EMOTIONAL PROCESSING IN HUMANS: A NEUROPHYSIOLOGICAL AND PSYCHOPHARMACOLOGICAL INVESTIGATION

A thesis submitted for the degree of

Doctor of Philosophy (Neuropsychopharmacology)

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Brain Sciences Institute (BSI), Swinburne University of Technology (SUT), Melbourne Australia.
EXAMINATION OF HOW THE BRAIN MEDIATES EMOTIONAL EXPERIENCE IS NOW AN AREA OF SIGNIFICANT AND INTENSE RESEARCH INTEREST. THIS IS AN IMPORTANT ENDEAVOUR CONSIDERING THAT EMOTION IS A KEY COMPONENT IN VULNERABILITY FACTORS GOVERNING RISK FOR MOOD AND ANXIETY DISORDERS. RECENT NEUROPSYCHOLoGICAL AND NEUROIMAGING STUDIES ARE ALSO BEGINNING TO EXPLORE THE EFFECTS OF ANTIDEPRESSANTS ON THE PROCESSING OF EMOTIONAL STIMULI IN HEALTHY PARTICIPANTS TO HELP UNDERSTAND THE ROLE OF NEUROCHEMICALS IN AFFECTIVE BEHAVIOUR MORE BROADLY. UNFORTUNATELY THE LITERATURE IS FRAUGHT WITH CONTRADICTIONS AND COMPLICATIONS RESULTING FROM THE TECHNIQUE USED, TASK INSTRUCTIONS, SELECTION OF STIMULI AND GENDER DIFFERENCES. THE AIM OF THE CURRENT THESIS THEREFORE, WAS TO INVESTIGATE EMOTIONAL PROCESSING IN HEALTHY PARTICIPANTS AND TO EXAMINE THE IMPACT OF SEROTONERGIC AUGMENTATION ON THIS PROCESSING THROUGH THE PRESENTATION OF VISUAL EMOTIONAL STIMULI AND EXAMINATION OF SELF REPORT, PERIPHERAL- AND NEUROPHYSIOLOGICAL MEASURES OF EMOTIONAL RESPONSIVENESS. SEVENTY-FIVE IMAGES LOW IN AROUSAL CONTENT, SELECTED FROM THE INTERNATIONAL AFFECTIVE PICTURE SYSTEM (IAPS) AND CATEGORISED AS PLEASANT, NEUTRAL AND UNPLEASANT, WERE PRESENTED TO PARTICIPANTS IN FOUR EXPERIMENTAL STUDIES.

FINDINGS SUPPORT PREVIOUS LITERATURE SUGGESTING THAT THERE IS SUBSTANTIAL OVERLAP IN FRONTAL NEURAL CIRCUITRY WHEN THE BRAIN PROCESSES EMOTIONAL IMAGES OF DIFFERENT VALANCE. GENDER DIFFERENCES IN THE PROCESSING OF VISUAL EMOTIONAL STIMULI WERE ALSO OBSERVED HOWEVER SUGGESTING THE NEED FOR FUTURE STUDIES TO TAKE SUCH FACTORS INTO ACCOUNT. IN PARTICULAR, FEMALES UNLIKE MALES DISPLAYED RIGHT-SIDED, FRONTAL, NEUROPHYSIOLOGICAL ACTIVATIONS IN RESPONSE TO UNPLEASANT RELATIVE TO NEUTRAL IMAGES. EMOTIONAL VALANCE WAS ALSO FOUND TO MODULATE HEART RATE (HR) THEREBY CONFIRMING THE RELIABILITY AND VALIDITY OF THE TASK-VIEWING PARADIGM. AUGMENTATION OF SEROTONIN WAS FOUND TO SUPPRESS ANY DIFFERENCES IN HR ACROSS THE THREE DIFFERENTLY VALANCED CATEGORIES OF IMAGES, WHILE NEUROPHYSIOLOGICAL RESPONSES WERE POTENTIATED DURING PLEASANT VALENCE BUT SUPPRESSED DURING UNPLEASANT VALENCE. IN SUMMARY, THE STUDIES INCLUDED IN THIS THESIS PROVIDE EVIDENCE FOR NEUROPHYSIOLOGICAL MODULATION BY EMOTIONAL CONTENT AND GENDER. IN ADDITION, THE STUDIES EMPLOY A MORE SYSTEMS-BASED APPROACH TO THE STUDY OF ANTIDEPRESSANT ACTION, THROUGH EXAMINATION OF THE NEUROPHYSIOLOGICAL RESPONSES TO VISUAL EMOTIONAL STIMULI. THIS APPROACH MAY LEAD TO GREATER UNDERSTANDING OF THE FUNCTIONAL CONSEQUENCES OF NEUROCHEMICAL MODULATION ON CORtical NETWORKS INVOLVED IN EMOTIONAL PROCESSING.
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 the 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 in the text of the thesis.

Signed....................................................

Dated......................................................
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<thead>
<tr>
<th>Term</th>
<th>Abbreviation</th>
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<tr>
<td>2,4 - methylenedioxymethamphetamine</td>
<td>MDMA</td>
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<tr>
<td>3-dimensional</td>
<td>3-D</td>
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<td>Analysis of Variance</td>
<td>ANOVA</td>
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<tr>
<td>Anterior Cingulate</td>
<td>AC</td>
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<td>Attention Deficit Hyperactivity Disorder</td>
<td>ADHD</td>
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<tr>
<td>Autonomic Nervous System</td>
<td>ANS</td>
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<tr>
<td>Beats per Minute</td>
<td>BPM</td>
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<td>Blood Flow</td>
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<td>Blood Oxygen Level Dependent</td>
<td>BOLD</td>
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<td>Brodman’s Area</td>
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<td>Cerebral Blood Flow</td>
<td>CBF</td>
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<td>Contingent Negative Variation</td>
<td>CNV</td>
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<td>Continuous Performance Task</td>
<td>CPT</td>
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<td>Dopamine</td>
<td>DA</td>
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<td>Dorsolateral Prefrontal Cortex</td>
<td>DLPFC</td>
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<td>Electrocardiogram</td>
<td>ECG</td>
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<td>Electroencephalogram</td>
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<td>Electromyogram</td>
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<td>Electrooculogram</td>
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<td>Event Related Desynchronisation</td>
<td>ERD</td>
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<td>Event-Related Brain Potentials</td>
<td>ERPs</td>
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<td>Fourier Coefficients</td>
<td>FCs</td>
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<td>Full Width Half Maximum</td>
<td>FWHM</td>
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<tr>
<td>Functional Magnetic Resonance Imaging</td>
<td>fMRI</td>
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<td>Gamma-aminobutyric Acid</td>
<td>GABA</td>
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<td>Glutamate</td>
<td>Glu</td>
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<tr>
<td>Glutamine</td>
<td>Gln</td>
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<td>Heart Rate Variability</td>
<td>HRV</td>
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<td>Heart Rate</td>
<td>HR</td>
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<tr>
<td>Inferior Temporal Cortex</td>
<td>ITC</td>
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<tr>
<td>International Affective Picture System</td>
<td>IAPS</td>
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<tr>
<td>Late Positive Potential</td>
<td>LPP</td>
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<tr>
<td>Lateral Geniculate Nuclei</td>
<td>LGN</td>
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<tr>
<td>Limbic-cortical-striatal-pallidal-thalamic</td>
<td>LCSPT</td>
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<td>Limbic-thalamo-cortical</td>
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</table>
Low Resolution Electromagnetic Tomography  LORETTA
Magnetic Resonance Imaging  MRI
Magnetic Resonance  MR
Magnetoencephalogram  MEG
Magnocellular  Mag
Mauchly’s Test of Sphericity  MTS
Mean  M
Medial Prefrontal Cortex  MPFC
Middle Temporal Area  MTA
Mismatch Negativity  MMN
Mood Induction Procedure  MIP
Neutral  N
Noradrenaline (Norepinephrine)  NA
Occipital Cortex  OC
Orbitofrontal Cortex  OFC
Parvocellular  Parv
Pictures of Facial Affect  PFA
Pleasant  P
Positive Affect Negative Affect Schedule  PANAS
Positron Emission Tomography  PET
Posterior Cingulate  PC
Prefrontal Cortex  PFC
Primary Visual Cortex  V1
Profile of Mood States  POMS
Quantitative Electroencephalogram  qEEG
Regional Cerebral Blood Flow  rCBF
Repeated Measures ANOVA  RANOVA
Secondary Visual Cortex  V2
Selective Noradrenaline Reuptake Inhibitor  NRI
Selective Serotonin and Noradrenaline Reuptake Inhibitor  SNRI
Selective Serotonin Reuptake Inhibitor  SRI
Self Assessment Maniken  SAM
Serotonin  5-HT
Serotonin Transporter  5-HTT
Single Photon Emission Tomography  SPECT
Standard Deviation  SD
Statistical Package for Social Science  SPSS
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<td>Steady State Probe Topography</td>
<td>SSPT</td>
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<td>Steady State Visually Evoked Potential-Event Related Partial Coherence</td>
<td>SSVEP-ERPC</td>
</tr>
<tr>
<td>Steady State Visually Evoked Potentials</td>
<td>SSVEPs</td>
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<tr>
<td>Sublenticular Extended Amygdala</td>
<td>SLEA</td>
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<td>Superior Colliculus</td>
<td>SC</td>
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<td>Tricyclic Antidepressants</td>
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<td>Tryptophan Depletion</td>
<td>TD</td>
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<td>Unpleasant</td>
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<td>Ventromedial Prefrontal Cortex</td>
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<tr>
<td>Visual Area 3</td>
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<tr>
<td>Visual Area 5</td>
<td>V5</td>
</tr>
<tr>
<td>α-methylparatyrosine</td>
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CHAPTER 1

1  EMOTION: A GENERAL INTRODUCTION

"Nature has placed mankind under the governance of two sovereign masters, pain and pleasure. It is for them alone to point out what we ought to do, as well as to determine what we shall do.... They govern us in all we do, in all we say, in all we think: every effort we can make to throw off our subjection, will serve but to demonstrate and confirm it"

Jeremy Bentham (1748-1832)
1.1 INTRODUCTION

Research on emotion has been conducted by researchers from a wide-spectrum of disciplines including psychology, psychiatry, the neurosciences, neurology, zoology, anthropology, sociology, and economics. The focus of this thesis is on affective neuroscience, which may be regarded as the subdiscipline of the biobehavioural sciences that examines the underlying neural bases of affective phenomena (Davidson, Pizzagalli, Nitschke & Kalin, 2003; Davidson, Pizzagalli, Nitschke & Putnam, 2002). More specifically, this thesis examines the neurophysiological processes, physiological responsiveness and subjective feeling that are associated with the viewing of visual emotional stimuli. Furthermore, the differences between males and females in the processing of emotional stimuli as well as the mechanisms underlying the effects of neurochemical modulation on this processing will be investigated.

This introductory chapter provides a review of the study of the biological basis of affective phenomena. Research has been particularly broad in scope; therefore the focus of this chapter is on studies that have employed neuroimaging techniques to investigate the underlying mechanisms of emotion and mood (see section 1.3 for a definition of these terms). Furthermore, this focus is specific to studies conducted on healthy subjects. The literature on the disorders of emotion such as depression will not be discussed, unless particular studies in the literature are relevant to the findings presented or issues discussed. It is also important to highlight that diverse paradigms have been used to evoke and explore affective states and that this thesis relates more specifically to the processing of emotional stimuli.

This chapter begins by highlighting some of the key theorists who have attempted to provide an explanation for the biological basis of emotion. These include William James, Walter Cannon, James Papez, Paul MacLean, Stanley Schacter, Jerome Singer and Joseph LeDoux. A number of affective phenomena are then specified and defined and some of the psychological constructs through which affective phenomena have been understood, are presented. Theorists have generally considered emotions as discrete phenomena or as phenomena which lie on a number of independent dimensions. These constructs lay a framework for understanding the results reported in studies employing the various neuroimaging techniques.
A variety of stimuli and techniques have been used to investigate the neural bases of affective phenomena. As the focus of this thesis is on the processing of visual emotional stimuli, two popular databases used in neuroimaging studies on emotion, the Pictures of Facial Affect (PFA) and the International Affective Picture System (IAPS), are described. An overview of the different neuroimaging techniques used in studies on emotion and mood is then provided. These techniques include electroencephalography, magnetoencephalography, single photon emission computed tomography, positron emission tomography, and functional magnetic resonance imaging. The findings reported in these studies are then reviewed and a description of some of the key brain structures implicated in the processing of emotional stimuli is provided.

A number of neurophysiological models that have guided our understanding of emotion are outlined. These include hemispheric specialisation models, anterior-posterior models, and cortical-subcortical models. These models continue to be developed and refined as technology develops and our understanding of the mechanisms involved improves. The monoamines, dopamine, serotonin and noradrenaline, are also described. These are viewed as being critically important in the mediation of mood and emotion and have been implicated in the pathophysiology and treatment of disorders of emotion such as depression. Some of the methodological difficulties with previous research are then discussed and key issues needing to be resolved are highlighted. Finally, this chapter concludes with a discussion of the general aims and a more general overview of the thesis.

1.2 **Biological Bases**

Many theories attempting to explain the biological basis of emotions have been proposed. Most notably, these have included William James (1884) who proposed that emotion was a direct function of feedback from the periphery or viscera; Walter Cannon (1929) who proposed that the viscera was too insensitive and slow to be the source of emotional feeling; James Papez (1937) who developed upon the ideas of Cannon and proposed two emotional pathways in the brain, comprising the stream of thinking (or neocortical pathways) and the stream of feeling (or hypothalamic pathways); Paul MacLean (1949, 1952) who built upon the Papez circuit and viewed the hippocampus as the centrepiece of emotion because this structure was seen to be responsible for integrating the external environment and bodily responsiveness to
stimuli; Stanley Schacter & Jerome Singer (1962) who demonstrated the importance of cognitive interpretations on situations and the subsequent emotion experienced; and more recently Joseph LeDoux (for a review see LeDoux, 1996) who used tracer substances to track the emotional pathways in the brain and emphasises that direct thalamic input to the amygdala may explain how fear responses can be elicited without the aid of the cortex. Though LeDoux’s work has been conducted mostly on animals, it has been crucial in understanding the brain mechanisms involved in certain mood disorders such as anxiety and specifically, how fear responses may be elicited without the aid of the cortex.

Research on emotion has derived from diverse sources including studies on animals, normal humans, and neurological and psychiatric patient populations. Following the advance of technology and the availability of a variety of neuroimaging methods (discussed in section 1.4), there has been a resurgence of interest in the study of the biological substrates of emotion. An increasing number of studies over the last decade have used neuroimaging methods to examine emotional processes in human beings. This research has helped to elucidate the regions involved in normal emotional processes, and these regions are now believed to include the PFC, amygdala, AC cortex, hippocampus, insula, and basal ganglia. Research examining emotional processes in healthy subjects is particularly important for both theoretical and pragmatic reasons. For example, various experimental manipulations in healthy subjects are proposed to be able to model certain aspects of emotional disorders such as depression and anxiety (Andreasen, 1997; Baker, Frith & Dolan, 1997; Lawrence & Grasby, 2001; Liotti et al., 2000; Mayberg et al., 1999).

1.3 AFFECTIVE PHENOMENA

Affective phenomena may be considered as an umbrella term comprising a variety of affective states ranging from stable, long lasting personality traits to brief episodes of synchronised responsiveness (see Table 1). These phenomena are extremely diverse and as such, different categorisations have emerged. Perhaps the simplest way of distinguishing these phenomena is on the basis of their temporal domain, whereby emotion is characterised as being very brief, mood more intermediate, whilst temperaments are sustained (Ketter, Wang, Lembke & Sachs, 2003). Emotion therefore is regarded, not as an all-encompassing term, but rather as a relatively brief episode of synchronised response (lasting seconds to minutes) involving multiple components including cognitive processes, physiological responses, motivational
changes, motor expression and subjective feeling. By contrast, mood is a term which reflects a more diffuse state, characterised by low intensity but relatively long duration (lasting hours to days), whilst temperament is a term reflecting a constitutional state and a lack of autonomic features (lasting years to decades) and is characterised as the most sustained and least intense state of the three phenomena. Other theorists however, have argued that appropriate understanding of the processes involved in human emotion requires distinction of phenomena that are likely to involve different underlying mechanisms (Scherer & Peper, 2001). The phenomena distinguished by these authors include emotion, mood, interpersonal stances, attitudes and personality traits.

This thesis introduces and focuses on emotional processing which is defined as the perception and evaluation of emotional stimuli that does not necessarily involve emotional experience. For example, emotional processing may involve recognition of emotional facial expressions (emotional perception), recollection of an emotional event (emotional experience), or the viewing of emotional film or images (emotional perception and experience). Importantly, research, to be discussed later in this introduction, has demonstrated that these processes are associated with different regional activations. (See Table 1 for a summary of these affective constructs).

**Table 1:** Psychological constructs defined under the umbrella term of affective phenomena. These range from stable, long lasting personality traits to brief episodes of synchronised responsiveness.  

<table>
<thead>
<tr>
<th>Psychological Construct</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Personality traits/dimensions</td>
<td>Emotionally laden, stable personality dispositions and behavioural tendencies</td>
</tr>
<tr>
<td>Attitudes</td>
<td>Relatively enduring, affectively coloured beliefs, preferences and predispositions</td>
</tr>
<tr>
<td>Mood</td>
<td>A more diffuse state characterized by low intensity but relatively long duration</td>
</tr>
<tr>
<td>Interpersonal stances</td>
<td>Affective stance taken toward another person in a particular situation</td>
</tr>
<tr>
<td>Emotion</td>
<td>A relatively brief episode of synchronized response of all or most organismic subsystems to affective stimuli</td>
</tr>
<tr>
<td>Emotional processing</td>
<td>Active processing of a stimulus which may or may not involve emotional experience</td>
</tr>
</tbody>
</table>

1.4 Psychological Constructs

Over the last decade, a clearly exciting marriage of disciplines has been that of psychology and the neurosciences. One of the most difficult questions for philosophers and psychologists to answer has been the question proposed by William James in 1884: ‘What is an emotion?’. Answering such a question gives rise to other related and equally important questions such as, are emotions best organised in terms of discrete emotions or in terms of particular dimensions? If emotions are to be classified on dimensions, how many dimensions should there be, to adequately describe the affective space and what are these dimensions? These issues are not just philosophical or semantic because the knowledge of affective phenomena would accumulate more rapidly if researchers could organize their thinking around one set of consensually held terms (Calder et al., 2001; Feldman-Barrett & Russell, 1999; Russell, 2003).

Russell, (2003) explains:

‘One of the mysteries of psychology is how it has been possible to define and construe emotion in such apparently incompatible ways.... If emotion were a well-defined natural kind with different theories of emotion competing head to head over the same territory, then scientific scrutiny should have rejected the false alternatives long ago. If, instead, the word emotion refers to a heterogeneous cluster of loosely related events, patterns and dispositions, then these diverse theories might each concern a somewhat different subset of events or different aspects of those events. Theories about different things are not in competition, and empirical scrutiny could easily find evidence for each.’ (p.167)

Emotions have been defined using different conceptual frameworks. For example, emotions have been considered as discrete individual emotions including happiness, surprise, sadness, anger, fear, disgust (Ekman & Friesen, 1975), interest and shame (Izard, 1971). Emotions have also been considered in a more general sense, in which they may be categorised on different independent dimensions. One of these models posits the dimensions of pleasantness (valence) and activation (arousal) (Larsen & Diener, 1992; Reisenzein, 1994; Russell, 1980; Schlosberg, 1941; Wundt, 1912/1924), whilst an alternative and competing model distinguishes two independent dimensions of valence (ie. positive affect and negative affect) that implicitly communicate activation (Watson & Tellegen, 1985). Conversely, Thayer
(1989) has defined two dimensions of activation, implicitly communicating valence. These latter models (based on the independence of valence) have been supported from a number of different perspectives including *independence by definition* (e.g. the Positive Affect Negative Affect Schedule or PANAS), *empirical independence* (i.e. observed correlations between pleasant and unpleasant affect are substantially weaker than -1), and *neuropsychological independence* (Feldman-Barrett & Russell, 1999). Regardless Feldman-Barrett and Russell have argued that affect should be measured using the pleasure and activation scales (rather than, for example positive affect and negative affect) as these sample more broadly, what is actually being measured. Furthermore, they suggest that even if neural processes of affect are independent, bipolarity is still likely to emerge when forming conscious affective feelings (See Feldman-Barrett & Russell, 1999 for a review of the different dimensional perspectives relating to the structure of affect).

Recently, a hierarchical model for the structure of affect has been proposed which integrates these different models of emotions into one comprehensive account (Tellegen, Watson & Clark, 1999). The authors reported that exploratory analysis yielded a three-level hierarchy in which at the top of this hierarchy lies a more general bipolar Happiness-Versus-Unhappiness dimension, at the level beneath lies the relatively independent positive affect and negative affect, and at the base lies the discrete emotions. This is consistent with the neuropsychological, hierarchical model of emotion proposed by Borod (1993) which involves four different aspects of emotion. These are: 1) processing modes which include perception, expression, experience, and physiological arousal, and lies at the top of the hierarchy; 2) communication channels such as facial, prosodic, lexical, content, gestural and postural channels, which lies at the next level in the model and reflects the different modalities in which emotion can be processed; 3) emotional dimensions such as pleasant-unpleasant and approach-avoidance and which lies at the next level in the hierarchy and 4) discrete emotions including happiness, disgust, anger, surprise, sadness, fear. Tellegen’s recent proposal however would encompass an additional level within this hierarchy which would distinguish between pleasantness-activation and positive affect and negative affect dimensions.

In order to answer the age-old questions such as “What is an emotion?”, psychologists are increasingly turning to psychophysiological and neuroscientific techniques for assistance. The debate over whether emotion may be characterised in a single unifying theory is ongoing however, and questions remain unanswered. For
example, valence and arousal dimensions have been associated with two distinct neural systems in the brain, which have been localised to the frontal lobes and right parieto-temporal regions respectively (Heller, 1990, 1993). Calder and colleagues (2001) however, report that it is still unclear whether Russell’s valence-arousal model or Watson and Tellegen’s positive-affect/negative-affect model (as described above) are able to account for the neuroimaging findings reported for fear and disgust emotions in patients with lesions in the amygdala, basal ganglia or the insula. For example, it was argued that if these dimensions are the foundation of human affect, then damage to just one of these dimensions should produce effects on a wide range of emotions. Instead, specific deficits in the perception and (possibly the) experience of fear are associated with amygdala damage while specific deficits in the perception and (possibly the) experience of disgust are associated with insula and basal ganglia damage.

1.5 Experimental Procedures for the Evocation of Emotion

Studies have employed a wide variety of visual and auditory stimuli to evoke and examine different affective states. Stimuli have included for example, facial expressions, scenes, film clips, music, hypnotic suggestion and combinations of different stimuli. Other techniques such as script rehearsal, imagery, autobiographical recall, self-statements (or Velten procedure) and psychopharmacological manipulations have also been effective elicitors of emotional states (see Martin, 1990 and Westermann, Spies, Stahl & Hesse 1996 for a review of the different procedures studies may use to manipulate emotion). Neuroimaging studies of emotion have generally focused on the generation of, or at least, the attention to, five key emotions, including happiness, fear, anger, sadness, and disgust (see Phan, Wager, Taylor & Liberonz, 2002 for review) though lower-order sensory or motor processes of emotion such as gustatory/olfactory or pain induction have also been utilised to manipulate affect (Small et al., 1999; Small, Zatorre, Dagher, Evans, & Jones-Gutman, 2001; Casey et al., 1994).

The primary focus of this thesis however is on the processing of visual emotional stimuli from the IAPS standardised database. The IAPS consists of over 600 images occupying a large portion of “two-dimensional affective space formed by covarying pleasure and arousal ratings” (Lang, Bradley & Cuthbert, 1998, p.1249). The IAPS images contain a variety of content including low arousal negative content such as cemeteries and dead animals, high arousal negative content such as mutilated
bodies and burn victims, low arousal positive content such as babies and scenery, and high arousal positive content such as pornography. Selected images from the IAPS have been used in multiple neuroimaging studies to evoke a broad range of emotions experienced outside the laboratory (e.g., Canli, Desmond, Zhao, Glover & Gabrieli, 1998; Irwin et al., 1996; Kalin et al., 1997; Kawasaki et al., 2001; Kemp, Gray, Eide, Silberstein & Nathan, 2002; Kosslyn et al., 1996; Lane, Fink, et al., 1997; Lane, Reiman, Bradley, et al., 1997; Lang, Bradley, Fitzsimmons et al., 1998; Liberzon et al., 2000; Liberzon, Phan, Decker, Taylor, 2003; Paradiso et al., 1999; Phan et al., 2003; Taylor et al., 1998; Taylor, Liberzon & Koenig, 2000; Taylor et al., 2003). In the experiments described in the current thesis, participants were presented with images that were drawn from the IAPS database; thus, additional detail on the IAPS will be provided in the four experimental chapters to follow. The IAPS is actually one of two popular databases of visual stimuli which are available for the study of emotion. The other standardised database is the PFA, which contains 110 photographs displaying the six basic emotions of fear, happiness, anger, surprise, disgust, and sadness (Ekman & Friesen, 1976). These photographs have been used in multiple neuroimaging studies of facial emotion evaluation (e.g., Blair, Morris, Frith, Perrett, & Dolan, 1999; Breiter et al., 1996; Kawasaki et al., 2001; Morris et al., 1996, 1998; Morris, Ohman & Dolan, 1999; Philips et al., 1997; Philips et al., 1998; Sprengelmeyer, Rausch, Eysel & Przentek, 1998; Whalen, et al., 1998).

The PFA and IAPS databases may be generally considered to explore emotional perception and emotional experience respectively, though it is critical to note that task instructions will have substantial impact on whether the participant experiences or perceives the emotional content contained in the images selected from these databases (e.g., Lane, Fink, Chau & Dolan, 1997; Taylor, Phan, Decker & Liberzon, 2003). (See section 1.8 for discussion on this point).

### 1.6 Neuroimaging Technologies and Key Findings

Improvements in technology and computing power have contributed to the rapid development of neuroimaging technologies and over time, researchers have begun to apply these technologies in order to understand the brain processes involved in emotion. The number of studies investigating emotional processing has grown exponentially and our understanding of the brain mechanisms involved has improved remarkably. This section will provide a brief overview of these techniques and
describe some of the key findings each technique has contributed to emotion research.

The primary techniques employed in emotion research include electroencephalographic (EEG) based techniques (ie. event-related potentials, ERPs and quantitative electroencephalography, qEEG), positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Other measures have included magnetoencephalography (MEG), single photon emission computed tomography (SPECT), and steady state probe topography (SSPT). All of these techniques, excepting SPECT and PET are non-invasive, though some researchers have recorded brain electrical activity from depth-electrodes in intra-operative patients (eg. Kawasaki et al., 2001).

Neuroimaging techniques may be characterised by the ability with which they are able to resolve detail on both spatial and temporal scales (see Table 2 for a summary of spatial and temporal resolutions of the above techniques). Spatial resolution refers to 'the ability of a method to distinguish two separate objects that are positioned close to each other' whilst temporal resolution refers to 'the ability of a system to detect two events that occur in close temporal proximity' (Mazziotta, 1996, pp.393-398). With increasing spatial resolution (ranging from EEG to SPECT to PET to MEG and to fMRI), techniques are able to identify increasingly smaller structures. With increasing temporal resolution ranging from minutes (SPECT and PET) to seconds and hundreds of milliseconds (fMRI) to milliseconds (SSPT, EEG and MEG), techniques are increasingly able to identify events which are comparable to the actual time frames of neuronal events.

**Table 2**: Summary of the neuroimaging techniques used in affective neuroscience and associated (approximate) resolutions.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Mechanism</th>
<th>Temporal Resolution</th>
<th>Spatial Resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERPs; qEEG; SSPT</td>
<td>scalp recordings of electrical activity</td>
<td>milliseconds</td>
<td>several centimetres</td>
</tr>
<tr>
<td>MEG</td>
<td>measurement of the magnetic fields generated by the weak electrical fields</td>
<td>milliseconds</td>
<td>2mm</td>
</tr>
<tr>
<td>fMRI</td>
<td>local changes in magnetic field due to changes in ratio of oxyhaemoglobin to deoxyhaemoglobin</td>
<td>few seconds (faster with event-related designs)</td>
<td>&lt;1mm FWHM*</td>
</tr>
</tbody>
</table>

*Note Table adapted from Gordon, 2002; Honey & Bullmore, 2002; Toga & Mazziotta, 1996; Zald, 2003.
H$_2^{15}$O-PET | detection of gamma rays as a result of emitted proton with an electron following decay of radio-labelled water | 30 – 90 seconds | 3.5-4mm FWHM

SPECT | detection of gamma emissions due to radionuclide decay | 3-4 mins | 6-7mm FWHM

FDG-PET | Detection of gamma rays as a result of collision of emitted proton with an electron following decay of radio-labelled glucose | >30 mins | 3.5-4mm FWHM

1 FWHM (full width half maximum): refers to the distance at which two separate foci may be distinguished

Studies using ERP, qEEG, MEG, SPECT, PET and fMRI will be reviewed below. These studies relate specifically to the investigation of emotional processes in healthy subjects and employ a variety of emotion-activating paradigms. Whilst the qEEG technique is the most frequently used for the investigation of such processes, the SPECT and MEG techniques are the least frequently used. The current thesis employs the SSPT technique which, prior to conducting the studies reported below, had not been utilised for the study of emotional processes. The SSPT technique will not therefore, be described below, but in chapter 2, as part of the general methods section. While, it is not the purpose of this section to exhaustively document each of the studies that have explored the neural bases of emotional processing in healthy subjects, key findings will be described in order to provide a foundation from which the experimental results presented in chapters 3, 4, 5 and 6, can be understood.

1.6.1 Electroencephalography (EEG)

EEG measures a spatio-temporal average of synchronous postsynaptic potentials produced in radially oriented pyramidal cells within cortical layers 3 to 5 and the accompanying distortion by volume conductance within tissue and through the skull. Recording the EEG generally involves placement of electrodes on the scalp in standardised positions (the International 10-20 positions). EEG activity is measured on a millisecond timescale which is comparable to the time frames of neuronal events. Spatial resolution however is in the vicinity of a few square centimetres at the cortical surface, although surface and depth electrodes are able to measure to a volume as small as 100µm/dimension (Binne & Prior, 1994; Toga & Mazziotta, 1996). A variety of EEG methods, have been employed in emotion research and involve both frequency (qEEG) and time domain (ERPs) parameters. A noticeable aspect of EEG research over the last decade which has corresponded with improvements in computing technologies, has been the increase in the numbers of electrodes used in studies. These have increased from as few as 4 to 8 electrodes (Carretié & Iglesias,
1995; Davidson, Ekman, Saron, Senulis & Friesen, 1990 respectively) to as many as 129 electrodes (Junghofer, Bradley, Elbert, & Lang, 2001; Keil et al., 2002; Schupp, Junghofer, Weike, & Hamm, 2003). This advance has been particularly important to better determine topography associated with human cognitive and affective processes.

1.6.1.1 Quantitative Electroencephalography (qEEG)

Quantitative electroencephalography or qEEG refers to the quantification of the EEG recording. Conventional analysis splits the power spectra into four specific bandwidths including: delta (less than 4Hz), theta (4-7Hz), alpha (8-12Hz) and beta frequency components (13-35Hz). However, research has demonstrated that functionally different rhythmic components exist in narrower bandwidths (see Barlow, 1993 for general discussion and Aftanas, Koshkarov, Pokrovskaja, Lotova, & Mordvintsev, 1996, Crawford, Clarke, & Kitner-Triolo, 1996 re this issue as it relates to EEG emotion research).

Hans Berger recorded the first human EEG in 1924 and first reported that decreases in the dominant rhythm of the EEG (alpha activity) were associated with mental arithmetic (Berger, 1929). Since this time reductions in wideband alpha activity (8 to 12 Hz) have been interpreted to reflect increases in mental processing. Over the last two decades, a large body of research has used the EEG to examine the effect of emotional responsiveness on alpha activity (Aftanas et al., 1996; Aftanas, Varlamov, Pavlov, Makhnev, Reva, 2001a,b; Ahern & Schwartz, 1985; Allen, Harmon-Jones & Cavender, 2001; Davidson et al., 1990; Ehlers, Wall, Garcia-Andrade & Phillips, 2001; Fox & Davidson, 1987, 1988; Hagemann, Naumann, Becker, Maier & Bartussek, 1998; Jones & Fox, 1992; Meyers & Smith 1986; Schutter, Putman, Hermans & van Honk, 2001; Sobotka, Davidson & Senulis, 1992; Wexler, Warrenburg, Schwartz & Janer, 1992).

Findings have generally demonstrated that emotion is lateralised within the frontal lobes of the brain. Specifically, lateralisation involves an increase in activation (decrease in the abundance of alpha) within the left hemisphere during positive affect and an increase in activation within the right hemisphere during negative affect within anterior frontal and anterior temporal electrodes (see Davidson, 1992, 1995, 1998; Davidson et al., 1990 for discussion). In addition, findings have also suggested that resting electroencephalographic anterior brain asymmetry may predict self-report responses to emotional elicitors, affective traits as measured by the PANAS, and

Some studies however, have either failed to support lateralisation for emotional affectivity or found more complex patterns of lateralization (Hagemann et al., 1998; Meyers & Smith, 1986; Smith, Kline & Meyers, 1990; Smith, Meyers, Kline, & Bozeman, 1987; Starkstein et al., 1990), whilst others have reported failure to distinguish depressed from non-depressed participants based on resting frontal EEG activity (Reid, Duke & Allen, 1998). In addition, measures of anterior alpha asymmetry have also failed to associate with measures of aggression, approach/withdrawal, and mood/depression (Ehlers et al., 2001). Other studies have reported that the effects of lateralisation are restricted to narrower alpha frequency bands and may also extend beyond the alpha bandwidth (Aftanas et al., 1996; Crawford et al., 1996). There has also been debate as to whether asymmetry is a trait or a state phenomenon (see Ehlers et al., 2001 for discussion). Some of the reasons for these discrepancies will be discussed in later in this chapter (see section 1.10).

1.6.1.2 Event-Related Brain Potentials (ERPs)

Event-related brain potential (ERP) components are derived from ensemble averages of the EEG. The spontaneous EEG activity is large in comparison with the ERP elicited by a single stimulus, thus a large number of averages are needed in order to be able to distinguish the evoked potential from the noise (background EEG activity). With an increasing number of averages (typically 25-100 trials), the mean of the EEG approaches zero, whilst ERP components aggregate as long as this has been time-locked to an event such as a tone or the onset of a presented stimulus. The result is a voltage versus time graph which consists of negative (N) and positive (P) deflections. These deflections have been interpreted as reflecting higher cortical excitability and cortical inhibition to a new sensory input, respectively (see Palomba, Angrilli & Mini, 1997 for discussion). Analysis of these deflections is conducted on the amplitude which reflects the intensity of certain processes, as well as the latency which reflects the time taken to activate these processes (Cacioppo, Crites, Gardner & Berntson, 1994; Gevins, 1996; Kok, 1997).

The ERP consists of multiple components which include prestimulus components such as the contingent negative variation (CNV) relating to attentional preparedness,
exogenous components such as the N1, P2 and mismatch negativity (MMN) relating to external factors, and endogenous components such as the PN, P3, N400 and late positivity (LP) relating to cognitive factors. Measurement of these components provides valuable information about timing and cortical distribution of the electrical activity associated with various cognitive and affective tasks. For instance, inferences may be made about such things as prestimulus preparation; encoding of stimulus features; operations such as matching or comparison of stimulus and memory codes; evaluation of the meaning of the stimulus; and response selection and execution (Gevins, 1996).

ERP research has employed both visually evoked (eg. Mini, Palomba, Angrilli & Bravi, 1996; Palomba et al., 1997) and auditory evoked (eg. Morita, Morita, Yamamoto, Waseda & Maeda, 2001; Schupp, Cuthbert, Bradley, Birbaumer & Lang, 1997) potentials to investigate the processing of affective stimuli. Studies which have investigated the visually evoked potential have used the picture onset as the time-locked event. Studies which have used auditory evoked potentials in emotion research have examined how these potentials are modulated by affective stimuli. Another variant on the auditory evoked potential is to examine ERP components time-locked to the evoked startle response (eg. Schupp et al., 1997). The elicited blink reflex is believed to be responsive to picture pleasantness, such that the viewing of unpleasant and pleasant pictures potentiates and inhibits the startle response, respectively. (For a review of the human startle response and it’s modification by cognitive and emotional processes, see Filion, Dawson & Schell, 1998). Instead, the ‘probe P3’ is regarded as responsive to picture arousal. Schupp and colleagues indicate that smaller ‘probe P3’ responses are elicited when viewing both pleasant and unpleasant stimuli relative to neutral stimuli. Examination of event related potentials associated with the presentation of affective stimuli allows for the assessment of processes relating to early affective discrimination.

Emotion research has employed this technique to examine the ERPs associated with the processing of rapidly presented affectively valent words (Bernat, Bunce & Shevrin, 2001; Vanderploeg, Brown & Marsh, 1987), attitudinal judgement (Cacioppo, Crites, Berntson & Coles, 1993; Cacioppo et al., 1994), discrimination of emotional facial expressions (Boucsein, Schaefer, Sokolov, Schroder & Furedy, 2001; Campanella et al., 2002; Carretié & Iglesias, 1995; Graham & Cabeza, 2001; Kawasaki et al., 2001; Lang, Nelson & Collins, 1990; Laurian, Bader, Lanares & Oros, 1991; Morita et al., 2001; Orozco & Ehlers, 1998; Pizzagalli et al., 2002; Vanderploeg et al., 1987), and
the processing of pictorial stimuli such as the IAPS (Carretié, Iglesias, Garcia & Ballesteros, 1997; Carretié, Martin-Loeches, Hinojosa & Mercado, 2001; Carretié, Mercado, Tapia, & Hinojosa, 2001; Cuthbert, Schupp, Bradley, Birbaumer & Lang, 2000; Cuthbert, Schupp, Bradley, McManis, & Lang, 1998; Diedrich, Naumann, Maier & Becker, 1997; Junghefer et al., 2001; Kayser et al., 1997; Kawasaki et al., 2001; Kayser, Bruder, Tenke, Stewart & Quitkin, 2000; Keil et al., 2001, 2002; Mini et al., 1996; Naumann, Becker, Maier, Diedrich & Bartussek, 1997; Palomba et al., 1997; Schupp et al., 2000, 2003; Surakka, Tenhunen-Eskelinen, Hietanen, & Sams, 1998). ERP research on emotional processes has employed a number of paradigms to explore affective states, including the oddball paradigm and context-free, random presentation, both of which are discussed below.

The oddball paradigm involves elicitation of an ERP response to a deviant stimulus which occurs in a sequence of frequent stimuli. The sequence of frequent stimuli which may be based on physical, semantic or other characteristics of the stimuli, establishes a ‘context’ for the subject and it is the presentation of the ‘oddball’ stimulus which differs along this dimension that elicits the ERP response. Some of the ERP responses elicited in the ‘oddball paradigm’ include ‘automatic’ detection of deviance in the auditory modality known as the MMN, the N2, which is elicited by attended deviants, whether or not they are targets, and the late positivities which include the P300, Slow Wave, Novelty P3, & P3a).

Emotion research has used a variation on the oddball paradigm to examine the modulation of the ERP by affective stimuli. For example, Cacioppo and colleagues have demonstrated that the late positive potential (LPP) is enhanced for stimuli evaluated as distant from an established affective context. In other words, a larger amplitude LPP is evoked for inconsistent stimuli (eg. a negative personality trait word embedded in a sequence of positive trait words) than by evaluatively consistent stimuli (ie. a positive trait word embedded in a sequence of positive trait words) (Cacioppo et al., 1993). In addition, the more inconsistent a deviant stimulus was, along the valence dimension, the larger the recorded potential (Cacioppo et al., 1994). In these studies the LPP has been interpreted as a long latency P300 component as the LPP shared many characteristics of the P300 component including the scalp distribution, eliciting conditions and latency. In addition, Campanella and colleagues (2002) have recently employed the oddball paradigm to examine the modulation of the ERP N2/P3a component, which is thought to relate to the orienting complex. This study demonstrated that the N2/P3a complex is associated with larger
amplitude to deviant stimuli. In addition, they demonstrate a delayed response to the deviant stimulus having the same emotion as the frequent stimulus, relative to the different-emotion deviant, suggesting a higher sensitivity and faster adaptive reactions to changes in emotional facial expressions.

