamine systems with regard to the modulation of the LDAEP, even though such interactions may have been postulated based on findings of previous investigations (Strobel et al., 2003, Pogarell et al., 2004). The issues previously discussed in relation to ATD (timing of testing, genetic factors, gender issues and amino acid mixture) may have similarly affected the impact of CMD on the LDAEP in the current study. However in addition to these mitigating factors, there are several studies in the literature outlining complex (including apposing) physiological and anatomical interactions between the serotonergic, dopaminergic and noradrenergic systems (Spoont 1992, Mongeau et al., 1997, Ressler and Nemeroff, 2000). Thus, it is conceivable that the simultaneous depletion of all three neuromodulators may have had competing effects on cortical generators of the LDAEP resulting in no net effect on the LDAEP.

There are methodological issues in the present investigation that warrant discussion. Firstly, dipole source analysis (DSA) was not utilised, rather, the LDAEP was examined from the $C_2$ electrode as has been the case in several studies (Croft et al., 2001; Gallinat et al., 2003; Lee et al., 2005). As discussed in Chapter 1, DSA is purported to allow for the separation of the auditory evoked N1/P2 component into subcomponents generated by the primary and secondary auditory cortices. It has been suggested that the LDAEP generated from the primary auditory cortex, which is highly innervated by serotonergic fibres
(Lewis et al., 1986; Hegerl and Juckel 1993), is more sensitive to serotonin function and may be isolated and analysed using DSA. However, the advantage of DSA in comparison to measurement from $C_Z$ has not been directly shown to date, with comparable results reported using both methods. (Tuchtenhagen et al., 2000, Croft et al., 2001, Norra et al., 2008, Guille et al., 2008). Accordingly this issue may warrant a separate methodological study to clarify this issue. It could be argued that the sample-size of the present study may have contributed to the reported lack of effect of selective and combined 5-HT and dopamine depletion on the LDAEP. Indeed, statistical analysis demonstrated small to moderate effect sizes ($\eta^2=0.077$, $\eta^2=0.245$ and $\eta^2=0.190$). It should however be highlighted that a very large sample size would be required ($>190$ subjects) (power of 0.8, alpha of 0.05) (Buchner et al., 1997) for significant effects to be noted with this study’s reported effect sizes. In addition, the sample size used in this investigation is similar to that of other studies testing for effects of ATD on the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Norra et al., 2008) as well as studies showing effects of serotonergic manipulation on the LDAEP (Nathan et al., 2006).

In the current experiment the selective and combined effects of 5-HT and dopamine depletion on the LDAEP in healthy male subjects were examined. The LDAEP was unaffected by acute depletion of 5-HT and dopamine providing further support that the LDAEP may be insensitive to acute changes in serotonergic neurotransmission and quite possibly monoaminergic neurotransmission in general.
Chapter 4: Dopamine receptor stimulation and the LDAEP

4.1 Introduction

The issue of specificity of the LDAEP for the 5-HT system is an area of some concern within the extant literature with several findings casting doubt as to its sensitivity to changes in 5-HT function alone (Von Knorring and Perris, 1981, Bruneau et al., 1986, Juckel et al., 1997; Pogarell et al., 2004, Nathan et al., 2005). In particular, the dopaminergic system has been implicated in the modulation of the LDAEP given the close interconnections between it and the serotonergic system.

For example, earlier studies on ERPs showed that a high loudness dependence of auditory and visual evoked potentials were related to low levels of dopamine metabolites (e.g. homovanillic acid) in CSF and urine (Von Knorring and Perris, 1981, Bruneau et al., 1986). Similarly, Juckel et al., (1997) found that the D₁/D₂ agonist apomorphine decreased the loudness dependence of AEPs in animals, though the effect was delayed, pointing toward an indirect effect of dopamine on the LDAEP, possibly via its actions on the serotonergic system. A recent single photon emission computed topography (SPECT) study using the radiotracer \([^{123}\text{I}]-2\beta\text{-carbomethoxy-3\beta-(4-iodophenyl)}\)tropane (\([^{123}\text{I}]\beta\text{-CIT}) found a correlation between the LDAEP and both serotonin transporter (SERT) binding and striatal dopamine transporter (DAT) binding in patients with obsessive compulsive disorder (Pogarell et al., 2004). It was reported that increased \([^{123}\text{I}]\beta\text{-CIT binding in the midbrain and pons regions (indicating elevated SERT availability and reduced serotonin), and in the striatum (indicating elevated DAT availability and reduced dopamine), was correlated with an increased LDAEP. Finally, an investigation of a functional polymorphism in the promoter region of the serotonin transporter gene (5-HTTLPR) found that individuals with the l/l genotype (long form of polymorphism that promotes gene transcription thereby leading to increased serotonin transporter levels and serotonin reuptake (and consequently decreased 5-HT) when compared to the short (s) forms), displayed

\(^2\)This chapter has been peer-reviewed and accepted for publication. The journal copy is included as Appendix 8 – O’Neill et al., 2006 (Psychopharmacology)
stronger loudness dependence when compared to individuals with the *I/s* genotype. Unexpectedly the effect was even more pronounced when the dopamine D₄ receptor gene (DRD4 exon III) was considered in the analysis (Strobel et al., 2003). Though the method by which the two polymorphisms interact to produce such a result is yet to be elucidated, the results are in line with the aforementioned studies indicating a possible dopaminergic influence on the LDAEP.

In the previous chapter acute dopamine depletion had no effect on the LDAEP, but the full extent of the dopaminergic sensitivity of the LDAEP is not yet elucidated. Accordingly the aim of this experiment was to examine the direct effects of modulation of the dopamine receptor system on the LDAEP in healthy subjects. Specifically, it aimed to examine the effects of the D₁/D₂/D₃ receptor agonist pergolide and the D₂/D₃ agonist bromocriptine (Perachon et al., 1999) on the LDAEP. It was predicted that in healthy volunteers, acute modulation of dopaminergic neurotransmission by stimulation of the dopamine receptors with pergolide and bromocriptine would decrease the LDAEP in accordance with reported preclinical and clinical findings (Bruneau et al., 1986, Juckel et al., 1997, Pogarell et al., 2004).

### 4.2 Methods

#### 4.2.1 Participants

A total of 14 healthy participants (All males; mean age = 23.8 ± 3.5 years) were recruited for the study.

#### 4.2.2 Study Design

The study used a double-blind, placebo-controlled, repeated measures design. Each subject underwent testing under three different acute (single dose) drug conditions [placebo, bromocriptine (2.5 mg) (D₂/D₃ agonist) and pergolide (0.1 mg) (non selective dopamine receptor agonist)] separated by at least a seven-day
washout period between each condition. The doses selected were based on: (i) previous research that found significant behavioural drug effects (Luciana et al., 1992, Muller et al., 1998) and (ii) minimizing the possibility of side-effects, such as nausea, which could confound the study results. To avoid the acute effects of alcohol or caffeine from interfering with test results all participants were required to abstain from alcohol and caffeinated products for 24 hours prior to testing. The timing of the testing sessions was kept constant across all participants for all sessions.

4.2.3 Procedure

Participants arrived on testing days and were first given 10 mg of domperidone (a peripheral dopamine D₂ receptor antagonist) in order to reduce the likelihood of any nausea following bromocriptine or pergolide administration. Subjects were then provided with a standard meal consisting of four slices of toast with jam or vegemite and a glass of orange juice. A second 10 mg dose of domperidone was administered approximately one hour and fifteen minutes after ingestion of the first domperidone tablet. Thirty minutes later participants were given their first capsule, containing bromocriptine, pergolide or placebo (Figure 4-1). The order of drug administration was randomized using a Latin Square design. Two and a half hours following drug administration electroencephalography (EEG) recording commenced to coincide with approximate peak plasma levels as well as behavioural effects of both pergolide (1-3 h; Muller et al., 1998) and bromocriptine (0.5-3.5 h; Luciana et al., 1992, Gibbs and D’Esposito 2005).

4.2.4 Data Acquisition

As described in Chapter 2

4.2.5 Data Analysis

As described in Chapter 2
4.2.6 Stimuli

As described in Chapter 2

4.2.7 Statistical Analysis

Repeated measures ANOVAs were employed predicting; (1) a main effect of stimulus intensity on N1/P2 amplitude, (2) a significant reduction in the slope of the N1/P2 in the bromocriptine condition relative to the placebo condition, and (3) a significant reduction in the slope of the N1/P2 in the pergolide condition relative to the placebo condition. An additional repeated measures ANOVA was employed to assess the effects of treatment order on the slope of the N1/P2. Slope means for the treatment conditions were expressed as mean ± standard deviation. There were a number of outliers present during analysis (defined as a slope value of <0.05 μV/10dB), and so in order to rule out effects that these might have had on the reported result, a second repeated measures ANOVA was employed after the removal of these data points. All statistical tests were performed using the SPSS 12.0 package for Windows (SPSS Inc. Chicago, USA)
### Table 4-1: N1/P2 slopes (expressed as mean ± SD) and amplitude range for the placebo, bromocriptine and pergolide treatment conditions separately.