The other paradigm commonly employed in ERP emotion research has been context-free, random presentation of pleasant, neutral and unpleasant images (eg. Cuthbert et al., 1995; Cuthbert et al., 2000; Diedrich et al., 1997; Palomba et al., 1997). These studies have demonstrated substantial late positive shifts to both pleasant and unpleasant pictures which may be sustained over a long period of picture presentation. Furthermore, these positive shifts are accentuated for high arousal pleasant and unpleasant images and are associated with increased autonomic responses and reports of greater affective arousal (Cuthbert et al., 2000).

Key findings from studies that have investigated ERP modulation to affective stimuli include a stronger involvement of the right hemisphere when processing emotional stimuli (Junghofer et al., 2001; Kayser et al., 1997, 2000; Keil et al., 2001, 2002); early onset of affective discrimination (≥80ms) (Junghofer et al., 2001; Kawasaki et al., 2001; Pizzagalli, Regard & Lehmann, 1999; Pizzagalli et al., 2002); and greater amplitude of the P300 component as well as a sustained later positivity to emotionally salient images (Cacioppo et al., 1994; Cuthbert et al., 1998, 2000; Keil et al., 2001; Laurian et al., 1991; Mini et al., 1996; Palomba et al., 1997; Schupp et al., 1997, 2000; Schupp et al., 2003). This latter effect has been theoretically related to motivational engagement and commitment of attentional resources to significant stimuli (Lang, Bradley & Cuthbert, 1997). There are, however, like the findings reported in the qEEG literature, conflicting reports. For example, Boucsein and colleagues (2001) regard the P300 component as reflecting cognitive processing. Furthermore, Carretié and colleagues (Carretié, Iglesias, Garcia & Ballesteros, 1997; Carretié, Iglesias & Garcia, 1997) have suggested that the P300 component is not modulated by emotional charge. In addition, ERP studies have reported different patterns of response to emotional faces (eg. Laurian et al., 1991; Vanderploeg et al., 1987). Some of the reasons for these issues will be discussed later in this chapter (see section 1.8).
1.6.2 Magnetoencephalography (MEG)

The weak electrical fields which are recorded by the EEG produce magnetic fields which are recorded by MEG. The EEG however, records the electrical activity produced in radially oriented pyramidal cells, whilst MEG records the magnetic field produced in tangentially oriented pyramidal cells. These magnetic fields are of extremely low magnitude and therefore require the use of a super-cooled device, known as a superconducting quantum interference device (SQUID), in rooms that are isolated from the external magnetic and electrical environment. This device is coupled to a sensor coil (gradiometer) which is placed just above the scalp. The signals measured on the scalp surface must then be interpreted and converted into information about the distribution of currents within the brain. This task involves methods that include the popular point current dipole, minimum norm methods, spatial filtering, beamformers, MUSIC, and Bayesian techniques. One of the major benefits of MEG is that magnetic fields are relatively invulnerable to distortion or smearing effects caused by the skull, as has hindered the recording of electrical potentials in EEG research. The technical limitations of MEG, however, are that it is unable to resolve radially oriented pyramidal cells and that it is not sensitive to deep sources of activity. Regardless, the spatial localisation for MEG is similar to that for surface and depth electrodes, whilst the temporal resolution is in the millisecond time frame, which is similar to that of EEG. (Chen, 2001; Gordon, 2002; Reite, Teale & Rojas, 1999; Toga & Mazziotta, 1996; Vrba & Robinson, 2001).

Very few studies have employed MEG to investigate emotional processes in the brain. This, in part, is due to the difficulty of measuring the tiny magnetic fields in the presence of high levels of instrumental and extraneous noise as well as the requirement of more sophisticated analysis methods than those routinely used (see Ioannides, Bolton & Clarke, 1990, Ioannides, Muratore, Balish & Sato, 1993 and Ioannides, 2001 for discussion of the biomagnetic inverse problem and magnetic field tomography). The studies that have used MEG in emotion research have investigated human pain (Kakigi, Watanabe, Yamasaki & Maeda, 1999; Kakigi et al., 2000; Kitamura et al., 1995), recognition of human emotional expressions (Halgren, Rajj, Marinkovic, Jousmaki & Hari, 2000; Ioannides, Liu, Kwapien, Drozdz & Streit, 2000; Liu, Ioannides & Streit, 1999; Streit et al., 1999; Streit et al., 2003), the viewing of emotional video or images (Ioannides, Liu, Theofilou et al., 2000; Northoff et al., 2000, 2002).
The MEG studies investigating the neural mechanisms underlying human pain, have mapped the somatosensory homunculus of SI and SII, and have reported pain-related source localisation, the effects of sensory/motor gating, as well as central modulation of the neural pain response. (See Chen, 2001 and Peyron, Laurent & Garcia-Larrea, 2000 for a review on neuroimaging of human pain). Key findings on the recognition of human emotional expressions suggest that affective discrimination begins very early in the brain (Halgren et al., 2000: 110ms; Streit et al., 1999: 160ms) and that this activity is separated from the activity associated with simple perception of faces (Halgren et al., 2000; Liu et al., 1999; Streit et al., 1999). In addition, interactions between the fusiform gyrus (FG) and the amygdala have been reported, which are thought to be primarily associated with face processing, and in the recognition of aspects of emotional expressions respectively (Liu et al., 1999). Studies which have investigated the processing of emotional images have reported a functional dissociation between medial and lateral orbital and PFC such that negative emotional processing was associated with stronger and more medially oriented orbitofrontal dipoles, whilst positive emotional processing was associated with weaker and more laterally oriented orbito-and prefrontal dipoles (Northoff et al., 2000). Furthermore, lorazepam (a GABA-A potentiator) was found to shift the early magnetic field dipole from the orbitofrontal cortex (OFC) to medial PFC (Northoff et al., 2002), suggesting that negative emotional processing in the OFC may be modulated by GABA-A receptors.

It is interesting to note that most of these MEG studies are characterised by small sample sizes. For example, in two studies conducted by Ioannides and colleagues, one subject only was used in their analyses (Ioannides, Liu, Kwapien, et al., 2000; Liu et al., 1999), whilst in another study by the same group, four subjects were used. Other groups have reported results using sample sizes of 5 (Kitamura et al., 1995) and 10 subjects (Halgren et al., 2000; Northoff et al., 2000). Although results have identified activity in similar regions to those reported in PET and fMRI studies, it has been reported that the reason for such small sample sizes is the demands of computational MEG analysis (see Ioannides, Liu, Kwapien et al., 2000 for discussion). This is a concern given that a recent review has highlighted the heterogeneous nature of cerebral organisation and suggested that small sample sizes may provide inadequate power to detect such heterogeneity or to detect changes (Ketter et al., 2003, p.937), particularly in smaller structures such as the amygdala in which there have been substantial contradictory findings reported in the literature (discussed below in section 1.7.2).
1.6.3 Single Photon Emission Computed Tomography (SPECT)

SPECT provides estimates of cerebral perfusion, blood volume and receptor distribution following intravenous administration or inhalation of radiopharmaceuticals. These radiopharmaceuticals include xenon-133 gas ($^{133}$Xe) SPECT, which is an older technique able to resolve rCBF from cortical regions only and technetium-99m hexamethyl-propylene-amine-oxime ($^{99m}$Tc-HMPAO) SPECT, which is better able to access deep structures within the limbic system. Following administration, the labelled radioisotope is trapped in or on neurons, in proportion to blood flow (BF) activity or receptor distribution and the rotating gamma camera(s) then acquires the counts of radioisotope distribution throughout the brain. Transverse, coronal and sagittal slices are then reconstructed to provide measures of cortical and subcortical activity (Gordon 2002; Ketter et al., 2003; Toga & Mazziotta, 1996, pp. 16-18).

Studies in healthy subjects using SPECT have investigated the differential effects of mood (De Raedt, D'haenen, Everaert, Cluydts & Bossuyt, 1997; Schneider, Gur, Jaggi & Gur, 1994), listening to military combat sounds such as explosions and small arms fire (Zubieta et al., 1999), and acute ethanol consumption (Tiihonen et al., 1994) on rCBF. Findings suggest cerebral dissociations between depressed mood 'within the realm of attention' and 'out of the realm of attention' using a modified Velten procedure (De Raedt et al., 1997) as well as between happy and sad mood inductions (Schneider et al., 1994). In addition, they suggest that the euphoria associated with acute ethanol intake is related to activation of the right PFC and the mediation by the endogenous opioid system (Tiihonen et al., 1994).

1.6.4 Positron Emission Tomography (PET)

Like SPECT, PET imaging requires the introduction of radiopharmaceuticals into the brain by either peripheral intravenous injection or inhalation. Radioactive isotopes, including oxygen-15 ($^{15}$O), carbon-11 ($^{11}$C), nitrogen-13 ($^{13}$N) or fluorine-18 ($^{18}$F), tag molecules of biological interest such as H$_2$O and deoxyglucose. Scanners then detect two simultaneous gamma rays (also called photons) emitted at 180° that result from the collision of an emitted proton with an electron. The site where the proton is annihilated by the electron is detected by the scanner. The distance between the site of annihilation which is imaged and the emitting nucleus however may be several millimetres (2mm for $^{18}$F and 3mm for $^{15}$O), which sets an absolute limit on the spatial resolution of PET scan images. After collection of events over a period of time, say 60s, it is possible, to reconstruct the 3-dimensional geometry of the source. PET may be used to measure regional glucose metabolism using $^{18}$F- deoxyglucose, cerebral blood-flow using H$_2$$^{15}$O as an indirect measure of local synaptic activity and the
distribution of a particular receptor in the brain using a radioligand. A radioligand is a
chemical which incorporates a positron emitting isotope into a molecule whose
pharmacokinetics is already known (eg. raclopride for D2 dopamine receptors)
(Berns, 1999; Saper, Iversen & Frackowiak, 2000; Toga & Mazziotta, 1996).

PET studies have examined emotional evaluation of facial, auditory, olfactory,
gustatory and lexical stimuli as well as emotion induction using physiological and
pharmacological methods and have been reviewed recently by two papers (including
one meta-analysis) (Ketter et al., 2003; Phan et al., 2002). A summary of the key
findings reported in the meta-analysis (Phan et al., 2002) will be described in section
1.4.6, whilst some of the studies that have been published subsequent to this meta-
analysis will be briefly reviewed.

1.6.5 Functional Magnetic Resonance Imaging (fMRI)

fMRI is a variant of magnetic resonance imaging (MRI) which examines local
changes in magnetic field due to changes in the ratio of oxyhaemoglobin to
deoxyhaemoglobin without requiring administration of radioactive isotopes as with
SPECT and PET imaging. An increase in BF to a particular region of the brain is
thought to result from an increase in local cerebral glucose metabolism within that
region as a result of increased neuronal activity. These changes in BF are
accompanied by smaller changes in oxygen consumption, such that the supply of
oxygen is not matched precisely by the demand of the activated brain region.
Consequently there is an increase in oxyhaemoglobin and a decrease in
deoxyhaemoglobin, which produces an increase in the magnetic resonance (MR)
signal. Deoxyhaemoglobin has paramagnetic properties and oxyhaemoglobin does
not, thus the two forms of haemoglobin have different effects on the dephasing of
protons and produce different MR signals (see Figure 1) (Berns, 1999; Casey,
Davidson & Rosen, 2002; Honey & Bullmore, 2002; Jueptner & Weiller, 1995;
Raichle, 1998, 2001; Toga & Mazziotta, 1996;).

Although the relationship between neural activity and haemodynamic response
remains unclear, recent work has substantially improved our understanding of this
relationship. One model relates an increase in blood oxygen levels to increases in
the processing of glutamate (Glu) in astrocytes (non-neuronal, glial cells) after
excitatory neurotransmission. As a result of excitatory neurotransmission, Glu is
released into the synapse. Glu reuptake then occurs in astrocytes to prevent
continued stimulation (and possible excitotoxicity). Astrocytes then convert Glu into
glutamine (Gln) and then return Gln to the neuron for recycling by the process of glycolysis (Magistretti, Pellerin, Rothman & Shulman, 1999; Shulman, Hyder & Rothman, 2001).

Logothetis and colleagues (Logothetis, Pauls, Augath, Trinath & Oeltermann, 2001) recorded electrical activity of neurons in the visual cortex of the monkey in conjunction with fMRI and found that the major determinant of the fMRI signal were local field potentials. These are the slowly varying electrical potentials arising from the input to, and integrative processes within the dendrites of neurons, such as the processing of Glu in astrocytes discussed above. Logothetis and colleagues concluded that: 1) a spatially localised increase in the blood oxygen level dependent (BOLD) signal directly reflects an increase in neural activity and 2) that the activation reflects the incoming input and the local processing in a given area rather than the spiking activity of action potentials (see Figure 1).

Figure 1: The neural basis of fMRI. (a) PET images associated with the viewing of a visual stimulus. (b) Metabolic and circulatory changes are driven by electrical potentials arising from the information processing that occurs within dendrites of neurons. (c) The BOLD signal may relate to the process of glycolysis in astrocytes. Note

fMRI measures neuronal activity indirectly through haemodynamic activity, thus interpretation of the BOLD signal is critically dependent on the nature of the underlying neural activity producing the haemodynamic response. Although recent work has improved our understanding of this relationship, a number of details still require explanation (see Casey et al., 2002; Harel, Lee, Nagaoka, Kim & Kim, 2002; Hutchinson et al., 1999; Phan et al., 2002; Raichle, 2001 for discussion). One of these is the interpretation of a deactivation in the BOLD signal. The physiologic mechanism underlying a negative BOLD signal change may relate to either a reduction in neuronal activity resulting in decreased CBF or a haemodynamic ‘stealing effect’ in which blood is directed to the most active areas, and consequently decreases blood supply to (still active, but less demanding) adjacent areas (Harel, et al., 2002). Harel and colleagues demonstrate that a prolonged negative BOLD response may be associated with a reduction in CBF but increased spike activities. In addition to the understanding of a negative BOLD signal, the relationship between the BOLD signal and changes in the brain’s major inhibitory neurotransmitter, y-aminobutyric acid (GABA) remain undetermined. Although the release of both inhibitory and excitatory neurotransmitters is associated with energy-consuming processes, the majority of the cortex consists of pyramidal cells which are excitatory in nature and therefore there may be lower metabolic demand during inhibition compared to excitation. Furthermore, CBF is noted to be unrelated to postsynaptic GABA (inhibitory) activity, whilst CBF is attenuated when Glu mediated (excitatory) processes are blocked. Although still speculative, inhibitory processes may influence the BOLD response less than excitatory processes. (See Arthurs & Boniface, 2002, for a discussion on the contribution of inhibitory processes to the BOLD signal).

Like PET, fMRI has been used in a variety of activation studies involving both emotional evaluation and induction using a wide range of stimuli including visual, auditory, olfactory, gustatory and lexical stimuli. Again, these studies have been recently reviewed (Ketter et al., 2003; Phan et al., 2002), therefore the key findings reported in these studies will be described below in the following section (section 1.6.6). In addition, key findings reported in more recent studies are briefly reviewed.

1.6.6 Summary of Findings Reported using PET and fMRI
A large number of PET and fMRI studies have recently investigated emotional processing in healthy subjects. These techniques unlike EEG, MEG and SPECT, have excellent spatial resolution ability and as a result, are able to image increasingly
small structures such as the amygdala and associated sub-nuclei, during the processing of emotion. Phan and colleagues (2002) have recently examined findings reported in 12 fMRI and 43 PET studies (published between January 1990 and December 2000) which employed a range of emotional tasks with and without cognitive demand in unmedicated healthy adults. The advantage of the meta-analysis is that it is not constrained by the nature of the task used or research design employed and is not limited by statistical power and sensitivity, as are individual studies. Some of the findings reported in this review will be briefly described below.

It was reported that the medial prefrontal cortex (MPFC) has a general role in the processing of emotional stimuli, and that this structure is not activated to a specific emotion or induction method. Activations within this area were reported to be particular to the ventral-rostral BA9 and 10 of the MPFC. This region is possibly involved in a number of processes common across a range of emotional tasks such as appraisal/evaluation of emotion, emotional regulation, and emotion-driven decision making, but it is important to note that this region is equally activated to emotional tasks with and without cognitive demand. Key studies implicating a more general role for the MPFC in emotional processing include Lane, Fink, et al., (1997), Lane, Reiman, Ahern, Schwartz and Davidson, (1997), and Reiman et al., 1997. Activity within the subcallosal cingulate however (localized to the ventral/subgenual AC in Brodman’s area, BA 25) was associated with sadness induced in recall as well as other induction methods (eg. Liotti et al., 2000; Mayberg et al., 1999).

Amygdala activation was found to be important in the processing of fear. In particular, the amygdala was implicated in the recognition of fearful facial expressions (eg. Adolphs, Tranel, Damasio & Damasio, 1995; Calder et al., 1996), but this response to fear was also noted to extend to other modalities such as words and vocalizations (Isenberg et al., 1999; Phillips et al., 1998 respectively). Phan and colleagues (2002) note that activation of the amygdala has recently been reported during presentation of a wide-range of pleasant and aversive/unpleasant stimuli (eg. Hamann, Ely, Grafton & Kilts, 1999; Taylor et al., 1998 respectively), and suggest that prior research has implicated fear more frequently than other emotions because fear is the most provocative of all human emotions. (See section 1.7.2 for further discussion on this point). Phan and colleagues note therefore that the amygdala may be involved in the processing of stimulus salience or emotional importance, rather than fear per se.
Phan and colleagues (2002) noted that activation of some regions is associated with particular induction methods. For example, activation of the occipital/visual cortex (mainly BA 18 and 19, but also occipital gyrus and fusiform gyrus) was almost exclusive to visually evocative stimuli (eg. Beauregard et al., 1998; Lang, Bradley, Fitzsimmons et al., 1998). Phan and colleagues note that emotion induced occipital activations may be a result of visual and semantic complexity, eye movements, presence of faces, arousal, and functional interactions with underlying subcortical structures such as the amygdala. Amygdala activation, like activation within the occipital cortex (OC), was also found to be preferential to emotional induction by visual stimuli (eg. Reiman et al., 1997; Teasdale et al., 1999). Phan and colleagues speculate that the amygdala is well positioned to alert the individual to visual threat following perception by the OC. A wide range of pictorial stimuli was used in the studies identified in the meta-analysis and included pleasant and aversive pictures, emotional faces and emotional films. By contrast, the AC and insula were found to be recruited during emotional recall and associated with emotional tasks that require cognitive demand (eg. Damasio et al., 2000; Reiman et al., 1997).

It is important to briefly mention some of the more recent findings (since January 2001) which have used PET and fMRI techniques. The relationship between cortical and subcortical structures in emotion has been investigated in detail (Beauregard, Levesque & Bourgouin, 2001; Hariri, Mattay, Tessitore, Fera & Weinberger, 2003; Iidaka et al., 2001; Keightley, Winocur et al., 2003; Killgore, Oki & Yurgelin-Todd, 2001; Levesque et al., 2003; Liberzon et al., 2003; Ochsner, Bunge, Gross & Gabrieli, 2002; Phan et al., 2003; Pochon et al., 2002; Posse et al., 2003; Taylor et al., 2003; Yamasaki, LaBar & McCarthy, 2002). For example, work by Beauregard and colleagues has demonstrated that when participants are requested to inhibit any emotional reaction to sexually arousing or sad films, the right dorsolateral prefrontal cortex (DLPFC) appears to inhibit activation of cortical and subcortical 'limbic' structures including the temporal pole and amygdala (Beauregard et al., 2001; Levesque et al., 2003, respectively). Studies have also begun to investigate the neural substrates for valence and arousal dimensions through careful selection of stimuli and methods of analysis (eg. Anderson et al., 2003; Garavan, Pendergrass, Ross, Stein & Risinger, 2001; Phan et al., 2003; Williams et al., 2001). For example, using functional MRI and an olfactory task, amygdala activation was found to be associated with arousal whilst activity within the OFC was found to be associated with valence independent of intensity (Anderson et al., 2003). Studies have also begun to examine the impact of different task instructions on resulting brain activation.
For example, differences between explicit and implicit emotional processing have been investigated (Gorno-Tempini et al., 2001; Keightley, Winocur et al., 2003) as have differences between passive viewing and explicit emotional judgment tasks (Lange et al., 2003; Taylor et al., 2003). In summary, PET and fMRI techniques have been crucial in understanding the specific neuronal structures and circuitry involved during emotional processing. Many of the findings reported by these studies are elaborated further in section 1.7 in which key structures involved in emotional processes are described.

### 1.7 Brain Structures and Regional Circuitry

In 1909, neurologist and neuroanatomist Korbinian Brodmann published his "Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues", which classified brain regions based on cellular organisation (or cytoarchitecture). The Brodmann atlas represents an impressive scientific achievement and is still widely used today, providing researchers who use PET and fMRI technologies with a common “frame of reference” when describing activations within distinct cortical regions. A number of areas defined by Brodmann have since been found to control specific brain functions. For example, Brodmann Area 4 (BA4, motor cortex) is known to be responsible for voluntary movement and BAs 1, 2 and 3 (primary somatosensory cortex) is known to receive information on bodily sensation. BA17 (primary visual cortex) and BAs 41 and 42 (primary auditory cortex) begin to decipher information relayed by the eyes and ears, respectively. More recently, a number of BAs have been strongly implicated in emotional processing and include BAs 9 and 10 (medial PFC), BA11 (ventromedial OFC), BA25 (the subgenual cingulate), BAs 24 and 32 (rostral ACC), and BAs 18 and 19 (occipital/visual cortex), in particular. These regions are frequently referred to in this thesis and will be discussed in more detail below (see also Figure 2).
Figure 2: Lateral (top) and medial (bottom) surfaces of the cerebral cortex as designated on the basis of cellular organisation by Korbinian Brodmann in 1909. 

In addition to these Brodmann areas, overlapping and additional neural structures have been regarded as comprising the neuronal substrates of emotion. In particular, two key structures, the PFC (comprising the ventromedial PFC, the dorsolateral PFC, and the OFC) and the amygdala, are presumed to govern positive and negative affect. Other important regions are believed to include the ACC, occipital/visual cortex (OC), hippocampus, insula, and the basal ganglia. Each of these structures may be considered to generate different subprocesses but to work together to

Note: Figure is from http://spot.colorado.edu/~dubin/talks/brodmann/brodmann.html. Reprinted with permission from Professor Mark Dubin.
process and generate emotional behaviour. The ventromedial OFC is reported to be involved in the representation of elementary positive and negative affective states; the dorsolateral PFC is involved in the representation of goal states toward which more elementary positive and negative states are directed; the ventrolateral OFC has been linked to rapid learning and unlearning of stimulus-incentive associations and interpreted as a key region for the regulation of emotion; the amygdala has a role in the processing of emotional salience; the ventral/subgenual cingulate (of the ACC) may interact with the MPFC to regulate cognition as well as emotion; the occipital/visual cortex may be involved in the mediation and appraisal of visual, emotionally arousing stimuli; the hippocampus has been implicated in the context-regulation of affect such as the memory of the context in which learning of a specific cue-punishment occurs; the insula is involved in the evaluative, experiential or expressive aspects of (predominantly) internally generated emotion; and the basal ganglia which may serve to coordinate appropriate action responses and guide the organism to a desired goal (see Davidson, 2000, 2002; Davidson & Irwin, 1999; Davidson et al., 2002; Phan et al., 2002 for reviews).

1.7.1 Prefrontal Cortex (PFC)
The PFC and its vast connections with both sensory and motor systems as well as subcortical structures involved in affect, memory and reward, have been extensively reviewed in a number of recent publications (Barbas, 2000; Krawczyk, 2002; Miller & Cohen, 2001). Moreover, the PFC is frequently implicated in studies of healthy and disordered emotional processes (see Davidson, 2000, 2002; Davidson et al., 2002; Mega, Cummings, Salloway & Malloy, 1997; and Phan et al., 2002 for discussion). The PFC is a heterogeneous structure and has been subdivided into different compartments based on cytoarchitecture and functional characteristics (see Figure 3).
Figure 3: Key regions of the prefrontal cortex (PFC) involved in emotion include (left) the ventrolateral orbital prefrontal cortex (green) and the ventromedial orbital prefrontal cortex (red), and (right) the dorsolateral prefrontal cortex (DLPFC) (blue).

At the most simplistic level, the PFC may be viewed in terms of ventral and dorsal compartments which are specialised for ‘vegetative-somatic’ functions and ‘attentional-cognitive’ functions, respectively (Mayberg, 1997). A more detailed differentiation of the PFC has been provided which distinguishes dorsolateral, dorsalmedial, and orbitofrontal sectors which are posited to have particular specialised functions, though it is acknowledged, that these sectors functionally interact (Davidson, 2000, 2002; Simpson, Drevets, Snyder, Gusnard & Raichle, 2001; Simpson, Snyder, Gusnard & Raichle, 2001). In addition, a region of agranular cortex on the most ventral extent of the AC cortex (the subgenual cingulate, BA25) has also been included as part of the (ventral) prefrontal cortex (Elliot & Dolan, 2003; Krawczyk, 2002; Phan et al., 2002).

The PFC is critically involved in ‘top down processing’; and contributes to control over a wide range of processes related to goal-directed behaviour (Miller & Cohen, 2001). The literature has begun to emphasise the importance of particular regions of the PFC in general emotional processing. For example, the ventral medial prefrontal cortex (MPFC) (BA 9 and 10) and the subgenual cingulate (BA rostral 24, anterior/ventral 32,33) are the most frequently activated brain areas in response to emotional stimuli (Phan et al., 2002) and are implicated in clinical depression (Drevets, 1997; Elliot & Dolan, 2003; Mayberg et al., 1999). Furthermore, the subgenual cingulate plays an important role in modulating monaminergic

neurotransmitter systems targeted by antidepressants (Goodwin & Jamison, 1990). Importantly, recent neuroimaging studies have begun to better distinguish the different compartments of the PFC in terms of their role in emotional processing (discussed in more detail below) (e.g. Gusnard, Akbudak, Shulman & Raichle, 2001; Keightley, Winocur et al., 2003; Northoff et al., 2000; Schaefer et al., 2003; Yamasaki et al., 2002).

The study conducted by Northoff and colleagues (2000) represents the first study to examine the spatiotemporal mechanisms of negative and positive emotional processing using fMRI and MEG techniques. The research group used positive, negative, ‘control’ neutral and ‘control’ gray pictures from the IAPS for emotional elicitation and reported that negative emotional processing was associated with strong medial orbitofrontal activation and more medially oriented dipoles, whilst positive emotional processing was associated with later and weaker activation in the lateral OFC, supporting the notion of a functional subdivision within the OFC.

A more recent study used fMRI in order to determine whether attentional and emotional functions are segregated into dissociable prefrontal networks (Yamasaki et al., 2002). Subjects discriminated infrequent and irregularly presented attentional targets (circles) from frequent standards (squares) while novel distracting unpleasant and neutral scenes, selected from the IAPS, were intermittently presented. The authors report that whilst attentional targets elicited a larger signal within the middle frontal gyrus, emotional distracters elicited stronger activation within the inferior frontal gyrus. The authors concluded that the ventrolateral PFC mediated emotional arousal, whilst the dorsolateral PFC mediated more attentional and cognitive processes. Their findings were interpreted as support for Mayberg’s dual-stream theory of mood regulation in healthy subjects.

Interestingly the Yamasaki study did not support the results reported by Northoff et al., 2000 (discussed above) who reported that negatively valent stimuli engage medial sectors, whereas positively valent stimuli engage lateral sectors. Instead, Yamasaki and colleagues report that externally triggered (negative) emotional states depend on lateral regions. They refer to a recent model of limbic function that combines phylogenetic, anatomical, functional, and clinical data to explain their results (Mega et al., 1997). This model distinguishes two parallel pathways by which emotionally arousing stimuli are processed in the amygdala and interface with the PFC. The first pathway links the basal amygdala with ventromedial OFC (BA 11),
rostral insula and subgenual portions of the AC gyrus (BA 25), whilst the second pathway interconnects the inferotemporal cortex and basal amygdala with ventrolateral PFC (BA 10/47) and rostral AC (BA 24/32). They suggest that the emotional distracters used in their study engaged this second pathway and that while internally generated emotional states and motivated behaviour may preferentially elicit ventromedial PFC (VMPFC), externally triggered emotional states may depend on lateral regions. It is important to note that an important difference between these two studies is that whilst IAPS images were presented as distracters in the Yamasaki study, IAPS images were presented without an additional cognitive task in the Northoff study.

Studies are also beginning to clarify the interactions between PFC compartments and connected regions using conventional subtractive methodology as well as several kinds of other analyses, such as correlational analyses and structural equation modelling (Beauregard et al., 2001; Hariri et al., 2003; lidaka et al., 2001; Keightley, Winocur et al., 2003; Killgore et al., 2001; Libezon et al., 2003; Ochsner et al., 2002; Phan et al., 2003; Pochon et al., 2002; Taylor et al., 2003; Yamasaki et al., 2002). In addition the role of the PFC and interconnected regions in terms of regulating the interplay between cognition and emotion has been further investigated (Keightley, Winocur et al., 2003; Lange et al., 2003; Levesque et al., 2003; Libezon et al., 2000; Gorno-Tempini et al., 2001; Simpson, Snyder, et al., 2001; Simpson, Drevets, et al., 2001; Taylor et al., 2003; Yamasaki et al., 2002). A number of these studies will be described briefly below, as these have potentially important implications for our understanding of the MPFC.

Simpson and colleagues (Simpson, Snyder et al., 2001) examined the relationship between attention-demanding cognitive performance and activity within the MPFC, using a task which required subjects to generate aloud, verbs for visually presented nouns. The authors reported that the expected decreases in BF were observed, but that these decreases were no greater than the less demanding task of word reading. Furthermore and quite surprisingly, the practice induced performance improvement was associated with greater reduction in BF within the ventral MPFC. The authors argued that this effect may have been due to performance anxiety as documented by self-report and changes in heart rate (HR). Performance anxiety was found to be greater during the cognitively demanding parts of the experiment, but that this decreased after practice. This study suggests therefore that performance anxiety substantially interferes with the decreased activity associated with cognitive task
performance. To examine this intriguing finding in more detail, the same authors examined transient anxiety associated with the anticipation of a painful shock to the fingers of one hand relative to an eyes-closed resting condition (Simpson, Drevets et al., 2001). As anticipated, the authors found an inverse correlation between BF and anxiety self-rating within two regions of the MPFC (BA 10/32 and 24/25), concluding that BF reductions in MPFC observed in cognitive tasks, reflects a dynamic balance between focused attention and subject anxiety.

Geday and colleagues (Geday, Gjedde, Boldsen & Kupers, 2003) have recently investigated the effects of neutral, positive, or negative emotional pictures of low (facial expressions) and high (persons in real-life situations) social complexity and report that activity within the medial PFC was lower during presentation of emotional compared to neutral images, which is at odds with previous studies. As the authors avoided the use of an explicit cognitive task and presented images for only 3-s in order to minimize idiosyncratic cognitive activity, they suggest that a reduction in activity within the medial PFC during presentation of emotional compared to neutral images is consistent with the hypothesis of an attentional role of the MPFC (Drevets & Raichle, 1998; Simpson, Drevets et al., 2001; Simpson, Snyder et al., 2001) (discussed above), which posits that the main task of the medial PFC is to maintain attention and to select between relevant inputs from other brain regions. In conclusion, these authors argue that their results support the notion of a crucial neural network used in empathic reactions and social interactions. This network involves the posterior fusiform and inferior occipital gyrus specialized in identifying emotionally important visual clues. Signals from these regions (and others) then converge to the medial PFC which then evaluates this information in terms of relevance for attention.

### 1.7.2 Amygdala

Like the PFC, the amygdala or ‘amygdaloid complex’ is not a homogeneous structure, as it includes several distinct groups of cells. (See Figures 7 and 9 for the location of the amygdaloid complex within the human brain). The amygdaloid complex is made up of the basolateral amygdala, and surrounding structures including the central, medial and cortical nuclei. The basolateral amygdala receives important afferent information from all sensory modalities and is involved in negative and positive affect. The central nucleus of the amygdala receives input from the basolateral amygdala and is involved in the mediation autonomic changes such as increases in HR, blood pressure and respiration via output pathways to the lateral hypothalamic and brain.
stem regions. (See figures 4 & 5 regarding outputs from these structures and possible functions of these connections). Although it has been argued that combining all nuclei into one anatomical entity does not make sense because of cell shape, content and projections (Amaral, Price, Pitkanen & Carmichael, 1992; Davis & Whalen, 2001; Swanson & Petrovich, 1998), Liberzon and colleagues (2003) refer to the sublenticular extended amygdala or SLEA, which comprises the amygdaloid nuclei, sublenticular nuclei and the nucleus accumbens and stress the role of this structure in detection or attribution of salience.

Figure 4: Outputs from the basolateral nucleus of amygdala to various target structures and possible functions of these connections.

Note Figure is from “The amygdala: vigilance and emotion,” by Davis & Whalen, 2001, Molecular Psychiatry, 6, p. 13-34. Copyright 2001 by Macmillan Magazines Ltd. Reprinted with permission.
The amygdala is primarily responsible for detecting, generating and maintaining fear-related emotions (see Davis & Whalen, 2001; Phan et al., 2002 for reviews). Indeed, Adolph and colleagues (Adolphs, Tranel, Damasio & Damasio, 1994; Adolphs et al., 1995) have suggested that bilateral amygdala damage in humans compromises the ability to detect facial expressions of fear whilst the ability to recognise facial identity remains relatively intact. In addition, Phillips and colleagues (Phillips et al., 1997) reported amygdalar activation for fearful faces but not disgust. Many recent studies also report that the amygdala is involved in both conscious and unconscious processing of fear (Critchley, Mathias & Dolan, 2002; Killgore & Yurgelun-Todd, 2001; Phelps et al., 2001; Williams et al., 2001). However there is also substantial evidence now to suggest that the amygdala is also responsive to a wide range of unpleasant stimuli (Irwin et al., 1996; Lane, Reiman, Bradley et al., 1997; Paradiso et al., 1999; Phan et al., 2003; Schneider et al., 1995) as well as pleasant stimuli (Beauregard et al., 2001; Breiter & Rosen, 1999; Garavan et al., 2001; Hamann et al., 1999; Hamann, Ely, Hoffman & Kilts, 2002; Schneider et al., 1997; Whalen et al., 1998).

Studies have demonstrated amygdala activation during both emotional perception (eg. Blair et al., 1999; Breiter et al., 1996; Gur et al., 2002; Hariri, Bookheimer &

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**Figure 5**: Outputs from the central nucleus to various target structures and possible functions of these connections.**Note**

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Mazziotta, 2000; Iidaka et al., 2001; Morris et al., 1996, 1998; Phillips et al., 1997; Whalen et al., 1998;) as well as emotional experience (eg. Aalto et al., 2002; Hariri et al., 2003; Liberonz et al., 2000, 2003; Posse et al., 2003; Schneider et al., 1998; Schneider, Habel, Kessler, Salloum & Posse, 2000; Taylor et al., 2000). However, studies have investigated the neural mechanisms underlying emotional experience and have completely failed to find amygdala activation (eg. Damasio et al., 2000; Geday et al., 2003; Mayberg et al., 1999; Teasdale et al., 1999). Amygdala activation therefore does not appear to correlate exclusively with emotional response, and it has been argued that the amygdala plays a more general role in the processing of meaningful stimuli (Cahill & McGaugh, 1998; Davis & Whalen, 2001; Liberonz et al., 2003; Phan et al., 2002; Schneider et al., 1997).

It is important to note however, that Phan and colleagues (2003) have reported that activation within the SLEA/Amygdala is detected only when individual ratings of emotional arousal are incorporated into the analysis of fMRI data. These findings suggest that the reason for the inconsistent findings regarding emotional experience could be due at least in part, to inter and intra individual differences during presentation of emotionally evocative stimuli. This finding is supported another recent study conducted by Posse and colleagues (2003), in which subjects performed a mood induction procedure (MIP) during scanning, and were provided with feedback regarding activation of their amygdala to reinforce mood induction. The authors report that in 78% of 120 trials, increased intensity of the left amygdala was associated with increases in self-rating of sadness.

Another issue that has recently been addressed in the literature is the question of amygdala asymmetry during emotional processing. For example, previous studies have reported unilateral left (Schneider et al., 1995, 1997), right-sided (Schneider et al., 2000) or bilateral amygdala (Garavan et al., 2001; Taylor, Liberonz, Decker & Koepppe, 2002) activations. There are a number of possible reasons for these contradictions in the literature. First, it is known that the amygdala habituates rapidly (Breiter et al., 1996; Irwin et al., 1996; Phillips et al., 2001) and that habituation may be greatest within the right amygdala (Wright et al., 2001). Second, it is possible that amygdala activation involves different stages of affective processing, (ie. an early stimulus evaluation stage and a later stage involving the subjective experience of induced mood) (Schneider et al., 2000; Wright et al., 2001). Third, failure to account for valence and arousal aspects of experimental stimuli further complicates comparisons between different studies (see Garavan et al., 2001 for a discussion of
this issue). Fourth, amygdala activation has been found to depend on arousal level (eg. Phan et al., 2003, discussed above). Williams and colleagues (2001) have also reported that amygdala activation to fearful faces is displayed only when electrodermal skin conductance responses (indexing arousal) are produced. (See also Anderson et al., 2003). Fifth, amygdala activation in response to happy facial expressions has been found to correlate positively and significantly with the degree of extraversion, suggesting that variability within amygdala associated with the processing of positively valent stimuli may have been due to studies not controlling for personality (Canli, Sivers, Whitfield, Gotlib & Gabrieli, 2002). Other reasons include gender differences between studies, small subject numbers in studies combining both males and females, strategies employed to complete a particular task (ie. language based strategies may account for left-amygdala activation), and possible asymmetries found in amygdala volume. Recently, Hariri and colleagues (2003) have reported bilateral amygdala activation in response to fearful stimuli and that the response of the left and right amygdala is positively correlated suggesting that the distinct collections of nuclei may work in concert, to produce an orchestrated response.

1.7.3 Anterior Cingulate (AC)

The cingulate cortex is also not a homogeneous structure, and like the PFC and the amygdala, a number of important subdivisions have been identified. The cingulate cortex comprises the ACC and the posterior cingulate (PC) cortex which have been characterised as ‘executive’ in function and ‘evaluative’ in function, respectively (Vogt, Finch & Olson, 1992). Furthermore, the ACC itself has been subdivided based upon cytoarchitecture, connectivity and function. The two major subdivisions are labelled as the dorsal cognitive division (ACcd; caudal area 24’ and 32’ and cingulate motor area) and the rostral-ventral affective division (ACad; BA 25, 32, 33 and rostral area 24) (see Figure 6 below).
Figure 6: Anatomy of the anterior cingulate (AC) cortex. The enlarged section (left) illustrates the cognitive division areas (red) and the affective division areas (blue). The upper right part of the figure displays a single cingulate gyrus between the cingulate sulcus and the corpus callosum.

The ACcd has strong reciprocal connections with the DLPFC (BA 46/9), parietal cortex (BA 7), premotor and supplementary motor areas (Devinsky, Morrell & Vogt, 1995; Bush, Luu & Posner, 2000). Various functions have been proposed for this compartment of the ACC, which include sensory or response selection (or both); monitoring competition, complex motor control, motivation, novelty, error detection and working memory; and anticipation of cognitively demanding tasks. Conversely, the ACad has extensive connections with limbic and paralimbic regions including the amygdala, nucleus accumbens, OFC, periaqueductal gray, hypothalamus, anterior insula, hippocampus (Devinsky et al., 1995; Bush et al., 2000). The ACad is involved in regulating visceral and autonomic responses to stressful behavioural and emotional events, emotional expression and social behaviour. A region within the ACad known as the subgenual ACC (BA 25) is particularly important for the

regulation of autonomic function (see Bush et al., 2000 and Davidson et al., 2002 for a review).

The recent meta-analysis conducted by Phan and colleagues (2002), reported that the ACC is specifically engaged during emotional tasks with a cognitive component as compared with more passive emotional conditions. The authors suggest that the ACC (together with the MPFC) may serve as top-down modulator of intense emotional responses, especially those generated by the amygdala. Activation of the ACC has been reported however, in a wide variety of experimental conditions, including pain, classical conditioning, transient mood, primal affect, Stroop task and perceiving facial expressions (see Davidson et al., 2002). The ACC is also frequently activated in studies on mood and anxiety disorders (Davidson et al., 2002; Elliot & Dolan, 2003). The implications of these findings have lead to proposals that the ACC is involved in the regulation of both emotional and cognitive behaviours (Devinsky et al., 1995; Ebert & Ebmeier, 1996; Elliot & Dolan, 2003; Mayberg, 1997; Vogt, Nimchinsky, Vogt, & Hof, 1995).

1.7.4 Occipital/Visual Cortex (OC)

The OC, including BA 18 and 19, occipital gyrus and fusiform gyrus is reported to be activated by visual emotional stimuli (see Phan et al., 2002 for a review). Although visual cortical activation may be driven by the physical characteristics of the stimulus (eg. colour, luminance), many studies that have explored the effects of emotionality on OC have also matched emotional and neutral stimuli for sensory load (see for example, Paradiso et al., 1999; Schupp et al., 2003; see also Phan et al., 2002 for review). Studies have also been conducted which asked participants to imagine, rather than view emotional stimuli and reported increased activation within OC (Kosslyn et al., 1996; Pietrini, Guazzelli, Brasso, Jaffe, & Grafman, 2000). In addition, a recent study explicitly tested whether presence of colour accounted for increased visual cortical activation to emotional images (ie. does the predominance of the colour red in images of mutilation and erotica in part explain increased activation), by presenting images in black and white and it was demonstrated that colour was not a relevant factor for activation within this region (Bradley, Sabatinelli, Lang, Fitzsimmons, King, & Desai, 2003). Studies have also been conducted which rule out the effect of differential eye movements between emotional and non-emotional stimuli (Lang, Bradley, & Fitzsimmons, 1998). All these studies support the notion that visual cortical activation may be driven by emotional content in addition to the processing of stimulus features. Two possible neurophysiological explanations have
been proposed for emotional activations within visual cortex (Bradley et al., 2003; Lang, Bradley & Cuthbert, 1998). The first explanation involves re-entrant projections from the amygdala back to primary visual cortex (V1). According to Amaral and colleagues, the amygdala in primates “projects to virtually all levels of the visual cortex” and these connections reciprocate, suggesting the presence of a reverberating processing loop. Increased activation within the visual cortex by emotional stimuli therefore may reflect activation within this processing loop facilitating motivational engagement, orientation towards relevant stimuli and autonomic and somatic behaviours. The second explanation involves projections from the anterior cingulate cortex which directs the processing within sensory systems (Posner & Petersen, 1990; Posner, 1996).

1.7.5 Other Structures

The hippocampus is located within the medial border of the temporal lobe and is critically involved in episodic, declarative, contextual and spatial learning and memory (Squire & Knowlton, 2000; Fanselow, 2000; Davidson et al., 2002). This structure contains a high density of glucorticoid receptors, is known to participate in the regulation of the hypothalamic-pituitary-adrenal axis, and is implicated in several stress disorders such as posttraumatic stress disorder and depression (see Figure 7 for location of the hippocampus) (see Davidson et al., 2002 for discussion). Although most studies up until now have implicated the hippocampus in cognitive function and in particular, declarative memory, it is now also thought to play a role in the context modulation of emotional behaviour (Davidson, 2000; Davidson et al., 2002). In particular, the hippocampus may involve memory of the ‘cold hard’ declarative facts and contexts related to a presented emotional stimulus. In this vein, a recent study has reported direct evidence for dissociation between human amygdala and hippocampal networks such that amygdala-medial frontal activity was observed only during skin conductance responses, and hippocampus-lateral frontal activity was observed only in the absence of these responses (Williams et al., 2001).
Figure 7: Location of the hippocampus (coloured purple) and amygdala (coloured orange) within a coronal slice of a human brain. Note

The insula cortex is located at the base of the lateral (Sylvian fissure) sulcus and is overgrown by the temporal, parietal and frontal lobes (see Figure 8). The insula cortex is regarded as part of the telencephalon, which refers to the anterior part of the brain, rostral to the midbrain and includes the cerebral cortex, basal ganglia, corpus striatum and olfactory bulb, as well as the insula cortex. The insula is known to play a role in gustatory function, the processing of taste information and pain (Calder et al., 2001; Sundsten & Mulligan, 1998).

Figure 8: View of the right hemisphere after removal of the superior temporal lobe and sections of the frontal and parietal lobes to reveal the insula. Note

Note Figure is from "Depression: Perspectives from Affective Neuroscience," by Davidson et al., 2002, Annual Review of Psychology, 53, p. 545-574. Copyright 2002 by Annual Reviews www.annualreviews.org. Reprinted with permission.
Functional imaging studies have implicated both the insula as well as the basal ganglia in the viewing of the facial expressions of disgust (see Calder et al., 2001 for a review). Bilateral, ventral insula activation has recently been reported to occur during passive viewing (but not during rating) of aversive (relative to non-aversive) IAPS images (Taylor et al., 2003). The authors concluded that the insula may provide an integration zone between the external sensory world and the internal milieu. Furthermore, it was suggested that the process of rating images may either reduce the resources available for processing emotional content or suppress the emotional response. The authors also suggest that the amygdala and insula may comprise a functional unit during the presentation of IAPS images, although this is speculative due to the limited resolving capacity of the PET technique. Activation within the vicinity of the anterior insular cortex, claustrum and lateral putamen has also been reported during recall-generated ‘sad’ emotion (Reiman et al., 1997). The authors suggest that the insular cortex is preferentially involved in the evaluation of potentially distressing cognitive and bodily sensations. In the recent meta-analysis on emotional processing, the insula was found to be activated in nearly 60% of emotional recall/imagery studies (involving multiple specific emotions such as happiness, sadness, fear and disgust) compared to less than 20% of studies which involved either visual or auditory inductions (Phan et al., 2002). It was noted that this structure was also involved during emotional tasks having a cognitive component.

The basal ganglia are also considered as an important region in the production of an emotional response (see Figure 9). The basal ganglia consist of several interconnected subcortical nuclei which include the striatum (putamen, caudate nucleus and nucleus accumbens), globus pallidus, subthalamic nucleus, ventral tegmental area and substantia nigra (Calder et al., 2001; DeLong, 2001). Phan and colleagues (2002) found that the basal ganglia were activated in nearly 70% of studies investigating the emotion, ‘happiness’ (eg. Damasio et al., 2000; Lane, Chua & Dolan, 1999) and 60% of studies investigating the emotion, ‘disgust’ (eg Phillips et al., 1997, 1998). These findings support the role of the basal ganglia in addictive behaviours, reward processing and enjoyable activities, as well as observations that patients with both Huntington’s Disease and obsessive-compulsive disorder have

impairments in recognizing facial expressions of disgust relative to other emotions (Calder et al., 2001; Phan et al., 2002).

1.8 NEUROPHYSIOLOGICAL MODELS

A number of models have been proposed to explain the biological mechanisms involved in emotion and include the hemispheric specialisation models, models relating to differential activations of anterior and posterior regions, and the cortical-subcortical models. These models, not necessarily in conflict with each other, will be discussed in the following sections.

1.8.1 Hemispheric Specialisation

Three models have been proposed to account for hemispheric specialisation during emotion. The right-hemisphere model maintains that the right hemisphere is specialised for emotion, regardless of valence (Borod, 1992; Buck, 1984; Heilman & Bowers, 1990; Heilman, Bowers & Valenstein, 1993; Ross, 1985); the valence-specific model maintains that the left hemisphere is specialised for positive emotions whereas the right hemisphere is specialised for negative emotions (Silberman &

Weingartner, 1986); and the hybrid model integrates the above two models, maintaining that subjective emotional experience displays a valence specific pattern of lateralisation but that the right-hemisphere is specialised for the perception of both valences (Bryden, 1982; Davidson, 1984; Hirschman & Safer, 1982). Recent conceptualisations of the hybrid model have added the approach and withdrawal distinction which is proposed to account for the role of the PFC in the experience of emotion (Davidson & Irwin, 1999; Davidson et al., 1990; Fox, 1991). This conceptualisation proposes that the left-anterior-hemisphere is specialised in the processing of approach-related emotions, such as happiness, whilst the right-anterior-hemisphere is specialised in the processing of withdrawal-related emotions, such as disgust. Davidson has developed this framework on the basis that emotional states are thought of in terms of motivational dispositions.

1.8.2 Anterior versus Posterior Regions
The literature suggests that anterior regions (the frontal lobes) mediate expressive functions, whilst posterior regions are implicated more in perceptual and arousal components of emotion (Borod, 1993; Davidson, 1984; Davidson et al., 1990; Fox, 1991; Heller & Nitschke, 1997; Heller & Nitschke, 1998).

With respects to anterior regions, Davidson’s approach and withdrawal framework has generated much research interest over the last decade, however brain asymmetries during the processing of emotional stimuli remain controversial (Borod, 1992; see also Davidson, 1988, 1998 for methodological issues regarding brain asymmetries and emotion). Although a large number of electrophysiological studies and, more recently, PET and fMRI techniques have supported Davidson’s approach and withdrawal (eg. Canli et al., 1998; Davidson et al., 1990; Jones & Fox, 1992; Sutton, Ward et al., 1997), a number of studies have also reported activation in overlapping areas and no frontal hemispheric laterality (eg. Baker et al., 1997; George et al., 1995; Lane, Fink, et al., 1997; Lane, Reiman, Ahern, Schwartz & Davidson, 1997; Lane, Reiman, Bradley et al., 1997; Pardo, Pardo & Raichle, 1993; Teasdale et al., 1999). This may in part be due to the notion that it is much harder to elicit positive affect in the laboratory environment (Davidson, 2000, 2001).

The hypothesis that unpleasant emotion is regulated by the right frontal hemisphere and that pleasant emotion is regulated by the left hemisphere is further contradicted by the different interpretations of resulting emotional states following damage to the right or left hemispheres (see Tucker, 1981, 1993, 2001 for discussion). The classical
findings of depressive symptoms following left hemispheric damage compared to either indifference or inappropriate cheerfulness following right-hemispheric damage have been interpreted as reflecting a release of the emotionality of the undamaged hemisphere (contralateral release hypothesis) (Gainotti, 1972; Robinson, Starr, & Price, 1984; Sackeim et al., 1982). Tucker (1993) however regards this interpretation as speculative and provides an alternative explanation supported by the neurological literature that the hemispheric lesion may result instead, in disinhibited, exaggerated emotional reactivity by the damaged hemisphere. In this view, unpleasant emotionality would be regulated by the left hemisphere (rather than the right) and pleasant emotionality would be regulated by the right hemisphere (rather than the left). Furthermore, Carson et al., 2000 have failed to find support for the hypothesis that depressive symptomatology is related to left hemispheric damage. Regardless, the prefrontal region is strongly implicated in emotional processing and the experience of emotion (Davidson & Irwin, 1999). These contradictions have however, lead to the development of recent models of emotion based on interactions between cortical and subcortical structures.

In contrast to the anterior regions, the posterior regions have been proposed to mediate perceptual functions (Ahern & Schwartz, 1985; Davidson 1984; Laurian et al., 1991; Ley & Bryden, 1981) and it is the right parieto-temporal region which is specifically implicated (Diedrich et al., 1997; Heller, 1993; Heller, Nitschke & Lindsay, 1997; Junghofer et al., 2001; Kayser et al., 1997, 2000; Liotti & Tucker, 1995). In addition, the posterior region has been related to the regulation of arousal functions (Aftanas et al., 2001a; Gainotti, 1987; Heller, 1993; Heller, Nitschke & Lindsay, 1997; Tranel & Damasio, 1994). Heller’s (Heller, 1990, 1993) model is based on neurophysiological and neuropsychological data, and holds that while the anterior regions are important for the valence dimension (pleasant - unpleasant) of emotional experience, the right parietotemporal regions mediate the arousal functions of emotional experience, in addition to emotional perception. Recent electrophysiological and fMRI data have supported this arousal role of the right parietotemporal region (Aftanas et al., 2001a; Lang, Bradley, Fitzsimmons et al., 1998).

1.8.3 Cortical versus Subcortical Regions

Models have also attempted to explain emotional processes in terms of cortical and subcortical interactions (Damasio, 1996; Davidson, Putnam & Larson, 2000; James, 1884; LeDoux, 1996; MacLean, 1949; Mayberg et al., 1999; Nauta, 1971; Papez,
1937; Rolls, 1990; Tucker, Luu & Pribram, 1995). Over a century ago, Hughlings Jackson (1879) introduced the principle of multiple representations which states that the brain is organised in parallel with its evolutionary history. In this framework, recently evolved functions are considered to subordinate and inhibit the more primitive levels. (See Tucker, 1993 for a review of ‘vertical integration’). Furthermore, the observations on decerebration and sham rage in cats and dogs reported in the classical studies such as those conducted by Cannon and Bard in the 1920s, have lead to the hypothesis that the cerebral cortex may serve to ‘inhibit unbridled expressions of emotion’ (Reiman, 1997).

As discussed above (in section 1.6.6), the use of PET and fMRI technologies in emotion research has helped to clarify the relationship between cortical and subcortical structures. Recent work by Beauregard and colleagues using fMRI has supported this notion of cortical inhibition of subcortical structures as it applies to emotional regulation of sexual arousal and sadness (Beauregard et al., 2001; Levesque et al., 2003, respectively). In these studies, the authors suggest that the right DLPFC may be involved in metacognitive/executive top-down processes used to inhibit the activation of cortical and subcortical ‘limbic’ structures including the VLPFC, the amygdala, the insula, the anterior temporal pole, and hypothalamus as well as the midbrain during positive and negative emotion.

Consistent with these studies, a recent model has been proposed by Mayberg and colleagues (Liotti et al., 2000; Liotti & Mayberg, 2001; Mayberg et al., 1999, 2000). This model proposes that normal emotional circuitry involves dorsal region inhibition of emotional responses via efferent connections to limbic-paralimbic regions (Liotti & Mayberg, 2001). Key structures in the dorsal region include the right prefrontal (BA 9), right inferior parietal (BA 40), right inferior temporal cortex (ITC) (BA 20/37), and the parahippocampal cortex whilst key regions in the limbic-paralimbic regions include the ventral cingulate (BA 25), insula, OFC, anterior temporal cortex and amygdala. The model emphasises the nature of cortical and subcortical interactions in the production of negative affect or transient sadness. This group employs a mood induction paradigm which involves the rehearsing of autobiographical scripts in order to induce either negative or neutral mood, and then scanning individuals (using PET) in the eyes closed resting state, once mood state had been acquired. Transient sadness in healthy participants is associated with increased activation within limbic-paralimbic regions and concomitant decreases within the connected neocortical regions.
Such models are not necessarily in conflict with models focusing on anterior laterality (see Davidson, 2002 for discussion), however, interpretations of hemispheric emotional valences have been regarded as incompatible with ‘emotional orientations associated with hemispheric cognition in personality and psychopathology’ (Tucker, 1981, 2001). Consequently, cortical-subcortical models have been proposed as alternatives to the hemispheric laterality models discussed in the previous section. Tucker (2001) has elaborated a core-and-shell model of corticolimbic architecture which further suggests new ways of thinking about the differential hemispheric specialisation for positive and negative emotion and the dense interconnectivity between right hemisphere neocortex and the paralimbic core of the cortex. It should be mentioned however, that if certain emotional states are thought of in terms of relative activation of brain regions, rather than regional specialisation for particular emotional states (ie. left frontal for positive emotion and right frontal for negative emotion), then such criticisms of anterior laterality models become moot (Heller, 1993).

1.9 **THE MONOAMINES AND AFFECTIVE STATES**

The monoamine neurotransmitters which include dopamine (DA), serotonin (5-HT) and noradrenaline (norepinephrine) (NA) are thought to be critically important in mediating mood and emotion and have been implicated in the pathophysiology and treatment of depression (Blier, 2001; Hirschfeld, 2000; Leonard, 1993; see also Leonard, 1996). For over thirty years, the leading theory on the biological basis of depression has been the ‘monoamine hypothesis of depression’ which proposes that depression may arise from a deficiency in the levels of one or more of these monoamines in the brain (Coppen, 1967; Delgado, 2000; Hirshfeld, 2000; Prange, 1964). Despite the important link between the monoamines and affective states, most (human) studies have been behavioural and correlational in nature. In addition, the neuroimaging studies that have investigated the relationship between the monoamines and affective states have focused almost exclusively on the disorders of emotion, generally failing to investigate this relationship in healthy individuals. A brief discussion of each of the monoaminergic systems is provided below.

The noradrenergic system has cell bodies located within the locus coreuleus which project to different regions in the brain including frontal and limbic cortices, the cerebellum and brainstem. Each of these pathways regulates different physiological
functions including mood and attention (frontal sites), emotions, energy, fatigue and psychomotor agitation or retardation (limbic sites), motor movements (cerebellum), and cardiovascular functions (brainstem) (Stahl, 1997, 2000). Research interest on emotion and the noradrenergic system has generally concentrated on the relationship between NA and psychiatric disorders such as post-traumatic stress disorder and depression (for reviews, see Bremner, Krystal, Southwick & Charney, 1996; Brunello et al., 2002 respectively). Unfortunately, our understanding of the effects of NA have been based on the tricyclic antidepressants (TCAs) such as desipramine and the selective 5-HT and NA reuptake inhibitors (SNRIs) such as venlafaxine, which increase the concentration of both NA and 5-HT. It has been difficult therefore to examine the selective effects of NA and after the introduction of selective 5-HT reuptake inhibitors (SRIs), research on NA was somewhat neglected. Recently however, the selective NA reuptake inhibitor (NRI) reboxetine has become available allowing research to be conducted that examines the impact of NA exclusively on emotional processing (eg. Harmer, Hill, Taylor, Cowen & Goodwin, 2003).

The dopaminergic system has cell bodies located within the midbrain ventral tegmental area (brainstem), substantia nigra of the brainstem, and the hypothalamus. There are well-defined DA pathways in the brain which include 1) the mesolimbic and 2) mesocortical pathways, both of which project from the midbrain ventral tegmental area to the nucleus accumbens and limbic cortex, respectively, 3) the nigrostriatal pathway which projects from the substantia nigra to the basal ganglia, and 4) the tuberoinfundibular pathway which projects from the hypothalamus to the anterior pituitary gland. Respectively, these pathways appear to have a role in mediating 1) pleasurable sensations, drug euphoria, and delusions and hallucinations of psychosis, 2) negative and cognitive symptoms of schizophrenia, 3) movement control, and 4) prolactin secretion which is associated with lactation during breast feeding, amenorrhea, and possibly sexual dysfunction (Stahl, 1997, 2000). A number of theories have been proposed implicating the dopaminergic system in affective states such as positive affect (eg. Ashby, Isen & Turken, 1999), and the personality trait of extraversion or positive affectivity (Depue & Collins, 1999; Deupue & Iacono, 1989; Depue, Luciana, Arbisi, Collins & Leon, 1994). For example, Ashby and colleagues (2001) propose that positive affect and its influence on cognition are related to increased brain DA levels. They do caution however, making the conclusion that DA release is directly responsible for positive affect as stressful or anxiety-provoking situations have also been found to increase DA levels in regions such as the PFC.
Instead, the authors argue that positive affect rather than negative affect or arousal, is associated with increased DA levels. Research studies are beginning to investigate the effects of dopaminergic modulation on emotional processing. For example, Hariri and colleagues investigated the effects of amphetamine, a potent monoaminergic agonist thought to primarily modulate DA neurotransmission, on the perceptual processing of angry and fearful facial expressions. The authors report increased amygdala response following dextroamphetamine and suggest that excitatory sensory input to the amygdala was potentiated while prefrontal input was inhibited (Hariri, Mattay, Tessitore, Fera et al., 2002).

The serotonergic system has cell bodies located in the raphe nucleus (brainstem) which project to the frontal cortex, the basal ganglia, the limbic area, the hypothalamus, and brainstem. Like the pathways of the noradrenergic and dopaminergic systems, the pathways of the serotonergic system mediate different physiological functions including mood (frontal sites), movements, obsessions and compulsions (basal ganglia), anxiety and panic (limbic sites), appetite and eating behaviour (hypothalamus), sleep (particularly slow wave sleep) and vomiting (brainstem) (Stahl, 1997, 2000). Of the monoamines, the current thesis focuses specifically on the serotonergic system (see the experimental chapters 5 and 6). This system has been implicated in a wide range of affective behaviours including mood and social behaviour (eg. Young & Leyton, 2002), impulsivity (eg. Depue, 1995; Spoont, 1992), aggression (eg. Berman, Tracy & Coccaro, 1997; Spoont, 1992) and personality (Cloninger, 1987). In addition the 5-HT system has been implicated in a wide range of disorders including depression, schizophrenia, generalised anxiety disorder, social phobia, obsessive compulsive disorder, panic disorder, post-traumatic stress disorder, migraine, pain, cognitive disorders, aggression, premenstrual dysphoria, chemotherapy and radiation-induced emesis, irritable bowel syndrome, obesity and appetite disorders, sexual dysfunction, alcoholism, drug dependence and abuse and autism. 5-HT research has had an enormous impact on medicine, yet many avenues remain unexplored (see Jones & Blackburn, 2002 for a review of the impact of 5-HT research). One of these avenues needing investigation is to focus on the physiological basis for the behavioural effects of 5-HT rather than simply correlating behavioural systems with levels of brain 5-HT.

Studies that have examined the relationship between 5-HT and affect suggest that enhancement of 5-HT with antidepressants such as the SRIs is associated with decreasing magnitude of negative emotional states in psychiatric patients (eg
Salzman et al., 1995; Steiner et al., 1995; Van Vliet, den Boer & Westenberg, 1994) and more recently, in healthy subjects (e.g. Knutson, et al., 1998; Moskowitz, Pinard, Zuroff, Annable & Young, 2002; Zald & Depue, 2001). In contrast to negative emotional states, the relationship between 5-HT and positive affect has been contradictory. For example, chronic administration of SRIs has been associated with increased levels of affiliative behaviour in healthy controls (Knutson, et al., 1998; Moskowitz et al., 2002). However measures of positive affect in healthy controls as assessed by the PANAS have been demonstrated to be either insensitive to serotonergic augmentation (Knutson et al., 1998) or negatively correlated with serotonergic functioning (assessed by maximum prolactin response to d-fenfluramine) (Zald & Depue, 2001). As hinted above however, it is critical for studies to be conducted that experimentally alter 5-HT and examine the corresponding behavioural changes, rather than simply correlating levels of serotonergic functioning with levels of positive and negative affect, in order to be able to directly investigate the effect of 5-HT on affective behaviour.

Studies have begun to examine the effects of serotonergic modulation on emotional responses and affective behaviour, through depletion of brain tryptophan (a precursor to 5-HT) (recently reviewed by Young and Leyton, 2002) as well as 5-HT augmentation using either SRIs (Knutson et al., 1998) or tryptophan (Moskowitz et al., 2002). This literature suggests that decreased 5-HT can predispose individuals to mood and impulse control disorders, whilst increased 5-HT may be involved in social affiliation and dominance. However, a limitation of these studies has been the use of indirect questionnaire based measures overlooking immediate responsiveness to, and processing associated with presentation of emotional stimuli.

Recently, a number of studies have examined the direct effects of serotonergic augmentation following administration of either the SRI citalopram (Harmer, Bhagwagar, et al., 2003; Harmer, Shelley, McTavish, Cowen & Goodwin, 2002) or administration of nutritionally-sourced tryptophan (Attenburrow et al., 2003), on recognition of and response times to morphed emotional-facial expressions. These studies reported increased detection of, and decreased response times to, expressions of happiness and fear but not to sadness, anger and disgust (Attenburrow et al., 2003; Harmer, Bhagwagar, et al., 2003), suggesting that augmentation of 5-HT can affect the neuropsychological processes involved in social and emotional processing. There remains however, little understanding on how enhancement of 5-HT mediate the physiological mechanisms underlying the
processing of emotional stimuli. Furthermore, studies are yet to examine how the
brain responds to the processing of emotional stimuli (pleasant and unpleasant)
following serotonergic augmentation. Interestingly, previous brain-imaging studies of
subjects in resting state have concluded that the cortical regions affected by
serotonergic augmentation overlap with those activated in mood induction paradigms
(Smith et al., 2002).

1.10 Methodological Difficulties and Some Key Issues

The issues involved in understanding how brain function is modulated by emotion
have been regarded as complex enough for different researchers to have reached
diametrically opposed conclusions from the same evidence (Tucker, 1981). Some of
the more important issues that should be considered by researchers who investigate
brain and peripheral correlates of emotional processing include the technique used,
task instructions, selection of stimuli and differences between males and females.
These methodological issues will not only contribute to the variability of research
findings but also the physiological explanations provided for the reported results.

Studies have used a variety of neuroimaging techniques (EEG, MEG, SPECT, PET
and fMRI), different independent and dependent variables (eg. location and number
of recording electrodes sites, frequency bandwidths, evoked potentials, rCBF,
metabolism and BOLD) and have reported both spatial measures (eg. PET, fMRI) as
well as temporal measures of brain activity (eg. EEG, ERPs, SSVEP’s, MEG). Thus,
it is a difficult process to make comparisons not only across studies using different
techniques (see Schneider et al., 1997 for discussion of discrepancies between fMRI
and PET data) but also across studies using the same technique (such as ERPs)
(see Boucsein et al., 2001 and Kayser et al., 1997 re discussion of potential
confounds in ERP research). Some of the potential confounds that have been
identified in ERP research on emotion, that may also relate to research using other
techniques, include detection of a specific target (Lang et al., 1990), manual
responses (Laurian et al., 1991), stimulus discrimination tasks (Carretié & Iglesias,
1995) and fixed stimulus intervals (ISIs) (Carretié & Iglesias, 1995; Vanderploeg,

Task instructions have also been identified as important components of variability
and are now known to result in differences in brain activation (eg. Lane, Fink et al.,
1997, Liberzon et al., 2000; Taylor et al., 2003). Studies have, for example;
presented images of facial expressions portraying different emotional states and requested subjects to either indicate the type of emotion expressed by a particular facial expression (eg. lidaka et al., 2001), or attempt to feel the emotion expressed by a particular facial expression (eg. George et al., 1995; Schneider et al., 1997). Similarly, studies have presented visual emotional stimuli (such as the IAPS images) to participants and have asked participants to either attentively view presented stimuli (eg. Mini et al., 1996; Muller, Keil, Gruber & Elbert, 1999) or requested participants to focus on emotional content and refrain from emotive inhibition (eg. Kemp et al., 2002; Lane, Reiman, Ahern et al., 1997). Other studies have not even reported on the instructions provided to participants (Lane, Reiman, Bradley et al., 1997).

Task instructions are important in terms of directing the participant to focus on particular aspects of their conscious state and consequently altering the brain’s response to the presented stimuli. Unless investigators make instructions to participants explicitly clear and report on these instructions in their published manuscripts, it is difficult to interpret the meaning of the reported findings. Clear description of instructions will help in untangling the related constructs such as attention, cognition and emotion. Probably the best evidence to cite regarding the importance of task instructions are those studies which have provided different instructions to participants within the same study on identical stimuli (eg. Beauregard et al., 2001; Lane, Fink et al., 1997; Liberzon et al., 2000; Taylor et al., 2003). For example, when participants are requested to attend to their subjective emotional responses, increased neural activity is elicited in the rostral AC. However, if participants are requested to attend to spatial aspects of the presented images activation is observed within the parieto-occipital cortex bilaterally (Lane, Fink et al., 1997).

Finally, most neuroimaging studies on emotion have often only recruited females for studies on emotional processing, and consequently brain gender-differences in emotional processes remain unclear. Although, there are an increasing number of studies investigating brain gender differences during the processing of emotional information (eg. George, Ketter, Parekh, Herscovitch & Post, 1996; Orozco and Ehlers, 1998; Pendergrass, Ross, Garavan, Stein & Risinger, 2003; Schneider et al., 2000), these studies have reported contradictory findings. For example, whilst it has been suggested that wider areas of the limbic system are involved in emotional processing in women (George et al., 1996), a more recent study concludes that greater subcortical processing is apparent in men (Schneider et al., 2000).
Furthermore, recent studies have suggested that males are more lateralised than females (eg. Killgore & Yurgelun-Todd, 2001). However Davidson’s approach and withdrawal theory which essentially posits that anterior activation is lateralised during discrete emotional states, has been based on previous studies in which participants have been only female. Clearly then, future research is needed to further explore gender differences on emotional processes in order to provide explanations to these questions.

1.11 GENERAL AIMS AND OVERVIEW OF THIS THESIS

The aim of the current thesis is to explore a number of issues that arose from an extensive search of the literature by examining steady state visually evoked potentials (SSVEPs), cardiovascular responses and subjective report associated with the processing of visual emotional stimuli in healthy males and females. The SSVEP was elicited using steady state probe topography (SSPT). (The SSPT technique is discussed in detail in chapter 2). This technique was selected for its ability to track rapid changes occurring in brain electrical activity during ongoing processing of stimuli. This characteristic is considered to be crucial for the investigation of phasic and transient processes associated with the viewing of emotional stimuli. Cardiovascular responses and subjective report were also examined in order to explore multiple response components associated with emotional responsiveness. Visual stimuli were selected from the IAPS database in order to investigate emotional processing. IAPS stimuli have been previously rated on valence and arousal dimensions and provision of these standardised ratings allow for systematic selection of images ranging in emotional content. Furthermore, this database has been specifically developed to evoke a broad range of emotions experienced outside the laboratory (Lang et al., 1997). The current thesis contains four experimental chapters, each of which include their own introduction, methods, results and discussion sections. All of these chapters have been peer-reviewed in international journals, and three have already been accepted for publication.

At the time the first experiment in this thesis was conducted, no previous studies had utilised the SSPT technique to investigate how the SSVEP is modulated by the processing of emotional stimuli. Consequently, the first experiment (positioned as chapter 3) was carried out to examine the steady-state visually evoked potential topography associated with the processing of emotional valence. Preliminary data from this study was presented as a poster at the Riken Brain Science Institute
Summer Program in Japan in 2001 (see appendix 9.6) and in 2002, the study was published in the journal, *NeuroImage* (see appendix 9.9 for a reprint of this article). Recently, a growing number of studies have reported that gender differences in brain activation exist during the processing of emotional stimuli. Therefore, in order to explore the findings from the first experimental chapter in more detail, the second experimental study (positioned as chapter 4) was conducted to explore the way in which the SSVEP associated with viewing of emotional stimuli, differed in males and females. This study is now currently ‘in press’ in the same journal as that for experiment one. (See appendix 9.11 for a reprint of the uncorrected proof). These two experimental studies represent the first studies to employ the SSPT technique in order to examine phasic emotional responses.

The first two experimental chapters only investigated electrophysiological and behavioural (subjective) responses to presented images however, and did not examine other physiological responses such as HR, which has long been regarded as a key component of visceral activity associated with an emotional response. In addition, little is known about the way in which serotonin modulates heart rate during emotional processing. This is particularly pertinent given that depression and anxiety have been associated with an increase in the likelihood of sudden cardiovascular death and that SRIs are now widely used for the treatment of many emotional disorders including depression. Therefore, the third experimental study (positioned as chapter 5) examined the impact of viewing emotional stimuli on heart rate as well as the impact of serotonergic augmentation on heart rate during viewing of these stimuli. This study has recently been accepted for publication in the *International Journal of Neuropsychopharmacology*. This particular chapter was conducted in part, to confirm the reliability and validity of the task viewing paradigm. This research also represents the first study to investigate the way in which heart rate, associated with the viewing of images differing on emotional valence, is modulated by 5-HT.

Although the serotonergic system is known to be one of the major neurochemical systems involved in the regulation of emotion, the neurophysiological mechanisms underlying the effects of 5-HT on emotional processing are relatively unknown. Therefore, the fourth experimental chapter (positioned as chapter 6) was conducted to explore the way in which the SSVEP associated with the viewing of differently valent images, is modulated by serotonergic augmentation. Preliminary gender differences were also examined in this study. Preliminary data from this study was published in 2003 in the journal, *Brain and Cognition*. (See appendix 9.10 for a
Data from this study has also been presented in poster format at the Emotions and the Brain conference held at the Rotman Research Institute in Canada in 2001 as well as the Australasian Society of Psychophysiology conference in Australia in 2003 (see appendix 9.7 and appendix 9.8, respectively). This research represents one of the first studies to explore modulation of neurochemicals on a neurophysiological measure of emotional processing.

This thesis concludes with a general discussion of the key findings and their broader implications. Results from the first two experimental studies are discussed as they relate to our understanding of emotional valence and the activation of the human brain. Moreover, results from recent studies are addressed and integrated into this understanding. In addition, results from the final two experimental studies are discussed in relation to the limited extant literature that has explored the relationship between 5-HT and emotional processing. Finally, two tentative models based on the results from the last two experimental chapters are provided. These models seek to explain both the underlying mechanisms of the SSVEP as well as neuropsychological effects associated with antidepressant administration following acute administration. The purpose of these models is to provide some direction for future research and assist in further elucidation of the underlying mechanisms of the SSVEP, heart rate and subjective report in response to emotional stimuli.
CHAPTER 2

2  STEADY STATE PROBE TOPOGRAPHY (SSPT): METHODOLOGY, AN INTERPRETATIVE FRAMEWORK, A BACKGROUND AND BRIEF REVIEW
2.1 **Steady State Probe Topography (SSPT)**

SSPT is a novel imaging technique developed within the Brain Sciences Institute over the last fourteen years. Silberstein et al., (1990) first described this technique in a paper which investigated the effects of a visual vigilance task on steady state visually-evoked potentials (SSVEPs). SSVEPs may be defined as evoked responses elicited by a repetitive stimulus fast enough to prevent the evoked response returning to baseline (Silberstein, 1995). The SSVEP is comprised of two components, amplitude and phase (latency), which relate to the size of the response and the delay between stimulus and response, respectively. The SSPT technique essentially involves the recording of SSVEPs during presentation of various cognitive tasks and is characterised by the recording of brain electrical activity from multiple electrodes, a short Fourier integration period and the Probe-ERP technique in which the visual flicker, acting as an ERP stimulus, is distinct from and irrelevant to the cognitive task presented to participants (Silberstein et al., 1990).

2.2 **Recording, Signal Processing & Analysis**

Each of the experimental chapters contained within this thesis includes a Methods section specific to each individual chapter; however a more general overview of SSPT methods will be described below with respects to the recording, signal processing and analysis of SSVEPs within our institute (as described previously by Line et al., 1998, Pipingas, 2003; Silberstein et al., 1990; Silberstein, Ciorciari & Pipingas, 1995).

2.2.1 **Stimulus**

SSPT is characterised by superimposing a diffuse 13Hz sinusoidal white flicker on the visual field by a pair of goggles (see image 1 in section 2.2.2) during the recording of brain electrical activity in order to elicit the SSVEP. A stimulus frequency of 13Hz is selected in the following experiments for a number of reasons. First, robust changes in 13Hz amplitude and latency SSVEP have been reported to be associated with various cognitive processes. (See section 2.5 for a review of key articles which employ SSPT methodology). Second, the selection of a low stimulating frequency is considered to evoke widespread SSVEP which is not limited to the visual cortex (Speckreijse et al., 1977). Importantly, this has been confirmed by a number of studies utilising SSPT employing a variety of paradigms (discussed in section 2.5). Third, 13Hz may be classified as falling within the high alpha or low beta
bandwidths. EEG studies have traditionally viewed the alpha bandwidth as a measure of ‘activity’, such that reduced alpha is suggestive of increased ‘activity’ (see Ray & Cole, 1985). This model has been applied to the assessment of brain activity during emotional processing (e.g. Davidson et al., 1990). Fourth, the amplitude of the 13Hz SSVEP may be interpreted as analogous to the amplitude of regional activity within the alpha frequency range (Silberstein, 1995a, b) and desynchronized upper alpha has been previously argued to reflect an enhancement of cognitive processing (e.g. Klimesch, Doppelmayr, Russegger, Pachinger & Schwaiger, 1998). Similarly, the latency of the 13Hz SSVEP (250-320ms) matches that of event related components (such as the P300) which are also known to be sensitive to cognitive components (Rizzolatti, Luppino & Matelli, 1998). Fifth, the 13Hz bandwidth is distinct from the prominent ‘alpha peak’ so as to optimise the signal to noise ratio. In this case ‘signal’ refers to the driven 13Hz activity by cognitive tasks whilst ‘noise’ refers to the prominent alpha peak in healthy human volunteers present during the recording of electroencephalographic activity (approximately 10Hz).

2.2.2 Recording

Image 1 displays a typical setup for a recording session using the SSPT technique, in which the subject is positioned within view of the computer on which the task is presented. The researcher (and author) is pictured (from left to right) altering the location of the specially designed goggles such that the half-mirrored strips fully cover the patient’s field of view; inserting gel into the electrode-cap; and examining the power spectra for interference and dud electrodes, respectively.

Figure 10: Typical setup for a recording session using the SSPT technique.
Brain electrical activity is recorded from 64 monopolar leads using a lycra electrode cap with chin strap. The averaged potential of both ears serves as a reference after each earlobe is separately buffered with unity gain, low noise amplifiers. This procedure removes the problems of unbalanced electrode impedances in linked earlobe references (Nunez, 1981; Silberstein et al., 1995). A nose electrode is used for a ground. Activity is amplified and bandpass filtered at 0.74Hz and 74Hz prior to digitization to 16-bit accuracy at a rate of 500Hz. The recording locations include all sites specified in the International 10-20 system as well as additional sites midway between. (See Figure 10 for locations of all 64 electrode sites used in the experimental studies). As discussed by Silberstein et al., (1990), the optimum number of recording sites to adequately sample scalp distribution is determined by the spatial variability of the recorded signal. 64 recording sites yields an average inter-electrode distance of 3.2cm, thereby significantly reducing the possibility of missing some of the smaller topographic features. This increased cortical spatial resolution is particularly important during the recording of activity associated with emotional processes.

Figure 11: Position of 64 scalp recording sites used in all experiments.

2.2.3 Signal Processing

SSVEP amplitude and phase are determined from the 13Hz cosine and sine Fourier coefficients and evaluated over a certain window length. SSVEPs are generally evaluated over ten 13Hz cycles however SSVEPs have been evaluated over much longer time periods such as 10 seconds (eg Silberstein et al., 1990). The duration over which SSVEPs are evaluated is a compromise between noise reduction and the ability to track rapid changes occurring in amplitude and phase during the ongoing processing of stimuli (Silberstein et al., 1990). Longer evaluation periods will
therefore increase the signal to noise ratio by rejecting more of the brain electrical activity not centred on the stimulus frequency, yet reduce the capacity to follow changes in amplitude and phase across time. Emotion has long been regarded as phasic in nature (Ekman, 1984) and understanding of the processes involved will benefit from an investigation of temporal processes (Schneider et al., 2000). In the experimental chapters contained in this thesis therefore, a window width of ten 13Hz cycles was selected, which yielded time series data with 13 points/sec corresponding to a temporal resolution of 0.77 seconds. A window width of ten cycles was thought to provide a temporal resolution appropriate for the investigation of temporal processes as well as a sufficient signal to noise ratio (Silberstein, 1999, 2000, personal communication). After time-series data are calculated, signal processing involves extraction of the SSVEP associated with specific stimuli, averaging across stimuli, averaging across subjects and then subtracting the averaged SSVEP associated with the ‘baseline’ task from that associated with the ‘activation’ task. Both amplitude and phase components of the SSVEP are normalised to account for the large inter-subject variations (as discussed in Silberstein et al., 1990 and described in more detail in chapter 3). In addition, changes in phase (measured in radians) are expressed in terms of latency (measured in milliseconds) (also described in chapter 3).

2.2.4 Artefact Detection and Compensation

It is important to note that a specific advantage of the SSVEP is its insensitivity to noise and artefact (Reagan, 1989; Silberstein et al., 1995). Consequently, it has been argued that it is possible to relax the rejection criteria for artefact contamination that would normally be employed in the evaluation of EEG power spectra (Line et al., 1998; Silberstein et al., 1995). All data however are checked for artefact by a number of methods, some of which have been described previously (Silberstein et al., 1995; Line et al., 1998). In the first phase of artefact screening, EEG amplitude spectra is displayed and assessed for gross deviations from normality prior to commencement of the recording at the beginning of each trial. The run-sheet is then used to record and identify the number and location of ‘dud’ electrodes for replacement. In the next phase, the SSVEP time series at each electrode are compared with the time series of its nearest neighbours during data analysis. The aim of this procedure was to identify sites where data significantly differed from that at surrounding sites. These were considered to be suspect because closely spaced electrodes within the 64-electrode system are expected to be highly correlated (Nunez, 1981). Electrodes identified as being contaminated with artefact in these tests are then replaced with a weighted
average time series of four adjacent electrodes that pass test criteria. Participants are generally excluded from the analysis if any more than 8 electrodes are listed for replacement.