<table>
<thead>
<tr>
<th></th>
<th>N1/P2 Slope (μV/10dB)</th>
<th>N1/P2 Amplitude range(μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0.23±0.14</td>
<td>0.08-0.53</td>
</tr>
<tr>
<td>Bromocriptine</td>
<td>0.22±0.14</td>
<td>0.04-0.50</td>
</tr>
<tr>
<td>Pergolide</td>
<td>0.24±0.16</td>
<td>0.04-0.51</td>
</tr>
</tbody>
</table>

4.3 Results

A repeated measures ANOVA found a linear increase in N1/P2 amplitude with increasing stimulus intensity ($F_{(1,13)}=38.38, p<0.001$). However, no significant decrease in the slope of the N1/P2 with increasing tone loudness was observed under the bromocriptine treatment condition relative to the placebo condition ($F_{(1,13)}=0.19, p=0.666$) (placebo: Mean: 0.23±0.14; range 0.08-0.53 and bromocriptine: Mean: 0.22±0.14; range 0.04-0.50) or under pergolide treatment relative to the placebo ($F_{(1,13)}=0.11, p=0.750$) (placebo: Mean: 0.23±0.14; range 0.08-0.53 and pergolide: Mean: 0.24±0.16; range 0.04-0.51) (Table 4-1) (Figure 4-2 and Figure 4-3). When these analyses were re-run without the inclusion of
the outliers, no significant decrease in the slope of the N1/P2 with increasing tone loudness was observed under bromocriptine treatment condition relative to the placebo condition ($F_{(1,10)}=0.87$, $p=0.382$) (placebo: Mean: 0.26±0.14; range 0.08-0.53 and bromocriptine: Mean: 0.24±0.14; range 0.09-0.50) or under pergolide treatment relative to placebo ($F_{(1,10)}=0.28$, $p=0.606$) (placebo: Mean: 0.26±0.14; range 0.08-0.53 and pergolide: Mean: 0.28±0.15; range 0.07-0.51) (Table 4-2). Further none of the findings were affected by order of treatment administration as there was no significant treatment x order interaction ($p>0.05$).

![Figure 4-2](image.png)

**Figure 4-2:** Mean N1/P2 amplitude plotted against stimulus intensity (loudness), for the placebo, bromocriptine and pergolide treatment conditions separately. Least-squares regression lines indicate no difference in slope for the placebo compared to the bromocriptine or pergolide conditions.

### 4.4. Discussion

Along with the previous chapter, these studies are the first to directly examine the effects of dopaminergic modulation on the LDAEP in healthy humans. In
this chapter acute manipulation of the dopaminergic system via stimulation of the D<sub>1</sub>/D<sub>2</sub>/D<sub>3</sub> receptors with pergolide or D<sub>2</sub>/D<sub>3</sub> receptors with bromocriptine had no significant effect on the LDAEP. These findings are contrary to the reported observations of several investigations that found a relationship between markers of dopamine neurotransmission and the LDAEP (Von Knorring and Perris, 1981, Bruneau et al., 1986, Juckel et al., 1997, Strobel et al., 2003, Pogarell et al., 2004) but are in agreement with those of Chapter 3.

The discrepancies between our findings and previous studies may be related to methodological differences between studies. Firstly, the study by Juckel et al., (1997) was performed in cats using intravenous administration of the dopamine agonist, apomorphine. Though the study found an effect of apomorphine on the LDAEP, the authors acknowledged that the results should be interpreted with caution due to interspecies differences and the large variability in the small numbers of animals used this study. Secondly, of the studies conducted in humans, two of the studies (Von Knorring and Perris, 1981, Bruneau et al., 1986) involved correlations with peripheral biochemical markers of dopamine (i.e. dopamine metabolites) and the reliability of these indirect methods and their validity as measures of central neurotransmitter function has been questioned (Auerbach et al., 1989). In addition, the latter study (Van Knorring and Perris, 1981) investigated visual evoked potentials which have been known to differ in their cortical processing when compared to the auditory modality (Raine et al., 1981). Finally the remaining studies in humans which found a relationship between dopamine and the LDAEP were confounded by possible interactions with the serotonin system. For example, a relationship between the D<sub>4</sub> receptor gene and the LDAEP was only observed when this was investigated in association with the l/l genotype of the serotonin transporter gene (5-HTTLPR) (Strobel et al., 2003). Similarly, while a correlation between the LDAEP and striatal dopamine transporter (DAT) binding was observed (Pogarell et al., 2004), the radioligand used in the study ([<sup>123</sup>I]β-CIT) lacked specificity for the DAT, as it also binds to the serotonin transporter (SERT). Furthermore the latter study was conducted in patients with OCD and it is possible that the findings may have been confounded by the underlying pathophysiology (including a serotonergic dysfunction). The first two investigations of this thesis are the only direct investigations into the effects of dopaminergic modulation on the LDAEP
in healthy human subjects. Hence, they are not confounded by interspecies differences, psychopathology, or direct interactions with the serotonergic system.
Figure 4-3: Grand mean auditory evoked potentials at Cz for placebo, bromocriptine and pergolide treatment conditions separately at 60 dB, 80 dB and 100 dB intensities of auditory stimuli.

Though methodological differences may have contributed to the discrepancy between our findings and the aforementioned studies which found a positive association between dopamine and the LDAEP, the neural mechanisms responsible for a possible modulation (including a dopaminergic interaction with the serotonergic system) is not known. Considering the laminar and regional distribution of dopamine receptors found on pyramidal cells and GABA interneuron’s in the cortex (Seamans and Yang, 2004, Trantham-Davidson et al., 2004), it is possible that dopamine may exert an indirect influence on auditory evoked potentials by modulating corticocortical connections arising from within the primary auditory cortex rather than the thalamocortical connections of layer IV, which are believed to be involved in the modulation of the LDAEP (Hegerl and Juckel 1993). It is also possible that the previously reported dopaminergic effects on the LDAEP could in part be due to the activation of the 5-HT system as both systems are thought to be closely interconnected. This is supported by the evidence that the relationship between the D₄ receptor gene and the LDAEP was only observed in association with the l/l genotype of the serotonin transporter gene (5-HTTLPR) (Strobel et al., 2003). Indeed, animal studies have reported interactions between the dopamine and 5-HT systems, with evidence that 5-HT output as well as the firing rate of serotonergic neurons in the dorsal raphé nucleus is enhanced following administration of D₂ receptor agonists (Ferre and Artigas 1993, Martin-Ruiz et al., 2001).

Despite the possibility for a functional interaction between the dopamine and 5-HT systems and a possible indirect effect of dopamine on the LDAEP, no such effect was observed in the current study. There are however further methodological factors in the present investigation which warrant discussion that may have contributed to the reported result.

It could be argued that the sample-size of the present study may have led to the reported result. However, statistical analysis demonstrated a low effect size ($\eta^2=0.016$) and subsequent power analysis indicated that in order to observe
significant results at 70% power, the sample size would need to be greater than 387 (Cohen, 1992), which suggests that the effect is too small to be of physiological significance. Finally, the findings of the present study need to be interpreted with some caution due to the limited dosage regimen employed. It is possible that the lack of effect of the dopamine receptor agonists may be related to factors such as dose, time of testing and duration of treatment. However, the dose of both drugs and the regimen utilised in the current study has previously been reported to modulate sensory (i.e. measures of sensory gating) (Abduljawad et al., 1998; Oranje et al., 2004) and cognitive (e.g. working memory) processes (Luciana et al., 1992, Muller et al., 1998) and neuroendocrine markers (e.g. prolactin) of dopamine function (Muller et al., 1998). It may be postulated that increasing the dose may produce the hypothesised effect on the LDAEP; however, higher doses of bromocriptine and pergolide have been associated with side effects including nausea (Markham and Benfield 1997), which may confound reported results. In addition, it has been suggested previously that there is substantial variation as regards the peak pharmacodynamic effects of pergolide and bromocriptine with reported maximal effects at both 1.5 h and 3 h post-drug administration (Bartholomeusz et al., 2003). It is therefore important that future studies with dopamine agonists investigate possible time related effects as well as considering the effects of chronic administration.