2.2.5 Topographic Mapping & Statistical Analaysis

Two dimensional maps are generally constructed for SSVEP amplitude, latency as well as Hotellings data using a spherical spline interpolation procedure (Cadusch, Breckon, & Silberstein, 1992; Nunez, Silberstein, Cadusch & Wijesinghe, 1993; Nunez et al., 1994). These maps contain 64 electrodes and are output as 1024 X 768 pixels having 256 colours, which display reduced amplitude and latency in the ‘activation’ task as warmer colours. This colour convention was adopted in early studies conducted within our institute to reflect the interpretation that amplitude and latency reductions are indicative of increased neural activity (or activation). The interpretation of SSVEP amplitude and latency is discussed in more detail below in section 2.4.

The statistical strength of the differences between the ‘activation’ task and the ‘baseline’ task is provided by the Hotellings $T^2$ parameter, which can be considered a multidimensional extension of the Student’s t-test (Picton, Vajsar, Rodriguez, & Campbell, 1982; Silbertsein et al., 1995). The Hotellings statistic is calculated from complex numbers and therefore the t-values obtained from the statistical analysis refer to a combination of both the SSVEP amplitude and latency. Mapping these $T^2$ statistics however, sometimes leads to small areas which contain very large values which consequently dominate the scale. This issue is dealt with by mapping the square root of the Hotellings $T^2$ ($\sqrt{T^2}$), which allows for much smoother contours in the topographic maps (Pipingas, 2003). For simplicity then, this statistic is referred to as Hotellings $T$. While this statistic illustrates within-subject task effects, it does not directly test between-subject effects (such as gender) or within-subject treatment effects (such as placebo and drug). Although topographic mapping of the Hotellings statistic does allow for a potentially useful visual comparison between different groups (eg. Silberstein et al., 1998; Silberstein, Line, Pipingas, Copolov & Harris, 2000), these other contrasts may be tested more directly by extracting amplitude and latency components using in-house software (developed by Dr. Peter Line) and then conducting repeated measures ANOVAs (RANOVAs) on these components separately (see method sections 4.2.4 and 6.2.5 for more detail on these procedures as they apply to analysis of gender and drug treatment respectively). It should be
noted however that there are limitations with these RANOVA procedures as they apply to SSVEP data (see discussion sections 4.4 and 6.4).

In the Hotellings $T^2$ topographic maps, warmer colours represent both a higher t-value and a lower level of probability that these effects occur by chance in order to provide a visual guide for the statistical strength of the effects. Specific levels of probability including the threshold for statistical significance (known as the alpha level), are identified in these maps using ‘contours’. An alpha level of 0.05 is usually set by experimenters as the threshold for statistical significance. This alpha level refers to the probability of obtaining observations as extreme as the ones actually observed. This value is regarded as a good compromise between the likelihood of reporting an effect when there is none (type 1 error) and reporting no effect when there is one (type 2 error). The likelihood of making a type 1 error however, is increased with an increasing number of comparisons. A Bonferroni correction is the technique usually applied to account for an increasing number of independent comparisons (Abt, 1983). In this approach, the alpha level is divided by the number of comparisons to yield adjusted P values thereby minimising the occurrence of a type I error. This approach however, has been regarded as extremely conservative and even incorrect when applied to EEG recordings on systems in which electrodes are closely spaced (Silberstein et al., 1995). As discussed above, most recordings using SSPT record from a minimum of 64 sites, therefore a Bonferroni correction would require that 0.05 is divided by 64. Correction in this way however overlooks the fact that individual electrodes are not independent because of high correlations between scalp recording sites (Duffy et al., 1990; Nunez, 1981; Silberstein & Cadusch, 1992; Silberstein et al., 1995). Instead, when correcting for the use of 64 electrodes, it has been recommended that the alpha level of 0.05 be divided by 5, therefore adjusting the alpha level from 0.05 to 0.01 (Silberstein et al., 1995). This value is derived from spatial principal components analysis which identified 5 independent factors to explain the data, and is regarded as more accurately representing the degree of independence between the 64 correlated locations (Silberstein & Cadusch, 1992; discussed in Pipingas, 2003). The issues relating to multiple comparisons will also be dealt with in each of the individual chapters, as different methods of presenting and reporting results have been used in the experimental chapters and the process of dealing with the issue of multiple comparisons therefore, has been discussed in a number of different ways.
A BRIEF DESCRIPTION OF THE VISUAL SYSTEM

An understanding of the visual system and associated neurophysiology is crucial for the understanding of the underlying mechanisms associated with the generation, projection and distribution of the SSVEP. The visual system includes photoreceptors, bipolar cells, retinal ganglion cells, the lateral geniculate nucleus (LGN) of the thalamus, the primary visual cortex (BA 17 or V1, also called the striate cortex), and the higher-order visual areas, also known as the extra-striate regions. Visual information is projected from the retina to three major subcortical targets, which include the superior colliculus (SC), located on the roof of the midbrain, the pretectum, located rostral to the SC, where the midbrain fuses with the thalamus and the LGN, located within the thalamus. The LGN however is the principal subcortical structure that carries visual information to the cortex. Fibres leaving the LGN, loop around the lateral ventricle in the optic radiation to reach V1 (Wurtz & Kandel, 2000a).

Retinal ganglion cells and the LGN respond primarily to contrast, whilst simple and complex cells of the visual cortex respond best to line segments and boundaries. A characteristic of the visual system is that cells at a higher level within the visual system display greater levels of abstraction than cells at lower levels such that ‘each complex cell surveys the activity of a group of simple cells, each simple cell surveys the activity of a group of geniculate cells, and each geniculate cell surveys the activity of a group of retinal ganglion cells. The ganglion cells survey the activity of bipolar cells that in turn, survey an array of receptors.’ (Wurtz & Kandel, 2000a, p. 536). This processing is considered an important step in analysing contours of objects, approximating the initial shape of a stimulus and may be sufficient for the recognition of an object. (For a detailed discussion of the pioneering studies on the visual cortex, see Hubel & Wiesel, 1977; reviewed by Sekuler & Blake, 1994 and Wurtz & Kandel, 2000a).

Two parallel and complementary pathways known as the parvocellular (Parv) and magnocellular (Mag) pathways project visual information from the retina to the cortex. The Parv pathway conveys information about contrast and spatial structure of slowly changing stimuli, whilst the Mag pathway conveys information about changes in the visual environment. In particular, the Parv pathway is thought to be important in perception of colour, spatial detail and texture whilst the Mag pathway is thought to be important in movement detection and flicker (Silberstein, 1995a; Ciorciari, 1999; Schiller & Logothetis, 1990; Sekuler & Blake, 1994). Segregation of visual
information begins with two types of retinal ganglion cells – small cells (P cells) and large cells (M cells). The two pathways project to the Parv and Mag layers of the LGN respectively which then project to separate regions within striate and visual association areas, thereby maintaining functional segregation of information within the different layers of the cortex (Livingstone & Hubel, 1988). The Mag pathway also projects to the superficial layers of the SC as well as the pulvinar nucleus of the thalamus both of which are involved in eye movement control (Robinson et al., 1989). The SC and pulvinar nucleus then convey information to several cortical areas including the striate cortex, visual association cortex, temporal cortex, primary somatosensory cortex and PFC (Silberstein, 1995a).

The neocortex is generally considered to be comprised of six layers and it is the fourth layer which principally receives inputs from the LGN. The upper subdivision of the fourth layer receives axons of cells from the Mag layer of the LGN whilst the lower subdivision receives axons of cells from the Parv layer of the LGN. The fourth layer is sometimes called the ‘sensory’ or input layer and is most prominent in primary sensory regions such as V1. The cortex is comprised of two basic classes of cells: the pyramidal cells, which are large and have long spiny dendrites, and non-pyramidal cells which are small and stellate in shape and have dendrites that are either spiny or smooth. The pyramidal and spiny stellate cells are excitatory in nature, whilst the smooth stellate cells are inhibitory in nature. The fourth layer of V1 is predominated by the spiny stellate cells, which receive input from specific thalamic relay nuclei and corticocortical fibres from neocortical pyramidal cells in layers 2 and 3. These stellate cells then deliver excitatory output to cells in layers 2 and 3 and to those in layers 5 and 6. The pyramidal cells feed axon collaterals to the different layers within V1 thereby integrating the activity within this structure and also project output to the extra-striate regions. Pyramidal cells within layers 2 and 3 project output to extra-striate regions, including V2, V3 and V4 (located in BA 18 and 19). Pyramidal cells within layer 4B project output to the middle temporal area; cells within layer 5 project to the SC, the pons and the pulvinar, and cells within layer 6 project to the LGN and the claustrum (see Sekuler & Blake, 1994; Silberstein, 1995b; Wurtz & Kandel, 2000a for reviews).

Different visual areas appear to be associated with different aspects of the visual world. For example, it has been argued on the basis of anatomical and functional evidence that the extrastriate visual areas are organised into a dorsal pathway, involving V1, the middle temporal area and the parietal temporal cortex, and a ventral
pathway involving V1, V4 and the inferior temporal cortex. It should be noted that the parietal pathway appears to be dominated by Mag input and the temporal pathway by both Parv and Mag input (Wurtz & Kandel, 2000b). Whilst the dorsal (parietal) pathway was argued to be concerned with localising where objects are, the ventral (temporal) pathway was argued to be important in the identification of what the objects are (Mishkin, Ungerleider & Macko, 1983; Ungerleider & Mishkin, 1982; reviewed by Kandel, 2000a; see also Burt, 1993; DeYoe et al., 1988; Van Essen et al., 1992). Both the dorsal and ventral pathways then project rostrally to different regions of the PFC (Wurtz & Kandel, 2000b).

These characteristics of the visual system have implications for the underlying mechanisms of SSVEPs. Different forms of visual stimuli will elicit different responses from the Mag and Parv pathways. These responses will depend on the different neuroanatomical and functional characteristics of these pathways. As described above, the Parv pathway conveys spatial detail whilst the Mag pathway conveys temporal detail. Silberstein (1995a) has argued that these differences may account for the observation that the SSVEP elicited by high spatial frequencies (such as a checkerboard) possesses a single maximum at 10Hz whilst the SSVEP elicited by low spatial frequencies (such as an unstructured visual flicker) possesses maxima at 10, 20 and 40Hz. It is possible to modulate the amplitude and latency of visually evoked potentials by altering stimulus frequency, luminance, amplitude and structure. For example, as the stimulus frequency increases from 8 to 13Hz the amplitude peaks and the phase exhibits an increased phase lag of approximately -2π radians, however if the stimulus frequency is fixed then an increase in the resonant frequency within neuronal networks appear as a phase advance (or latency decrease) (Silberstein, Nunez, Pipingas, Harris & Danieli, 2001; see also Ciorciari, 1999 and Silberstein, 1995a for discussion). The next section lays an interpretative framework for understanding the SSVEP elicited by the SSPT technique.

2.4 AN INTERPRETATIVE FRAMEWORK

SSPT examines changes in 13Hz SSVEP amplitude and phase (latency) which relate to the size of the response and the delay between stimulus and response, respectively. Silberstein and colleagues have proposed a preliminary neurophysiological model by which the genesis of driven EEG rhythms in the 8-18Hz range may be understood (Silberstein, 1995b, 1998; Silberstein et al., 2001). This model has been devised with specific regard to 13Hz SSVEP amplitude and latency.
components and highlights the importance of re-entrant feedback and feed-forward cortico-cortico and thalamo-cortico fibres in the generation of the SSVEP. In this model, changes in the synaptic transmission efficiency and number of synchronously active neuronal elements in re-entrant loops (loop gain) will be associated with changes in SSVEP amplitude whilst changes in the synaptic and axonal transmission times of the re-entrant loop (loop-time) will be associated with changes in SSVEP phase (Silberstein et al., 2001).

It has been suggested that SSVEP amplitude may be compared to alpha activity in association with cognitive tasks (Silberstein, 1995a,b). A reduction in alpha is generally associated with an increase in vigilance, and interpreted as a surrogate measure of activation. Interpretation of alpha activity in this way is based on a traditional arousal model dating back to the beginnings of EEG research (eg. Adrian & Matthews, 1934) and is supported by a wealth of evidence (eg. Shagass, 1972). In addition, this model continues to provide a basis for the understanding of the underlying dynamic mechanisms in emotion research (eg. Davidson et al., 1990; Davidson, 1992). However, the model of alpha amplitude as an unambiguous indicator of activation has been questioned. For example, Ray and Cole (1985) made the distinction between intake tasks and rejection tasks. Intake tasks refer to tasks in which one must pay attention to an external aspect of the visual environment. These tasks are invariably associated with a decrease in the amplitude of alpha activity. By contrast, rejection tasks such as mental arithmetic require that information from the environment is rejected and internal representations of information are attended to. These tasks are associated with an alpha increase.

The amplitude of alpha has generally been considered to be dependent on levels of depolarisation in cells located within the reticularis nucleus of the thalamus (Buzsaki, 1991; Silberstein, 1995b; Steriade, 1990, 1991; Steriade, Dossi & Nunez, 1991; Steriade, Dossi, Pare & Oaken, 1991). Cells within this structure may fire in either single unit firing mode or burst firing mode depending on the level of depolarisation or hyperpolarisation and the frequency of the bursting is then transmitted to the cortex and the corresponding frequency is recorded in the EEG. Other models however highlight the complementary roles played by the neocortex in addition to the thalamus (see Silberstein, 1995a & 1995b for discussion). Paul Nunez (1981, 1989) brought attention to the possibility of travelling and standing waves which are mediated by long corticocortico fibres and are dependent on features of cortical neuroanatomy and ‘damping’. While standing waves may occur more often in a
weakly ‘damped’ environment, strong ‘damping’ reduces the possibility of wave interference making it more likely that travelling waves will be produced (Silberstein, 1995a, p. 281). For example, a structured sinusoidal stimulus (high spatial frequency) such as a checkerboard appears to preferentially activate the Parv pathways and is more likely to yield travelling waves due to a more restricted projection to the striate cortex. By contrast, an unstructured sinusoidal stimulus (low spatial frequency) appears to preferentially activate the Mag pathways and is more likely to yield standing waves due to having more extensive cortical projections and therefore more likely to allow interference (Silberstein, 1985a, p. 280). These differences between an unstructured and structured sinusoidal stimulus appear to relate to the differences in cortical projections between the Mag and Parv pathways.

The other component of the SSVEP is known as phase (latency) and refers to alterations in the temporal expression of the SSVEP waveforms. Latency reductions may reflect increased functional coupling between neural networks (or increased resonant frequency) as a consequence of increased synaptic excitatory processes. Conversely latency increases may reflect reduced synaptic excitation (or increased synaptic inhibition) (Silberstein et al., 2001). This interpretation is consistent with the demonstration that reaction time in visual vigilance tasks such as the CPT correlates with frontal SSVEP latency (Silberstein, Cadusch, Nield, Pipingas & Simpson, 1996; Silberstein, Line, et al., 2000). Silberstein and colleagues (2000) report this as being analogous to the observation that magnitude of the CNV (thought to index cortical excitability) is negatively correlated with reaction time (Naatanen & Gaillard, 1974; Roctroh et al., 1989). In addition, the interpretation that latency reductions may reflect increased levels of cortical excitability is consistent with animal studies, which have demonstrated that stimulation of the major cholinergic nucleus of basalis in cats reduces the time it takes for an electrical stimulus applied to the thalamus to actually reach and cause depolarisation within the cortex in regions where the acetylcholine is released (Methrate & Ashe 1993).

The latency of a visual stimulus to the response recorded from the scalp is around 250-320 msecs, however transmission from the optic nerve to the LGN is in the order of 45 – 50msecs, whilst thalamocortical transmission time is in the order of 30msecs or less (Regan, 1989; Silberstein, 1995). This rules out the possibility that the signal is a direct projection from the retina through the LGN to the scalp recording site. Instead, it has been suggested that neocortical resonances determine EEG rhythms including the SSVEP within the 9-17Hz range. For example, ‘two reciprocally
connected regions separated by 5cm and 4 to 7 synapses yielding axonal delays of
10 to 20 milliseconds with synaptic delays of 2 to 35 milliseconds may be expected to
produce resonant frequencies corresponding to approximately twice the loop time or
9 to 17Hz.’ (Silberstein, 1995b, p. 595-596)

The physiological basis for these effects is based on excitatory and inhibitory
neuronal synaptic transmission. An excitatory process is associated with a reduction
in nerve cell membrane potential (eg. from -65mV to -55mV) (known as
depolarisation) thereby enhancing the ability of the cell to produce an axon potential.
By contrast, an inhibitory process is associated with an increase in nerve cell
membrane potential (eg. from -65mV to -75mV) (known as hyperpolarisation) thereby
making the cell less likely to produce an action potential (Kandel, 2000b). Nerve cells
receive input signals which are graded in amplitude and duration and are proportional
to the eliciting stimulus. These signals are then integrated and once the threshold
has been reached, the action potential is produced. The action potential is then
propagated along the axon to the synaptic terminal, which in turn releases the
chemical neurotransmitter. The neurotransmitter then diffuses across the synaptic
cleft to receptors in the membrane of the post-synaptic neuron which causes the
post-synaptic cell to generate a synaptic potential (Kandel, 2000b). The speed of
axonal transmission is in the order of to 6-15meters per second and is a function of
myelination and the diameter of the fibre. The main effects on the delay therefore,
with regards to SSVEP phase change, relate to whether the activated post-synaptic
receptor is inhibitory or excitatory. If the cell is inhibited, it is going to take longer for
the cell to produce its action potential. If, on the other hand, the cell is excited, the
cell will take a much shorter time to produce its action potential. The firing rate of the
post-synaptic cell will depend therefore on the level of inhibition or excitation.

The difference between a cell producing an action potential and not producing one is
actually very small, and in some cases less than a millisecond. The phase changes
reported however are large variations in the order of up to 15ms and in some cases
6-7msecs. Thus, for such large variations to occur, long chain multi-synaptic
pathways must be involved by which the signal eventually reaches the recording site.
Each of the synapses involved will make a small variation, but with an increasing
number of synaptic diffusions it is possible that quite large variations in phase will be
observed even though each of the individual steps is relatively small. It is possible to
interpret therefore that a phase advance or an apparent latency reduction is a
manifestation of increasing excitatory processes within the cells that generate the
signal. Conversely, a phase delay or an apparent latency increase is a manifestation of increased inhibitory processes of the cell populations that generate the signal. It is important to note however that an increase in excitation and a decrease in inhibition are neurophysiologically equivalent as are a decrease in excitation and an increase in inhibition, thus a latency decrease may be the result of either increased excitation or decreased inhibition and a latency increase, the result of either reduced excitation or increased inhibition.

In the following experimental chapters SSVEP data is displayed that relate to the presentation of visual emotional stimuli (task condition) relative to visual neutral stimuli (baseline condition). Based on the considerations presented above we interpret reductions in the SSVEP amplitude and latency during the task condition relative to the baseline condition as increases in activity, whereby reductions in amplitude are viewed as an increase in the number of desynchronised neural elements (increased desynchronisation), whilst latency decreases may be interpreted as an increase in the resonant frequency or reductions in loop time within regional networks. Data exist which suggest that there may be a high degree of concordance between SSPT and hemodynamic imaging techniques. For example, Papanicolaou and colleagues (1987) examined the relative contribution of the left and right parietal areas during a mental rotation task and report that both evoked potentials to strobe flashes and rCBF revealed greater right parietal activation. More recently, another group compared the relationship between SSVEPs and fMRI data for a working memory task and report ‘a striking overlap’ of activation with respect to a right frontal locus (Perlstein et al., 2003). It is important to note that SSPT cannot match the high spatial and 3-dimensional information offered by PET and fMRI techniques. However, a number of researchers have argued that both inhibitory and excitatory processes are associated with increased rCBF (see Gray, Kemp, Silberstein & Nathan, 2003 for discussion). By contrast, SSPT appears to provide information relating to increased excitatory or inhibitory processes and therefore is potentially able to shed new light on the nature of neurophysiological mechanisms underlying emotional processes.

2.5 SSPT: A BACKGROUND AND BRIEF REVIEW

Since the publication of Silberstein’s seminal paper which described the effects of visual vigilance on the SSVEP, a number of key studies using the SSPT technique conducted within our institute have been published in international journals. These studies investigated visual vigilance and attention (Silberstein et al., 1998; Silberstein,
Line et al., 2000), cognitive-set change (Silberstein et al., 1995), working memory (Silberstein et al., 2001), long-term recognition memory (Silberstein, Harris, Nield & Pipingas, 2000), mental rotation (Silberstein, Danieli & Nunez, 2003), emotional processing (Kemp et al., 2002), and expectation of electric shock (Gray et al., 2003). Furthermore, SSPT has been used to investigate cognitive processes in clinical groups such as children diagnosed with Attention Deficit Hyperactivity Disorder (Silberstein et al., 1998) and Schizophrenia (Line et al., 1998; Silberstein, Line et al., 2000).

Silberstein et al. (1995) described the modulation of the SSVEP by the Wisconsin card-sorting test, a widely used neuropsychological test of prefrontal lobe function. The authors reported that the prefrontal, central and right parieto-temporal regions displayed pronounced attenuation in SSVEP amplitude and an increase in phase lag (latency increases), 1-2 seconds following the occurrence of the cue for change in the sort criterion. Importantly, the authors discard the possible confounding factors of pupil size and eye movement effects. An increase in visual vigilance is known to be associated with pupil dilation which could in turn increase retinal illuminance thereby increasing the effectiveness of the SSVEP stimulus and increasing SSVEP amplitude (Janisse, 1977; Silberstein et al., 1990). A reduction in the SSVEP amplitude therefore is opposite to that which would be expected if SSVEP were to be confounded by pupil size. The effects of eye movement were discarded because a previous study conducted at our institute with 40 subjects demonstrated that large and frequent eye movements failed to modulate SSVEP topography (Silberstein, Pipingas, Ciociri & Schier, 1991). Silberstein et al. (1995) demonstrate that activation within specific regions peak at points in time in which a change in the cognitive-set is required.

Line et al. (1998) describe changes in the SSVEP topography associated with the onset of auditory hallucinations in eight schizophrenic patients. For this study, patients completed two tasks, a hallucination task and a control task. In the hallucination task, patients were required to press a button box to indicate onset of any auditory hallucination and to release this switch when this hallucination had ceased. For the control task, patients were required to press a button box on the appearance of a cross on the screen and to release this switch on the appearance of a square. For each condition, topographic maps were presented which represented 0.69 seconds prior to report of hallucination or response in the control task. The mean (M) of the entire 1s preceding these responses for each respective condition were
used as the baseline as a baseline comparison. The authors report that a large decrease in SSVEP latency within the right temporo/parietal region occurred in the second prior to the report of an auditory hallucination whilst the same effects were not present for the control task. The importance of this study is the demonstration of the ability of rapid time-scale technologies to aid in the functional analysis of auditory hallucinations.

Silberstein et al., (1998) investigated the effects of a continuous performance task (CPT-X; the reference task; CPT-AX; the activation task) on the SSVEP in normal boys and boys with attention deficit hyperactivity disorder (ADHD). While the CPT reference task required participants to press a button box on the unpredictable appearance of the letter X, the CPT-AX required participants to press the button box only when the letter X was preceded by the letter A. In the interval between the appearances of the A and the X, the authors reported that controls displayed transient reductions in SSVEP latency at right prefrontal sites whilst boys with ADHD displayed either no change or an increase in prefrontal SSVEP latency at right-prefrontal sites. Importantly, these results were obtained from correct trials only, thereby making it unlikely that findings were a consequence of ADHD group deficits in performance. Furthermore, differences between controls and boys with ADHD were also apparent when controlling for differences in IQ. It was concluded that control boys display increased speed of prefrontal neural processing relative to boys with ADHD and this conclusion is consistent with a range of neuropsychological and functional neuroimaging findings illustrating prefrontal deficits in ADHD.

Silberstein, Line et al., (2000) examined the effects of a visual vigilance task, (CPT-AX task as used in the Silberstein et al., 1998 study) on SSVEP latency in patients diagnosed with schizophrenia and normal controls. The authors reported transient SSVEP latency reductions at parietal and prefrontal sites in normal controls. By contrast, SSVEP latency increases were displayed in these regions in the patient group. Furthermore, prefrontal latency changes 500ms following the appearance of the ‘X’ were correlated with individual reaction time in both the control and patient groups. It is of particular interest that latency was found to correlate with reaction time, as this finding supports the interpretation that this SSVEP component may reflect speed of neural information processing. The importance of this study lies in the finding that schizophrenic patients are unable to transiently activate prefrontal processes during specific cognitive tasks and supports previous studies which have
argued that a core abnormality of schizophrenia may be an inability to maintain an appropriate distribution of excitation and inhibition (eg. Fletcher et al., 1998).

Silberstein, Harris et al., (2000) examined the relationship between posterior frontal SSVEP latency changes and recognition of a random array of 40 advertising frames selected from advertisements shown 7 days previously. This study recorded brain electrical activity from sites over cortical regions thought to participate in long-term memory. These included sites between C3 and F7 over the left hemisphere (C3-F7) and homologous sites over the right hemisphere. Activity was also recorded from six other sites including a site located half way between Fp1 and F7 (Fp1-F7), a site halfway between Fp2 and F8 (Fp2-F8) and F3, F4, O1 and O2. The authors report that frames coinciding with posterior frontal latency minima were more likely to be recognised than frames coinciding within frontal latency maxima (58.7% versus 45.3% recognition respectively). Furthermore, the authors indicate that the only electrode site to correlate with recognition performance was within the left posterior frontal region. The importance of this study is the demonstration that the SSPT technique is useful in assessing the strength of long-term memory encoding of naturalistic stimuli.

Silberstein et al., (2001) examined the effects of a high demand version of an object working memory task relative to a low demand version on the SSVEP. The authors report on the effects of both the perceptual and hold components of the working memory task. The perceptual component refers to the initial presentation of the objects, whilst the hold component refers to the 4.2 second delay prior to the subject responding as to whether or not the presented object matched one of the objects presented in the initial presentation. The authors report that the perceptual component was associated with SSVEP amplitude reductions at left and right parieto-occipital sites, whilst the hold or memory component of the task was associated with load-dependent SSVEP amplitude increases at frontal and occipito-parietal sites, and SSVEP latency reductions at central and left frontal sites. The authors concluded that holding information in short-term working memory is associated with SSVEP amplitude increases. The importance of this study is the proposal of a neurophysiological model of the SSVEP generators. In this model SSVEP amplitude and latency are discussed with respects to changes in cortico-cortical and thalamo-cortical loops utilising cortical layer I. In addition, this paper distinguishes between modulation of the SSVEP by visual vigilance and working memory tasks, such that SSVEP amplitude during visual vigilance tasks is similar to the reductions in alpha EEG amplitude associated with cognitive and motor tasks,
whilst SSVEP amplitude during working memory tasks, exhibits an increase during the hold component. Amplitude increases were reported to be consistent with EEG memory studies in which increases in alpha activity have been reported (eg. Klimesch, Doppelmayr, Schwaiger, Auinger & Winkler, 1999; Krause, Lang, Laine, Kuusisto & Porn, 1996).

Kemp et al. (2002) describe the effects of processing differently valent emotional stimuli on the SSVEP. Seventy-five images were selected from the IAPS and presented to healthy participants whilst brain activity was recorded from 64 electrodes. The SSVEP corresponding to the neutral images was subtracted from the SSVEP associated with both pleasant and unpleasant images and the averaged and time-series data were then examined. This particular study was conducted by the author of this thesis and is included as the first experimental chapter. The study reported that transient, widespread and bilateral frontal SSVEP latency and occipital amplitude reductions are associated with the processing of pleasant and unpleasant relative to neutral visual stimuli. In addition, unpleasant relative to neutral images were associated with left temporal and right occipitotemporal latency reductions, a bilateral anterior frontal amplitude reduction and centroparietal amplitude increases. One of the benefits of the SSPT technique is the ability to track rapid and continuous brain-surface electrical activity. This research represents the first study to employ this technique in order to examine phasic emotional responses.

Silberstein et al., (2003) examined the effects of a sequential version of the Shepard and Metzler mental rotation task (Shepard & Metzler, 1971) on the SSVEP-event related partial coherence (SSVEP-ERPC). This task required participants to determine whether a second shape presented 3 seconds after the first shape was either identical to the first shape (except for a rotation about the vertical axis) or a mirror image of the first shape. During the 180º rotation, compared to the 60º rotation, the authors report increased synchronisation between bilateral prefrontal and parieto-occipital sites proposing that that this increase in coherence may relate to the working memory component of the task. The authors also report increased synchronisation between left frontal and right parietal sites, and speculate that this activity may relate to the increased interaction thought to be associated with the completion of a number of visuo-motor tasks. This study sheds new light on the functional coupling between frontal (including the motor and supplementary motor cortex) and parietal cortices that is engaged during mental rotation.
Gray et al. (2003) examined the temporal dynamics of the SSVEP whilst participants conducted the CPT-AX task during anticipation of an electric shock relative to a relaxed condition in which there was an assurance of no electric shock. The authors report that anticipatory anxiety was associated with SSVEP latency increases within medial anterior frontal electrodes, left dorsolateral prefrontal and bilateral temporal sites. In addition, SSVEP amplitude increases and latency decreases were observed within the occipital region. Importantly, this study selected a subset of subjects to examine the effects of jaw clench on SSVEP activity in order to insure that the results from this study were not contaminated by electromyographic noise. Results from this analysis indicated no statistically significant difference between the baseline and the EMG condition at any electrode site, confirming that the findings were not due to the presence of jaw clench during anticipation of electric shock.

All of these studies illustrate the utility of SSPT when examining rapid and continuous changes in brain activity during not only visual vigilance and attentional tasks (Silberstein et al., 1995, 1998, 2003; Silberstein, Line et al., 2000), but also working and long-term memory (Silberstein, Harris et al., 2000; Silberstein et al., 2001), emotional processing (Kemp et al., 2002) and anticipatory anxiety (Gray et al., 2003). Furthermore, SSPT is documented to be especially resistant to common artefacts such as electro-oculograms (EOG) and electro-myograms (EMG). (See Silberstein, 1995 for discussion, Silberstein, Pipingas et al., 1991 and Silberstein, Schier, Pipingas & Ciocciari, 1991 for analysis of EOG artefact, and Gray et al., 2003 for analysis of EMG artefact). The reason for this is that SSPT analysis involves the examination of the spectral characteristics of the SSVEP through Fourier analysis, rather than the calculation of ensemble averages of EEG associated with a time-locked stimulus as in ERP analysis. While the power of artefact such as EOG and EMG is distributed across a range of frequencies, Fourier analysis of the SSVEP focuses on the signal power of the stimulus frequency. SSPT therefore, is ideally suited to the tracking of phasic emotional responses associated with the processing of emotional stimuli.
CHAPTER 3

3 STEADY-STATE VISUALLY EVOKED POTENTIAL (SSVEP) TOPOGRAPHY DURING PROCESSING OF EMOTIONAL VALENCE IN HEALTHY SUBJECTS.
3.1 Introduction

The investigation of emotional processing and examination of how the brain mediates emotional experience is once more an area of significant research interest (LeDoux, 2000). The brain mechanisms involved in emotional processing however are no longer explained by sole reference to the limbic brain (MacLean, 1949; MacLean, 1952; Papez, 1937), but by a number of regions and their interconnections. These include the DLPFC, VMPFC, OFC, amygdala, hippocampus, ACC and the insular cortex (Davidson, 2000).

A number of studies have reported anterior lateralization during discrete emotional states. This has generally involved increases in right-sided activation during unpleasant affect (eg. disgust) and left-sided activation during pleasant affect (eg. happiness). Cortical lateralization has been demonstrated in EEG studies using video clips (eg. Davidson et al., 1990; Jones & Fox, 1992) as well as images from the IAPS (Aftanas et al., 2001a & 2001b) and these findings are supported in studies using PET and fMRI (Sutton et al., 1997a; Canli et al., 1998 respectively). Following presentation of images selected from the IAPS, researchers report hemispheric lateralization when arousal is similar between differently valenced conditions (Canli et al., 1998). However, Canli et al. (1998) also indicate that valence related lateralization was only partially evident and that hemispheric laterality is a fragile phenomenon.

Other studies have reported activation in overlapping areas and no frontal hemispheric laterality (Baker et al., 1997; George et al., 1995; Lane, Fink et al., 1997; Lane, Reiman, Ahern et al., 1997, Lane, Reiman, Bradley et al., 1997; Pardo et al., 1993; Teasdale et al., 1999). For example, a study using PET reported increased activation within the thalamus and MPFC (Brodmann’s area 9) for happiness, sadness and disgust emotions induced by film as well as recall (Lane, Reiman, Ahern et al., 1997). Similarly, another PET study using the IAPS distinguished pleasant and unpleasant emotions from neutral emotion by increased activation within the MPFC, thalamus, hypothalamus and midbrain (Lane, Reiman, Bradley et al., 1997). These findings have been supported by an fMRI study, using picture-caption pairs (Teasdale et al., 1999).

Findings, in terms of frontal hemispheric laterality have been varied and contradictory and could in part, be accounted for by failing to control for the levels of arousal within
and between valenced categories. Although it has been suggested that arousal is associated with the activation of the right-parieto-temporal region (Aftanas et al., 2001a; Heller et al., 1998), frontal regions including the OFC (Barbas, 2000), the VMPFC, the DLPFC and the ACC have also been associated with the modulation of emotional autonomic responses such as skin conductance (Damasio, Tranel & Damasio, 1990; Tranel & Damasio, 1994; Zahn, Grafman & Tranel, 1999). It should be highlighted that this measure of emotional responsivity has been reported to covary positively with judged emotional arousal (Lang, Bradley & Cuthbert, 1998).

It is recognized that research must focus on well-defined aspects of emotion (LeDoux, 2000), in order to properly investigate emotional processing. The IAPS is an increasingly popular means to investigate emotional processing in neuroimaging studies. This task has been developed to selectively activate appetitive and defensive motivational systems and is believed to be able to evoke a broad range of emotions experienced outside the laboratory (Lang et al., 1997).

The IAPS contains emotional visual stimuli that have been rated on valence and arousal dimensions, which allows research to systematically manipulate these variables to determine the associated processing. However, many imaging studies using this task have not attempted to control for the effects of one dimension whilst varying the other. For example, pleasant content has previously ranged from sexual content to ice cream and smiling babies whilst unpleasant image content has ranged from mutilations to a gun aimed at the viewer (eg. Lane, Flnk et al., 1997; Lane, Reiman, Bradley et al., 1997; Mini et al., 1996). Such examples confound emotional valence with emotional arousal which may lead to differences in brain activation.

A number of studies have systematically varied either valence or arousal (eg. Canli et al., 1998 and Garavan et al., 2001 using fMRI and Taylor et al., 2000 using PET respectively), however due to the nature of the techniques used, these studies focused on the regions involved rather than temporal dynamics. The varied and contradictory findings reported in the literature may also be due in part, to not investigating the temporal processes associated with the processing of emotional stimuli. The phasic nature of emotional processing has long been understood (Ekman, 1984), however studies are only beginning to investigate the temporal processes. It has been suggested that hemispheric asymmetry may be more prominent early on in image processing (Roschmann & Wittling 1992) and that differential hemispheric laterality for pleasant and unpleasant conditions cannot be
distinguished when data is averaged across the entire film period (Davidson et al., 1990). Studies that have focused upon the temporal dynamics have largely focused on event related potential (ERP) activity associated with the initial processing of emotional stimuli (eg. Junghofer et al., 2001; Kawasaki et al., 2001). ERP studies have also been characterized by failing to control for arousal when varying valence (eg. Mini et al., 1996).

SSPT is a technique that is able to track rapid changes occurring in brain electrical activity during the ongoing processing of stimuli (Silberstein et al., 1990, 1995, 1996, 1998, 2000, 2001). SSPT examines changes to the 13Hz SSVEPs and offers not only, relatively high temporal resolution compared with PET and fMRI, but also temporal continuity. The SSVEP is characterized by two components. These are SSVEP amplitude, which may be compared to alpha activity in association with cognitive tasks (Silberstein, 1995a, b) and SSVEP latency, which has been proposed to index changes in the neural information processing speed (Silberstein et al., 1996, 2000).

The aim of the present study therefore was to use SSPT to examine the statiotemporal characteristics of the SSVEP associated with the processing of pleasant and unpleasant images low in arousal content. It was hypothesized therefore that the effects of emotional stimuli on the SSVEP would be most evident when observing the time-series data and that the valence-related effects on the SSPT would be predominantly frontally distributed. It was also hypothesized that emotional images, after subtraction of the processing associated with the neutral images would induce valence specific lateralization within the frontal regions.

3.2 Methods

3.2.1 Participants

16 healthy subjects, consisting of 11 males (M = 24.18 yrs, SD = 5.51) and 5 females (M = 25.40, SD = 6.07), participated in the current study. All participants were right handed as assessed by the Edinburgh Inventory (Oldfield, 1971), drug free and had no history of epilepsy, head injury, stroke, psychiatric illness, neurological disorders, or alcoholism. (See Appendices 9.1 for the personal information questionnaire completed by all participants).
3.2.2 The International Affective Picture System (IAPS)

75 IAPS images were chosen based upon the standardized valence and arousal ratings provided in the IAPS manual (Lang, Bradley & Cuthbert, 1999), and were selected so that valence was varied (unpleasant, neutral and pleasant) whilst arousal remained relatively low for all categories. Chosen images were not rated greater than 6 on the arousal scale which ranged up to a score of 9. The unpleasant, neutral and pleasant categories were characterized with valence ratings ranging between 1.8 and 3.47; 4.46 and 5.46; 7.02 and 8.34, respectively and arousal ratings ranging between 3.52 and 5.5; 1.55 and 4.27; 2.67 and 5.94, respectively. A one-way analysis of variance (ANOVA) was conducted on SPSS software (SPSS Inc., 1999) to assess for any differences in brightness and contrast (as determined by luminosity histogram plots in Adobe Photoshop) between pleasant, unpleasant and neutral picture-categories. No significant differences were found between any categories of images for brightness \( F(2,72)=0.67, p=0.52 \) and contrast, \( F(2,72)=0.83, p=0.44 \), respectively. (The images selected for presentation in the experimental chapters contained in this thesis are displayed in Appendices 9.3, 9.4 and 9.5).

3.2.3 Procedure

The SSPT recording technique was explained and the recording only took place when participants were completely relaxed and comfortable, and when they had a complete understanding of how to rate each image. IAPS images were presented to participants in 3 blocks (25 unpleasant, 25 neutral and 25 pleasant). Each image was presented once only (6-seconds duration) and was followed by the Self-Assessment Manikin (SAM) rating scale. SAM enables participants to rate each image for valence and arousal (Lang et al., 1999). (SAM was modified to display the valence and arousal scales only).

Participants were randomly allocated to a group that had either the unpleasant or pleasant category presented first. All participants were presented each of the 3 categories of images, and the neutral category was always presented between the pleasant and unpleasant categories. Participants were asked to focus on emotional content, to refrain from emotive inhibition, and to rate each image (using SAM) as they actually felt whilst viewing it. (See Appendix 9.2 for explicit instructions provided to participants).

Brain electrical activity was recorded by 64 monopolar leads using a lycra electrode cap with chin strap, with linked ears as the reference and a nose electrode used for
ground. The sites were located in International 10/20 positions as well as sites midway between these positions. Electrode impedance generally remained lower than 5 KOhms. The bandpass filter was set at 0.74Hz and 74Hz prior to digitization to 16-bit accuracy at a rate of 500Hz. A diffuse 13Hz sinusoidal white-flicker, superimposed on the visual field by a pair of goggles, elicited the SSVEP and subtended a horizontal angle of 160° and a vertical angle of 90°.

3.2.4 Signal Processing and Analysis

The major features of the signal processing procedures within our institute have already been described (see chapter 2), thus, only procedures specific to the present study’s analysis will be discussed. Figure 11 summarises the major methodological steps taken to analyse the IAPS SSVEP data and to present the topographic maps. SSVEP’s were produced for all electrodes from the 13Hz Fourier coefficients (FC) and were then evaluated using a 10 unit window. For each stimulus cycle, this evaluation period averaged overlapping blocks of 10 FCs and the coefficients recalculated for this overlapping period. This yields a time series with 13 points/sec corresponding to a temporal resolution of 0.77 seconds. This procedure was conducted for the entire recording period for all 3 image-categories. A target averaging technique was then used to select the SSVEP epochs that corresponded with the presentation of an image and all selected epochs for each of the 3 categories were then averaged.

Both components of the SSVEP (amplitude and phase difference between the sinusoidal visual stimulus and the SSVEP) were normalized. Amplitude was normalized by first calculating a M for each of the 64 electrodes for the neutral category across the length of the time series data and then averaging these 64 values to yield a single normalization factor (NF). The amplitude from all categories from each electrode for each individual was then divided by the NF. In addition, SSVEP phase was normalized by first calculating a M for each of the 64 electrodes in the SSVEP time series for the neutral condition (reference task). These values (one for each of the 64 electrodes) were then subtracted from the phase for all categories for each of the corresponding electrodes. Changes in phase (expressed as radians) are expressed in terms of latency (milliseconds) using the formula: (change in phase/2π) x (1000/13).
The SSVEP epochs for each individual were then averaged together to form averages of pleasant, neutral and unpleasant images. The SSVEP epoch corresponding to the neutral images was then subtracted from both emotional categories to yield the activity associated with either pleasant or unpleasant valence. The neutral condition was used as a reference task for both emotional conditions in order to remove aspects of processing unrelated to the processing of either pleasant or unpleasant valence as conducted previously (e.g., Lane, Reiman, Bradley et al., 1997; Northoff et al., 2000).

Figure 12: Methodological steps taken to analyse the recorded data for the IAPS task.

3.2.5 Topographic Mapping and Statistical Analysis

The complex SSVEP time series was then displayed in topographic maps produced using a spherical spline interpolation procedure (Nunez et al., 1994). The topographic maps express the SSVEP in terms of amplitude and the latency differences following subtraction of the neutral reference condition. The statistical strength of the differences between the emotional conditions and the neutral condition were examined using the Hotellings $T^2$ parameter and each presented topographic map was corrected for 5 multiple comparisons (as discussed in section 2.2.5).
Topographic maps representing unpleasant and pleasant valence are presented for the average of the 6-second data (Figure 12). For these reasons a strict alpha level of 0.005 was chosen to counter for the multiple comparisons being made both in terms of the number of electrodes used as well as the two types of emotional activation. In addition, a time-point was chosen for display of temporal characteristics (Figure 13). The literature suggests that it is the anterior frontal locations that are most important in the emotional processing of valence, therefore a frontal electrode was chosen. A time-point at which unpleasant and pleasant categories are maximally discriminated from the neutral condition, was then selected from the time-series (of the selected electrode) (see Figure 12) and then re-presented in additional topographic maps (Figure 13).

### 3.3 Results

#### 3.3.1 Behavioural Results

All participants were required to rate each image on valence and arousal scales. The means and standard deviations for the ratings of valence and arousal made by participants are detailed in Table 3.

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Valence M (SD)</th>
<th>Arousal M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpleasant</td>
<td>16</td>
<td>3.50 (0.17)</td>
<td>3.81 (0.45)</td>
</tr>
<tr>
<td>Neutral</td>
<td>16</td>
<td>5.08 (0.08)</td>
<td>1.96 (0.19)</td>
</tr>
<tr>
<td>Pleasant</td>
<td>16</td>
<td>6.14 (0.14)</td>
<td>3.20 (0.35)</td>
</tr>
</tbody>
</table>

A univariate repeated measures ANOVA (RANOVA) was conducted for both the valence and arousal dimensions separately. Multivariate results are reported for the valence dimension as Mauchly’s Test of Sphericity (MTS) was violated for this dimension. (Visit: http://www.statsoft.com/textbook/stanman.html#assumptions for a discussion on MTS).

Significant category effects were found for both valence [Pillai’s = 0.91, F(2,13) = 63.18, p<0.001, \textit{partial eta squared} = 0.907] and arousal [F(2,13) = 19.60, p=<0.001, \textit{partial eta squared} = 0.583]. For the valence rating scale, planned comparisons revealed significant differences between the unpleasant and neutral categories,
[F(1,14)=116.73, p<0.001, partial eta squared = 0.893] as well as pleasant and neutral categories, [F(1,14)=36.68, p<0.001, partial eta squared = 0.724]. For the arousal rating scale, significant differences between the unpleasant and neutral categories, [F(1,14)= 32.79, p<0.001, partial eta squared = 0.701], pleasant and neutral categories, [F(1,14)=27.50, p<0.001, partial eta squared = 0.663] were also revealed, but not for pleasant and unpleasant categories, [F(1,14)=3.65, p=0.077].

3.3.2 SSPT Results

The amplitude and latency SSVEP’s for affective (unpleasant and pleasant) relative to neutral images is displayed in Figure 12. These maps reflect the SSVEP activation that corresponds to the M of the 6-second time series (78 13Hz cycles). Warmer colours in these maps indicate reduced amplitude and reduced latency during the presentation of affective images.

Figure 12 demonstrates that pleasant valence is associated with a frontal amplitude increase and latency decrease bilaterally as well as an amplitude decrease and latency increase within the occipital region (Hotellings T (15) > 3.29, P< 0.005). SSVEP during the processing of unpleasant valence displayed an amplitude increase and latency reduction in the left temporo-parietal, posterior frontal, and right-anterior temporal regions (Hotellings T (15) > 3.29, P< 0.005).

The time-series for electrode 0 (located in the left anterior prefrontal region) is displayed in Figure 12. Latency was best able to differentiate between emotional and neutral images at frontal sites. In particular it was electrode 0 (left prefrontal) which displayed the maximal latency difference. A time-point was selected which corresponded to the point at which emotional images were maximally discriminated from the neutral condition and this time-point is displayed topographically in Figure 13. This difference was significant at an alpha level of 0.005 at the 1462 msec time-point for both emotional conditions relative to the neutral condition.
Figure 13 indicates that unpleasant valence is associated with a bilateral anterior-frontal amplitude decrease (particularly pronounced within the left anterior frontal), an amplitude increase within the centro-parietal region, an amplitude decrease within the occipital region, as well as latency reductions within the frontal, left temporal and right occipito-temporal regions. These effects are statistically significant within the left anterior-frontal, the right posterior frontal, left anterior temporo-parietal, and right occipito-temporal regions (Hotellings $T^2(15) = 3.29, P< 0.005$). Pleasant valence produced a reduction in amplitude within the occipital region, and frontal latency.

Figure 14: Topographic presentation of the 13Hz SSVEP (amplitude and latency) for unpleasant and pleasant valence at 1462ms (temporal resolution, 0.77s).
reductions. These effects were significant in the left anterior frontal, right lateral posterior frontal and occipital regions (Hotellings $T^2(15) = 3.29$, $P< 0.005$).

### 3.4 Discussion

The current study investigated the statiotemporal characteristics of the SSVEP associated with processing of low arousal, unpleasant and pleasant valence.

Behavioral results for valence confirm that the allocation of images to pleasant, neutral or unpleasant categories based upon IAPS standardized values was appropriate for the current Australian sample. In addition, behavioral results indicate that although pleasant and unpleasant categories do not differ from each other on the arousal dimension, both emotional conditions differ from the neutral condition. As illustrated in a recent paper (Bradley, Codispoti, Cuthbert & Lang, 2001), when IAPS images are distributed on a 2-dimensional plot according to valence and arousal ratings, the resulting plot forms a boomerang shape in which the arms reach toward the high arousal quadrants. This indicates that it is difficult to select either positively or negatively valenced images which are equivalent to neutral images on the arousal dimension. It is important to note however, that no category of images presented to subjects in the current study contained a mixture of low and high arousal content, unlike in previous studies (eg. Lane, Finke et al., 1997; Lane, Reiman, Bradley et al., 1997; Mini et al., 1996).

A number of key findings are reported which demonstrate the spatiotemporal characteristics of the SSVEP during emotional processing (Figure 13). Firstly, latency reductions were displayed within the frontal regions for both unpleasant and pleasant valence. In addition, latency reductions were also displayed within the left temporal and right occipito-temporal regions for unpleasant valence. Secondly, a number of amplitude effects were identified. Both pleasant and unpleasant valence was associated with an amplitude reduction within the occipital region. In addition, an amplitude reduction within the bilateral anterior frontal and an amplitude increase within centro-parietal regions were displayed for unpleasant valence. Results also demonstrate differences between averaged and transient activity (discussed below) emphasizing the importance of focusing on transient activity when investigating the nature of emotional processing.
The SSVEP is believed to reflect neuronal activity primarily within the pyramidal cells of the neo-cortex and is characterised by two components. The first component is amplitude, which has been previously interpreted as analogous to the amplitude of regional activity within the alpha frequency range (Silberstein, 1995a, b). In this framework, an amplitude reduction may be regarded as an electrophysiological correlate of activated cortical areas and is known as event related desynchronisation (ERD) (See Pfurtscheller & Lopes da Silva, 1999 for a review). Previous findings, which have demonstrated reductions in SSVEP amplitude within occipito/parietal and centro/parietal regions during a visual vigilance task (Silberstein et al., 1990) and also within prefrontal sites during the set change of the Wisconsin Card Sort Task (Silberstein et al., 1995), support this interpretation. Latency, the second component of the SSVEP, may be interpreted as reflecting neural information processing speed. This interpretation is again supported by previous studies, which have demonstrated that reaction time in a visual vigilance task CPT A-X correlates with frontal SSVEP latency (Silberstein et al., 1996, 2000). Increases in neural information processing speed may be related to increased levels of cortical excitability as indicated by animal studies which have shown reductions in thalamo-cortical transmission time following the release of the excitatory neurotransmitter ACh (eg. Methrate & Ashe, 1993). In addition, latency reductions may also be related to cortico-cortico processing as proposed in a recent model (Silberstein et al., 2001).

Before discussing the significance of our findings, it is important to emphasize that the time-point (1462msecs) discussed in the present paper (and presented topographically in Figure 13) corresponds to the point in the time series in which the emotional conditions are maximally discriminated from the neutral condition. Previous studies have reported much earlier emotional discrimination. These have used for example, scalp recorded event related potentials (150ms-260ms) (Junghofer et al., 2001) and in-dwelling electrodes (120-160ms) to record from single-neurons (Kawasaki et al., 2001). It is indicated by the authors that these effects are related to initial conceptual or encoding processing of the images, rather than conscious processing of stimuli, likely to be reflected in the SSVEP (Silberstein et al., 1990). The present study does not investigate the temporal aspects of early emotional discrimination processes, but rather the spatiotemporal characteristics associated with ongoing emotional processing with specific regard to the point of maximal difference in SSVEP between the emotional and neutral categories.
All methodologies have their strengths and weaknesses and while event related potential (ERP) techniques are useful for investigating the processing immediately following stimulus presentation, they do not allow for investigation of time-extended processes (Silberstein et al., 1990). SSPT by contrast, does not have as good a temporal resolution as ERP’s, however it allows for an examination of the temporal continuity of cortical activations, and as a result, may be useful for investigating the conscious, ongoing processing of emotion. Interestingly, our findings support the proposition that the PFC will be important for guiding the processing of emotional stimuli at not only pre-conscious but additional temporal scales (Kawasaki et al., 2001).

The current paper reports a number of interesting SSPT findings, and these are discussed in the following paragraphs. Statiotemporal characteristics for the latency data indicate increases in excitatory processes within frontal regions during processing of both unpleasant and pleasant valence, suggesting that processing within these regions is not specific to valence type. This finding supports previous studies (Lane, Reiman, Ahern et al., 1997; Lane, Reiman, Bradley et al., 1997; Reiman et al., 1997) that have shown substantial overlap in emotional activation irrespective of valence. Our findings indicate, temporally at least, that anterior neural mechanisms for pleasant and unpleasant affect are closely linked.

Both unpleasant and pleasant valence also displayed transient amplitude reductions within the occipital regions. In addition, unpleasant valence displayed transient latency reductions within the right occipito-temporal region. While the literature suggests that emotional processing involves areas within the PFC (for a comprehensive review see, Davidson and Irwin, 1999), the role of posterior regions in emotional processing are yet to be clarified. As cortical and sub-cortical structures are involved in emotional processing, the interpretation of the data within regions posterior to frontal locations is tentative because the SSPT technique does not allow investigation of sub-cortical activation.

However, our findings support previous studies which demonstrate activation within visual cortical areas in response to emotional images of both unpleasant and pleasant valence. For example, in an fMRI study, Lang and colleagues compared emotional to neutral images and reported activation within the occipital gyrus bilaterally, the right fusiform gyrus and the right inferior and superior parietal lobules and that this activation was not due to eye movement artefact (Lang, Bradley,
Fitzsimmons et al., 1998). More recent studies have confirmed visual cortical activation in response to emotional stimuli (Hamman et al., 2002; Lane et al., 1999), and suggest that this activity may be modulated by the amygdala (Cahill & McGaugh, 1998; Morris et al., 1998; Morris et al., 1999). Studies are beginning to suggest that pleasant valence may modulate the amygdala and visual cortical activity in addition to unpleasant valence, though this still appears more extensive with unpleasant valenced images (Hamman et al., 2002). This may account for our finding that in addition to amplitude reductions, unpleasant valence was also associated with a transient latency reduction in the right occipito-temporal region.

A number of spatiotemporal differences were also apparent between unpleasant and pleasant valence. In comparison to neutral images, unpleasant images are associated with a transient bilateral anterior frontal amplitude reduction. These effects were not evident for pleasant images relative to a neutral reference. While our findings demonstrate that positively and negatively valenced images compared with neutral images are associated with increases in neural information speed (reductions in latency), unpleasant images compared with neutral images are also associated with increased activation (reductions in amplitude) within anterior-frontal regions. This latter finding is consistent with the findings reported in a previous study which demonstrate that positive emotional processing is associated with weaker activation (compared with negative emotional processing) within the lateral orbitofrontal/prefrontal cortex (Northoff et al., 2000).

Unpleasant images also displayed significant activation within the left anterior temporo-parietal cortex. Again, it is important to be cautious regarding the interpretation of activation posterior to the frontal lobes, however, left temporo-parietal activation during unpleasant valence may be related to increased left amygdala activation, previously associated with the viewing unpleasant images (Lane, Reiman, Bradley et al. 1997) as well as sad mood induction (Grodd, Schneider, Klose & Nagele, 1995; Levesque et al., 2003; Posse et al., 2003; Schneider et al. 1995, 1997) and depression (Drevets, Videen, Price et al., 1992). Furthermore, Lane and colleagues report that activation of the left medial temporal lobe (including the amygdala and parahippocampal gyrus) distinguished unpleasant from neutral as well as pleasant emotions. Significant activation within this same region remains when averaging the entire image-viewing period for unpleasant images compared to the average for neutral images (Figure 12). In addition, significant frontal activation was not displayed during the average of the picture.
viewing period for unpleasant valence suggesting that frontal activation maybe more of a transient phenomenon when processing unpleasant images low on arousal. By contrast, the averaged SSVEP for the pleasant valence over this same period (Figure 12) however, is associated with a bilateral frontal activation. These findings emphasize the importance of focusing on transient, phasic activity associated with the processing of emotional valence.

The use of the IAPS task in brain imaging studies is becoming established as a useful way to probe the brain during the processing of emotional stimuli. However, it is important that valence and arousal are carefully controlled in the design of activation studies. Our data demonstrate that both pleasant and unpleasant pictures low in arousal content are associated with bilateral frontal latency reductions supporting previous studies that have shown substantial overlap in processing of unpleasant and pleasant valence. Furthermore our results support previous research which, after subtracting activation associated with neutral images from that associated with both pleasant and unpleasant images, reported an increase in cerebral blood flow (CBF) within the MPFC (Lane, Reiman, Bradley et al., 1997).

The current findings imply that overlapping frontal activations for unpleasant and pleasant valence are not due to the failure of some studies to control for the levels of arousal within and between valenced categories. Although frontal regions (such as the OFC) have been implicated in arousal functions, a body of evidence suggests that the frontal lobes may mediate expressive functions, whilst the posterior regions mediate perceptual (Ahern & Schwartz, 1985; Ley & Bryden, 1981) and arousal functions (Aftanas et al., 2001a; Heller et al., 1998). In addition to the current findings therefore contradictions, in terms of frontal laterality specifically, may be due to other factors such as the degree with which stimuli are able to elicit approach or withdrawal behaviors. For example, anger, despite its negative valence is regarded as an approach-related emotion and has been associated with left frontal activation (Harmon-Jones & Allen, 1998). In addition, adult emotions such as sadness have been described as complex blends of approach and withdrawal emotions (Jones & Fox, 1992). The current results support the notion that anterior laterality is not related to differences in valence after possible effects of arousal have been controlled for. It is also possible, however, that the selected time point reflects conscious and voluntary regulatory processes in addition to affective elicitation.
While the current study investigated brain activation in response to images differing on emotional valence, there are other factors that influence emotional processing which include emotional arousal and gender differences. In order to fully understand the neurophysiology of emotional processing, future studies will need to address the specificity of these issues to the processing of affective images. Given the relative ease with which IAPS images can be systematically selected, it would be particularly interesting for future studies to investigate the effects of images varying in terms of arousal on regions believed to mediate the processing of emotional arousal, primarily the right parieto-temporal region (Heller et al., 1998) but also frontal regions which may modulate the SCR in specific situations having emotional significance (Zahn et al., 1999). With respects to gender, very few studies have investigated differential effects on emotional processing. This may in part, be due to the perception of women’s increased responsiveness to emotional stimuli (eg. Canli et al., 2001; Lane, Reiman, Ahern et al., 1997; Lane, Reiman, Bradley et al., 1997). It is important to note however, that although men and women may differ in terms of global, memory based measures of emotion, they do not differ when documenting their emotional reactions on a moment-to-moment basis (Barrett et al., 1998).

In summary, the current study suggests that bilateral frontal latency reductions are associated with both pleasant and unpleasant valence (in terms of transient activation for images rated low on arousal), and supports previous literature that suggests substantial overlap in frontal emotional activation irrespective of emotional valence. In addition, unpleasant images were associated with a transient bilateral anterior-frontal amplitude decrease, which may reflect differences in degree of activation. Finally, regional posterior differences were noted for unpleasant and pleasant valence and these may be related to connections with subcortical areas.
CHAPTER 4

4 GENDER DIFFERENCES IN THE CORTICAL ELECTROPHYSIOLOGICAL PROCESSING OF VISUAL EMOTIONAL STIMULI
4.1 INTRODUCTION

Gender differences in the brain have been well characterised in animals and to a lesser extent, in humans (Cooke, Hegstrom, Villeneuve & Breedlove, 1998; Rabinowicz, Dean, Petetot & de Courten-Myers, 1999; Rabinowicz et al., 2002; Supprian & Kalus, 1996). Although the functional significance of these differences are unclear (Rabinowicz et al. 1999; Supprian & Kalus, 1996), research is now beginning to examine the gender differences in emotion. This is an important endeavour considering that emotion has been described as the key component in personality and vulnerability factors governing risk for psychopathology (Davidson, 2002). With regards to the disorders of emotion, it is known that the life-time risk for depression is 10-25% for women but only 5-12% for men (American Psychiatric Association, 1994). A more recent survey based on the ICD-10 classification found 6% of adults to suffer from depressive disorders and that twice as many females as males experience depression (Australian Bureau of Statistics, 1997). Differences in biology as well as gender-related environmental experiences are regarded as the key to understanding these gender-related differences in depression (Kessler, 2003).

‘Emotion’ may be defined as a relatively brief episode of synchronised response involving multiple components including cognitive processes, physiological responses, motivation changes, motor expression and subjective feeling (Borod, 1993; Ekman, 1984, 1992; Lang, 1968, 1984; Scherer & Peper, 2001). By contrast, ‘mood’ is generally considered to be a more diffuse state, characterised by low intensity, but relatively long duration (lasting hours to days) (Ekman, 1992; Ketter et al., 2003; Scherer & Peper, 2001). Although, these terms relate to different behavioural constructs, it is possible that chronic or repeated activation of certain underlying neurophysiological mechanisms may be the connection between emotional experience and mood disorders such as depression and anxiety. For example, theories have been proposed which describe certain neurophysiological processes as they relate to longer lasting affective phenomena such as depressed and anxious mood (eg. Heller et al., 1993). Furthermore, brain activation resulting from the use of certain mood induction techniques in healthy participants is regarded as similar to that seen in mood disorders such as depression (see Lawrence & Grasby, 2001 for discussion).

A limited but growing number of studies have investigated whether gender differences in brain activation exist on tasks designed to assess a broad range of
emotional processes. These studies are important to enable researchers to move beyond employment of behavioural methodologies which have been criticised for their inability to illuminate processes inaccessible to consciousness (e.g. Davidson et al., 2003). Studies that have investigated emotional perception have reported either no gender differences (Meyers & Smith, 1986), subtle differences between males and females (Morita et al., 2001; Wildgruber, Pihan, Ackermann, Erb & Grodd, 2002), or sex-specific areas of brain activation (Killgore et al., 2001; Lee et al., 2002). Tasks that involve more the experience of emotion (e.g. Del Parigi et al., 2002; George et al., 1996; Pardo et al., 1993; Schneider et al., 2000; Pendergrass, Ross, Garavan, Stein & Risinger, 2003) have reported more consistent gender differences. Healthy women have been shown to display more activity (i.e. larger number and more widespread significant differences between transient induced negative mood states and baseline) than healthy men in anterior limbic structures such as the inferior frontal, orbital and prefrontal cortices, during transient induced sadness (e.g. George et al., 1996 & Pardo et al., 1993). These studies have also demonstrated that women show more bilateral activation without asymmetries during induced sadness. For example, in a female-only study, increases in activity were reported within the thalamus and medial prefrontal using both film as well as recall induced emotional states (Lane, Reiman, Bradley et al., 1997). Pardo and colleagues (1993) demonstrated left-sided activation of inferior frontal and orbitofrontal cortices in males, whilst bilateral activation of these areas was reported in females. In addition, sadness has been associated with amygdala activation in males but not females (Schneider et al., 2000). The authors suggested that females produce less concentrated and less lateralised brain activation than males. Bilateral findings in females are consistent with a widely held neuropsychological theory on the organisation of the brain which posits that females are more bilateralised than men (Iaccino, 1993; Levy & Heller, 1992; McGlone, 1986). While these studies examining emotional experience have shown gender differences, inconsistent differences have been reported. For example, George and colleagues (1996) report that women activate a greater portion of their limbic system than men during transient sadness, whilst Schneider and colleagues (2000) report that processing of sadness is more focal and subcortical in men. The literature focusing on transient happiness has also been inconsistent. For example, decreases as well as increases in activity have been reported for happiness (see George et al., 1995 and Lane, Reiman, Bradley et al., 1997 respectively). Gender differences in transient happiness however may be more subtle. This is supported by previous studies which report either slight or no differences for happiness (George et al., 1996; Schneider et al., 2000, respectively).
It should be noted however that a number of problems arise when attempting to compare studies that have investigated gender differences in emotion. First, brain imaging studies have used a variety of different paradigms. These paradigms have included recollection of sad events (Pardo et al., 1993), perception and experience of human emotional non-verbal sounds (Meyers & Smith, 1986; Smith et al., 1995), recollection of affect-specific events (George et al., 1996), viewing of faces with either happy or sad facial expressions to aid mood induction (Schneider et al., 2000), the recognition of facial affect (Kesler-West et al., 2001; Killgore et al., 2001; Lee et al., 2002; Morita et al., 2001), the processing of emotionally evocative images (Canli, Desmond, Zhao & Gabrieli, 2002; Pendergrass et al., 2003), the detection of emotional intonation (Wildgruber et al., 2002), and the experience of hunger and satiation (Del Parigi et al., 2002). Second, there are a large number of different neuroimaging techniques used, which range from PET (Del Parigi et al., 2002; George et al., 1996; Pardo et al., 1993), fMRI (Canli, Desmond et al., 2002; Kesler-West et al., 2001; Killgore et al., 2001; Lee et al., 2002; Pendergrass et al., 2003; Schneider et al., 2000; Wildgruber et al., 2002) and different electroencephalographic techniques (Meyers & Smith, 1986; Morita et al., 2001; Smith et al., 1995).

The IAPS has become increasingly used amongst brain imaging studies to investigate emotional processes as it allows for systematic selection of images that range in emotional content. Specifically, these images are associated with standardised ratings for valence and arousal which allows researchers to easily replicate published findings for a specific selection of images and also aid interpretation of and allow conclusions to be drawn from multiple studies using this task. Previous studies using the IAPS have investigated emotional processing with haemodynamic imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (eg. Canli et al., 1998; Canli, Desmond et al., 2002; Lane, Fink et al., 1997, Lane, Reiman, Bradley et al., 1997; Lang, Bradley, Fitzsimmons et al., 1998; Paradiso et al., 1999; Pendergrass et al., 2003; Taylor et al., 1998; Wrase et al., 2003), electroencephalographic based techniques (eg. Aftanas et al., 2001a,b; Aftanas, Varlamov, Pavlov, Makhnev & Reva, 2002; Junghofer et al., 2001; Kawasaki et al., 2001; Kemp et al., 2002, also included as chapter 3; Mini et al., 1996; Palomba et al., 1997; Schupp et al., 2000, 2003) as well as magnetoencephalography (Northoff et al., 2000, 2002).
However few of these studies have examined gender differences (Canli, Desmond et al., 2002; Pendergrass et al., 2003; Wrase et al., 2003). These studies suggest there to be gender differences in a number of neural structures including the insular, prefrontal and parietal cortices, bilateral visual processing areas, thalamic nuclei, amygdala, caudate, putamen and pons regions, and the postcentral and parahippocampal gyri, during the processing of visual emotional stimuli. By contrast, a recent behavioural study which examined gender differences in IAPS images in terms of valence and arousal ratings, facial EMG, skin response and HR, suggests that ‘remarkable congruence’ was displayed in the physiological profile between the two genders when viewing images having less arousing appetitive and defensive contexts (Bradley, Codispoti, Sabatinelli & Lang, 2001). In addition, Wrase and colleagues (2003) have recently reported that although no significant differences were found between males or females in valence, arousal, skin conductance response and startle modulation, men displayed stronger brain activity for positive visual stimuli in the inferior and medial frontal gyrus whilst women displayed stronger brain activity for negative visual stimuli in the anterior and medial cingulate gyrus. These findings suggest that although males and females may not differ in terms of behavioural and peripheral physiological measures of emotional responsivity, the two genders may well differ in neurophysiology.

The present study focuses on aspects of emotion which relate to the processing of emotional stimuli or emotional processing. Emotional processing may be defined as the perception and evaluation of emotional stimuli which may or may not involve emotional experience. For example, emotional processing may involve recognition of emotional facial expressions (emotional perception), recollection of an emotional event (emotional experience), or the viewing of emotional film or images (emotional perception and experience). Note however, that such categorisations are a simplification as studies have clearly demonstrated the presence of autonomic arousal during tasks involving recognition of emotional facial expressions (eg. Williams et al., 2001). Although the present study involves presentation of images rated low on the dimension of arousal, these pictures do appear to involve aspects of emotional experience, such as alterations in HR (Kemp & Nathan, in press, also included as chapter 5).

It has been suggested that techniques with a superior temporal resolution may better address gender differences in emotional processing (eg. Schneider et al., 2000). Event-related potential techniques however are unable to elucidate time-extended
processes following stimulus presentation (Silberstein et al., 1990). Two other
techniques, magnetoencephalography (MEG) and SSPT provide such information,
however, relatively few studies, using these techniques have investigated how the
brain differentially responds to emotional stimuli over time. In the current study, SSPT
was used to investigate gender differences during the viewing of emotional stimuli
selected from the IAPS. SSPT may be characterised by three features which include
1) the presentation of a rapid and repetitive visual flicker distinct from and irrelevant
to the cognitive task undertaken by the participants, 2) the recording of brain
electrical activity from 64 scalp-electrode sites within the area defined by the
International 10-20 system and 3) a relatively short integration period which enables
the rapid changes in brain electrical activity as well as the time-extended processes
following stimulus presentation to be tracked (Gray et al., 2003; Kemp et al., 2002,
also included as chapter 3; Silberstein et al., 1990). We have previously shown that
transient widespread and bilateral frontal SSVEP latency and occipital amplitude
reductions are associated with the cortical processing of pleasant and unpleasant
emotional stimuli in a mixed gender sample (Kemp et al., 2002, chapter 3). The aim
of the current study was to investigate how cortical SSVEPs recorded using the
SSPT technique, are modulated by pleasant and unpleasant images (relative to
neutral images) in a larger sample size, and to investigate whether this processing
diffs between males and females. Based on the extant literature reviewed above,
we hypothesise 1) that males and females will not differ in subjective verbal report, 2)
that females will display bilateral frontal latency reductions during the processing of
both unpleasant and pleasant images relative to neutral images, and 3) that males
will display more focal changes.

4.2 METHODS

4.2.1 Participants
Thirty healthy participants (M age 23.00 ± 4.21 years), consisting of 15 males (M age
23.73 ± 5.04 years) and 15 females (M age 22.27 ± 3.20 years), were included in the
current study. All participants were right handed as assessed by the Edinburgh
Inventory (Oldfield, 1971), non-smokers, drug free, and had no history of epilepsy,
head injury, stroke, psychiatric disorders, neurological conditions or alcoholism. A
medical examination was conducted by a physician who, on the basis of physical and
question based assessment, screened and excluded potential participants according
to these exclusion criteria. Participants were recruited by advertising on noticeboards
and word of mouth, were generally from a university population and gave informed consent to participate in the current study.

4.2.2 Procedure
All participants were informed that they should not drink alcoholic or caffeinated beverages in the 12 hours prior to the experiment being conducted. Participants then arrived for testing in the morning at approximately 8 am after which a standard breakfast was provided. Participants were then brought to the testing room and the recording procedure explained. Participants completed a short in-house questionnaire relating to standard demographics such as age, gender, years of education and exclusion criteria (see appendix 9.1), the Oldfield handedness inventory (Oldfield, 1971), and the Profile of Mood States (POMS; McNair, Lorr & Droleman, 1988) prior to the SSPT recording (see Table 4). Participants indicated how they felt “RIGHT NOW” on the POMS questionnaire, to determine current mood state. As outlined by Kemp and colleagues (2002) (chapter 3), 75 images selected from the IAPS were then presented to participants in 3 blocks (categorised as pleasant, neutral or unpleasant) of 25 images. The images were carefully selected so that images were relatively low on the dimension of arousal and did not contain high arousal content such as violent death and erotica. Pleasant (P) stimuli included kittens, puppies, babies, flowers, sailing etc; unpleasant (U) stimuli included cemeteries, smoke, garbage, dead cows, handicapped individuals, etc; neutral (N) stimuli included mushrooms, animals, abstract art, buildings, kitchen objects, etc. Neutral images were always presented between pleasant and unpleasant image-categories, and the presentation order of the categories was counter-balanced (ie. P,N,U or U,N,P). There were no statistical differences in brightness and contrast between any of the image categories. Following each image, the Self Assessment Maniken (SAM) valence rating scale, ranging from 1 (maximally unpleasant) to 9 (maximally pleasant), and the SAM arousal rating scale ranging from 1 (low arousal) to 9 (high arousal), appeared on the computer screen requiring the participant to rate each image corresponding to how they felt whilst viewing the previously presented image. Participants had been specifically asked to focus on emotional content and to refrain from emotive inhibition. Recording of brain electrical activity was made from 64 electrodes, located in International 10/20 positions and sites midway between these positions, while participants viewed the images and a diffuse 13Hz sinusoidal white visual flicker was presented over the visual field.
4.2.3 Signal Processing and Presentation of Data

The major methodological steps taken to analyse and present the SSVEP data are presented in Figure 14. Signal processing involved calculation of the 13Hz Fourier coefficients (FC) for each stimulus cycle, smoothing the subsequent time-series by averaging overlapping blocks of 10FCs, extracting the 6-second SSVEP associated with each image, averaging across categories, averaging across subjects and then subtracting the averaged SSVEP associated with the neutral category from that associated with the emotional categories to produce activity interpreted as reflecting emotional valence.

Data is presented firstly to efficiently summarise the activity associated with the electrophysiological processing of unpleasant and pleasant valence. Rather than selecting a specific time-point through identification of the maximal difference between the emotional categories and the neutral category as described in the previous study (Kemp et al., 2002, included as chapter 3), time-series plots (including both amplitude and latency SSVEP components) and statistical cluster plots (Hotellings T²) are now presented, which display all 64 electrodes and all 78 13Hz time-points. These time-series and statistical cluster-plots reflect the SSVEP associated with the processing of emotional valence for the entire 6-second image presentation. In all plots and topographic maps, warmer colours reflect reduced amplitude and latency during the presentation of emotional images relative to neutral images as well as larger t-values in the Hotellings maps. The time-series as well as the statistical

Figure 15: Methodological steps taken to analyse and present SSVEP data.
cluster plots present electrodes on the y-axis and time-points on the x-axis. Amplitude is always located in the top row, latency on the second row and the Hotellings statistical cluster plots on the third row. Electrode numbers have been demarcated as having either frontal (including electrodes Fp1, Fp2, F7, F3, Fz, F4 and F8), centro-parieto-temporal (including electrodes T3, C3, Cz, C4, T4, T5, P3, Pz, P4 and T6) and occipital locations (including electrodes O1, Oz and O2) to aid in interpretation of these plots.

Statistical clusters of surrounding electrodes and consecutive timepoints were identified and used as a guide for determining epochs of interest within the larger 6-second epoch. Consecutive timepoints within these epochs were then averaged and re-presented in the form of topographic maps using a spherical spline interpolation procedure (Nunez et al., 1994). These epochs were normalised using normalisation factors (NFs) which were identical to those used for the amplitude and latency components in the 6-second epoch in order for the newly epoched data to be directly comparable to the 6-second epoched data. (For description of normalisation procedures, see Kemp et al., 2002 also included as chapter 3). The topographic maps display amplitude and latency components of the SSVEP for emotional images relative to neutral images (emotional valence), as well as the statistical strength of these differences.

Results are presented in the following way. Firstly, Figure 15 displays the time-series and statistical cluster plots for the entire sample (n=30), while Figure 16 displays the epochs of interest in the form of topographic maps. Figure 17 presents time-series and statistical cluster plots for males (n=15) and females (n=15) separately, while Figure 18 presents male and female epochs of interest in the form of topographic maps. Results summarise all significant effects into the following regions: 1) left frontal region, 2) right frontal region, 3) left temporal, central and parietal regions, 4) right temporal, central and parietal regions and the 5) occipital region. In summarising the results displayed in the topographic maps, if an SSVEP component (amplitude or latency) did not appear to be either increased or decreased in the emotional condition relative to the neutral condition (as indicated by topographic difference maps) within the statistically significant region (as indicated by the Hotellings maps), then the other SSVEP component is reported only and it is this component that is interpreted as being responsible for this effect.
4.2.4 Statistics

Separate independent-sample t-tests were firstly conducted on behavioural variables including age, education and each of the POMS subscales to determine whether differences existed between males and females. In addition, between-groups RANOVAs were conducted on participant’s valence and arousal ratings to determine whether gender modified the main effects of these two variables. The statistical strength of the SSVEP differences between the emotional images (unpleasant, pleasant) and the neutral images were examined using the Hotellings $T^2$ parameter and presented in both statistical cluster plots and topographic maps. Due to the exploratory nature of this study an alpha criterion for the Hotellings $T$ was arbitrarily set at $p=0.01$ (uncorrected for multiple comparisons) for the SSVEP data. It is assumed that real effects will have some degree of statistical continuity both in terms of surrounding electrodes and consecutive timepoints and that if one particular electrode or point in time is statistically significant then adjacent electrodes and consecutive timepoints will also be significant. The rationale that clusters of statistical significance are likely to reflect real effects has been used previously (eg. Gray et al., 2003; Guthrie & Buchwald, 1991; Murray et al., 2002).

The Hotellings $T$ statistic illustrates statistical differences of within subject effects. Although topographic mapping of within-subject statistical differences allow for a potentially useful, visual comparison between different groups (eg. Silberstein et al., 1998, 2000), the Hotellings $T$ statistic does not directly compare differences between males and females. Therefore, in addition to the Hotellings $T$ statistics, the SSVEP was also analysed using six, 2 (male, female genders) X 3 (neutral, pleasant, unpleasant categories) X 2 (left, right hemispheres) X 2 (frontal, posterior regions), mixed between and within-subject, RANOVAs for early, middle and late time points separately. Separate tests were conducted for each time-period in order to complement the effects displayed in the topographic maps and provide some means of comparison between the two statistical tests (and topographic maps) conducted on the SSVEP. In order to reduce the number of within-subject dependent variables (ie. electrodes) entered into these analyses, three standard electrodes within each quadrant were selected and averaged, yielding four SSVEP (amplitude and latency) values per quadrant (ie. left frontal: Fp1, F7, F3; right frontal: Fp2, F8, F4; left posterior: O1, P3, T5; right posterior: O2, P4, T6). Although Hotellings T statistics were conducted on complex numbers (combination of amplitude and phase) using in-house software, no in-house software is available at present for conducting repeated
measures statistics on such data. Therefore RANOVA statistics were performed on normalised amplitude and latency data (separately), which enabled the tests to be run using the Statistical Package for Social Sciences (SPSS) V.10 (SPSS Inc., 1999). In total, 6 RANOVAs were conducted to examine effects of gender in amplitude and latency data at each of the three time-periods. As these tests were run to examine effects of gender and in order to (partially) minimise the impact of experiment-wise type 1 error resulting from the running of multiple statistical tests, only gender associated effects and their interactions are reported in this paper. An alpha level of 0.05 uncorrected for multiple comparisons was arbitrarily set for the RANOVAs conducted.

4.3 Results

4.3.1 Behavioural Results

A series of independent-samples t-tests were conducted on age, education, and each of the POMS subscales separately, to determine whether any differences exist between males and females. None of these variables were found to significantly differ between males and females (p>0.05). Means (M), standard deviations (SD), and range are provided for age, years of education and all 6 POMS subscales for males and females (grouped as well as separated by gender) in Table 2.

Table 4: Participant age, education and mood state (M ± SD and range).

<table>
<thead>
<tr>
<th></th>
<th>Grouped Sample (n=30)</th>
<th>Males (n=15)</th>
<th>Females (n=15)</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>23.00 ± 4.21 (18-39)</td>
<td>23.73 ± 5.04 (18-39)</td>
<td>22.27 ± 3.20 (19-31)</td>
</tr>
<tr>
<td>Education (years)*</td>
<td>15.88 ± 1.97 (12-21)</td>
<td>15.80 ± 2.30 (12-20)</td>
<td>15.96 ± 1.62 (14-21)</td>
</tr>
<tr>
<td>POMS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tension-Anxiety</td>
<td>6.07 ± 4.03 (0-14)</td>
<td>5.67 ± 3.66 (0-11)</td>
<td>6.47 ± 4.47 (0-14)</td>
</tr>
<tr>
<td>Depression-Dejection</td>
<td>5.17 ± 5.68 (0-21)</td>
<td>5.07 ± 5.35 (0-16)</td>
<td>5.27 ± 6.18 (0-21)</td>
</tr>
<tr>
<td>Anger-Hostility</td>
<td>4.37 ± 5.32 (0-26)</td>
<td>5.47 ± 6.46 (0-26)</td>
<td>3.27 ± 3.79 (0-13)</td>
</tr>
<tr>
<td>Vigor</td>
<td>15.27 ± 6.44 (0-28)</td>
<td>15.67 ± 6.81 (4-27)</td>
<td>14.87 ± 6.27 (0-28)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>5.93 ± 5.21 (0-20)</td>
<td>4.87 ± 4.64 (0-15)</td>
<td>7.00 ± 5.68 (1-20)</td>
</tr>
<tr>
<td>Confusion-Bewilderment</td>
<td>6.10 ± 4.23 (0-16)</td>
<td>5.33 ± 3.92 (0-13)</td>
<td>6.87 ± 4.52 (0-16)</td>
</tr>
</tbody>
</table>

*Missing data for 1 female

Participants rated each image on valence and arousal scales. These ratings were averaged for each subject (25 images per category) and then averaged across subjects. Means (M), standard deviations (SD), and ranges for these ratings are provided below in Table 5 for the group averaged data, as well as males and females separately.

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Table 5: Male and female valence and arousal behavioral ratings (SAM) for pleasant, neutral and unpleasant categories (M ± SD and range).