In closing, the results reported in the current chapter suggest that acute modulation of the dopaminergic system via stimulation of D₁, D₂ or D₃ receptors does not modulate the LDAEP. This result taken together with those of the previous chapter suggest the LDAEP may be insensitive to acute dopaminergic modulation and possibly to monoaminergic modulation in general.
5.1 Introduction

As described in Chapter 1 the particular anatomic structures involved in the generation of the LDAEP are yet to be fully elucidated (Hegerl and Juckel 1993). It is thought the amplitude of cortical evoked potentials such as the LDAEP reflect the global activity of cortical pyramidal cells resulting from summation of inhibitory as well as excitatory post-synaptic potentials in cortico-cortico and thalamo-cortical loops (Zemon et al., 1986, Simpson and Knight 1993, Hegerl and Juckel 1993). In addition to possible neuromodulation by monoaminergic systems as discussed in Chapter 1, 3 and 4 these evoked potentials may also be modulated by the phasic release of excitatory (i.e. glutamate) and inhibitory (i.e. γ-aminobutyric acid - GABA) neurotransmitter systems in the cortex (Zemon et al., 1986; Simpson and Knight 1993).

Within the glutamate system, N-methyl-d-aspartate (NMDA) receptors play a critical role in modulating pyramidal cell activity (Frick et al., 2001, Lewis and Moghaddam, 2006). NMDA receptors are widely distributed throughout the CNS (Dansyz and Parsons, 1998, Coyle and Tsai, 2004), including the cortex, where these receptors are found on various cortical layers and primarily located on the cell bodies and apical dendrites of pyramidal cells (Bowen et al., 1993). While the focus on the neurochemical basis of the LDAEP has been on the serotonergic system, the role of the glutamate system, and in particular the NMDA receptor system, is yet to be determined. In the current study we examine the effects of NMDA receptor modulation on the LDAEP. NMDA receptors have multiple agonist sites including a neuromodulatory site for the amino acid glycine (Figure 5-1) (see Dansyz and Parsons, 1998, Coyle and Tsai, 2004 for review). Normal activation of NMDA receptors requires the presence of this essential co-factor, glycine, in moderate concentrations (Parsons et al., 1998). The subsequent non-competitive binding of glycine to its glycine modulatory site (GMS) causes allosteric conformation changes of the receptor.
to promote the binding of glutamate to enhance NMDA receptor function (D’Souza et al., 1995, Millan 2005).

**Figure 5-1:** Stylised depiction of an activated NMDA receptor. Glutamate is bound to the glutamate binding site and glycine is bound to the glycine binding site. Allosteric sites that would cause inhibition of the receptor are not occupied. NMDA receptors require the binding of glycine in order to function.

In support, clinical studies have shown that high dose glycine (0.8g/kg), by enhancing NMDA mediated neurotransmission, can improve negative and cognitive symptoms in patients with schizophrenia (Heresco-Levy et al., 1999, Javitt et al., 2001). Accordingly, we hypothesized that; acute administration of high-dose oral glycine would result in an increased LDAEP, via activation of NMDA receptors and subsequent stimulation of cortical pyramidal cells within cortico-cortico and cortico-thalamic loops.
5.2. Methods

5.2.1 Participants

Sixteen healthy participants (All males; aged 19-36 years, mean = 23 ± 4.1 years) were recruited through university advertisements for the study, however only 14 participants completed the study.

5.2.2 Study Design

The study was a double-blind, placebo-controlled repeated measures design in which each subject was tested under two acute treatment conditions, separated by a minimum five-day washout period. The treatment conditions were; (1) placebo and (2) glycine (0.8 g/kg) (Hebei Donghua Jian Chemical Co. Ltd, China). The dose selected was based on previous research that found it to be well tolerated in clinical populations (Leiderman et al., 1996, Heresco-Levy et al., 1999, Javitt et al., 2001). To avoid the acute effects of alcohol or caffeine from interfering with test results all participants were required to abstain from alcohol and caffeinated products for 24 hours prior to testing and to fast on the morning of the recording session. The timing of the testing sessions was kept constant across all participants for all sessions. Individual assignment to order of treatment condition was randomized and counterbalanced to ensure that equal numbers of subjects are tested under each treatment condition on the two testing days.

5.2.3 Procedure

The study was conducted at the Brain Sciences Institute, Swinburne University of Technology. On testing days participants arrived in the morning and were prepared for electrophysiological recording (Table 5-1). This was followed by administration of either the glycine or placebo mixture. The glycine and placebo (Corn flour) powders were mixed with 200 ml of orange juice several minutes prior to oral administration. Upon completion of ingestion of the mixture, participants were given forty-five minutes rest before beginning electrophysiological recording, which lasted approximately forty-five minutes.
Electrophysiological recording coincided with approximate peak plasma levels of oral glycine (30-45mins) (Truong and Fahn 1988, Gannon et al., 2002). Subjective side effect questionnaires (see Appendix 2) were administered before the glycine/placebo mixture (Baseline), fifteen minutes after mixture ingestion (time 1) and finally ninety minutes following mixture ingestion (time 2). Due to experimental difficulties (i.e. loss of electronic data), side effect data was only obtained from eleven participants.

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:00 am</td>
<td>Participant arrives, completion of demographic questionnaires</td>
</tr>
<tr>
<td>10:30 am</td>
<td>EEG set-up and side effect questionnaire (Baseline)</td>
</tr>
<tr>
<td>10:40 am</td>
<td>Glycine administration (0.8g/kg)</td>
</tr>
<tr>
<td>10:55 am</td>
<td>Side effect questionnaire (time1)</td>
</tr>
<tr>
<td>11:15 am</td>
<td>EEG recording begins</td>
</tr>
<tr>
<td>12:00 pm</td>
<td>EEG recording completed and final side effect questionnaire (time2)</td>
</tr>
</tbody>
</table>

**Table 5-1: Timetable of each testing session**

5.2.4 *Data acquisition*

As described in Chapter 2.

5.2.5 *Data Analysis*

As described in Chapter 2.
5.2.6 Stimuli

As described in Chapter 2.

5.2.7 Statistical Analysis

Repeated measures ANOVAs were used to determine (1) the effect of acute glycine treatment on self reported side effect symptoms, with Treatment (glycine; placebo) and Time (baseline, time 1, time 2) the independent variables, and side effect symptoms (excluding the nausea score) the dependent variable, and (2) the effect of glycine administration on nausea, with Treatment (glycine; placebo) and Time (baseline; time 1, time 2) the independent variables, nausea scores the dependent variable. In addition, correlations were conducted between the changes in LDAEP slope values’ for the glycine treatment condition and the corresponding changes in nausea rating scores in order to determine the possible contribution of nausea to any drug induced changes of the LDAEP.

A repeated measures linear contrast was employed to determine whether N1/P2 amplitude (dependent variable) increased linearly with stimulus intensity (independent variable), in the placebo condition. In addition, non-parametric Wilcoxon signed rank tests were utilised to investigate the effect of glycine administration on N1/P2 amplitude across the five individual intensities. Finally, paired samples t-tests were employed to test for; a significant difference in the LDAEP between the glycine and placebo conditions for the least squares linear regression slope across the 60-100db range, 80-100db range and the 60-80db range. Slope means for the treatment conditions were expressed as Mean ± Standard deviation. All statistical tests were performed using the SPSS 14.0 package for Windows (SPSS Inc. Chicago, USA).
5.3 Results

5.3.1 Adverse effects

Within the group that received the side effect questionnaires ($n=11$), there was no difference between the treatment conditions in subjective side effect total scores [$F(1,10)=4.74$, $p>0.05$], no significant effects of time [$F(1,10)=2.50$, $p>0.05$] and no significant Time x Score interaction [$F(1,10)=3.61$, $p>0.05$]. Examination of the nausea scale indicated a significant increase in nausea scores under glycine treatment [$F(1,10)=5.00$, $p=0.049$] compared to placebo, a significant effect of Time [$F(1,10)=11.26$, $p=0.007$] for baseline versus time 2 and a significant Time x Score interaction at time 2 [$F(1,10)=6.11$, $p=0.033$] (Table 5-2). However, there were no significant correlations between changes in LDAEP slope values and changes in nausea scores. [$r = 0.03$, $p=0.927$]