<table>
<thead>
<tr>
<th>Category</th>
<th>Males &amp; Females (n=30)</th>
<th>Males (n=15)</th>
<th>Females (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpleasant: Valence</td>
<td>3.38 ± 0.65 (1.88-4.24)</td>
<td>3.57 ± 0.51 (2.52-4.24)</td>
<td>3.18 ± 0.72 (1.88-4.20)</td>
</tr>
<tr>
<td>Unpleasant: Arousal</td>
<td>3.94 ± 1.40 (1.16-7.16)</td>
<td>3.97 ± 1.49 (1.96-7.16)</td>
<td>3.90 ± 1.35 (1.16-6.44)</td>
</tr>
<tr>
<td>Neutral: Valence</td>
<td>5.15 ± 0.28 (4.28-5.80)</td>
<td>5.15 ± 0.32 (4.28-5.68)</td>
<td>5.16 ± 0.26 (4.80-5.80)</td>
</tr>
<tr>
<td>Neutral: Arousal</td>
<td>1.75 ± 0.62 (1.00-3.48)</td>
<td>1.87 ± 0.58 (1.08-3.08)</td>
<td>1.63 ± 0.66 (1.00-3.48)</td>
</tr>
<tr>
<td>Pleasant: Valence</td>
<td>6.33 ± 0.64 (5.28-7.84)</td>
<td>6.32 ± 0.64 (5.28-7.72)</td>
<td>6.35 ± 0.66 (5.44-7.84)</td>
</tr>
<tr>
<td>Pleasant: Arousal</td>
<td>3.23 ± 1.43 (1.12-6.04)</td>
<td>3.10 ± 1.45 (1.12-6.04)</td>
<td>3.36 ± 1.44 (1.64-5.40)</td>
</tr>
</tbody>
</table>

To determine whether gender modified the main effects for either valence or arousal, a between-groups repeated-measures ANOVA was conducted for both valence and arousal (separately). The tests revealed no significant Valence X Gender interaction (F(1.19,33.31)=1.51, p=0.30) (Greenhouse-Geisser adjusted), or Arousal X Gender interaction (F(1.58,44.24)=0.41, p=0.62) (Greenhouse-Geisser adjusted), indicating that gender did not modify the significant main effect of valence (F(1.19,33.31)=175.18, p<0.001) (Greenhouse-Geisser adjusted) or arousal (F(1.58,44.24)=30.52, p<0.001) (Greenhouse-Geisser adjusted). For the valence rating scale, planned comparisons revealed differences between pleasant and neutral categories, (F(1,28)=119.86, p<0.001, partial eta squared = 0.81) as well as unpleasant and neutral categories (F(1,28)=170.72, p<0.001, partial eta squared = 0.86). For the arousal rating scale, significant differences between pleasant and neutral categories, (F(1,28)=41.42, p<0.001, partial eta squared = 0.60) as well as unpleasant and neutral categories (F(1,28)=68.92, p<0.001, partial eta squared = 0.71) were also revealed, but not for pleasant and unpleasant categories (F(1,28)=4.08, p=0.053). Finally, no main effects of gender were observed for ratings of valence or arousal.

4.3.2 SSPT Results

On examination of the amplitude, latency and Hotellings plots in Figure 15, distinct significant clusters of activation differ between pleasant and unpleasant valence. To better examine these activations in terms of spatial scalp location, the data presented in the time-series plots below, was averaged into three 2-second time-periods (specified as early (0-2 seconds), middle, (2-4 seconds), and late components (4-6 seconds)), and examined topographically (Figure 16). These maps reflect the SSVEP that correspond with the mean difference (emotional category (-) neutral category) of early, middle and late components (each map containing an average of the 26 13 Hz cycles) as demarcated in the time-series plots in Figure 15. The statistically...
significant findings displayed in these topographic maps are summarised in Table 6 and discussed below.

Pleasant and unpleasant valence display similarities as well as differences across the 6-second epoch, suggesting that activation of both unique and common cortical areas occurs during the processing of pleasant and unpleasant stimuli (see Figure 16 and Table 6). During both pleasant and unpleasant valence, reductions in amplitude and latency are observed within the left frontal region (latency only) and the occipital region. A number of differences are also observed and these indicate that pleasant valence unlike unpleasant valence is associated with increases in amplitude within left frontal and decreases in amplitude within right frontal regions; more persistent and widespread frontal latency decreases; and amplitude and latency changes within both L and R temporal, central and parietal regions. Finally, latency reductions during unpleasant valence occur during the early time-period only, thus appearing as more of a transient phenomenon compared to pleasant valence in which latency reductions occur throughout all time-periods.

Figure 16: Illustration of amplitude (row 1) and latency (row 2) time series for pleasant and unpleasant valence across time (x-axis) for each of the 64 electrode positions (y-axis) for 30 participants. In addition, plots of Hotellings T values (row 3) illustrate the statistical significance of these effects.
To examine potential gender differences, amplitude and latency time-series and statistical cluster plots are displayed in Figure 17 for males and females for both pleasant and unpleasant valence. Inspection of the Hotellings $T$ plots, particularly for unpleasant valence, suggests that the number and size of statistically significant clusters of activation differ between males and females. Again, to allow a better spatial scalp profile of these findings, early, middle and late epoch components are presented in the form of topographic maps (Figure 18). Statistically significant results displayed in these maps are summarised in Table 7 and discussed below.
Table 6: Summary of the statistically significant SSVEP findings (amplitude and latency) as indicated by the Hotellings $T^2 (p<0.01)$ for pleasant and unpleasant valence for 30 participants, where Amp = amplitude and Lat = latency.

<table>
<thead>
<tr>
<th>Valence</th>
<th>Region</th>
<th>Time Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Early-Component (0-2seconds)</td>
</tr>
<tr>
<td>Pleasant</td>
<td>left frontal region</td>
<td>Amp ↑; Lat ↓</td>
</tr>
<tr>
<td></td>
<td>right frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>Amp ↑; Lat ↓</td>
</tr>
<tr>
<td></td>
<td>right temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>occipital region</td>
<td>Amp ↓; Lat ↓</td>
</tr>
<tr>
<td>Unpleasant</td>
<td>left frontal region</td>
<td>Lat ↓</td>
</tr>
<tr>
<td></td>
<td>right frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>Amp ↓; Lat ↓</td>
</tr>
<tr>
<td></td>
<td>right temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td>occipital region</td>
<td>Amp ↓; Lat ↓</td>
</tr>
</tbody>
</table>

Males and females display both similarities as well as differences in the processing of emotional valence suggesting that unique as well as common cortical areas are activated when males and females process emotional stimuli. During the processing of pleasant valence, both males and females display reductions in latency within the right frontal and left temporal regions. However, males display left frontal increases in amplitude and reductions in latency as well as reductions in occipital amplitude, whilst females display reductions in latency within left and right temporal, central and parietal regions and increases in occipital latency. During the processing of unpleasant valence, both males and females display amplitude increases within the right temporal region during the middle epoch, although males display latency increases and females display latency decreases. A number of differences are also observed indicating that females display frontal SSVEP changes (predominantly latency reductions and distributed primarily in the right hemisphere), whilst males do not display frontal SSVEP changes. In addition, females display reductions in both amplitude and latency in left temporal, central and parietal regions whilst males display occipital reductions in both amplitude and latency. In summary, the processing of pleasant valence was associated with latency reductions within right as well as left frontal regions in males, and latency reductions within right frontal regions (during the late time-period only) in females. By contrast, the processing of
unpleasant valence was associated with latency reductions within right frontal and temporal regions in females only.

**Figure 18:** Illustration of amplitude (row 1) and latency (row 2) time series for pleasant and unpleasant valence across time (x-axis) for each of the 64 electrode positions (y-axis) for 15 males and 15 females. In addition, plots of Horellings T values (row 3) illustrate the statistical significance of these effects.
Figure 19: Topographic presentation of the 13Hz SSVEP (amplitude and latency) and Hotellings $T$ values for pleasant and unpleasant valence in males and females. Three time-periods are presented which relate to early (0-2 seconds), middle (2-4 seconds) and late (4-6 seconds) components of image viewing.
Table 7: Summary of the statistically significant SSVEP findings (amplitude and latency) as indicated by the Hotellings $T^2$ ($p<0.01$) for pleasant and unpleasant valence for 15 males and 15 females, where Amp = amplitude and Lat = latency.

<table>
<thead>
<tr>
<th>Valence</th>
<th>Gender</th>
<th>Region</th>
<th>Time Periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Early-Component</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(0-2seconds)</td>
</tr>
<tr>
<td>Pleasant</td>
<td>Males</td>
<td>left frontal region</td>
<td>Amp ↑; Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>right frontal region</td>
<td>Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>right temporal, central and parietal regions</td>
<td>Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>occipital region</td>
<td>Amp ↓</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>left frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>left temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>occipital region</td>
<td></td>
</tr>
<tr>
<td>Unpleasant</td>
<td>Males</td>
<td>left frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>left temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right temporal, central and parietal regions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>occipital region</td>
<td>Amp ↑; Lat ↑</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>left frontal region</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>right frontal region</td>
<td>Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>Amp ↓; Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>right temporal, central and parietal regions</td>
<td>Amp ↑; Lat ↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>occipital region</td>
<td>Amp ↑; Lat ↓</td>
</tr>
</tbody>
</table>

A series of mixed-, between- (gender) and within-subject (category, hemisphere, region) repeated measures ANOVAs were conducted for amplitude and latency data at early, middle and late time points in order to directly compare between males and females. These RANOVAs revealed a significant Category X Hemisphere X Gender interaction ($F(2, 56)=3.172, p=0.050$, partial eta squared = 0.102) for amplitude during the middle time-period, a significant Category X Region X Gender interaction ($F(2, 56)=3.307, p=0.044$, partial eta squared = 0.106) for latency during the middle
time-period and a significant Category X Hemisphere X Gender interaction (F(2, 56)=3.899, p=0.026, partial eta squared = 0.122) for amplitude during the late time-period. No other gender associated effects, nor any overall between-subject effects (main effects of gender) were found to be significant.

The Category X Hemisphere X Gender interaction for amplitude during the middle time-period indicates that Category modified a Hemisphere X Gender interaction. Tests of within-subject contrasts indicated that Category modified the Hemisphere X Gender interaction during presentation of unpleasant images relative to neutral images (F(1,28)=4.324, p=0.047, partial eta squared = 0.134), but not during pleasant images relative to neutral images (F(1,28)=0.402, p=0.531). To investigate this effect further, two Hemisphere X Gender RANOVAs were conducted on neutral (F(1,28)=0.262, p=0.613) and unpleasant (F(1,28)=6.830, p=0.014, partial eta squared = 0.196) images separately. These findings indicate that amplitude within the right hemisphere (anterior and posterior) in females is greater than the left hemisphere while viewing unpleasant images. This effect however, was not present in the male sample.

The Category X Region X Gender interaction for latency during the middle time-period indicates that Category modified a Region X Gender interaction. Tests of within-subject contrasts indicated that Category modified the Region X Gender interaction during presentation of unpleasant images relative to neutral images (F(1,28)=6.000, p=0.021, partial eta squared = 0.176), but not during pleasant images relative to neutral images (F(1,28)=0.073, p=0.788, partial eta squared = 0.003). To investigate this effect further, two Region X Gender RANOVAs were conducted on neutral (F(1,28)=2.753, p=0.108) and unpleasant images (F(1,28)=3.144, p=0.87, partial eta squared = 0.101) separately. The results for the latter ANOVA indicate a trend for a Region X Gender interaction during viewing of unpleasant images. As the initial ANOVA was significant however, further examination of the associated SPSS profile plot (not displayed) was warranted. This plot indicated that females may display latency reductions within the frontal regions, while males displayed small latency increases. An independent sample t-test conducted on the average of left and right frontal locations indicated that this effect was significant (T(28)=2.242, p=0.033).

Finally, the Category X Hemisphere X Gender interaction for amplitude during the late time-period indicates that Category modified a Hemisphere X Gender interaction.
Tests of within-subject contrasts indicated that Category modifies the Hemisphere X Gender Interaction during the presentation of unpleasant images relative to neutral images (F(1,28)=7.110, p=0.013, partial eta squared = 0.203) but not during the presentation of pleasant images relative to neutral images (F(1,28)=0.452, p=0.507). In order to investigate this effect further two Hemisphere X Gender RANOVAs were conducted on neutral (F(1,28)=0.006, p=0.939) and unpleasant (F(1,28)=7.726, p=0.010, partial eta squared = 0.216) images separately. Like those reported for the middle time-period, these findings indicate that amplitude within the right hemisphere (anterior and posterior) in females is greater than the left hemisphere while viewing unpleasant images. This effect again was not present in males.

In summary, results indicate no overall between-subject (gender) differences, however findings for amplitude during both the middle and late time-periods indicate that only females display increased amplitude within the right hemisphere relative to the left hemisphere (after collapsing across frontal and posterior locations). In addition, findings for latency during the middle time-period indicate that only females display latency reductions within frontal locations. All these effects were significant during viewing of unpleasant images only.

4.4 DISCUSSION

The current study investigated the spatiotemporal characteristics of the SSVEP associated with the processing of low arousal, unpleasant and pleasant images relative to neutral images in males and females using the SSPT technique. SSVEP results support our previous study (Kemp et al., 2002, also included as chapter 3) which reports that processing of emotional valence (pleasant and unpleasant) is associated with frontal SSVEP latency and occipital amplitude reductions, however substantial gender differences exist particularly within the frontal regions during the processing of unpleasant valence and these are discussed below. Key findings demonstrate: 1) that electrophysiological differences between males and females exist despite there being no differences in subjective mood (POMS questionnaire) or behavioural ratings (valence and arousal dimensions), 2) that the processing of pleasant valence is associated with left and right frontal latency reductions in males, but not in females, 3) that the processing of unpleasant valence is associated with widespread frontal latency reductions (predominantly right sided and most apparent during the middle time-period) in females, but not in males, and 4) that only females
display increased amplitude within the right hemisphere relative to the left hemisphere during unpleasant images in the middle and late time-periods.

The SSVEP amplitude and latency components have been interpreted previously as being analogous to the amplitude of regional cortical activity within the alpha frequency range and as reflecting neural information processing speed, respectively (Kemp et al., 2002; Silberstein et al., 1990; Silberstein et al., 1995; Silberstein et al., 1996; Silberstein et al., 2000). In this framework, reductions in SSVEP amplitude may be considered comparable to the transient reduction in alpha activity, known as ERD (see Pfurtscheller & Lopes da Silva, 1999 for review), whilst reductions in SSVEP latency may be considered as increased neural information processing speed or more generally as either increased excitation or reduced inhibition. Supporting this interpretation, increases in visual attention are associated with decreases in SSVEP amplitude at occipital and prefrontal sites (Silberstein et al., 1990; Silberstein et al., 1995) and variations in reaction time in a visual vigilance task correlates with frontal SSVEP latency changes (Silberstein et al., 1996, 2000).

The literature implicates anterior frontal locations more in emotional valence and emotional experience whilst posterior regions are implicated more in perceptual and arousal components of emotion (Davidson, 1992, 1998; Davidson & Irwin, 1999; Heller, 1990, 1993). This background in terms of the neural structures underlying experiential and perceptual components of emotion is important for the interpretation of the gender differences presented in the results, particularly within frontal regions. Right frontal activation during unpleasant emotion (eg. disgust), and left frontal activation during pleasant emotion (eg. happiness) has been reported extensively in the EEG literature, and activation of these regions may be specialised for withdrawal and approach behaviours respectively (for review see Davidson, 1998). These EEG findings have more recently been supported by PET, fMRI and electrophysiological studies (Sutton et al., 1997; Canli et al., 1998; Aftanas et al., 2001a & 2001b, respectively), though others have reported overlapping activation and no hemispheric asymmetries (Baker et al., 1997; George et al., 1995; Kemp et al., 2002; Lane, Fink et al., 1997; Lane, Reiman, Ahern et al., 1997; Lane, Reiman, Bradley et al., 1997; Pardo et al., 1993; Teasdale et al., 1999). Regardless, the prefrontal region is strongly implicated in emotional processing and the experience of emotion (Davidson & Irwin, 1999).
The processing of unpleasant valence is associated with widespread frontal latency reductions (predominantly right sided) in females but not in males. It is possible that males were less responsive to unpleasant stimuli, supporting previous studies which have reported greater activations particularly in frontal regions, in females relative to males. For example, Pardo and colleagues (1993) reported that whilst women displayed bilateral inferior and orbitofrontal activation in response to recalled sad mood, males displayed left-sided activation only. In addition, George and colleagues (1996) reported that whilst women displayed increased activity in the bilateral AC, left MPFC, left insula and thalamus during the transient sadness, males activated only the left insula and right caudate, completely failing to activate the PFC. Furthermore, women in this latter study displayed a greater number of significant changes from neutral to sadness tasks. It is important to note that SSPT is unable to acquire functional information from subcortical structures and it is possible therefore that the processing of unpleasant valence in males may be more subcortical than in females. This interpretation has been made previously in an fMRI study (Schneider et al., 2000), in which, the authors speculated that men may have displayed increased amygdala involvement because they exerted greater effort to experience sadness.

These results are particularly interesting given that females are more susceptible to lowered mood as demonstrated in tryptophan depletion (TD) studies (eg. Booij et al., 2002; Ellenbogen, Young, Dean, Palmour & Benkelfat, 1996; Smith, Clifford, Hockney, Clark & Cowen, 1997) and, more generally, that females are more likely to experience depression and anxiety (see Darlington, 2003 for review). Nishizawa and colleagues (1997) reported that 5-HT synthesis in normal females was 52% lower than normal males, indicating that females may be less able to maintain adequate stores of the 5-HT neurotransmitter, particularly under stressful situations. In addition, it has been argued on the basis of differing social and gender roles that females are more likely to experience feelings of sadness, hurt and disappointment, which is likely to lead to excessive ruminating and clinical depression (Brody, 1993, 2001; Hankin & Abramson, 2001; Noelen-Hoeksema, 1991). Hankin & Abramson (2001) have posited a cognitive vulnerability-transactional stress theory to explain the ‘big fact’ that more girls become depressed than boys after the age of 13 or ‘middle puberty’ and that this difference persists throughout adulthood. On the basis of experimental data, these authors indicate that adolescent girls are more likely than boys to encounter negative life events, experience cognitive vulnerabilities to depression, personality traits such as neuroticism and environmental adversity such as sexual abuse. The model suggests that these experiences will lead to increased
negative, anxious and depressive affect, which in turn generates more dependent negative life events, eventually leading to depression. Recent neuroimaging evidence has provided a direct relationship between depressed mood and regions of the frontal cortex following modulation of certain neurochemical systems. For example, PET suggests that both tryptophan and α-methylparatyrosine (AMPT)-induced return of depressive symptoms lead to decreases in metabolism within common brain circuitry including the orbitofrontal, dorsolateral and thalamus (Bremner et al., 1997, 2003). Interestingly, Davidson (2002) has suggested that greater relative right-sided prefrontal metabolism is associated with higher metabolic activity within the amygdala and that such activations have been associated with mood disorders such as anxiety and depression. It is possible therefore that the gender differences observed within right frontal and anterior temporal regions reflect decreased and increased responsiveness in males and females respectively to unpleasant images (relative to neutral) despite similar ratings on verbal report.

By contrast, the processing of pleasant valence is associated with left and right frontal latency reductions in males but only the right frontal region (during one time-period) in females. The literature has indicated that positive affect is much harder to elicit in the laboratory (Davidson, 2002) and given that the present study presented images previously rated as low on the arousal dimension, it is possible that our sample of females did not engage the cortical areas involved in the experiential components of emotion to the same extent as males. It could be argued that females do not need the same degree of cortical activation in order to have a subjective feeling comparable to those of males. However, a number of recent studies suggest that this is not the case. For example, a previous study which investigated sex differences in hunger and satiation concluded that men may have a brain response producing greater hedonic effects from eating and more rewarding feelings associated with satiation (Del Parigi et al., 2002). While not directly comparable, it does suggest that brain responses in males may be more sensitive to pleasant valence than females. Most recently, another study using fMRI to investigate gender differences in the viewing of IAPS images reported that men display a stronger brain activity for positive visual stimuli than women within the inferior and medial frontal gyrus as well as the amygdala (Wrase et al., 2003). If we consider pleasant and unpleasant valence to lie on a continuum as previously hypothesised (Feldman-Barrett & Russell, 1999), it is possible that females are more orientated towards the unpleasant pole on this continuum further supporting female susceptibility to lowered mood. Although, the Hotellings statistics provide support for the conclusion that
males activate frontal regions more consistently than females during viewing of pleasant images, no gender differences were evident in the RANOVA statistics for these images. Instead, the gender differences (as reported in the RANOVA) in both amplitude and latency were specific to unpleasant emotional stimuli, suggesting perhaps that differences in males and females may relate more to unpleasant than pleasant stimuli. Gender differences therefore, relating to the neurobiological mechanisms underlying the processing of pleasant images and pleasant emotion more generally, will need verification in future studies.

A number of similarities between males and females were also observed in the processing of emotional valence. This is consistent with previous reports that there are both similarities and differences between males and females in resting state (eg. Gur et al., 1995) as well as in the processing of emotional stimuli (eg. Del Parigi et al., 2002; Pendergrass et al., 2003). During pleasant valence both males and females display activations (reduced latency) within right frontal and left temporal regions. In addition, the left frontal region in females was close to, but did not reach significance within the late component of image viewing. Left temporal activation during the processing of pleasant valence may relate to findings reported in a previous study in which a more posterior distribution of activity, located in the region of the pre- and post-central gyri, was associated with an extended-picture presentation to evoke positive mood (Sutton et al., 1997). However in the current study, females did not engage the frontal structures to the same extent as males during pleasant valence, which may reflect a lower responsiveness of neural structures involved in the experiential components of emotion in our female subjects. Finally, during unpleasant valence, both males and females display reduced activation (amplitude increases) within right temporal regions.

As discussed above, SSVEP amplitude may be interpreted in exactly the same way as the amplitude of regional cortical activity within the alpha frequency range. In this framework, an amplitude enhancement has been interpreted as a deactivated state in which the brain region is neither receiving nor processing sensory information and that this may be important for the introduction of inhibitory effects (Pfurtscheller & Lopes da Silva, 1999). Consistent with this model, males also display latency increases (increase in inhibitory processes) within this region, possibly reflecting a compensatory response to over-ride expressions of emotion generated by limbic-subcortical structures (as discussed in Liotti et al., 2000). Unlike males however, the processing of unpleasant valence in females is associated with widespread
reductions in latency (increases in excitatory processes) which may reflect increased responsiveness to unpleasant (relative to neutral) images and possibly an inability to successfully suppress activation associated with the presentation of unpleasant stimuli. This interpretation is consistent with the purported role of the right hemisphere in inhibitory control (Garavan et al., 1999) and its dense interconnectivity with the paralimbic core (Tucker, 2001).

The finding that only females displayed a more general increase in amplitude during viewing of unpleasant images within the right hemisphere deserves some comment as it could be argued that this finding contradicts traditional theorised patterns in the processing of emotion. For example, one of the oldest theories of emotions in the brain is the key role of the right hemisphere in the processing of emotion (eg. Levine & Levy, 1986; Ross & Mesulam, 1979; Sackeim, Gur & Saucy, 1978). In terms of brain activation and on the basis of the ‘arousal’ model of alpha amplitude, in which reductions in alpha amplitude are thought to reflect increases in activation (eg. Lindsley & Wicke, 1974; Ray & Cole, 1985), it could be argued that decreases in amplitude within the right-hemisphere should be displayed rather than the increases found in the current study. However, given that participants were requested to focus on emotional content and refrain from emotional inhibition, it is possible that as part of this process females primed particular emotional circuits through imagining and remembering similar emotional events. Amplitude findings could therefore be considered to be consistent with previous reports of an increase in alpha activity associated with mental imagery (Ray & Cole, 1985; Tesche, Uusitalo, Ilmoniemi & Kajola, 1995) and also the finding that this alpha increase is specific to the right hemisphere (Ray & Cole, 1985).

Finally, some limitations of the study are worth noting. Firstly, participant’s ratings of arousal indicate that pleasant and unpleasant images significantly differed from neutral images, thereby making the interpretation, that difference maps (emotional versus neutral images) reflect only ‘valence’, difficult. It is important to mention however, that pleasant and unpleasant images did not significantly differ from each other and also that this finding may reflect the more general difficulty of selecting positively or negatively valenced images which are equivalent to neutral images on the arousal dimension. Moreover, the current study did not select images containing high arousal content such as ‘violent death’ and ‘erotica’, which would have otherwise confounded emotional arousal with emotional valence. Secondly, the calculation of RANOVA statistics required data reduction procedures which limited
the potential conclusions able to be drawn from the results of these tests. Although these statistics confirmed differences between males and females with respects to the processing of unpleasant images, the fact that no statistical differences were displayed for the pleasant stimuli does not rule out that differences for such stimuli may exist (as reported recently by Wrase et al., 2003). Thirdly, it is possible that a range of variables including psychological, cognitive, and social variables, could in part, account for the observed effects. Authors have argued for example, that women may employ more cognitive strategies and internal cues while men may focus on the external stimulus in order to generate emotion (eg. Schneider et al., 2000). Future studies should examine these issues in more detail when investigating gender differences in emotional processing.

In summary, electrophysiological differences in the processing of pleasant and unpleasant valence between males and females were observed despite there being no differences in subjective mood or ratings of pleasant, neutral or unpleasant images. These results suggest that gender differences do exist in the processing of visual emotional stimuli, and illustrate the importance of taking these differences into account during investigations of emotional processing. The main gender difference reported in the current study relates to the processing of unpleasant valence which is associated with widespread frontal latency reductions (predominantly right sided) in females but not in males. This finding is consistent with the interpretation that females rather than males are more susceptible to negative life experiences and lowered mood, and may have implications for the pathophysiology of mood disorders such as depression.
CHAPTER 5

5 ACUTE AUGMENTATION OF SEROTONIN SUPPRESSES CARDIOVASCULAR RESPONSES TO EMOTIONAL VALENCE
5.1 INTRODUCTION

Emotion is regarded as consisting of multiple components including cognitive processes, physiological responses, motivational changes, motor expression and subjective feeling. William James proposed that an emotion was a direct function of feedback from the periphery or viscera (James, 1884) and a key component of such visceral reactivity to emotional states are changes in HR. Changes in HR have been reported to be able to differentiate pleasant from unpleasant visual stimuli (Aftanas et al., 2001b; Greenwald, Cook & Lang, 1989; Palomba et al., 1997) and load onto a valence dimension (Lang, Greenwald, Bradley & Hamm, 1993). Previous studies have generally reported larger HR decelerations to unpleasant stimuli compared to pleasant stimuli relative to a blank screen prior to image onset and these deceleratory changes in HR are believed to reflect an orienting reaction which purportedly reflects milder emotional stimulations (see Palomba et al., 1997 for discussion).

Although the cardiovascular response to images differing on emotional valence has been demonstrated, little is known about how these responses are modulated by neurochemicals including 5-HT. Although results from extensive retrospective analyses demonstrate a small reduction in HR (4-8 beats per minute, BPM) following chronic SRI treatment (Rasmussen, Overo & Tanghoj, 1999), cardiovascular reactivity to emotional stimuli following administration of SRIs is unknown. This is an important research area given that 1) HR is a key component in emotional responsiveness, 2) the serotonergic system is implicated in a range of disorders including depression, anxiety, social phobia, and premenstrual dysphoria (for a review, see Jones & Blackburn, 2002), 3) depression and anxiety have been associated with an increase in the likelihood of sudden cardiovascular death (Roose, 2001; Sheps & Sheffield, 2001) and 4) SRIs have been reported as having ‘cardio-protective effects’ (Yeragani, Pesce, Jayaraman, & Roose, 2002).

The aim of the present study was therefore to investigate how increases in 5-HT with a SRI (citalopram) modulates the HR associated with the viewing of differently valent images selected from the IAPS. Previous literature suggests that HR is able to differentiate differently valent, thus in this study we will employ a within subjects repeated measures design to investigate differences between categories during placebo and citalopram conditions. We hypothesise that HR will differentiate differently valent images during both treatment conditions. Specifically, we
hypothesise that HR during unpleasant images will be less than HR during pleasant images. We also hypothesise that there will be a small reduction in the HR for all image categories during the citalopram condition.

5.2 METHODS

5.2.1 Participants
16 healthy, non-smoking, medication-free (minimum 1 month drug-free) volunteers (8 males and 8 females) participated in the current study (M age ± SD = 22.94 ± 4.75 years). Potential participants were carefully screened by a medical physician and excluded from the study if found to have a history of cardiovascular, hepatic, gastrointestinal, endocrine, neurological or psychiatric conditions. The study was approved by the Swinburne Human Research Ethics Committee at the Swinburne University of Technology.

5.2.2 Procedure
All participants were requested not to eat breakfast or to drink any caffeinated beverage prior to arrival for testing. Participants arrived at the institute at 8am and were then provided with a standard breakfast in order to control for diet variability between and within subjects across both testing sessions of the study. The study was a double-blind placebo controlled design in which participants were tested under two single dose treatment conditions: placebo and citalopram (20mg). Participants were tested 2 hours following administration of either substance to coincide with approximate peak plasma levels of citalopram (Noble & Benfield, 1997) and the order in which either placebo or citalopram were administered was counter-balanced. These conditions were separated by a minimum washout period of 1-week.

75 images were selected from the IAPS, categorised as either pleasant (P), neutral (N) or unpleasant (U) (25 images in each category) and presented to participants in 3 blocks (P,N,U or U,N,P). Following the 6-second presentation of each image, the participant was presented with the SAM rating scales for valence and arousal dimensions, which allow participants to rate each image in terms of how they actually felt during the presentation of the previous image (see Lang et al., 1999 regarding the IAPS and SAM). These scales range from 1 – 9, in which valence reflects the degree of pleasantness (1: unpleasant – 9: pleasant) and arousal, the degree of stimulation or excitement (1: low arousal – 9: high arousal). A thorough description of image selection and task instructions has been provided chapter 3.
Valence and arousal ratings for each image were averaged across categories so that each subject was associated with three valence and three arousal ratings which were then used in RANOVA statistics. HR was recorded from the upper left arm and referenced to linked ear electrodes as part of the set up for the recording of brain electrical activity. After the recording, cardiac interbeat intervals during picture presentation were converted to BPM in half second bins for each image-category as conducted previously (Graham, 1980; Lang et al., 1993). BPM over the six-second epochs were then averaged for each category and treatment condition creating 6 BPM per subject for use in a RANOVA statistic.

5.2.3 Statistics

Behavioural SAM ratings were analysed using a 2 (placebo, citalopram) X 3 (pleasant, neutral, unpleasant) within-subjects, RANOVA for valence and arousal dimensions separately to determine whether citalopram modified participant’s reports of how they felt whilst they viewed differently valent images. HR data was also analysed using a 2 (Treatment) X 3 (Category) within-subjects, RANOVA to determine whether citalopram modified HR to the viewing of differently valent images. In addition, three paired measures t-tests were conducted to examine the citalopram-treatment effects on each of the different valent image categories separately. One-tailed t-tests will be used for the HR analysis, as explicit hypotheses were made for differences between valent categories (RANOVA) as well as between treatment conditions (RANOVA & paired measures t-tests). (α therefore is set at 0.10, two-tailed).

5.3 Results

Means and standard deviations for participant’s ratings of pleasant, neutral and unpleasant images (valence and arousal) on the SAM rating scale for both placebo and citalopram treatment conditions are provided in Table 8.
Within-subjects RANOVA was conducted for both valence and arousal dimensions separately. Significant category effects were found for both valence (F(1.19, 17.78) = 109.45, p < 0.001, partial eta squared = 0.88) (Greenhouse-Geisser adjusted) and arousal (F(2,30) = 23.72, p < 0.001, partial eta squared = 0.61) ratings. However, no main treatment effect or a category X treatment effect was found for either valence or arousal rating scales, indicating that treatment had no effect on ratings generally, nor did treatment modulate participant’s ratings to specific image categories. For the valence dimension, planned comparisons revealed that participant’s ratings of both pleasant and unpleasant images were significantly different from ratings of neutral images (F(1,15) = 91.10, p < 0.001, partial eta squared = 0.86 and F(1,15) = 94.31, p < 0.001, partial eta squared = 0.86, respectively). For the arousal dimension, planned comparisons revealed that ratings of arousal for both pleasant and unpleasant images were significantly different from ratings of neutral images (F(1,15) = 14.16, p = 0.002, partial eta squared = 0.49 and F(1,15) = 45.95, p < 0.000, partial eta squared = 0.75, respectively). In addition, ratings of arousal for unpleasant images were significantly different to those of pleasant images (F(1,15) = 9.56, p = 0.007, partial eta squared = 0.39).

Means and standard deviations for participant’s HR during viewing of pleasant, neutral and unpleasant images for both placebo and citalopram treatment conditions are provided in Table 9.

Table 9: Heart rate (HR) means and standard deviations for pleasant, neutral and unpleasant images in placebo and citalopram treatment conditions

<table>
<thead>
<tr>
<th></th>
<th>Pleasant</th>
<th>Neutral</th>
<th>Unpleasant</th>
</tr>
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<tbody>
<tr>
<td>Placebo</td>
<td>69.30 (8.96)</td>
<td>68.05 (8.87)</td>
<td>66.45 (10.56)</td>
</tr>
<tr>
<td>Citalopram</td>
<td>66.48 (11.53)</td>
<td>67.20 (10.70)</td>
<td>67.17 (10.29)</td>
</tr>
</tbody>
</table>
A 2 (Treatment) X 3 (Category) within subject RANOVA on BPM revealed a significant main effect for category (F(2,30)=3.92, p=0.031, partial eta squared =0.21) and a treatment X category interaction (F(1.43, 21.51)=4.54, p=0.033, partial eta squared =0.23) (Greenhouse-Geisser adjusted). No main effect for treatment was evident. Planned comparisons for category indicated a significant difference between HR during viewing of unpleasant images and HR during viewing of pleasant images (F(1,15)=9.00, p=0.009, partial eta squared = 0.38); a significant difference between HR during viewing of unpleasant images and HR during viewing of neutral images (F(1,15)=3.81, p=0.070, partial eta squared = 0.20); and no statistical difference between HR during pleasant images and HR during viewing of neutral images.

Post-hoc one-way within-subject analysis of variance statistics were conducted on placebo and citalopram treatment conditions separately to determine the nature of the treatment X category interaction. Significant category effects were found for the placebo treatment condition (F(2,30)=6.69, p=0.004, partial eta squared=0.31), but not for the citalopram condition, suggesting that although HR is modulated by differently valent images within the placebo condition, the ability for differently valent images to modulate HR during the citalopram treatment condition is suppressed. Planned comparisons for category within the placebo treatment condition revealed a significant reduction in HR during viewing of unpleasant images relative to that during the viewing of pleasant images (F(1,15)=8.88, p=0.009, partial eta squared=0.37); a significant reduction in HR during viewing of unpleasant images relative to that during the viewing of neutral images (F(1,15)=4.53, p=0.050, partial eta squared=0.23); as well as a significant reduction in HR during viewing of neutral images relative to that during the viewing of pleasant images (F(1,15)=4.47, p=0.052, partial eta squared=0.23) (see Figure 19).

Paired-sample t-tests were conducted on each of the three valent categories separately to examine the differences between placebo and citalopram treatment conditions. Differences between treatment conditions for unpleasant and neutral images were not significantly different, however there was a trend for a statistically significant difference between placebo and citalopram treatment conditions for pleasant images (t(15)=1.61, p=0.129). Figure 19 displays the average HR for all image categories in both placebo and citalopram treatment conditions as well as the significant differences between these conditions.
5.4 DISCUSSION

This study examined the modulatory effect of serotonergic augmentation on cardiovascular, HR changes associated with the viewing of differently valent images selected from the IAPS. The major findings were 1) that HR was able to differentiate differently valent images within the placebo condition and, 2) that HR was no longer able to differentiate differently valent images after increasing 5-HT with the SRI citalopram. In addition, although we hypothesised there would be a reduction in the HR for all image categories, results suggest that there was no main effect for citalopram.

During the placebo condition, HR differentiated all three valent image categories. HR during viewing of unpleasant images was significantly less than that during the viewing of neutral images and the HR during the viewing of neutral images was significantly less than that during the viewing of pleasant images. These findings support previously reported effects of emotional valence on HR, believed to reflect a mild emotional stimulation (Aftanas et al., 2001b; Greenwald et al., 1989; Lang et al., 1993; Palomba et al., 1997). As indicated above (in section 5.1), previous studies that have examined HR response to IAPS slides have generally reported heart rate change relative to a blank screen (which is presented immediately prior to image onset) and these changes are described as an acceleration or deceleration. The
behavioural significance of heart rate change has long been considered to reflect changes in information processing (Lacey & Lacey, 1970, 1974). According to this model, attending to environmental events (stimulus intake) produces heart rate deceleration whilst rejection of external stimuli (stimulus rejection) produces heart rate acceleration. Elicitation of HR changes by different emotional states have been considered in light of this model, whereby mild emotional stimulations produce an orienting response and heart rate deceleration, whilst more intense emotional states produce a defense reaction and heart rate acceleration (Cook & Turpin, 1997; Graham & Clifton, 1966; Sokolov, 1963; Turpin, 1986). For instance, whilst the experience of fear would be expected to be associated with HR increases, the viewing of unpleasant scenes is thought to evoke milder emotional states, an orienting reaction and HR decreases. In the current study, there is no indication as to whether a HR acceleration or deceleration occurred because blank screens were not presented before IAPS stimuli. Nonetheless, it would not be overly speculative to suggest that the present findings (for all valent categories) reflect a HR deceleration considering previous authors have concluded that “cardiac deceleration prevails over acceleratory changes” (Palomba et al., 1997, but see also Aftanas et al., 2001b who report acceleratory changes for all valent categories).

During the citalopram condition, no main treatment effect was found for HR which suggests that the dosage of citalopram administered in the current study (20mg) may have been too low for this effect to appear or that the HR reductions hypothesised in the current study are limited to chronic administration. Interestingly, we did identify a trend for citalopram to reduce HR during the viewing of pleasant images and although this finding initially appears counter intuitive given the use of SRIs to alleviate depressed mood, SRI-associated decreases in HR (as previously reported by Rasmussen et al., 1999) may be specific to pleasant images because these images were associated with the highest HR. More importantly, the citalopram condition was found to modulate the ability for HR to differentiate differently valent stimuli. These findings suggest that the HR changes associated with emotional stimuli are suppressed with acute enhancement of 5-HT. This is a particularly important finding given the relationship between emotion and the cardiovascular system. For example, negative feelings have been associated with arterial wall thickening in treated hypertensive men at high cardiovascular risk (Agewall, Wikstrand, Dahllof & Fagerberg, 1996). Studies suggest that a diagnosis of major depressive disorder in patients with cardiovascular disease is not only associated with poor prognosis (see Yeragani et al., 2002a for discussion), but that the
depression is also associated with an increase in the likelihood of sudden cardiovascular death (Roose, 2001; Sheps & Sheffield, 2001). SRIs appear to be associated with less cardiovascular side effects compared to tricyclic antidepressants (TCAs) (Roose, 2001; Yeragani et al., 2002a) and have even been regarded as having cardio-protective effects especially in patients with cardio-vascular disease (Yeragani et al., 2002a). Our findings imply that 5-HT may have a protective effect on the heart with regards to the fluctuations in HR associated with differently valent stimuli.

Although the mechanisms underlying these effects are unknown, it is unlikely that our findings are the result of a direct 5-HT induced modulation of the noradrenergic (NA) system. Firstly, if 5-HT was to directly modulate NA, HR change would be non-specific to image category, and a main effect for treatment would be reported. Secondly, animal studies have shown that acute manipulation of 5-HT does not affect NA synthesis (Bymaster et al., 2002; Hajos-Korcsok, McTavish & Sharp, 2000; Thomas, Nutt & Holman, 1998). Finally, human studies using acute 5-HT agonists have found no effect on NA based neuroendocrine responses (melatonin secretion) (Nathan, Norman & Burrows, 1996; Norman, Nathan & McNulty, 1995). On the other hand, it is well-known that the brain modulates HR from basic brain-stem reflexes as well as from descending influences of regions involved in emotional processing, such as the amygdala and the PFC (for reviews, see Bernston, Cacioppo & Karen, 1991; Loewy & McKellar, 1980; Richardson & Chiu, 1983; Thayer & Siegle, 2002). Furthermore, both animal (Freedman & Shi, 2001; Sadikot & Parent, 1990) as well as human research (Gurevich & Joyce, 1996; Pazos, Probst & Palacios, 1987; Smith et al., 2002) have demonstrated that 5-HT is widely distributed throughout the brain. It is therefore possible that 5-HT may modulate the cardiovascular system through actions at multiple sites in the emotional circuitry.