5.3.2 LDAEP

Two subjects withdrew from the study due to nausea and their electrophysiological data were not analysed; the remaining sample ($n=14$) is presented here. A repeated measures ANOVA found a linear increase in N1/P2 amplitude with increasing stimulus intensity in the placebo condition ($F_{(1,13)}=80.26$, $p<0.001$). Non-parametric analysis failed to reveal a difference in N1/P2 amplitude between glycine and placebo treatment conditions at 60dB ($Z=-.220$, $p=0.83$), 70dB ($Z=-.220$, $p=0.83$), 80dB ($Z=-.973$, $p=0.33$), 90dB ($Z=-.471$, $p=0.64$) and 100dB ($Z=-1.41$, $p=0.16$). However, a significant decrease in the slope of the LDAEP (60-100db) was observed under the glycine treatment condition relative to the placebo condition ($t(13)=-2.8$, $p=0.016$) (placebo: Mean: 0.25±0.10; range 0.08-0.44 and glycine: Mean: 0.14±0.07; range 0.09-0.34) (Figure 5-2(a) and (b); Figure 5-3). Statistical analysis also demonstrated moderate effect size ($\eta^2=0.37$). This attenuation was significant at the higher (80-100 db) intensity range ($t(13)=-2.4$, $p=0.030$) but not the lower (60-80 db) intensity range ($t(13)=-1.9$, $p>.05$).
Figure 5-2: (a) Mean N1/P2 amplitude (µV) plotted against stimulus intensity (loudness (dB)), for glycine and placebo treatment conditions separately. Least-squares linear regression lines indicate a significant decrease in slope for the glycine condition compared to placebo. (b) Mean Values (Mean ± Standard deviation) of LDAEP (µV/10dB) at Cz for placebo and glycine treatment conditions. [*p<.05]
<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo</th>
<th>Baseline</th>
<th>90mins Post</th>
<th>Glycine</th>
<th>Baseline</th>
<th>90 mins Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>1.3 (0.5)</td>
<td>1.2 (0.4)</td>
<td></td>
<td>1.1 (0.3)</td>
<td>1.6 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>1.4 (0.9)</td>
<td>1.3 (0.6)</td>
<td></td>
<td>1.2 (0.4)</td>
<td>1.4 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Hot</td>
<td>1.0 (0.0)</td>
<td>1.0 (0.0)</td>
<td></td>
<td>1.0 (0.0)</td>
<td>1.1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Dizzy</td>
<td>1.1 (0.3)</td>
<td>1.2 (0.4)</td>
<td></td>
<td>1.2 (0.4)</td>
<td>1.7 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Sweaty</td>
<td>1.0 (0.0)</td>
<td>1.0 (0.0)</td>
<td></td>
<td>1.0 (0.0)</td>
<td>1.1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td>1.1 (0.3)</td>
<td>1.0 (0.0)</td>
<td></td>
<td>1.0 (0.0)</td>
<td>1.1 (0.3)</td>
<td></td>
</tr>
<tr>
<td>Dry Mouth</td>
<td>1.2 (0.4)</td>
<td>1.2 (0.4)</td>
<td></td>
<td>1.4 (0.5)</td>
<td>1.2 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Stomach Pains</td>
<td>1.0 (0.0)</td>
<td>1.2 (0.6)</td>
<td></td>
<td>1.0 (0.0)</td>
<td>1.5 (0.8)</td>
<td></td>
</tr>
<tr>
<td>Blurred Vision</td>
<td>1.1 (0.3)</td>
<td>1.3 (0.6)</td>
<td></td>
<td>1.1 (0.3)</td>
<td>1.5 (1.0)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>1.2 (0.4)</td>
<td>1.3 (0.5)</td>
<td></td>
<td>1.0 (0.0)</td>
<td>2.1 (1.2)*</td>
<td></td>
</tr>
</tbody>
</table>

*Table 5-2: Effects of high dose oral glycine on subjective physiological symptoms (n=11). Data are mean (±SEM). Numbers indicate mean subjective ratings (on a scale of 1-5). Lower values indicate less adverse effects. (*p<0.5*)*
Figure 5-3: Grand mean auditory evoked potentials at Cz for placebo and glycine treatment conditions separately at 60 dB, 80 dB and 100 dB intensities of auditory stimuli.
5.4 Discussion

To the author’s knowledge this is the first investigation examining the effects of glycine administration on the LDAEP in healthy humans. As evoked potentials such as the LDAEP are thought to originate from synchronous activity of cortical pyramidal cells within cortico-cortico and thalamo-cortical loops (Zemon et al., 1986, Simpson and Knight, 1993, Hegerl and Juckel 1993), we hypothesised that directly increasing the activity of these cells, by targeting the glycine modulatory site of the NMDA receptor with high dose glycine, would result in an increased LDAEP. This scenario would be in accordance with several clinical studies that have utilised glycine treatment to reverse a hypothesised NMDA receptor hypofunction in schizophrenic patients resulting in improved positive and negative symptoms (Heresco-Levy et al., 1999, Javitt et al., 2001). Contrary to our hypothesis, glycine reduced the LDAEP when compared to placebo. While the exact mechanism by which high dose glycine reduced the LDAEP is unknown, its actions may be explained by a number of possible mechanisms, which are discussed below.

Firstly, while enhancing NMDA receptor function has traditionally resulted in excitatory activation; recent studies have illustrated a possible dual excitatory-inhibitory role of NMDA receptors in certain brain regions (Taber et al., 1996, Javitt et al., 2005). It is noted in these studies that the glutametergic system may modulate neurotransmitter release by NMDA receptors located on synaptic terminals or on GABA interneurons. In the current study, glycine may have reduced the LDAEP through activation of NMDA receptors located on GABAergic interneurons, thereby stimulating the release of GABA and a subsequent inhibition of pyramidal cell activity. Evidence for such NMDA receptor facilitated GABA release has been reported by Javitt and colleagues (2005).

Secondly, the effect of glycine on NMDA receptor function may also be dependent on the saturability of the GMS on the NMDA receptor. The affinity of the GMS for glycine is in the micromolar range (D’Souza et al., 2000, Martina et al., 2004) and glycine transporters which are co-localised with NMDA receptors (Smith et al., 1992) are efficient in maintaining glycine at low,
non-saturating concentrations (approximately 150nM under normal conditions (Roux and Supplisson 2000)) in the synapse (Danysz and Parsons, 1998). Consistent with this, *in vitro* studies (Berger et al., 1998, Chen et al., 2003) suggest that the GMS located on the NMDA receptor is not saturated under normal conditions. However in the current study the saturability of the GMS is unknown. A dose related effect of glycine on NMDA receptor function has been shown with high doses of glycine impairing NMDA mediated currents in neurons *in vitro* (Martina et al., 2004). The authors attributed this attenuation of NMDA receptor function to the high saturating doses of glycine at the synapse leading to NMDA receptor internalisation by endocytosis (Martina et al., 2004). It is thus possible that the GMS may have saturated under the current dosage regimen with single high dose glycine administration (which may increase glycine concentrations beyond physiological levels) leading to desensitisation of the NMDA receptor (Martina et al., 2004) and as a consequence, a reduction in global pyramidal cell activity and the slope of the LDAEP.

Thirdly, the inhibitory effects of glycine on the LDAEP may be explained by the possible pharmacological actions of glycine via stimulation of inhibitory glycine receptors (GlyRs). Glycine receptors are located throughout the CNS, including pyramidal cells in the primary sensory cortex (Rampon et al., 1996, Waldvogel et al., 2003). In addition to its role in excitatory neurotransmission at the NMDA receptor, glycine has a major inhibitory role in the mammalian CNS by binding to these strychnine-sensitive inhibitory glycine receptors (Laube et al., 2002, Kirsch 2006, Betz and Laube, 2006). Glycine’s affinity for GlyRs extends across the millimolar to micromolar range (D’Souza et al., 2000, Breustedt et al., 2004) and these receptors are often co-localised with GABA_A receptors to act synergistically as inhibitory receptors (Jonas et al., 1998). GlyRs have been reported to be located on the apical dendrites and cell bodies of pyramidal neurons in various layers of the cortex (Naas et al., 1991, Waldvogel et al., 2003) and throughout the brainstem auditory nuclei such as the cochlear nuclei, inferior colliculus and the superior olivary complex (see Wenthold 1990 for review). Potentially at high doses, glycine may have activated GlyRs at any one of these sites and resulted in an attenuation of auditory processing and thus the LDAEP.
Finally, as discussed in Chapter 1 a neuronal model proposed by Grossberg and Gutowsk (1987), suggests that the ability of neurons to increase output with increasing stimulus intensity may be reduced in a neuronal layer with a high level of unspecific pre-activation (see Hegerl and Juckel 1993). Accordingly, it is possible in the current investigation, that glycine induced NMDA receptor activation (resulting in increased unspecific activation) may have led to an increase in N1/P2 amplitude while having no effect, or even attenuating the LDAEP (due to ceiling effects). This hypothesized scenario may explain the current findings supporting the action of glycine via the GMS of the NMDA receptor. However, we did not observe an effect of glycine on N1/P2 amplitude at any intensity arguing against any unspecific activation and subsequent ceiling effects.

There may be some methodological issues in the current study that warrant discussion. Firstly, in accordance with the LDAEP theory, glycine effects were more prominent at higher intensities (80-100 dB) in comparison to lower intensities (60-80dB). Though the result may not have been as significant when grouping into high or low intensities as compared to over the five intensities ($p=0.030$ vs. $p=0.016$), it is possible that at these higher intensities (i.e. reflecting greater baseline global activity of pyramidal cells within cortico-cortical and thalamo-cortical loops), global activity of pyramidal cells are more sensitive to neuromodulation by both excitatory and inhibitory neurotransmitter systems (e.g. serotonin, dopamine, noradrenaline, glutamate and GABA). Indeed previous studies have demonstrated that the effects of dopaminergic modulation of the frontal cortex depends on both an optimal level of stimulation and baseline dependent modulation of pyramidal cell activity (Sawaguchi and Goldman-Rakic 1994; Yang et al., 1999) and this may extend to AEPs and the LDAEP and may need to be considered when interpreting future investigations involving the LDAEP.

Thirdly, it is possible that nausea may have influenced our findings as some subjects reported subjective nausea (Table 5-2). However, this is unlikely as we did not observe any relationship between changes in LDAEP slope values and subjective nausea scores. Furthermore, in the same group of subjects, glycine did not impair any cognitive processes (i.e. attention, psychomotor speed,
information processing and memory) (findings published elsewhere; Palmer et al., 2008), providing further evidence that objective or subjective nausea is likely to have had minimal influence on physiological and behavioural processes, including the LDAEP. Finally, as a dose related effect of glycine on NMDA receptor function has been shown, with high doses impairing NMDA function in vitro (Martina et al., 2004), it may be beneficial in future studies involving the LDAEP to utilise lower, possibly non-saturating doses, that could result in optimal NMDA receptor activation and an increase in the LDAEP. The use of these alternative dose-response approaches may avoid the issue of receptor saturation and provide an improved method for investigating NMDA receptor function in relation to the LDAEP. In addition, increased NMDA receptor activity has been reported using glycine reuptake inhibitors (Chen et al., 2003; Martina et al., 2004) and partial GMS agonists (Tsai et al., 1999) and their use in future studies investigating the LDAEP and NMDA receptor function may also be warranted.

In conclusion, the findings reported in this chapter suggest that the LDAEP may be attenuated by high dose glycine. These findings add to the growing literature exhibiting modulation of the LDAEP by multiple neurochemical systems in addition to the serotonergic system. While the mechanisms responsible for the neuromodulatory effects of glycine or 5-HT on the LDAEP are unknown, it is possible that effects are mediated by stimulation of specific excitatory or inhibitory receptors located on pyramidal cells or GABA interneurons within the vicinity of pyramidal cells, in cortico-cortico or cortico-thalamic loops.
6.1 Summary of findings

This thesis is comprised of a series of studies designed to investigate the pharmacology of loudness dependence of the auditory evoked potential (LDAEP). More specifically this thesis aimed to elucidate the sensitivity of the LDAEP to pharmacological manipulation of multiple neurotransmitter systems and the specificity of the LDAEP as a marker of 5-HT function, by assessing the effects of acutely modulating monoamines (i.e. 5-HT and dopamine) and neurotransmitter (glutamate) systems in healthy volunteers.

The first experiment of this thesis examined the effects of global changes in 5-HT and dopamine neurotransmission on the LDAEP. This study examined for the first time, the effects of acute 5-HT depletion (via tryptophan depletion) and dopamine depletion (via tyrosine/phenylalanine depletion) alone, and simultaneously (to examine the effects of synergistic interactions) on the LDAEP in healthy male subjects. Despite achieving significant depletion of amino acid precursors, no effect was observed on the LDAEP under each experimental condition. Previous investigations involving the LDAEP and acute tryptophan depletion (ATD) have reported similar results to this thesis with no effect of ATD on the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Norra et al., 2008). Conversely, this was the first investigation to study the effects of acute dopamine and combined serotonin/dopamine depletion on the LDAEP. Together the results of this study indicate that acute manipulation of 5-HT and dopamine neurotransmission alone or simultaneously has no effects on the LDAEP. These findings confirm growing evidence (including studies conducted in our laboratory) (see Appendix 6, Guille et al., 2008), indicating that the LDAEP may be insensitive to acute changes in 5-HT and dopamine neurotransmission.

The second investigation aimed to examine the effects of directly modulating the dopamine D1, D2 and D3 receptors on the LDAEP. Previous research has indicated a possible dopaminergic influence on the LDAEP but was potentially confounded by interspecies differences, psychopathology, or direct interactions
with the serotonergic system (Von Knorring and Perris, 1981, Juckel et al., 1997, Strobel et al., 2003, Pogarell et al., 2004). Accordingly in this thesis I utilised the dopaminergic receptor agonists, bromocriptine (D_2/D_3 receptor agonist) and pergolide (D_1/D_2/D_3 agonist), in healthy humans in an attempt to directly modulate the LDAEP without the aforementioned confounds. Despite previous evidence to the contrary, acute stimulation of dopaminergic receptors had no effect on the LDAEP when compared to placebo.

The majority of previous research involving the LDAEP has focused on investigating monoaminergic systems and the LDAEP (particularly 5-HT), but little is known about other neurotransmitter systems. The third and final experiment, like the previous studies, was a novel study investigating the effects of acutely stimulating excitatory glutamate receptors (NMDA receptors) using high dose glycine on the LDAEP. This study sought to examine the role of glutamate (NMDA receptors) on the LDAEP given the association between glutamatergic neurotransmission and evoked potentials within cortico-cortico and cortico-thalamic circuitry (Zemon et al. 1986, Simpson and Knight, 1993). Contrary to findings with acute changes in 5-HT and dopamine, a marked decrease in the LDAEP was observed following acute administration of high dose glycine. The exact mechanisms responsible for such a result are not fully known but this data demonstrates an acute sensitivity of the LDAEP to neurotransmitter systems other than 5-HT.

In summary, the results of the present thesis demonstrate that the LDAEP is insensitive to acute changes in both 5-HT and dopaminergic neurotransmission and possibly to monoaminergic neurotransmission in general. In contrast, acute stimulation of either NMDA receptors or glycinergic receptors using high dose glycine decreased the LDAEP, suggesting that the LDAEP may be sensitive to changes in glutamatergic or glycinergic neurotransmission. This result when taken together with previous indirect evidence in preclinical and clinical investigations (Juckel et al., 1997, Strobel et al., 2003, Pogarell et al., 2004) may suggest that the LDAEP is sensitive to neurotransmitter systems other than the serotonergic system. As such, the findings of this thesis, together with the findings outlined in the literature, as discussed in Chapter 1, suggest that the LDAEP may not be reliably modulated by acute changes in monoaminergic
neurotransmission and also questions the selectivity of the LDAEP as a marker of central 5-HT function.

6.2 Discussion

The majority of evidence for a direct relationship between the LDAEP and 5-HT function is derived from animal studies (Juckel et al., 1997, 1999). To date, direct evidence linking the LDAEP and 5-HT function in healthy humans has largely failed to identify a link between changes in 5-HT function and the LDAEP (Dierks et al., 1999, Debener et al., 2002, Massey et al., 2004, Nathan et al., 2006, Uhl et al., 2006, Norra et al., 2008, Guille et al., 2008). Additionally, there have been reports of other neuromodulators influencing the LDAEP (Strobel et al., 2003, Pogarell et al., 2004, O’Neill et al., 2007 – also included as Chapter 5). The discrepancies found throughout the literature with regard to the sensitivity and selectivity of the LDAEP for 5-HT function may be dependent on numerous variables which may have also influenced the results of this thesis. These factors are discussed in the following sections.

6.2.1 Acute versus chronic modulation of neurotransmitter systems

The results of this thesis are in line with the observations outlined above, with acute tryptophan depletion having no effect on the LDAEP; a result which is consistent with previous acute studies (Nathan et al., 2006, Uhl et al., 2006, Norra et al., 2008, Guille et al., 2008) and may lead one to believe that the LDAEP is insensitive to acute changes in serotonergic neurotransmission. This result coupled with the observation that acute modulation of the dopaminergic receptor system and acute depletion of the amino acid precursors for dopamine, had no effect on the LDAEP, suggest that the LDAEP may be insensitive to acute monoaminergic modulation.