Recently, there has been an increasing interest in the relationship between depression and cardiac mortality using measures of heart rate variability (HRV) by analysing fluctuations between normal heartbeats using time and frequency domain methods. The usefulness of nonlinear measures has also been reported though these measures still require critical experimentation. Decreased HRV as indicated by an increase in cardiac sympathetic function or a decrease in vagal function and reported in patients with depression, is now viewed as an important predictor of cardiac mortality (Carney et al., 2001; Stein & Kleiger, 1999; Task Force of the European Society of Cardiology and the North American Society for Pacing and
Electrophysiology, 1996; Yeragani, 2000; Yeragani, Pesce et al., 2002; Yeragani, Rao et al., 2002). Although most studies have employed these measures over a long time-frame (usually 24 hours), research is beginning to examine the utility of this technique in healthy subjects during the processing of emotional stimuli. For example, two recent studies have investigated the effects of emotional film on measures of HRV (Lane, Reiman, Ahern & Thayer, 2001; Sakuragi, Sugiyama & Takeuchi, 2002); the former study also examined HRV and rCBF during emotional recall conditions. Lane and colleagues report a preliminary study which correlated the high frequency-HRV component (an index of vagal tone) with regional cerebral blood flow (rCBF). The authors report that decreases in HF-HRV correlate with decreases in brain activation within the MPFC and left posterior orbitofrontal/anterior insular cortices during emotional arousal, concluding that these findings are consistent with the proposed inhibitory role of the MPFC on the cardiovascular system (Lane et al., 2001; Thayer & Siegle, 2002 for discussion). Importantly, Lane and colleagues demonstrate that HRV may be used to index activity within neural structures associated with emotional processing, although how these measures relate to levels of 5-HT remains to be examined.

Finally, we should note a few limitations of the present study and provide some directions for future research. Firstly the sample size of the current study is small and is less than previous studies which have investigated HR response to emotional images (eg. Aftanas et al., 2001b; Lang et al., 1993; Palomba et al., 1997). Secondly, we were unable to provide measures of ‘deceleration’ (as reported in previous studies) as the protocol for the current study did not involve presentation of a blank screen prior to slide onset. Despite these limitations however, our findings display a similar trend which suggests that unpleasant images are associated with a lower HR than pleasant images with neutral images falling in between. In order to more fully understand the role of cardiovascular responsiveness to emotional stimuli and the effects of neurochemicals on this processing, it will be important for future research to examine other HR measures, such as HR variability.

In conclusion, the current findings indicate that in addition to the known psychotropic effects, citalopram may modulate the HR associated with the viewing of emotional stimuli. Specifically acute enhancement of 5-HT appears to suppress the normal fluctuations in HR associated with different emotional stimuli. These findings suggest a possible neurophysiological mechanism which may contribute to the safe cardiovascular profile of SRIs.
CHAPTER 6

6 AUGMENTATION OF SEROTONIN ENHANCES PLEASANT AND SUPPRESSES UNPLEASANT CORTICAL ELECTROPHYSIOLOGICAL RESPONSES TO VISUAL EMOTIONAL STIMULI IN HUMANS
6.1 INTRODUCTION

The indoleamine, 5-hydroxytryptamine, or serotonin (5-HT) was discovered over 50 years ago (Erspamer & Asero, 1952; Rapport et al., 1948; Twarog & Page, 1953), and since then its role in the pathophysiology of emotional disorders and mechanism of action of antidepressants has been subject to considerable research. Selective serotonin re-uptake inhibitors (SRIs) augment 5-HT in the brain and are now one of the major pharmacological treatments of a wide range of mood disorders including depression. Studies have demonstrated that enhancement of 5-HT with antidepressants such as the SRIs is associated with a decreasing magnitude of negative emotional states in psychiatric patients (eg. Salzman et al., 1995; Steiner et al., 1995; Van Vliet, Den Boer & Westenberg, 1994; see also Delgado et al., 1990). While SRIs are now widely used for the treatment of many emotional disorders, little is known about the neurophysiological mechanisms underlying the effects of serotonin on affective phenomena including emotional behaviours, mood and emotional processing, which may contribute towards their therapeutic mechanism of action.

Early correlational research on emotional behaviours and mood suggested that serotonergic abnormalities are associated with a variety of psychiatric and personality disturbances involving emotional dysfunction, including depression and impulse control disorders (see Coccaro et al 1989; Heninger 1995). Low 5-HT has been shown to be associated with violent and impulsive suicidal behaviour (Cremniter et al 1999; Spreux-Varoquaux et al 2001), impulsive aggression (Spoont 1992), and personality measures of hostility and aggression (Cleare & Bond 1997; Manuck et al 1998). In contrast, high 5-HT has been shown to be associated with harm avoidance, behavioural inhibition and reduced levels of positive and negative affect (Depue 1995; Hansenne & Ansseau 1999; Hennig et al 2000; Zald & Depue, 2001). This has lead to the proposal that 5-HT may act as a general constraint system, where its primary function is to inhibit the information flow of neural systems that mediate affective and motivational processes (Depue & Spoont 1986; Spoont 1992; Zald & Depue, 2001).

Recent studies have directly compared the effects of serotonergic manipulation via 5-HT pre-cursor depletion (see reviews: Moore et al 2000; Reilly et al 1997; Van der Does 2001) or serotonin enhancing agents (e.g., SRIs) (Knutson et al 1998) on mood and emotional behaviour. 5-HT depletion via 5-HT precursor depletion (ie. tryptophan
depletion) has been shown to reduce mood in those with a genetic predisposition to affective disorders in a number of studies (Benkelfat et al 1994; Ellenbogen et al 1996; Klaassen et al 1999; Quintin et al 2001). In contrast, chronic (4 week) serotonergic enhancement with the SRI paroxetine, was correlated negatively with measures of hostility, assaultiveness and negative affect and positively with social affiliation (Knutson et al 1998). Furthermore, chronic (2 week) ingestion of the serotonin precursor, L-tryptophan, enhanced social functioning by decreasing quarrelsome behaviour and increasing dominant behaviour in normal volunteers (Moskowitz et al 2001).

While most studies have examined 5-HT-mediated changes over long time frames (such as mood and personality traits), and have used questionnaire and behavioural based measures, little is known about the role of 5-HT in the mechanisms involved in the processing and responding to emotional stimuli. Recently, Harmer et al., (2003a) reported that acute administration of the SRI, citalopram (10mg i.v) facilitated the processing of happy facial expressions as evidenced by greater accuracy and reduced response times under citalopram relative to the placebo treatment. This finding was replicated in a subsequent study by the same authors after administration of tryptophan (Attenburrow et al., 2003). In addition, repeated administration of citalopram was associated with reduced recognition of the negative facial expressions, fear and disgust (Harmer et al., 2002). These findings, together with those from studies investigating emotional behaviour and mood, suggest a negative association between serotonin and unpleasant affect and a positive association with positive affect.

An alternative method for examining effects of neurochemical manipulation on emotional processing is neurophysiological methods incorporating brain imaging. For example, a recent study employing functional magnetic resonance imaging (fMRI), found that acute amistration of lorazepam (a GABA-A/benzodiazepine receptor agonist) decreased the activation associated with negative affective stimulation (both intensity of signal and number of voxels), but increased the activation associated with positive affective stimulation within the orbitofrontal cortex (Northoff et al., 2002). Similarly, a preliminary fMRI study in depressed subjects reported that global brain activation to unpleasant images was decreased, but the activation to pleasant images was enhanced within the right secondary visual cortex following chronic venlafaxine (a combined noradrenaline and 5-HT reuptake inhibitor) (Kalin et al., 1997). However these findings were not reported in a subsequent article based on
the same study/sample (Davidson et al., 2003). In this latter study, activations associated with the negative versus neutral stimuli trial only, were modified by chronic venlafaxine in depressed subjects (decreased activations in the insular and anterior cingulate cortex). It is important to note however that these latter two studies were conducted in depressed patients and not control subjects and there was no placebo comparison making it difficult to interpret the findings. These findings do however suggest possible system or network based neurophysiological mechanisms for the modulation of emotional processes by antidepressants.

The mechanisms involved in the modulatory effects of serotonin on emotional processing are yet to be examined. This is particularly relevant given that the cortical regions affected by serotonergic manipulation also overlap with those regions associated with emotion (Smith et al., 2002). The current study therefore examined the effects of acute serotonergic augmentation (with the SRI, citalopram) on cortical electrophysiological responses to the processing of pleasant and unpleasant visual emotional stimuli. Emotional responses are phasic in nature, and consequently require a technique that is able to track transient electrophysiological changes associated with the processing of emotional stimuli. Therefore, steady state probe topography (SSPT) was used. This technique offers the ability to track transient cortical electrophysiological changes with relatively high temporal resolution (usually 0.77 seconds) (Silberstein et al., 1995c, 1998). SSPT examines changes in 13Hz steady-state visually evoked potentials (SSVEPs) which comprise of two components: amplitude and latency (phase), and previous work has demonstrated that these components are sensitive to emotional manipulation (Kemp et al., 2002, also included in chapter 3; Kemp et al., in press, also included in chapter 4). These studies demonstrated that transient widespread and bilateral frontal SSVEP latency and occipital amplitude reductions are associated with the cortical processing of pleasant and unpleasant emotional stimuli. Based on the literature, it was hypothesised that following citalopram, unpleasant images relative to neutral images will be associated with a reduction in significantly activated cortical brain regions (especially within the frontal and occipital regions) whilst pleasant images relative to neutral images will be associated with enhancement of activation.
6.2 METHODS

6.2.1 Participants

17 healthy subjects (8 males and 9 females) participated in the current study (mean age, 22.88; SD, 4.61; mean education, 15.69; SD, 1.30). All participants were right-handed, (the Edinburgh Inventory, Oldfield, 1971), non-smokers, not on any medication (minimum 1 month drug-free) or illicit drugs. All successfully completed a medical examination involving physical and question based assessment, conducted by a physician who screened and excluded individuals with a history of past and present cardiovascular, hepatic, gastrointestinal, endocrine, neurological or psychiatric conditions. Participants were recruited by advertising on university notice boards and gave informed consent to take part in the study, which was approved by the Swinburne Research Ethics Committee.

6.2.2 Procedure

The study employed a randomised double-blind, placebo controlled design, in which subjects were tested under two acute treatment conditions: oral administration of placebo and citalopram (20mg), each of which were separated by a minimum washout period of 1-week. The study involved presentation of a series of images, selected from the International Affective Picture System (IAPS) (Lang et al., 1999), under both treatment conditions. These images were chosen based on standardized valence and arousal ratings published in the IAPS Instruction Manual. Images selected were categorized as unpleasant, neutral and pleasant and standardised ratings for valence ranged between 1.8 and 3.47; 4.46 and 5.46; 7.02 and 8.34 respectively, whilst standardised ratings of arousal ranged between 3.52 and 5.5; 1.55 and 4.27; 2.67 and 5.94 respectively. A more detailed description of task construction has been described previously (Kemp et al., 2002).

Subjects were instructed to focus on emotional content, to refrain from emotive inhibition, and to be prepared to rate each image on valence and arousal dimensions using the Self-Assessment Manikin (SAM) (Lang et al., 1999). The task involved presentation of selected images for a duration of 6-seconds, with each image followed by a valence, then an arousal rating scale in order to obtain verbal responses as to how subjects felt whilst they viewed the presented images. Images were categorised according to the predefined valence of the images (unpleasant (U), neutral (N) and pleasant (P); with each block containing 25 images) and presented to participants in 3 blocks (P,N,U or U,N,P).
Subjects arrived for testing on each of the two testing days at approximately 8am after which a standard breakfast was provided. Subjects were then brought to the recording room, which was soundproofed and dimly lit and instructed on how to complete the IAPS task. Subjects were tested 2 hours following administration of either placebo or citalopram. This 2 hour delay was chosen to coincide with approximate peak plasma levels of citalopram (Noble and Benfield, 1997). The administration of either placebo or citalopram on a particular day was counterbalanced across subjects.

A diffuse 13Hz sinusoidal white flicker, which was superimposed onto the visual field, elicited the SSVEPs whilst images and rating scales were presented to subjects from a computer monitor. The visual flicker was presented through a set of modified goggles and subtended a horizontal angle of 160° and a vertical angle of 90°, and had a modulation depth of 45% when viewed against the background. Brain electrical activity was recorded by 64 monopolar leads (impedances generally <5 KOhms), positioned in International 10/20 positions and sites between these positions, using a lycra electrode cap. Linked ear electrodes were used as a reference and a nose electrode was used for ground. One of the monopolar leads was sacrificed in order to record the electrocardiogram (ECG) from the upper left arm, which was also referenced to the linked ear electrodes. Recorded activity was bandpass filtered from 0.74Hz to 74Hz and digitized at a rate of 500Hz with 16-bit accuracy.

6.2.3 Signal Processing

The key features of SSPT signal processing analysis and associated artifact detection procedures have been described previously (eg. Silberstein et al., 1995c). SSVEP’s were produced for all electrodes by calculating the 13Hz fourier coefficients (FC) for each cycle and then smoothing the FC time-series by averaging overlapping blocks of 10 FC’s. This methodology was applied to all image categories and for each treatment condition. A target averaging technique was used to select the SSVEP associated with each image and then, average all epochs for each category. Epochs for each individual were then averaged to form a cross-subject-averaged 6-second epoch for each image category. The SSVEP epoch corresponding to the neutral images was then subtracted from both emotional categories yielding activity interpreted as being associated with pleasant or unpleasant valence.
The functional significance of the SSVEP amplitude and latency modulations has been discussed previously (Silberstein, 1995b, 1998; Silberstein et al., 2001). To summarise, re-entrant feedback and feed-forward cortico-cortico and thalamo-cortico fibres have been proposed to underlie the generation of the SSVEP. In this framework, decreases in the number of synchronised neural elements (or increases in the number of desynchronised neural elements) within the re-entrant loop (loop gain) will be associated with decreases in SSVEP amplitude whilst decreases in the synaptic and axonal transmission times of the re-entrant loop (loop-time) will be associated with SSVEP latency decreases.

6.2.4 Presentation of Data

SSVEP results are initially presented in terms of amplitude and latency time-series plots, as well as Hotellings $T$ statistical cluster plots (Figure 21). Electrodes (presented on the y-axis of these time-series and cluster plots) are compartmentalised into regions approximately associated with frontal (electrodes 0 – 20, including Fp1, Fp2, F7, F3, Fz, F4 and F8), centro-parieto-temporal (electrodes 21 – 52, including T3, C3, Cz, C4, T4, T5, P3, Pz, P4, and T6) and occipital (electrodes 53 – 63, including O1, Oz and O2) electrode placement to aid interpretation of these plots. Display of statistical cluster plots have been used previously in order to efficiently summarise the comparison of multiple data sets as well as determine robust effects through identification of consecutive statistical spatiotemporal clusters (Murray et al., 2002; Gray et al., 2003; Kemp et al., in press). These plots illustrate levels of statistical significance (indicated by colour values), across the two treatment conditions (placebo, citalopram) for all electrodes across all time-points.

Based on this initial examination we then averaged the SSPT into different time periods (identified in the cluster plots) and present these averaged time-periods as topographic maps using a spherical spline interpolation procedure (Nunez et al., 1994). These maps display the two components of the SSVEP: amplitude and latency for each emotional condition relative to the neutral images, and the statistical strength of the differences (Hotellings $T$ statistic). The SSVEP topographic maps are scaled to the highest/lowest values for each valence category (pleasant, unpleasant) and each SSVEP component (amplitude, latency), as the purpose of the current paper is to explore the differences between treatment conditions (placebo, citalopram).
6.2.5 Statistical Issues

Behavioural SAM ratings were analysed using a within-subjects Treatment X Category repeated measures analysis of variance (RANOVA) for valence and arousal (separately) to determine whether treatment modified participant’s emotional ratings of the presented images. In addition, analysis of heart rate corresponding with the presentation of all image categories within placebo and citalopram conditions was conducted in the following way. Cardiac interbeat intervals (ibi) during picture presentation were converted to beats per minute (bpm) in half-second bins. In the current study we averaged these half second bins for each six-second epoch associated with the three valenced categories and conducted a within-subjects Treatment (placebo, citalopram) X Category (unpleasant, neutral, pleasant) RANOVA in order to allow investigation of treatment effects.

The statistical strength of the SSVEP differences between the emotional images (unpleasant, pleasant) and the neutral images were examined using the Hotellings $T^2$ parameter and presented in statistical cluster plots as well as topographic maps. An alpha criterion for the Hotellings $T$ was arbitrarily set at $p=0.01$ (uncorrected for multiple comparisons) for the SSVEP data as used previously (Gray et al., 2003; Kemp et al., in press). In addition to Hotellings statistics, a series of post-hoc RANOVA tests were conducted in order to directly test differences between the two drug conditions. 18 electrodes (9 within the left-hemisphere and 9 within the right-hemisphere) were entered for both frontal and posterior locations and included Fp1, Fp2, F3, F4, F7, F8 for frontal locations and T5, T6, P3, P4, O1 and O2 for posterior electrode locations as well as electrode locations midway between these standard positions. Midline electrode sites were excluded. As the within-subject factors of Drug (Placebo, Citalopram), Category (Neutral, Pleasant, Unpleasant image categories) and Hemisphere were of primary a-priori interest, RANOVAs employing a customised experimental design were applied (separately) for amplitude and latency SSVEP components. Applying a customised experimental design allows for selection of fewer effects to be reported regardless of the number of factors entered. This method therefore minimises the generation of false positive findings (Type 1 error), while focusing on the effects of most interest. The within-subject factors of Drug, Category, Hemisphere and Electrode and a between-subjects factor of gender were defined, however the within-subjects model included only Drug X Category and Drug X Category X Hemisphere interactions. The between-subjects factor of gender was also included in the model in order to investigate possible confounding effects of
gender on the basis of our recent study which reports on electrophysiological gender effects following presentation of identical images to those presented in the current study (Kemp et al., in press).

6.3 RESULTS

6.3.1 Behavioural Data
The means and standard deviations for ratings of valence and arousal made by subjects are presented below in Table 10.

Table 10: Means and standard deviation are presented for Valence and Arousal SAM ratings, for pleasant, neutral and unpleasant categories in placebo and citalopram treatment conditions.

<table>
<thead>
<tr>
<th></th>
<th>Pleasant</th>
<th>Neutral</th>
<th>Unpleasant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Citalopram</td>
<td>Placebo</td>
</tr>
<tr>
<td>Valence</td>
<td>6.37±0.62</td>
<td>6.12±0.51</td>
<td>5.04±0.22</td>
</tr>
<tr>
<td>Arousal</td>
<td>3.31±1.70</td>
<td>2.82±1.30</td>
<td>1.88±0.94</td>
</tr>
</tbody>
</table>

Significant category effects were found for both valence \((F(1.17,18.77)=110.38, p<0.001, \text{partial eta squared} = 0.87)\) (Greenhouse-Geisser adjusted) and arousal \((F(2,32)=26.41, p<0.001, \text{partial eta squared} = 0.62)\). For the valence dimension, planned comparisons revealed that participant’s ratings of both pleasant and unpleasant images were significantly different from ratings of neutral images \((F(1,16)=95.92, p<0.001, \text{partial eta squared} = 0.86\) and \(F(1,16)=93.72, p<0.001, \text{partial eta squared} = 0.85\), respectively). For the arousal dimension, planned comparisons revealed that ratings of arousal for both pleasant and unpleasant images were significantly different from ratings of neutral images \((F(1,16)=15.93, p=0.001, \text{partial eta squared} = 0.50\) and \(F(1,16)=51.05, p<0.001, \text{partial eta squared} = 0.76\), respectively). In addition, ratings of arousal for unpleasant images were significantly different to those of pleasant images \((F(1,16)=10.49, p=0.005, \text{partial eta squared} = 0.40)\). No main effects for treatment were demonstrated, nor were participant’s ratings of valence or arousal modified by treatment.

6.3.2 ECG Data
Analysis of heart rate (HR) using a 2 (Treatment) X 3 (Category) within subject, RANOVA design revealed a significant main effect for category \((F(2,32)=3.39, p=0.046, \text{partial eta squared} = 0.18)\), and although the main effect for treatment was not significant, treatment was found to modify the main effect for category \((F(1.43,22.80)=4.12, p=0.042, \text{partial eta squared} = 0.21)\) (Greenhouse-Geisser
adjusted) (see Figure 20, for a visual display of this interaction). Planned comparisons for category revealed that HR during viewing of unpleasant images was significantly different from HR during viewing of pleasant images ($F(1,16)=6.79$, $p=0.019$, partial eta squared $= 0.30$); a trend towards statistical significance for HR during viewing of unpleasant images relative to HR during viewing of neutral images ($F(1,16)=4.16$, $p=0.058$, partial eta squared $= 0.21$); and no statistical difference between HR during pleasant images and HR during viewing of neutral images.

RANOVA post-hoc tests were conducted on the placebo and citalopram treatment groups to further understand the category X treatment interaction effect. Previous literature suggests that heart rate is able to discriminate differently valenced images (eg. Lang et al., 1993; Palomba et al., 1997), therefore RANOVA’s were chosen to investigate this interaction rather than paired-sample t-tests on each of the valenced categories, because we were interested in (1) whether heart-rate was able to discriminate the three valenced categories of images in placebo treatment and (2), how citalopram altered this effect.

Significant category effects were found for the placebo treatment condition ($F(2,32)=5.70$, $p=0.008$, partial eta squared=0.26), but not for the citalopram condition ($F(2,32)=0.96$, $p=0.394$), suggesting that the ability for heart rate to distinguish between differently valenced images under no drug condition was suppressed by the citalopram treatment condition. Planned comparisons for category within the placebo treatment condition revealed that heart rate during viewing of unpleasant images was significantly less than heart rate during viewing of pleasant images ($F(1,16)=7.38$, $p=0.015$, partial eta squared=0.32); a trend towards a statistically significant reduction in heart rate during viewing of unpleasant images relative to neutral images ($F(1,16)=4.51$, $p=0.05$, partial eta squared=0.22); no significant differences between neutral and pleasant images.
6.3.3 SSVEP Data

Amplitude and latency time-series plots, representing the differences between emotional and neutral categories (emotional valence), as well as the statistical significance of these differences are presented in Figure 21. The Hotellings $T$ time-series plots display significant clusters of electrodes and time-points during the processing of both pleasant and unpleasant valence under placebo and citalopram conditions. Figure 21 indicates that, for pleasant valence, the placebo condition is associated with a smaller number of significant time points relative to the citalopram condition during the middle and late time-components (visual comparison between the first and second statistical cluster plots). In addition, Figure 21 indicates for unpleasant valence, that the placebo condition is associated with a greater number of significant time points relative to the citalopram condition, during all three components (early, middle, late), particularly within the posterior region (visual comparison between the first and second statistical cluster plots). The locations of these differences as well as statistical significance of these differences are displayed in topographic maps and examined in three equal 2-second time-periods (Figures 22 and 23).
Figure 22: Illustration of amplitude (row 1) and latency (row 2) time series plots for pleasant and unpleasant valence in placebo and citalopram conditions across time (x-axis) for each of the 64 electrode positions (y-axis). In addition, plots of Hotellings t values (row 3) illustrate the statistical significance of these effects. Each plot has been divided into early (0-2 seconds), middle (2-4 seconds) and late (4-6 seconds) time periods.

The effects of placebo and citalopram on the processing of pleasant valence are illustrated in Figure 22. After administration of placebo, the processing of pleasant valence is associated with amplitude decreases and latency increases in frontal regions throughout the six-second epoch and in posterior regions during the middle component. After administration of citalopram, the effects observed under placebo condition during the early and middle epochs were attenuated; while there was an augmentation reflected by a large parieto-occipital amplitude decrease suggesting
increased activation within this region. This amplitude decrease begins within the 2-4 second epoch and extends into frontal regions during the 4-6 second epoch. The associated Hotellings \( T \) topographic map revealed widespread significant posterior activations for the 4-6 second epoch. In summary, while citalopram reduces the significant differences between pleasant and neutral images within early and middle components of processing pleasant stimuli, the greatest effects (amplitude reductions) are displayed within the late component, suggesting increased activity within parieto-occipital regions.

The effects of placebo and citalopram on the processing of unpleasant valence are illustrated in Figure 23. After administration of placebo, the processing of unpleasant valence is associated with widespread significant reductions in amplitude and latency, particularly within the posterior region. After administration of citalopram, unpleasant valence is associated with a suppression of these amplitude and latency reductions (reduced activity), within both frontal and posterior regions, and this suppression is particularly prominent during the early and middle epochs. In addition, latency is noted to demonstrate significant latency increases within the right anterior frontal (early epoch), left anterior temporal regions (middle epoch) and occipital regions (late epoch) during the citalopram condition. Finally, the late component of the citalopram condition is characterized by significant and widespread decreases in posterior amplitude. In summary, administration of citalopram is associated with a suppression of the frontal and posterior amplitude and latency reductions (reduced activity) as well as latency increases (reversal of activity seen under placebo) when compared with the placebo condition.
Figure 23: Topographic presentation of the 13Hz SSVEP data (amplitude and latency) and Hotellings $T$ values for pleasant valence in placebo and citalopram conditions. Three time-periods are presented which relate to early (0-2 seconds), middle (2-4 seconds) and late (4-6 seconds) components of image viewing.
A preliminary analysis was conducted to examine whether males (n=8) and females (n=9) differ in the processing of pleasant and unpleasant valence and the effects on this processing following acute administration of citalopram. Results of this preliminary investigation are displayed in Figure 24 and suggest that following citalopram, pleasant valence is associated with greater reductions in amplitude within centro-parieto-temporal and occipital regions in males (also corresponding with an increase in the number and size of statistical clusters within these regions). This profile however was not displayed by females. Results also suggest that following
administration of citalopram, unpleasant valence is associated with a suppression of both amplitude and latency reductions in females (also corresponding with a reduction in the number and size of statistical clusters). This profile however was not displayed by males. In summary, the effects of citalopram on pleasant valence (when males and females are combined) appear to be predominantly influenced by males, whilst effects of citalopram on unpleasant valence (when males and females are combined) appear to be predominantly influenced by females.

Finally, a series of RANOVAs were conducted on both anterior and posterior electrode locations to more directly test for statistical differences between placebo and citalopram. Frontal effects during the early time-period were investigated because the most interesting effects, according to the Hotellings maps, appear to occur during this epoch, particularly for the latency component in the unpleasant (-) neutral comparison and citalopram condition. In contrast, posterior effects during the late time-period were investigated because the most interesting effects, according to the Hotellings maps, appear to occur during this epoch, particularly for the amplitude component in the pleasant (-) neutral comparison and citalopram condition. The RANOVAs conducted on amplitude and latency in anterior locations during the early time-period revealed no Drug X Category, Drug X Category X Gender, Drug X Category X Hemisphere, Drug X Category X Hemisphere X Gender interactions suggesting that Drug condition does not modify the effects of Category at anterior electrode locations. The RANOVA conducted on amplitude in posterior locations for the late time-period, revealed no statistically significant effects although the Drug X Category X Hemisphere (F(2,30)=2.869, p=0.072, partial eta squared = 0.161) reached trend levels of significance. The RANOVA conducted on latency in posterior locations for the late time-period revealed a significant Drug X Category interaction (F(2,30)=3.845, p=0.033, partial eta squared = 0.204). Tests of within-subjects contrasts identified a significant effect of drug for the contrast of unpleasant images versus neutral images (F(1,15)=5.926, p=0.028, partial eta squared = 0.283) but not for pleasant images versus neutral images (F(1,15)=1.627, p=0.221). To explore this finding further, a follow-up, repeated measures ANOVA was conducted for the placebo and citalopram conditions separately, for the difference between unpleasant and neutral images across all 18 posterior electrodes. Only the within-subjects factor of Category was included in the model therefore exploring the effects of Category after collapsing across Electrode. Findings suggest that posterior latency in the placebo condition during presentation of unpleasant images is less than that during presentation of neutral images (F(1,16)=5.794, p=0.029, partial eta squared = 0.266).
By contrast, posterior latency in the citalopram condition during presentation of unpleasant images did not significantly differ from that during presentation of neutral images (F(1,16)=0.573, p=0.460). Gender was not found to modify the Drug X Category or Drug X Category X Hemisphere interactions for either anterior or posterior electrode locations. In summary, RANOVA results confirm the Hotellings findings that latency reductions during the processing of unpleasant images (relative to neutral images) within posterior regions are suppressed in the citalopram condition, and suggest a trend for a modifying role of citalopram on the processing of pleasant images (relative to neutral images) within posterior regions but failed to confirm any effects of citalopram within anterior electrode locations.

**Figure 25:** Unpleasant and pleasant time-series plots for placebo and citalopram conditions associated with male and female genders.
6.4 DISCUSSION

Acute augmentation of serotonin with citalopram was found to differentially affect the electrophysiological responsiveness to pleasant and unpleasant images relative to neutral images. Two key findings in terms of the SSVEPs should be highlighted. Firstly, in the citalopram condition in contrast to the placebo condition, processing of pleasant valence was predominantly associated with increased parieto-occipital activity (characterised by widespread reductions in amplitude), whilst processing of unpleasant valence was predominantly associated with a suppression of anterior-frontal and occipital activity (characterised by suppression of amplitude and latency reductions). Secondly, different spatio-temporal statistically significant patterns emerge following administration of citalopram in contrast to placebo. In particular, the processing of unpleasant valence (i.e. in the placebo condition) was associated with significant parieto-occipital activations (amplitude and latency reductions) throughout the three time periods, and frontal activations during the middle time-period. Citalopram’s predominant effects were the attenuation of these findings. The processing of pleasant valence however was associated with significant frontal activations (amplitude reductions) throughout the three time periods and left temporoparietal activations during the middle time-period. Citalopram’s predominant effect was to enhance processing within parieto-occipital regions in the middle and late time points.

The present study employed SSPT to examine the effects of citalopram on emotional processing. This technique examines changes in 13Hz SSVEPs, which primarily reflect neuronal activity within pyramidal cells of the neo-cortex (which are the principal source of cortical glutamate). SSVEP is comprised of two components, amplitude and phase (latency) and previous work has demonstrated that these components are sensitive to cognitive and emotional manipulation (eg. Gray et al., 2003; Kemp et al., 2002, also included as chapter 3; Kemp et al., in press, also included as chapter 4; Silberstein et al., 1996, 2000). We have suggested that amplitude may be compared to alpha activity in association with cognitive tasks (Silberstein, 1995a,b), whilst latency reductions (or increased processing speed) may be a consequence of increased synaptic excitatory processes in these networks, and conversely latency increases may be a consequence of reduced synaptic excitation (or increased synaptic inhibition) (Silberstein et al., 2000, 2001). Hence amplitude and latency reductions are interpreted as increased cortical activation. While the exact mechanisms associated with SSVEP changes are not known, there is strong
evidence for interactions between serotonin and glutamate in neocortical pyramidal cells (see Marek & Aghajanian, 1998 for a review). In addition, it is possible that the responsiveness to serotonergic augmentation, of certain excitatory (5-HT$_{2A}$) and inhibitory (5-HT$_{1A}$) receptors within pyramidal cells may vary depending on the emotional circuitry involved in the processing of pleasant or unpleasant valence. Consequently, activation of these excitatory and inhibitory receptors may lead to amplitude and latency changes observed during the processing of pleasant and unpleasant valence, respectively. These mechanisms are supported by evidence that most antidepressants enhance post-synaptic 5-HT$_{1A}$ hyperpolarisation (see Barnes & Sharp, 1999 for a review of central 5-HT receptors) and the finding that 5-HT induces a marked increase in excitatory postsynaptic currents within layer V of the pyramidal cells through activation of 5-HT$_{2A}$ receptors (Marek & Aghajanian, 1998).

In the present study, the processing of pleasant valence after citalopram (relative to placebo) was characterized by diffuse, amplitude reductions within parieto-occipital regions which are suggestive of increased activity. We have previously reported that amplitude reductions within occipital regions are associated with increases in visual attention (eg. Silberstein et al., 1990). In addition, a study using fMRI, reported increased activity in response to positive stimuli within the right occipital region, following chronic administration of venlafaxine (Kalin et al., 1997). These authors suggested that the increased responsiveness to positive stimuli following venlafaxine treatment may be associated with an increase in attention. Harmer and colleagues have recently reported that enhanced perception of affiliative signals occurring with citalopram administration may be an aspect particular to the action of SRIs (Harmer et al., 2003a). The authors suggest that enhanced perception of affiliative signals may facilitate approach behaviours such as social affiliation and dominance. This interpretation supports earlier studies which reported an enhancement of these behaviours following chronic SRI administration (Knutson et al 1998; Moskowitz et al 2001). These previous findings suggest that the strong amplitude reductions displayed after citalopram in the present study may reflect a cortical neurophysiological mechanism relating to enhanced perception to or orienting towards the pleasant images.

The processing of unpleasant valence after citalopram, was associated with an attenuation of the cortical activation seen during placebo treatment (i.e. amplitude and latency reductions) within anterior frontal and occipital regions. This attenuation with citalopram was more pronounced within right-anterior frontal and left anterior
temporal regions in which latency increases were observed (decreased activation). Interestingly, the right prefrontal cortex has been previously implicated in both transient and chronic changes in negative mood (eg. Kemp et al., in press; Mayberg et al., 1999 respectively) as well as withdrawal related behaviour (eg. Davidson & Irwin, 1999). Our findings are consistent with studies that have reported that antidepressant treatment decreases activation within the orbital cortex and ventrolateral PFC (Drevets et al., 1992; Drevets, 1999; Brody et al., 2001b; Mayberg et al., 1999; Nobler et al., 1994). In addition, it has been previously reported that antidepressant treatment is associated with a reversal of pre-treatment brain activity in depressed patients (Mayberg et al., 1999; Brody et al., 2001a; Drevets et al., 2002). The increased activation within the occipital cortex (observed under placebo) is likely a function of feedback from the amygdala (Amaral et al., 1992; Davidson et al., 2003) and may relate to a heightened sensitivity to visual stimuli with emotional relevance (Lane et al., 1999; Lang et al., 1998). Findings suggest the citalopram may reduce this sensitivity possibly by modulating the amygdala response to negative stimuli. Decreases in activation within frontal and occipital areas may be related to the theory that reduced excitatory transmission within limbic-thalamo-cortical (LTC) and limbic-cortical-striatal-pallidal-thalamic (KCSPT) circuitry plays a role in the mechanisms of antidepressant treatment (Drevets et al., 2002).

The findings of this study may demonstrate a neurophysiological mechanism for the effects of serotonergic antidepressants such that increasing 5-HT leads to an enhancement of pleasant but a suppression of unpleasant cortical electrophysiological responses to visual emotional stimuli. As discussed in section 6.1, theoretical models have suggested that increases in 5-HT are associated with the behavioural dimensions of harm avoidance and constraint. Results from the current study suggest that increased levels of 5-HT may “constrain” neurophysiological processing of unpleasant stimuli. The results also suggest that initial processing of pleasant stimuli may be enhanced. Harmer and colleagues have posited a tentative hypothesis for antidepressant action that suggests enhanced perception of affiliative signals may precede and even facilitate approach behaviour and social interaction (Harmer et al., 2003c). It is possible therefore that antidepressants such as SRIs shift the attentional bias from negative to positive stimuli and that these effects are apparent even after an acute dosage. Our findings also suggest that the use of brain imaging techniques may better explain immediate responsiveness to affective stimuli than simply correlating the association between 5-

Gender differences have been previously reported in studies on emotional processing (eg. Bremner et al., 2001; Lee et al., 2002; Kemp et al., in press, also included as chapter 4; Killgore, 2000; Pendergrass et al., 2003; Wrase et al., 2003). Therefore, preliminary gender differences in the processing of pleasant and unpleasant valence and the effects on this processing following acute administration of citalopram were examined in the current study. Results suggest that the effects of citalopram on pleasant valence (when males and females are combined) appear to be predominantly influenced by males, whilst the effects of citalopram on unpleasant valence (when males and females are combined) appear to be predominantly influenced by females. This suggests that citalopram may potentiate male’s responsiveness to pleasant stimuli, whilst suppressing female’s responsiveness to unpleasant stimuli. These findings are consistent with our previously published study that reported that while males may be more responsive (electrophysiologically) to pleasant images (relative to neutral images), females may be more responsive (electrophysiologically) to unpleasant images (relative to neutral images). It should be noted however, that the gender differences (reported in the current study) are preliminary findings and based on a small sample size. Future studies should further investigate therefore not only how males and females differ on emotional processing, but neurochemical modulation of this processing.

While differences in the electrophysiological responses to emotional valence were observed in the current study, no changes were observed in subjective behavioural ratings (SAM ratings). This finding supports observations of a previous study which showed changes on objective measures (i.e. detection of a higher number of facial expressions of happiness with reduced response times), but lack of changes in subjective state (using visual analogue scales and the Befindlichkeits Scale) following acute serotonergic enhancement with citalopram (Harmer et al., 2003a). These findings suggest that emotional processing may occur independently or at a lower threshold, than overt changes in mood. In contrast to the subjective behavioural findings, physiological heart rate was shown to be modulated by citalopram. More specifically, the findings in the placebo condition demonstrate that heart rate during the viewing of unpleasant images was less than that during the viewing of pleasant images (supporting previous findings eg. Lang et al., 1993; Palomba et al., 1997; Aftanas et al., 2001), and that this effect disappeared following
administration of citalopram. Importantly, our heart rate data provides a physiological measure of emotional responsiveness to the presented images and in addition, that this emotional responsiveness is eliminated by serotonergic enhancement with an antidepressant. While it is uncertain what the exact mechanism responsible for this effect is, it is likely that the effects of citalopram on emotion modulated HR may occur through descending influences of regions known to be involved in emotional processing, such as the prefrontal cortex and amygdala (see Thayer & Lane, 2000 for a discussion of the functional networks which mediate psychophysiological resources in attention and emotion).

We have previously reported that transient, widespread and bilateral frontal SSVEP latency and occipital amplitude reductions are associated with the cortical processing of pleasant and unpleasant valence in 16 participants using the same IAPS stimuli (Kemp et al., 2002, also included as chapter 3). More recently, we confirmed the finding that frontal latency and occipital amplitude reductions are associated with the processing of pleasant and unpleasant valence in 30 participants (Kemp et al., in press, also included as chapter 4). Previous studies in healthy subjects using fMRI and PET imaging have reported increased activity within the medial prefrontal cortex and visual cortical areas during tasks that examine emotional processing (see recent meta-analyses, Phan et al., 2002; Wager et al., 2003). It is important to note however that increased activity within the medial prefrontal cortex is in contrast to the findings of mood induction and depressed state in which decreased activation is reported within dorsomedial/dorsolateral regions (eg. Mayberg et al., 1999; Drevets et al., 2002). It is likely that such a discrepancy relates to differences between emotional processing and emotional induction paradigms, internally and externally generated emotions as well as the modulatory effects of cognitive processes.

In the current study, the processing of unpleasant valence was associated with a decrease in SSVEP amplitude and latency in frontal and occipital cortices, while pleasant valence was associated with widespread amplitude decreases within frontal and temporo-parietal cortices. While the findings reported in the current study relating to the processing of unpleasant valence under placebo are generally consistent with our two previously published studies (under drug-free conditions), the SSVEP latency increases during the processing of pleasant valence was not. It is possible that this difference may relate to a number of factors. Firstly, compared to our previous studies, the current study examined emotional processing after the administration of placebo. Indeed, administration of placebo has been reported to produce specific
brain modulated activation (Mayberg et al., 2002). It is possible that the differences observed in the processing of pleasant valence between this study and our previous studies, may be related to a placebo effect. Secondly, there were differences in experimental design (i.e. participants in the two previous studies had only viewed the IAPS stimuli once, whilst participants in the current study may have seen the images up to three times, which included prior baseline and treatment recordings).

Interestingly, Phan and colleagues have suggested that the rostral anterior cingulate cortex, medial prefrontal cortex, hippocampus and amygdala activations may habituate with repeated exposure (Phan et al., 2003). It is possible that participants under placebo had habituated to the pleasant stimuli as the present study presented images that were classified as low on the arousal dimension. In addition, participants may have been less likely to habituate to unpleasant images due to a heightened sensitivity to negative stimuli, a phenomenon known as the negativity bias (see Cacioppo & Gardener, 1999 for discussion).