The third investigation of this thesis found that acute modulation of the glutamate system (possibly via NMDA receptors) decreased the LDAEP, a result which contrasts to those involving acute monoaminergic modulation but adds to reports of multiple neurochemical systems, in addition to the
Chapter 6 General discussion and conclusions

Barry O’Neill

serotonergic system, modulating the LDAEP. Nevertheless the majority of studies involving acute pharmacological challenges and the LDAEP suggest this electrophysiological biomarker is insensitive to acute changes in serotonergic neurotransmission. This observation combined with the more consistent findings in conditions characterised by longer term serotonergic dysfunction (i.e. predicting treatment response in depression (Buchsbaum et al., 1971, Gallinat et al., 2000, Juckel et al., 2004, Lee et al., 2005, Juckel et al., 2007a) and serotonergic impairments in MDMA users (Croft et al., 2001; Tuchtenhagen et al., 2000, Daumann et al., 2006)), suggest that the LDAEP may not be reliably sensitive to acute changes in 5-HT neurotransmission but may be a more effective marker of chronic changes in the serotonin system. In support, a preliminary chronic study conducted in our laboratory, using the SSRI sertraline, observed a decrease in the LDAEP in healthy volunteers (Simmons et al., 2003). However, such a possibility requires validation before one could conclude on the potential use of the LDAEP as a marker of chronic 5-HT function. This is particularly critical given that the application of the LDAEP would normally be in relation to disorders that are associated with chronic 5-HT dysfunction or in relation to examining the effectiveness of pharmacological agents on 5-HT function following chronic administration (i.e. as a measure of efficacy).

6.2.2 Genetic polymorphisms of the 5-HT system

As discussed in Chapter 1 the LDAEP has been investigated in an attempt to elucidate the influence of genetic variations of various 5-HT system components on this purported “direct” serotonergic marker. Results to date have been mixed with reports of an association between a particular polymorphism in the promoter region of the serotonin transporter gene (5HTTLPR) and both a reduced (Gallinat et al., 2003) and increased LDAEP (Strobel et al., 2003, Hensch et al., 2006). In a more recent study, investigators randomly selected a community-based sample of healthy volunteers from the general population and analysed the association between the LDAEP and the 5HTTLPR and extended the analysis to include four further variations in the SERT gene. This study reported no association between the LDAEP and any of the variables tested (Juckel et al., 2007c). This series of results led the authors to speculate that the
LDAEP may be more closely related to genetic variants controlling 5-HT release (5-HT autoreceptors) and synthesis (tryptophan hydroxylase), rather than re-uptake (Juckel et al., 2007c). In line with this suggestion, the same research group recently investigated a single nucleotide polymorphism (SNP) in the gene coding for the 5-HT$_{1B}$ receptor and its relationship to the LDAEP (Juckel et al., 2007b). The 5-HT$_{1B}$ receptor can control 5-HT release in terminal areas and may also influence serotonergic neurons in the raphé nuclei (Moret and Briley, 2000, Sari, 2004) and could conceivably play an important role in the modulation of the LDAEP. In support, Juckel and co-workers reported that 5-HT$_{1B}$ alleles were related to an increased LDAEP (Juckel et al., 2007b).

The results of these studies provide evidence for a genetic influence of serotonergic system components on the LDAEP. As studies of this thesis, and most in the literature, have not included analysis of genetic components, the influence of genetic variations within the serotonergic system on the investigations of this thesis and those in the literature cannot be ruled out. Furthermore, the influence of genetic variations within other neurotransmitter systems may also need to be considered. Strobel and colleagues (2003) reported an influence of the dopamine D$_4$ receptor gene (DRD4exon III) on the LDAEP and recently an association between genetic variants in the catechol-O-methyltransferase gene (COMT – enzyme involved in the inactivation of synaptic dopamine) and the LDAEP has been noted (Juckel et al., 2008). Further studies are needed in this area to analyse each of the possible genetic variants in turn and their influence on the different neurotransmitter system components and consequently on the LDAEP.

### 6.2.3 Gender and LDAEP methodologies

Several studies throughout the literature have utilised mixed populations as well as homogenous samples, but men and women have been shown to differ greatly in markers of 5-HT function including 5-HT synthesis rates. For example, CSF studies have reported an increased serotonergic metabolism in females when compared to males (Young et al., 1980, Agren et al., 1986), while in a study
using positron emission tomography (PET) the mean rate of synthesis in males was reported to be 52% greater than that of females (Nishizawa et al., 1997). Gender has also been shown to influence evoked potential research, with auditory evoked potential (AEP) amplitude shown to be greater in females compared to males (Camposano and Lolas, 1992). Additionally, modulation of LDAEP components in females during their menstrual cycle differs from males with a significant reduction in N1/P2 amplitude during the luteal (high oestrogen) phase (Walpurger et al., 2004). Gender differences in serotonergic sensitivity may need to be considered when comparing investigations of this thesis with those of the extant literature.

When analysing studies involving the LDAEP, methodologies specific to auditory evoked potentials may need to be considered. As discussed previously, dipole source analysis (DSA) is purported to allow for the separation of the auditory evoked N1/P2 component into subcomponents generated by the primary and secondary auditory cortices. It has been suggested that the LDAEP generated from the primary auditory cortex, which is highly innervated by serotonergic fibres (Lewis et al., 1986; Hegerl and Juckel 1993), is more sensitive to 5-HT function and may be isolated and analysed using DSA. However, the advantage of DSA in comparison to measurement from Cz has not been directly shown to date, with comparable results reported using both methods. (Tuchtenhagen et al., 2000, Croft et al., 2001, Norra et al., 2008, O’Neill et al., 2008 (also included as Chapter 3)). A recent study conducted in our laboratory compared DSA and Cz analysis in the same population of healthy volunteers under acute serotonergic manipulation (SSRIs - escitalopram, citalopram and sertraline) (Guille et al., 2008). None of the SSRIs had any effect on the LDAEP independent of the analysis method utilised. This result is in agreement with a similar study in which the authors found no effect of acute citalopram administration on the LDAEP after both Cz analysis and DSA in healthy volunteers (Uhl et al., 2006). Taken together these studies suggest there is no advantage in using DSA over Cz analysis in acute studies involving the LDAEP and as such, the choice of technique utilised is an unlikely explanation for disparities between studies.
Apart from the technique used for analysis of the LDAEP, stimulus intensity may single-handedly affect the outcome of a study. High intensities (e.g. those 100 dB or greater) have been known to lead to a paradoxical decrease in evoked potentials (Prescott et al., 1984, Brocke et al., 1999) and may therefore affect the derivation of linear regression slopes such as those used in the LDAEP. It is possible that individuals that display an increase in AEP amplitude at moderate intensities may reach the point of paradoxical reduction sooner, while individuals displaying a decrease in AEP amplitude at moderate intensities could still show a linear increase at higher intensities. This scenario could possibly lead to a completely different outcome of a given study and in support; studies involving the LDAEP and 5HTTLPR have reported both a decreased and increased LDAEP (Gallinat et al., 2003, Strobel et al., 2003, Hensch et al., 2006). In these studies, two utilised a maximum intensity of 96 dB (Strobel et al., 2003, Hensch et al., 2006) while the other group used a significantly higher stimulus level of 113 dB (Gallinat et al., 2003). This higher intensity level may have caused a paradoxical reduction as discussed above and lead to the opposing results reported (Hensch et al., 2006). Stimulus presentation order has also been shown to affect the amplitude of the AEP with tones delivered in a pseudorandomised order resulting in greater amplitudes of evoked potentials than those delivered in ascending or descending blocks (Carrillo-de-la-Peña, 1999). Other methodologies relevant to ERP research such as the number of intensities examined, interstimulus interval and EEG recording sites may also play a role in the outcome of an investigation and vary considerably across studies in the current literature (Table 6-1). The methodologies included in this thesis are similar to those used in several studies throughout the recent literature, however, assessing the serotonergic sensitivity of the LDAEP or exploring its relationship to other neurotransmitter systems remains a difficult task as long as the effects of diverse methodologies are not well understood (Beauducel et al., 2000).
6.3 Implications for future research

Previous research, along with the initial investigations of this thesis, has outlined a significant weight of evidence in favour of an insensitivity of the LDAEP to acute serotonergic modulation and quite possibly acute monoaminergic modulation. The future of LDAEP research may consist of studies involving chronic serotonergic manipulations (in both patient and control populations) before one can make conclusive statements about the use of the LDAEP as a valid non-invasive marker of *in vivo* central 5-HT function.

<table>
<thead>
<tr>
<th>Study</th>
<th>Stimulus intensity levels (dB)</th>
<th>ISI (secs)</th>
<th>Presentation order</th>
<th>Analysed sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dierks et al., 1999</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>DSA</td>
</tr>
<tr>
<td>Gallinat et al., 2000</td>
<td>54, 64, 74, 84, 94</td>
<td>1.8-2.2</td>
<td>p-r</td>
<td>DSA</td>
</tr>
<tr>
<td>Debener et al., 2002</td>
<td>59, 71, 79, 88, 92, 96</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>C3, Cz, C4</td>
</tr>
<tr>
<td>Kähkönen et al., 2002</td>
<td>50, 60, 70, 80</td>
<td>3.5</td>
<td>r</td>
<td>Fz</td>
</tr>
<tr>
<td>Gallinat et al., 2003</td>
<td>79, 87.5, 96, 104.5, 113</td>
<td>1.8-2.2</td>
<td>Not reported</td>
<td>Cz</td>
</tr>
<tr>
<td>Strobel et al., 2003</td>
<td>59, 71, 79, 88, 92, 96</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>C3, Cz, C4</td>
</tr>
<tr>
<td>Juckel et al., 2004</td>
<td>50, 60, 70, 80, 90</td>
<td>1.8-2.2</td>
<td>p-r</td>
<td>DSA</td>
</tr>
<tr>
<td>Linka et al., 2005</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Fz, FCz, C3, Cz, C4</td>
</tr>
<tr>
<td>O’Neill et al., 2006</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Cz</td>
</tr>
<tr>
<td>Nathan et al., 2006</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Cz</td>
</tr>
<tr>
<td>Uhl et al., 2006</td>
<td>79, 87.5, 96, 104.5, 111</td>
<td>1.8-2.2</td>
<td>p-r</td>
<td>Cz and DSA</td>
</tr>
<tr>
<td>O’Neill et al., 2007</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Cz</td>
</tr>
<tr>
<td>Juckel et al., 2007a</td>
<td>60, 70, 80, 90, 100</td>
<td>1.8-2.2</td>
<td>p-r</td>
<td>Cz</td>
</tr>
<tr>
<td>Guille et al., 2008</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Cz and DSA</td>
</tr>
<tr>
<td>O’Neill et al., 2008</td>
<td>60, 70, 80, 90, 100</td>
<td>1.6-2.1</td>
<td>p-r</td>
<td>Cz</td>
</tr>
</tbody>
</table>

**Table 6-1:** Methodological variation between studies involving the LDAEP. Abbreviations: ISI = interstimulus interval, p-r = pseudo-random, r = random, DSA = dipole source analysis
In addition, the issue of specificity of the LDAEP for serotonergic function is as yet far from a clear cut issue. The third and final study of this thesis demonstrates a sensitivity of the LDAEP for acute glycine administration possibly mediated through excitatory glutamate neurotransmission by the activation of the NMDA receptor system. ERPs are thought to directly reflect postsynaptic effects of cortically released inhibitory (i.e. GABA) and excitatory (i.e. glutamate) neurotransmitters and indirect modulatory effects of neuromodulators (i.e. 5-HT, dopamine) on cortical neuronal function. The results of Chapter 5 are thus not surprising given the role of glutamate and the NMDA receptor system in the generation of cortical ERPs (Frick et al., 2001, Lewis and Moghaddam, 2006). But this result, when taken together with previous reports of several neurochemical systems modulating the LDAEP (Juckel et al., 1997, Strobel et al., 2003, Pogarell et al., 2004), does raise the question as to whether the LDAEP can be labelled a reliable marker for 5-HT function alone. This question is particularly pertinent when one considers the multiple components involved in the generation of ERPs (i.e. excitatory/inhibitory neurotransmitter systems and neuromodulators) where each component may interact with the next and each one may influence each other mutually. Future direct studies (chronic pharmacological manipulation, genetic studies of monoaminergic components of synthesis, release and re-uptake) in humans aimed at further unravelling the respective roles of neurotransmitter and neuromodulatory systems previously examined with respect to the generation of the LDAEP (i.e. serotonin, dopamine, glutamate) are warranted. Indeed, further trials (acute, chronic and genetic studies) are needed for those neurochemical systems not yet investigated (i.e. noradrenaline, GABA, acetylcholine). In addition to this unravelling of the neurochemical foundations of the LDAEP, the exact neural generators of this ERP are not yet fully elucidated. As such, future studies may also consider utilising imaging techniques (i.e. functional magnetic resonance imaging and magnetoencephalography) in conjunction with the LDAEP, in an attempt to pinpoint the specific area of the cortices involved in the generation of the LDAEP, thus allowing for more accurate spatial and temporal information. Until such a time, the results of this thesis, combined with the majority of the extant literature, seem to support the assertion that the LDAEP is not reliably sensitive or selective for 5-HT, and cannot be considered a direct acute marker of in vivo central 5-HT function.
Despite this observation the LDAEP has displayed an ability to accurately predict a favourable outcome to antidepressant treatment in depressed individuals. Firstly, in several studies utilising SSRIs, patients possessing a steeper pre-treatment slope (indicating reduced 5-HT) exhibited a greater reduction in depressive symptoms suggesting that an increased pre-treatment LDAEP (implying low 5-HT) may be a useful indicator of a favourable response to SSRI treatment (Gallinat et al., 2000, Lee et al., 2005). In a similar investigation a correlation between an LDAEP component and favourable antidepressant response was observed when using the selective noradrenergic reuptake inhibitor (NRI) reboxetine (Linka et al., 2005). In that study, lower N1 amplitudes (possibly implying increased 5-HT) prior to treatment with reboxetine, were correlated with a greater decrease in depressive symptomology in patients with major depressive disorder (Linka et al., 2005). This study by Linka and colleagues (2005), when considered with the above SSRI investigations, raises the issue of the differential predictive ability of the LDAEP (i.e. decreased vs. increased baseline LDAEP), for serotonergic and noradrenergic antidepressants respectively. However, this study needs to be interpreted with caution due to the reported result of reduced N1 amplitudes rather than the N1/P2 component as reported in the SSRI investigations.

In an attempt to further investigate the differential predictive ability of the LDAEP a recent study in unmedicated acutely depressed patients randomly assigned patients to treatment with either the SSRI, citalopram, or the NRI, reboxetine (Juckel et al., 2007a). Following 4 weeks of treatment it was discovered that those patients who responded favourably to citalopram treatment had a significantly increased baseline LDAEP (implying lower 5-HT) when compared to non-responders. Additionally the baseline LDAEP of reboxetine responders was comparable to that of citalopram non-responders (i.e. reduced baseline LDAEP) and also reduced when compared to citalopram responders (Juckel et al., 2007a). These results are in line with those of previous studies suggesting a predictive ability of the LDAEP for favourable treatment response to both serotonergic and noradrenergic antidepressants (Gallinat et al., 2000, Lee et al., 2005, Linka et al., 2005). To date, selection of an antidepressant for sufferers of depression is based on a trial and error approach with 30-50 % of patients not responding to the first or second medication administered (Thase,
2003, Adli et al., 2003). Accordingly the LDAEP may offer clinicians an important tool to aid in their decisions as regards treatment strategies for different patients and as such further research is needed in this area to replicate these encouraging preliminary results.

6.4 Conclusions

This thesis has provided evidence that acute changes in 5-HT and dopamine neurotransmission have no effect on the LDAEP, supporting previous evidence in the literature. These findings raise questions as to the acute sensitivity and selectivity of the LDAEP for central serotonergic neurotransmission in humans. Firstly, acute depletion of the amino acid precursor for 5-HT had no effect on the LDAEP. Secondly, modulation of the dopaminergic receptor system as well as depletion of the amino acid precursors for dopamine alone and in conjunction with those of 5-HT had no effect on the LDAEP. The demonstrated sensitivity of the LDAEP to acute glycine administration in the final investigation of this thesis, displays a sensitivity of the LDAEP to neurochemical systems other than 5-HT. This result, together with those of the monoaminergic studies in this thesis, indicate the LDAEP may not be a reliable sensitive or selective acute marker of central serotonergic function. Before disregarding the LDAEP as a specific indicator of serotonergic function altogether, the chronic sensitivity and selectivity of this electrophysiological biomarker needs to be elucidated. Nevertheless, at present the most promising utility of the LDAEP is as a clinical marker of initial antidepressant treatment response. This approach warrants further investigation in an effort to realise the full potential of the LDAEP as a possible important diagnostic aid.
References


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References


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dependence of auditory evoked potentials in a community-based sample of healthy volunteers. *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*. Published online: DOI 10.1002/ajmg.b.30628


Leiderman E, Zylberman I, Zuki SR, Cooper TB and Javitt DC (1996) Preliminary investigation of high-dose oral glycine on serum levels and


Mehta MA, Gumaste D, Montgomery AJ, McTavish SF and Grasby PM (2005) The effects of acute tyrosine and phenylalanine depletion on spatial working memory and planning in healthy volunteers are predicted by
changes in striatal dopamine levels. *Psychopharmacology (Berl)* **180**(4):654-663.


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Appendix 1a – Personal information/screening questionnaire
Used in chapters 3, 4 and 5
Subject Demographic information

Name: ...........................................................................................................

Contact address: ...........................................................................................

Phone: (Home)......................  (Mobile)..........................................

Email address: ..............................................................................................

Subject No: .................

Date: .....................

Time: .....................
## Exclusion criteria:

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smokers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug free (6 months)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart and circulatory disease</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aged 18-45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein Drinks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
INSTRUCTIONS: This questionnaire will help in understanding problems that you may have. It may be necessary to ask you more questions about some of these items. Please make sure to tick a box for every item. Your doctor will keep this information confidential.

<table>
<thead>
<tr>
<th>During the PAST MONTH, have you been bothered A LOT by...</th>
<th>During the PAST MONTH...</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. stomach pain</td>
<td>12. constipation, loose bowels, or diarrhoea</td>
</tr>
<tr>
<td>2. back pain</td>
<td>13. nausea, gas, or indigestion</td>
</tr>
<tr>
<td>3. pain in your arms, legs, or joints (knees, hips, etc)</td>
<td>14. feeling tired or having low energy</td>
</tr>
<tr>
<td>4. menstrual pain or problems</td>
<td>15. trouble sleeping</td>
</tr>
<tr>
<td>5. pain or problems during sexual intercourse</td>
<td>16. your eating being out of control</td>
</tr>
<tr>
<td>6. headaches</td>
<td>17. little interest or pleasure in doing things</td>
</tr>
<tr>
<td>7. chest pain</td>
<td>18. feeling down, depressed, or hopeless</td>
</tr>
<tr>
<td>8. dizziness</td>
<td>19. “nerves” or feeling anxious or on edge</td>
</tr>
<tr>
<td>9. fainting spells</td>
<td>20. worrying about a lot of different things</td>
</tr>
<tr>
<td>10. feeling your heart pound or race</td>
<td></td>
</tr>
<tr>
<td>11. shortness of breath</td>
<td></td>
</tr>
</tbody>
</table>
Please provide the following information:
(If you do not want to answer a question please leave it blank.)

1. Age: years ______ months ______
2. Gender: Female  Male
4. Handedness (circle):  Left  Right  Both
5. Have you ever suffered an epileptic seizure?  Yes / No
   If yes, please specify: ___________________________________________
6. Have you ever suffered any serious head injuries or periods of unconsciousness?  Yes / No
   If yes, please specify: ___________________________________________
7. Do you have any hearing problems?  Yes / No
   If yes, please specify: ___________________________________________
8. Are you currently taking any form of medication?  Yes / No
   If yes, please specify: ___________________________________________
9. Have you ever had to see a psychologist or psychiatrist (or are you concerned about
   your psychological well-being)?  Yes / No
   If yes, please specify: ___________________________________________
10. Has anyone in your family had any psychological or psychiatric illnesses? Yes / No
    If yes, please specify which family member and what illness:

11. Has a relative of yours died or experienced a life-threatening illness in the past three months? Yes/No
    If yes, please specify which family member and what illness

12. Is English your first language?  Yes / No
    If no, nationality: ___________________________________________

13. Ethnic Background ___________________________________________
Please fill out the following information about your use of the following substances. 
Note: If the questions are not applicable to you please write N/A.

14. Have you ever smoked tobacco? Yes / No

14.1 When was the last time you smoked tobacco? Please specify

- Hours [ ]
- Days [ ]
- More than 1 week ago [ ]
- More than a month ago [ ]

15. Have you ever consumed alcohol? Yes / No

15.1 When was the last time you consumed alcohol? Please specify

- Hours [ ]
- Days [ ]
- More than 1 week ago [ ]
- More than a month ago [ ]

15.2 How often do you consume alcohol? Please specify approximately how many times per day/week etc.

- Daily [ ]
- Weekly [ ]
- Monthly [ ]
- More than a month ago [ ]

15.3 How many standard drinks do you consume in a session? ______________________

15.4 How many standard drinks do you consume in a week? ______________________

15.5 How often during the last year have you needed a drink in the morning to get yourself going after a heavy drinking session? Please Specify

- Never [ ]
- Less than monthly [ ]
- Monthly [ ]
- Weekly [ ]
- Daily [ ]

15.6 How long have you been drinking alcohol for? ______________________

16. Have you ever consumed caffeine (e.g. tea, coffee, coke)? Yes / No

16.1 When was the last time you consumed caffeine? Please specify

- Hours [ ]
- Days [ ]
- More than 1 week ago [ ]
- More than a month ago [ ]
17. Have you ever had cannabis?  
   Yes / No

17.1 When was the last time you had cannabis? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

18. Have you ever had ecstasy?  
   Yes / No

18.1 When was the last time you had ecstasy? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

19. Have you ever had cocaine?  
   Yes / No

19.1 When was the last time you had cocaine? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

20. Have you ever had amphetamines?  
   Yes / No

20.1 When was the last time you had an amphetamine? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

21. Have you ever had heroin?  
   Yes / No

21.1 When was the last time you had heroin? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

22. Have you ever used inhalants (petrol, glue, etc)?  
   Yes / No
   Please specify what type

22.1 When was the last time you used an inhalant? Please specify
   Hours □ □ Days □ □ More than 1 week ago □
   More than a month ago □

Fit the criteria for the study:  Yes  No
Appendix 1b – Medical exam conducted by physician
Used in chapters 3, 4 and 5
**Medical History**

Participant Number

Date:

Background / concurrent disease:

Medications:

<table>
<thead>
<tr>
<th>Yes</th>
<th>No</th>
<th>If yes, give details below</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allergic History</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ophthalmologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory</td>
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<td></td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatobiliary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal / Genitourinary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic / Endocrine</td>
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<td></td>
</tr>
<tr>
<td>Neurological</td>
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<td></td>
</tr>
<tr>
<td>Musculoskeletal</td>
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<tr>
<td>Dermatological</td>
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<tr>
<td>Haematological</td>
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</tr>
<tr>
<td>Neoplastic</td>
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</tr>
<tr>
<td>Other (specify)</td>
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<td></td>
</tr>
</tbody>
</table>
## Medical Examination

<table>
<thead>
<tr>
<th>Body System</th>
<th>Normal / abnormal</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chest</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Abdomen</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Nervous System</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Lymph nodes</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>ENT and Eyes</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Extremities</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Skin</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
<tr>
<td><strong>Other (specify)</strong></td>
<td>☐ ☐</td>
<td></td>
</tr>
</tbody>
</table>

**Baseline Obs:**

- **BP standing**: ______
- **BP sitting**: ______
- **Pulse**: ______
- **T°**: ______
- **Height**: ______
- **Weight**: ______

**Comments:**

Signature __________________________
Appendix 2 – Physiological symptom checklist
As used in chapter 5
**Symptoms evaluation before EEG recording**

Form given at: (10:30am)  
Participant:  
Session:  

Please indicate how you currently feel on the symptoms listed below using the following ratings:

1 = Not at all  2 = Somewhat  3 = Unsure  4 = Moderately  5 = Very much

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Not at all</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>I have a headache</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel cold</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel hot</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel Dizzy</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I am sweating more than usual</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have palpitations</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I feel nauseous</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have dry mouth</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have stomach pains</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>I have blurred vision</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Comments:
Appendix 3 – Visual analogue Mood Scales (VAMS) (Adopted from Bond and Lader 1974) As used in chapter 4
Visual Analogue Mood Scale

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*

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
Appendix 4 – Reprint of O’Neill et al., 2008
(Human Psychopharmacology: Clinical and Experimental)
PLEASE NOTE

Appendix 4 is unable to be reproduced online. Please consult print copy held in the Swinburne library or click on the link below.

DOI: 10.1002/hup.926
Appendix 5 – Reprint of Guille et al., 2008
(Human Psychopharmacology: Clinical and Experimental)
PLEASE NOTE

Appendix 5 is unable to be reproduced online. Please consult print copy held in the Swinburne library or click on the link below.

DOI: 10.1002/hup.922
Appendix 6 – Reprint of O’Neill et al., 2007
(Psychopharmacology)
PLEASE NOTE

Appendix 6 is unable to be reproduced online. Please consult print copy held in the Swinburne library or click on the link below.


DOI: 10.1007/s00213-007-0870-4
Appendix 7 – Reprint of O’Neill et al., 2006
(Psychopharmacology)
PLEASE NOTE

Appendix 7 is unable to be reproduced online. Please consult print copy held in the Swinburne library or click on the link below.


[DOI: 10.1007/s00213-006-0501-5]
Appendix 8 – Poster presented at the Collegium Internationale Neuropsychopharmacologicum (CINP) meeting in Chicago (July 2006)
PLEASE NOTE

Appendix 8 is unable to be reproduced online. Please consult print copy held in the Swinburne library.
Appendix 9 – Publications arising from the current thesis
Publications arising from work in this thesis:


Published abstracts