Finally, a number of limitations of the study are worth noting. Firstly, although participants were instructed to refrain from emotive inhibition, it should be recognised that the SSPT technique examines the conscious, ongoing processing of emotion (see Kemp et al., 2002 for discussion). Therefore the results within frontal locations may reflect conscious, cognitive regulatory processes in addition to affective elicitation. Secondly, the SSPT technique used in the present study only provides information on modulation of amplitude and latency components of the 13Hz frequency, therefore the specificity of these findings is unclear. Thirdly, RANOVA statistics confirm the Hotellings findings that latency reductions during the processing of unpleasant images (relative to neutral images) within posterior regions, are suppressed in the citalopram condition, and suggest a trend for a modifying role of citalopram on the processing of pleasant images (relative to neutral images) within posterior regions. However, RANOVA statistics failed to confirm any effects of citalopram within anterior electrode locations as is suggested by the Hotellings statistics from a visual comparison between placebo and citalopram conditions. It is important to note that while the Hotellings T statistics were conducted on complex numbers (combination of amplitude and phase) using in-house software, no in-house software is available at present for conducting repeated-measures statistics on such data. RANOVA statistics were therefore conducted on amplitude and latency data separately, to allow for tests to be run using SPSS V.10 (SPSS Inc., 1999). These procedural differences may in part account for differences between the results of the two statistical tests reported in the current study. It is also possible that the failure to
confirm any effects of citalopram within frontal regions by the RANOVA, may indicate that frontal changes are more pronounced following chronic administration.

Overall, our findings suggest that acute enhancement of serotonergic function with the SRI, citalopram modulates neurophysiological processing of emotionally valent stimuli such that cortical response to pleasant valence is potentiated and cortical response to unpleasant valence is suppressed. These findings are consistent with the interpretation that antidepressants may enhance perception of affiliative signals, whilst constraining perception of negative signals even after an acute dosage (Harmer et al., 2003c). Our study moves beyond the examination of cellular and neurochemical mechanisms of antidepressant action and employs a more systems-based approach to the study of antidepressant action, through examination of the neurophysiological responses to visual emotional stimuli (Nathan et al., 2003; Harmer et al., 2003b). This approach may lead to greater understanding of the functional consequences of neurochemical modulation on cortical networks involved in emotional processing.
CHAPTER 7

7 GENERAL DISCUSSION AND CONCLUSIONS
In early 2000, active researchers were invited by the Brazilian Society of Neuroscience and Behaviour (SBNeC) to discuss advances that had been made over the last 10 years in the domain of affective neuroscience. This symposium involved discussion of a number of critical issues facing researchers interested in the neurobiology of emotion, some of which have been explored in the current thesis. Some of the issues that were raised and subsequently explored in this thesis include: 1) a concern that much research has focused on aversive emotional activations and has ignored positive emotional states; 2) the importance of neurochemistry in emotional processes as well as the modulation of these processes; 3) to investigate not only ‘what’ neuronal centers are involved in emotion, but ‘how’ these compute during an apparent emotional state, and 4) the relation between self-report and brain systems (see Blanchard et al., 2001 for more details on this forum).

This thesis reports on and discusses the effects of emotional processing on brain electrical activity, HR and self-report variables, such as the POMS and SAM ratings. In each of the studies included in this thesis, participants viewed 75 pleasant, neutral and unpleasant images, which had been selected from the IAPS. Participants were asked to focus on emotional content, to refrain from emotive inhibition, and to rate each image as they actually felt whilst viewing it. Given that the IAPS task is able to evoke a broad range of emotions experienced outside the laboratory (Lang et al., 1997) and that the images selected from the IAPS for presentation to our participants were rated low on the arousal dimension, the results reported in each of the four experimental chapters are interpreted as reflecting both perceptual and mild experiential components.

### 7.1 Summary of Key Findings and Implications

The aim of the first experimental chapter was to examine SSVEPs associated with the processing of pleasant and unpleasant images relative to neutral images. Results demonstrated that transient, widespread and bilateral frontal SSVEP latency and occipital amplitude reductions were associated with the cortical processing of pleasant and unpleasant emotional stimuli. In addition, unpleasant relative to neutral images were associated with left temporal and right occipitotemporal latency reductions, a bilateral anterior frontal amplitude reduction and centroparietal amplitude increases. These findings support previous literature in terms of there being substantial overlap in frontal neural circuitry when the brain processes pleasant and unpleasant images relative to neutral images. In addition, findings within
posterior locations support those of previous studies which have demonstrated activation within visual cortical areas in response to emotional images having both pleasant and unpleasant valence. This research represents the first study to employ the SSPT technique in order to examine phasic emotional responses.

The first experimental chapter however, reported findings from a mixed gender sample. To date, few studies have investigated the differential effects of gender on emotional processing. The aim of the second experimental chapter therefore was to determine how the processing of IAPS images differed between males and females. Results demonstrate again that pleasant and unpleasant images relative to neutral images (pleasant and unpleasant valence, respectively) are associated with reductions in frontal latency and occipital amplitude. In addition, electrophysiological gender differences were observed in the processing of images with emotional content despite there being no differences between males and females on subjective mood (POMS) or behavioural ratings of presented images (valence and arousal dimensions). Key gender differences indicated that the processing of pleasant valence is associated with left and right frontal latency reductions in males but not in females, whilst the processing of unpleasant valence is associated with widespread frontal latency reductions (predominantly right sided) in females but not in males. Results suggest that gender differences do exist in the processing of visual emotional stimuli, and illustrate the importance of taking these differences into account during investigations of emotional processing. In addition, these gender differences may have implications for the pathophysiology of mood disorders such as depression.

The images selected for presentation to participants in all experimental studies reported in this thesis were identical and constrained along the dimension of arousal to allow for the corresponding electrophysiological activation to be interpreted along the valence dimension. The IAPS images are believed to be able to evoke a broad range of emotions experienced outside the laboratory, however the images selected for presentation were rated relatively low on the dimension of arousal. It could be argued therefore that the electrocortical activations reported in the first two experimental chapters relate more to a ‘perception’ of the emotional content rather than an ‘experience’. Emotion is generally regarded as consisting of multiple response components including cognitive processes, physiological responses, motivational changes, motor expression and subjective feeling. The first two experimental chapters however, only investigated electrophysiological and behavioural (subjective) responses to presented images and did not examine other
physiological responses such as HR, which has long been regarded as a key component of visceral activity associated with an emotional response. Previous studies have demonstrated that changes in HR load onto a valence dimension and are able to differentiate pleasant from unpleasant visual stimuli.

The aim of the third experimental chapter therefore was to investigate whether the images chosen for presentation to participants produced visceral responsiveness (HR), in addition to the electrocortical response reported in the first two experimental chapters. In addition to exploring the cardiovascular response to images differing on emotional valence, the modulation of these responses by 5-HT was examined. Monoamine neurotransmitters such as 5-HT are thought to be critically important in the regulation of mood and emotion and the serotonergic system is now one of the major systems targeted in the pharmacological treatment of a wide range of mood disorders including depression. A second aim for the third experimental chapter therefore was to investigate how increases in 5-HT modulates the HR associated with the viewing of differently valent images. Results indicate that HR was able to differentiate differently valent images supporting the interpretation that viewing of the selected IAPS images was sufficient to evoke experiential (or feeling) as well as perceptual components. In addition, results indicated that 5-HT may modulate the cardiovascular HR response to visual emotional stimuli and suggest that 5-HT may have some protective effect on the cardiovascular responses to emotional stimuli. This research represents the first study to investigate HR responses associated with emotional images following serotonergic modulation.

Findings reported in the third experimental chapter suggested that differences in HR between differently valent images were eliminated following 5-HT augmentation, however the effect of 5-HT on electrocortical activation associated with the viewing of these same images remained unclear. The aim of the fourth and final experimental chapter, therefore, was to investigate how 5-HT acutely modulates SSVEPs associated with the viewing of the IAPS images. Results for pleasant valence demonstrate that, citalopram relative to placebo potentiated cortical activity (as indicated by reductions in amplitude) within parieto-occipital regions. For unpleasant valence, citalopram relative to placebo suppressed cortical activity (as indicated by diminished amplitude and latency reductions) within anterior-frontal and occipital regions. These results were observed despite the finding that serotonergic augmentation did not alter participants' subjective responses to emotional images. In addition, preliminary analysis demonstrated that the effects of citalopram on pleasant
valence (when males and females are combined) appear to be predominantly influenced by males, whilst effects of citalopram on unpleasant valence (when males and females are combined) appear to be predominantly influenced by females. This study moves beyond examination of cellular and neurochemical mechanisms of antidepressant action and employs a more systems based approach to the study of antidepressant action.

In summary, findings support previous literature suggesting that there is substantial overlap in frontal neural circuitry when the brain processes emotional images of different valence. Gender differences in the processing of visual emotional stimuli were observed however suggesting the need for future studies to take such factors into account. In particular, females unlike males displayed right-sided frontal latency reductions in response to unpleasant images (relative to neutral images). Emotional valence was found to modulate HR thereby confirming the reliability and validity of the task-viewing paradigm. Augmentation of 5-HT was found to suppress any differences in HR across the three differently valenced categories of images, while electrophysiological responses were potentiated during pleasant valence but suppressed during unpleasant valence. This research provides evidence for modulation of the SSVEP by emotional content, as well as modulation of emotion-related SSVEP by 5-HT.

7.2 EMOTIONAL VALENCE, EMOTIONAL AROUSAL AND HUMAN BRAIN ACTIVATION

The IAPS database contains a wide variety of emotional content which have been independently rated on valence and arousal dimensions. These dimensions refer to pleasantness and intensity respectively, and have been regarded as dimensions which explain the principal variance in emotional meaning (Osgood, Suci, & Tannenbaum, 1957; Russell, 1979; Smith & Ellsworth, 1985). A wealth of behavioural and psychophysiological evidence exists to support the notion of distinct valence and arousal dimensions. This evidence comprises viewing times, subjective evaluations, physiological markers such as HR and skin conductance and facial electromyographic measurements (Greenwald et al., 1989; Lang et al., 1993; Russell & Bullock, 1985). Using the IAPS images, Lang and colleagues have conducted much experimental work to examine the patterns of human emotion and report that a number of behavioural and physiological systems significantly covary with either valence or arousal. For instance, it is reported that while facial muscle activity and HR during picture viewing is related to affective valence, other dependent variables
such as viewing time, reaction times, skin conductance and cortical slow-wave EEG response are related to the intensity of the affective state (see Lang, Bradley & Cuthbert, 1998 for discussion). Importantly, the psychophysiological and behavioural responses elicited by these images are regarded as being fundamentally similar to those experienced outside the laboratory.

As mentioned above, valence and arousal dimensions have even been associated with two separate neural systems in the brain, which have been localised to the frontal lobes and right parieto-temporal regions of the right hemisphere respectively (Heller, 1990, 1993). In Heller’s model, the frontal cortices are argued to modulate the emotional valence of experience, while the parieto-temporal region of the right hemisphere is argued to modulate the autonomic and behavioural arousal in emotional states. Although recent meta-analyses suggest a more complicated pattern of brain activation during emotional perception and experience (Phan et al., 2002; Wager, Phan, Liberzon & Taylor, 2003), the findings reported in this thesis are, in part, consistent with such a model. Findings support the notion that: 1) emotional valence is mediated largely by frontal cortical activation; 2) emotional processing is a dynamic and transient process and 3) neurophysiological processes are sensitive to both individual differences (such as gender) and neurochemical modulation.

Over the last decade or so, Davidson and colleagues have amassed a wealth of evidence suggesting that experience of emotion is associated with differential left-right anterior cortical asymmetry (eg. Tomarken et al., 1990; Wheeler, Davidson, Tomarken & Kinney, 1993; for review see Davidson, 1998). In particular, increased activity (decreased alpha amplitude) within the left frontal region is associated with pleasant affect, while increased activity within the right frontal region is associated with negative affect. These data employing EEG technology have also been supported in studies using PET and fMRI technologies (Sutton et al., 1997; Canli et al., 1998, respectively). The first experimental chapter however, does not support these previous studies. Instead, activation associated with the viewing of both pleasant and unpleasant images relative to neutral images, within frontal regions, is widespread and bilateral. Nevertheless, this data does support a large number of studies which have also reported overlapping activation and no hemispheric asymmetries (eg. Baker et al., 1997; George et al., 1995; Lane, Fink et al., 1997; Lane, Reiman, Ahern et al., 1997; Lane, Reiman, Bradley et al., 1997; Pardo et al., 1993; Teasdale et al., 1999). Furthermore, a recent meta-analysis on 65 studies of emotion utilising PET and fMRI techniques concluded that there was only 'limited
support for valence-specific lateralisation of emotional activity in frontal cortex’ (Wager et al., 2003). Still, it should be noted that PET and fMRI technologies are generally much less able to track rapid functional activations across very rapid time-scales. Interestingly, Aftanas and colleagues (2001b) demonstrated that valence-specific hemispheric asymmetries over anterior locations (temporal sites) were time-dependent and specific to the theta and alpha-3 bandwidths. In addition, findings reported in the second experimental chapter suggest that gender is an important factor to take into consideration when examining anterior hemispheric laterality. Specifically, these findings support the role for the involvement of right prefrontal cortex in the processing of unpleasant stimuli in females but not in males.

One of the limitations of the studies contained in this thesis is that the image viewing paradigm did not contain categories of differently valenced images having high arousal content, in addition to the categories containing images with low arousal content. (See Garavan et al., 2001 and Lane et al., 1999 for studies which selected images on the valence dimension as well as the arousal dimension, and also controlled for differing levels of valence and arousal within the same category). Consequently, it is not clear whether posterior electrocortical activations reported in the experimental chapters in this thesis were the result of valence or arousal processes. Notwithstanding, findings reported by previous studies do lend support to the hypothesis that posterior activations are related more to emotional arousal (eg. Aftanas et al., 2002; Heilman, Schwartz & Watson, 1978; Heller et al., 1997; Junghofer et al., 2001, Lane, Reiman, Bradley et al., 1997; Lane et al., 1999; Lang, Bradley, Fitzsimmons et al., 1998; Nitschke, Heller, Palmieri & Miller, 1999). For example, Lang, Bradley, Fitzsimmons et al. (1998) report extensive visual cortical activity when viewing both pleasant and unpleasant images (relative to neutral images). Most recently, Aftanas and colleagues (2002) reported that posterior regions of the right hemisphere are involved in the modulation of emotional arousal (theta and alpha-3). Lang, Bradley & Cuthbert (1998) propose two possible neurophysiological explanations for visual cortical activity during the processing of emotional stimuli. The first explanation involves re-entrant projections from the amygdala back to V1 and is based on findings from animal research (Amaral et al., 1992). The second explanation involves projections from the cingulate cortex that prime visual cortex as part of a posterior attentional network as described by Posner (1996).
Unfortunately, studies have generally failed to adequately control for differences in the arousal and valence qualities of experimental stimuli (see Anderson et al., 2003, Garavan et al., 2001 and Hamann, 2003 for discussion) and although studies are beginning to take such considerations into account, these have been few in number (eg. Anderson et al., 2003; Canli et al., 1998; Garavan et al., 2001; Kemp et al., 2002; Lane et al., 1999). Interestingly, Anderson and colleagues demonstrated dissociation between valence and arousal dimensions of unpleasant and pleasant olfactory stimuli. Whilst the amygdala was found to be associated with the intensity (‘arousal’), the OFC was found to be associated with the ‘valence’ of olfactory stimuli. Hamann (2003) remarks on the striking similarities in the brain’s response to differently valenced olfactory stimuli reported in the Anderson article. Furthermore, Hamman recommends future studies to investigate whether other modalities (ie. visual or auditory) are able to neurophysiologically dissociate these two dimensions, provided that the studies are able to disentangle arousal from pleasantness (‘valence’). This data together with recent imaging findings that have shown amygdala activation in response to both pleasant and unpleasant stimuli (eg. Garavan et al., 2001) as well as findings which reported that increased intensity of amygdala activation (predominantly left sided) corresponds with self-rating of sadness (Posse et al., 2003), implicate this structure in emotional arousal. Furthermore, this data lend support to the notion that it is the reentrant projections from amygdala back to visual cortices, rather than from AC cortex, that modulate visual cortical activity during emotional relative to neutral conditions.

In the experimental chapters presented in this thesis, images were selected so that valence was varied (unpleasant, neutral and pleasant), while arousal remained relatively low for all categories. In other words, an explicit attempt was made to control for the level of arousal within and between valenced categories. It should be noted however, that it is more difficult to dissociate arousal from valence in visual stimuli than it is in olfactory stimuli due to complex differences in perceptual and semantic content (Anderson et al., 2003). It is also important that valent categories are matched on physical characteristics as well as on the level of arousal, in order to reduce the chance that differences between conditions reflect physical differences between conditions as well as differences in emotional content. In Chapter 3, it was reported that there were no differences between any categories of images for brightness and contrast (see section 3.2.2). However other stimulus characteristics such as color, presence of faces, and visual complexity should also be examined. One limitation of the current thesis not yet discussed, is that groups of picture stimuli
were not matched for visual complexity. Post-hoc perusal of the images presented to participants (also displayed in appendices 9.3 to 9.5), suggest that emotional pictures may be more visually complex than neutral pictures, and these differences may have implications for the SSPT findings particularly within the occipital cortex. Results however, do support previously published studies that report modulation of both frontal and posterior cortices by emotional stimuli. In conclusion, it is highly likely that researchers will eventually be able to untangle differences between valence and arousal for visual emotional stimuli, through careful selection of stimuli and focusing on well-defined, objective aspects of emotion such as the dimensions of valence and arousal.

7.3 EMOTIONAL VALENCE, SEROTONIN, PERIPHERAL PHYSIOLOGY AND HUMAN BRAIN ACTIVATION

While there is a large body of literature implicating a relationship between 5-HT levels and mood states, aggression and personality variables there is limited data regarding how 5-HT modulates immediate, real time responses to explicit affective stimuli. This is important research which is currently missing from the literature. The research presented in this thesis is in part, intended to bridge this gap in our understanding. As discussed in the introduction, it is important to distinguish between different affective phenomena. For example, whilst emotions are generally brief and elicited in response to explicit stimuli, moods are longer lasting and their causes are often ambiguous and difficult to recognize (Gainotti, 2001; Ketter et al., 2003; Scherer and Peper, 2001). The findings reported in the third and fourth experimental chapters contained in this thesis suggest that while differences in HR between differently valenced images were eliminated following 5-HT augmentation, cortical response to pleasant images (relative to neutral images) was potentiated, but suppressed to unpleasant images (relative to neutral images). These results were displayed despite the finding that serotonergic augmentation did not alter participant's subjective responses (behavioural ratings as well as subjective mood) to emotional images and suggest that different components of the emotional response (i.e. electrophysiology and peripheral physiology) are modulated differently by 5-HT.

Researchers have only recently begun to examine emotional processing in healthy participants by manipulating 5-HT levels and then measuring certain aspects related to this processing. Studies have measured emotional processing by behavioral responses such as reaction time to and recognition of facial expressions of emotion
(Attenburrow et al., 2003; Harmer et al., 2002; Harmer, Bhagwagar et al., 2003; Harmer, Cowen et al., 2003) as well as reaction times to an affective go/no-go task (Murphy, Smith, Cowen, Robbins & Sahakian, 2002), and psychophysiological measures such as electromyography, electrocardiography and startle response (Simmons and colleagues, 2003, personal communication). The studies which have augmented 5-HT generally report that the processing of pleasant stimuli is enhanced, rather than suppressed. Harmer and colleagues for example, examined the acute effects of an SRI, citalopram (10mg i.v) relative to placebo on the recognition of facial expression in healthy female volunteers (Harmer, Bhagwagar et al., 2001). Following administration of citalopram, performance was facilitated (as measured by greater accuracy and reduced response times) to facial expressions of happiness. This finding is replicated by the same authors following administration of nutritionally-sourced tryptophan (which increase 5-HT) in a double-blind placebo controlled study (Attenburrow et al., 2003). Harmer and colleagues also report that repeated administration of citalopram (20mg/day for 7 days) was associated with reduced recognition of negative facial expressions (fear and disgust) in healthy male and female volunteers (Harmer et al., 2002). Furthermore, these effects were independent of changes in ratings of subjective mood.

In addition, the acute effects of the SRI citalopram (20mg) on the electrophysiological responses associated with the processing of unpleasant images from the IAPS in 13 healthy individuals have recently been investigated (Kemp, Gray, Line, Silberstein & Nathan, 2003, included as appendix 9.10. This study comprised preliminary results from the study presented in chapter 6.) It was reported that citalopram inhibits the increases in activity associated with the viewing of unpleasant images relative to neutral images. These effects were particular to regions known to be important in the processing of emotion, including frontal (Davidson, 1998; Kemp et al., 2002), left temporal (Lane, Reiman, Bradley et al., 1997) and right parieto-temporal (Heller et al., 1993) regions and it was suggested that acute administration of 5-HT modulates the processing of negative affect. In an extension of this study, the fourth experimental chapter of this thesis reports that while cortical activity during viewing of unpleasant images (relative to neutral images) following an acute administration of citalopram was suppressed, cortical activity during viewing of pleasant images (relative to neutral images) was potentiated. Like the studies conducted by Harmer and colleagues, no changes in self-reported mood were observed.
Findings suggest therefore that acute enhancement of serotonergic function modulates neuropsychological responses such as recognition and reaction time as well as the cortical processing of affective stimuli such that response to positive valence is potentiated and response to negative valence is suppressed. Interestingly, another study, which employed ATD, report that TD increases response times for happy but not sad targets in an affective go/no-go task without influencing depressive symptomatology or subjective ratings of mood (Murphy et al., 2002). These results may be considered a converse of those findings reported in serotonergic augmentation studies and are also consistent with those findings reported in depressed patients (Murphy et al., 1999).

The findings on emotional processing should be discussed in more detail however, as findings have also been inconsistent. For example, chronic fluoxetine administration is associated with the constraint of attentional biases towards socially threatening stimuli relative to placebo, but no effect of fluoxetine on psychophysiological indices of affective response such as facial muscle activity, HR, self-report, or affective startle modulation were observed (Simmons and colleagues, 2003, personal communication). However, the third experimental chapter in this thesis reported that acute administration of citalopram suppressed the differences in HR activity associated with the viewing of both pleasant and unpleasant images. Possible reasons for these differences are study design (between-group design in the Simmons study versus within-group design in the current thesis), differences in the images selected and the analysis of HR data as well as acute (current thesis) versus chronic effects (Simmons and colleagues). Furthermore, findings differ within the different domains of emotional responsivity (ie. heart-rate versus electrophysiology relating to the third and fourth experimental chapters in the current thesis, respectively). Whilst an increase in HR to pleasant images and a decrease in HR to unpleasant images (relative to neutral) appear to be abolished following 5-HT, differential electrophysiological responsiveness to pleasant and unpleasant images is noted following serotonergic augmentation. Possible reasons for this may relate to differences between more low level emotional (or visceral) responsiveness to emotional stimuli and cognitive processes associated with attention to emotional stimuli. The implications of these findings will be elaborated below (see section 7.5).

Other findings within the same study have also appeared initially inconsistent. For example, in the study conducted by Harmer and colleagues (Harmer, Bhagwagar et al., 2003) (discussed above) performance was facilitated not only to facial
expressions of happiness but also to facial expressions of fear. (This finding was also reported following augmentation of 5-HT by tryptophan in Attenburrow et al., 2003). Although this enhancement of a negative emotion following augmentation of 5-HT levels may at first appear counterintuitive, it is possible that there are differences between acute and chronic augmentation of 5-HT on the processing of emotional stimuli. Indeed, and as discussed above, repeated administration of citalopram was associated with reduced recognition of the negative facial expressions, fear and disgust (Harmer et al., 2002).

In another recent study by Harmer and colleagues (Harmer, Cowen et al., 2003) the effects of lowered 5-HT (via ATD) on the recognition of facial expressions in healthy male and female volunteers is reported. Results showed that although ATD did not affect subjective ratings of mood, the recognition of fearful facial expressions decreased in female, but not male, volunteers. Although again, these findings appear counterintuitive, the authors note that this finding is the opposite pattern to that reported in their paper in which female volunteers were tested following serotonergic augmentation (Harmer, Bhagwagar et al., 2003). Moreover, the authors highlight that initial treatment of SRIs may be associated with increases in generalized anxiety and agitation prior to the therapeutic effects becoming evident and that this mechanism may relate to differential modulation of the amygdala through either desensitization of 5-HT₂ receptors or effects on other neurochemical or neural systems. This further illustrates the importance of acute versus chronic effects of serotonergic manipulation of emotional processing.

In summary, the findings reported in the current thesis suggest that 5-HT modulates emotional functioning at a neurophysiological level. The few studies which have so far investigated the real time responses to explicit affective stimuli suggest that serotonergic augmentation may be associated with constraint of the processing of negative stimuli (fourth experimental chapter; Harmer et al., 2002; Kemp et al., 2003). It is also possible that serotonergic augmentation may be associated with constraint of the more low level emotional (or visceral) responsiveness (as indicated by HR measures) (third experimental chapter), though the lack of such findings in other studies (Simmons and colleagues, 2003, personal communication) suggests that these effects be interpreted cautiously prior to further investigation. In addition, the studies suggest an enhancing effect of 5-HT on the cognitive processes underlying emotional perception (ie. attention towards social threat; posterior cortical electrophysiological responses; reaction time to and recognition of facial expressions...
The extent to which these cognitive processes influence subsequent affective behaviours or moods, however remains unclear. Future studies should therefore investigate the role of the serotonergic system in the processing of emotion by examining neurophysiological responses associated with the viewing of visual emotional stimuli following chronic serotonergic augmentation.

### 7.4 Toward a Neurophysiological Model of Antidepressant Action

SSPT is an electrophysiological technique and therefore reflects neuronal activity produced primarily within the pyramidal cells of the neocortex (see Westbrook, 2000 p.914 for an explanation of the underlying sources of the EEG). SSPT examines changes in 13Hz SSVEPs which are comprised of two components: amplitude and phase (latency). Amplitude relates to the size of the response while the phase (latency) relates to the delay between stimulus and response. In the experimental studies reported above, results were displayed as topographic maps which represent the activation associated with the viewing of emotional images (relative to neutral images). While amplitude reductions are interpreted as analogous to an event-related desynchronisation commonly associated with the alpha bandwidth, latency reductions are interpreted as decreases in the delay between the incoming stimulus and cortical response to this stimulus which may reflect increases in the functional coupling between neural networks and cortical excitability. Amplitude and latency reductions observed during the processing of pleasant and unpleasant valence therefore are interpreted as increased activation within pyramidal cell networks.

While the exact mechanisms underlying the modulation of electrophysiological responses by neurochemicals are unknown, there is strong evidence for interactions between 5-HT and Glu in neocortical pyramidal cells (see Marek & Aghajanian, 1998 for a review). In addition, it is possible that responsiveness to serotonergic augmentation, of certain excitatory (5-HT$_{2A}$) and inhibitory (5-HT$_{1A}$) receptors within pyramidal cells will vary depending on the emotional circuitry involved in the processing of pleasant or unpleasant valence. Consequently, activation of these excitatory and inhibitory receptors may lead to amplitude and latency changes observed during the processing of pleasant and unpleasant valence respectively.

A tentative model describing the effects of citalopram on pleasant and unpleasant valence is described in Figure 25. In this model changes in both SSVEP amplitude as well as latency will reflect functional activity within cortical networks.
Figure 26: A hypothetical model describing the actions of citalopram on SSVEP during the processing of unpleasant pleasant valence. Note that the responsiveness between 5-HT\textsubscript{1A} and 5-HT\textsubscript{2A} is proposed to be dependent on unpleasant and pleasant valence respectively.

In this model SSVEP changes associated with the processing of unpleasant valence following administration of citalopram may be the result of direct activation of 5-HT\textsubscript{1A} receptors on layer 5 pyramidal cells or indirect activation of GABA interneurons innervating pyramidal cells. Activation of 5-HT\textsubscript{1A} receptors is supported by evidence that most antidepressants enhance post-synaptic 5-HT\textsubscript{1A} hyperpolarisation (see Barnes & Sharp, 1999 for a review of central 5-HT receptors). Activation of GABA interneurons innervating pyramidal cells is supported by studies showing: (1) that the majority of 5-HT synapses within the cortex are located on GABA interneurons (Smiley & Goldman-Rakic, 1996), (2) increased GABA concentrations within the OC, following chronic administration of SRIs in the treatment of depression (Sanacora et al., 2002) and (3) that lorazepam (a GABA-A receptor potentiator) reduced the activation (signal increases) associated with negative affective stimulation within the OFC (Northoff et al., 2002).
SSVEP changes associated with processing of pleasant valence following administration of citalopram may also be the result of specific 5-HT receptor activation. Changes in the responsiveness of particular receptors, however, may alter the excitatory or inhibitory nature of the pyramidal cells. In this model, the circuitry involved in the processing of pleasant valence may be associated with activation of excitatory 5-HT$_{2A}$ receptors. While not directly comparable, hallucinogens which increase pleasant affect (ie. euphoria) potentiate pyramidal cell firing through activation of 5-HT$_{2A}$ receptors (Aghajanian & Marek, 1999), implicating a possible relationship between 5-HT$_{2A}$ receptors and pleasant valence. The findings for pleasant valence reported in the fourth experimental paper suggest that these receptors may increase responsiveness within the parieto-occipital region during the late component of image-viewing. These tentative conclusions on the underlying mechanisms of the SSVEP are testable by administering 5-HT$_{1A}$ and GABA agonists (such as buspirone and lorazepam, respectively) and examining the corresponding SSVEP activation.

This tentative model is certainly an oversimplification, yet the utility of this model is to provide some direction for future research in order to further elucidate the underlying mechanisms of the SSVEP response. In addition to exploring the SSVEP responsiveness to emotional visual stimuli and the modulation of this responsiveness by neurochemicals, future studies should also utilise imaging technologies with greater spatial resolution in order to help elucidate the mechanisms associated with the increased parieto-occipital activity displayed when the brain processes pleasant valence following serotonergic augmentation. Like most research endeavours, findings reported in this thesis appear to raise more questions than answers. Some of the more obvious questions that could be raised from the results reported in the fourth experimental chapter include the following. Are the posterior responses to pleasant stimuli (relative to neutral stimuli) following administration of citalopram related to activation within the amygdala or the ACC? Why is the activation associated with pleasant valence specific to posterior regions? Would such regionally specific activation occur during viewing of images rated higher on the dimension of arousal? Could the responses to both pleasant and unpleasant valence be modulated by altering the arousal levels of the emotional stimuli? What happens to neurophysiological responsiveness following chronic modulation of the serotonergic system? Would depletion of 5-HT using a TD paradigm result in a reversal of these findings? Are these responses specific to the serotonergic system? How does manipulation of other neurochemical systems affect the neurophysiological emotional
response? Perhaps one of the most important questions that needs to be asked is how the effects of neurochemical modulation on emotional processing relate, over time, to more overt changes in mood? By answering these questions, the functional consequences of neurochemical modulation on networks involved in emotional processing will be better understood and it is possible that novel antidepressants based on emotional brain networks could be created.

7.5 **Toward a Neuropsychological Model for Antidepressant Action**

The serotonergic system has been implicated in a variety of affective phenomena including positive and negative mood, impulsivity, aggression, personality and social behaviour and is now one of the major systems targeted in the pharmacological treatment of a wide range of mood disorders including depression (Blier & de Montigny, 1994). Involvement of 5-HT in such a wide range of behaviours has lead to speculation that it may exert a general constraining influence on behaviour, by means of inhibitory control over neuronal as well as behavioural systems (see Spoont, 1992 for a review). Increased 5-HT levels have been correlated with behavioural dimensions of "constraint" and "harm avoidance", whilst low levels of 5-HT have been correlated with "unsocialised impulsivity" (Cloninger, 1987; Depue and Spoont, 1986; Spoont, 1992; Zuckerman, 1995). However, before models such as these are able to have any more than heuristic utility, studies need to focus on the physiological basis for the behavioural effects of 5-HT rather than correlating behavioural systems with levels of brain 5-HT (Lucki, 1998).

The results reported by studies investigating emotional processing generally support the hypothesis of serotonergic constraint (or inhibition) of the processing of unpleasant stimuli. However, the results also suggest that initial processing of pleasant stimuli may be *enhanced*. A number of questions should be raised therefore regarding these traditional models of 5-HT and constraint. How is it possible to reconcile the neuropsychological models with the findings reported by studies on emotional processing? Furthermore, how is it possible to reconcile the seemingly contradictory findings reported in the third and fourth experimental chapters with regards to the different domains of emotional responsivity (HR versus brain electrical activity)? A possible answer to these questions is that the posterior electrophysiological activations reflect an enhanced perception to or orienting towards the pleasant stimuli, rather than an enhancement of experiential aspects
associated with the viewing of these stimuli which would presumably be associated with more frontally distributed neural systems.

There is substantial evidence to suggest that posterior regions are implicated in attentional functions. First, the parietal lobe has been identified as an important region in attention, spatial awareness and possibly working memory (reviewed by Mesulam, 1998; Posner, 1994; and Posner & Dehaene, 1994). Second, the posterior association area, which spans the parietal, temporal and occipital lobes, is known to be a convergence zone of information from several sensory modalities for perception and language (eg. Saper et al., 2000). Third, researchers have proposed that emotional expression is mediated by the frontal lobes whilst emotional perception is mediated by posterior structures (Ahern & Schwartz, 1985; Ley & Bryden, 1981; Ross, 1985). Fourth, authors have suggested that the right parieto-temporal region mediates not only perceptual but arousal functions as well (Aftanas et al., 2001a; Heller, 1993; Lane et al., 1999). Fifth, neuroimaging research has demonstrated visual cortical activation to emotional images relative to neutral images (for a meta-analysis see Phan et al., 2002). The authors suggest that activation within the OC (mainly BA 18 and 19, but also occipital and fusiform gyri) may represent top-down modulatory effects on the amygdala. Furthermore, the authors suggest that a component of such modulatory action may include selective attention which is known to drive the modulation of visual processing. Sixth, ERP research demonstrates that this natural selective attention to emotional picture content begins around 150ms after picture onset and that the main neural sources for these effects are located over the OC bilaterally and the right parietal cortex (Junghofer et al., 2001). This supports findings from an fMRI study which reported extensive activity for emotional pictures over occipital and parietal sites (Lang, Bradley, Fitzsimmons et al., 1998).

Increased activation associated with the processing of pleasant stimuli within posterior regions, therefore, may reflect enhanced perception to or orienting towards pleasant stimuli. Recent studies that have administered antidepressants that increase 5-HT (eg. citalopram) as well as those that increase NA (eg. reboxetine) have both shown increased efficiency in the processing of positively valenced material in nondepressed volunteers (Harmer, Bhagwagar et al., 2003; Harmer, Hill, et al., 2003). Furthermore, chronic administration of SRIIs has been associated with increased levels of affiliative behaviour in healthy controls (Knutson, et al., 1998; Moskowitz et al., 2002). Harmer and colleagues have posited a tentative hypothesis for antidepressant action that suggests enhanced perception of affiliative signals may
precede and even facilitate approach behaviour and social interaction. Consistent with this idea, Simmons and colleagues have also concluded that following chronic fluoxetine administration, attentional biases towards socially threatening stimuli are constrained (Simmons and colleagues, 2003, personal communication). Hence, it is possible, that antidepressants such as the SRIs shift the attentional bias from negative stimuli to positive stimuli and that these effects are apparent even after an acute dosage. The results of the fourth experimental chapter demonstrated enhanced processing of pleasant stimuli (relative to neutral stimuli) within posterior locations, but suppressed processing of unpleasant stimuli within frontal and posterior locations. Consequently, these findings are also consistent with the interpretation that antidepressants may enhance perception of affiliative (approach) signals, whilst constraining perception of negative or avoidance signals.

Findings from both the second and fourth experimental papers indicate that substantial electrophysiological differences exist between males and females. Preliminary findings reported in the fourth experimental chapter suggest that the effect of 5-HT on the processing of emotion is modulated differently by males and females. The findings presented in the second experimental chapter suggest that these differences may relate to the finding that males are generally more responsive to pleasant stimuli while females are generally more responsive to unpleasant stimuli. These latter findings are supported by a number of previous studies which also investigated gender differences in the processing of visual emotional stimuli (eg. Lang, Bradley, Fitzsimmons et al., 1998; Wrase et al., 2003). Together, these findings are consistent with the hypothesis that pleasant and unpleasant valence lie on a continuum (see Feldman-Barrett & Russell, 1999) and that, females are oriented towards the unpleasant pole on this continuum. After administration of an SRI however it is possible that females move closer to the pleasant pole on this continuum. In addition, the findings are also consistent with the ‘fact’ that more females become depressed than males after the age of 13 (Hankin & Abramson, 2001) and that this may be related to the finding that 5-HT synthesis in normal females is 52% lower than normal males (Nishizawa et al., 1997).

In conclusion, increased levels of 5-HT may be associated with the behavioural dimensions of “constraint”. However results presented in the fourth experimental chapter lend support to initial data from emotional processing studies which suggest that increased levels of 5-HT may also be associated with enhanced affiliative signals which may precede approach behaviour and improved sociability. Future studies
could employ PET and fMRI neuroimaging techniques to better determine the underlying nature of visual cortical activation to pleasant stimuli following acute administration of SRIs. For example, functional connectivity between visual cortical activation and the ACC for example could implicate mechanisms relating to attentional processes, whilst connectivity between visual cortical activation and the amygdala could implicate an underlying mechanism relating to emotional arousal. Future studies should also consider the role of other important neurotransmitter systems (such as NA) in the modulation of emotional processing in order to provide a more comprehensive model of antidepressant action. Preliminary work using the new antidepressant reboxetine (an NRI) has reported very similar findings to those reported for SRIs. The findings reported in chapter 6 suggest therefore suggest that an enhancement of activation within parieto-occipital regions may also be observed for NRIs, though studies which explore the neurophysiological mechanisms of these antidepressants remain to be conducted. These tentative hypotheses should be further investigated in order to understand more completely, the functional consequences of neurochemical modulation on cortical networks involved in emotional processing.

### 7.6 Future Research Directions

Future studies should consider utilising the SSPT technology in combination with Low Resolution Electromagnetic Tomography (LORETTA) in order to investigate 3-dimensional (3-D) time-extended processes associated with emotional processing. The combination of SSPT’s ability to examine time-extended processes and LORETTA’s ability to localise electrical sources in the brain from scalp recordings using a direct 3-D solution would considerably improve our knowledge of not only the neural substrates underlying emotional processes but also enable researchers to investigate the time course of processing within these structures at high temporal resolution (relative to PET and fMRI techniques). Although no studies have yet been conducted which combine the SSPT and LORETTA technologies, this opportunity has recently become available (Richard Silberstein, personal communication, 2003). Another advance on the SSPT technique would be to examine SSVEP coherence associated with tasks that engage the emotional circuitry. SSVEP coherence has recently been examined during a mental rotation task (Silberstein et al., 2003), but remains to be investigated during emotion tasks. Increases in coherence are considered to reflect long-range synchronisation of neural networks critical for cognition (Silberstein et al., 2003) and examination of SSVEP coherence during
emotional processing therefore may help to improve our understanding of the functional coupling between frontal and posterior neural systems during these states.

More broadly, future studies need to explore the relationship between brain activations (within regions such as the PFC) and measures of personality or mood in order to improve our understanding of the basis of such activations during emotional processing. Although studies have only recently begun to investigate the effects of personality variables on neural emotional responsiveness, initial results suggest that personality variables in part, underlie individual differences in the biological basis of emotion (e.g., Canli et al., 2001; Canli, Sivers et al., 2002; Keightley, Bagby, Seminowicz, Costa & Mayberg, 2003). Utilising fMRI, Canli and colleagues (2001) reported that positive (relative to negative) pictures correlated significantly with participant's ratings of extraversion within frontal and temporal cortical regions as well as amygdala, caudate and putamen subcortical structures. In addition, negative (relative to positive) pictures were found to correlate significantly with participants ratings of neuroticism within left frontal and temporal cortical regions. More recently, the same group has demonstrated that amygdala activation in response to happy facial expressions is positively and significantly correlated with the degree of extraversion (Canli, Sivers et al., 2002). In addition, Keightley and colleagues utilised PET to investigate differences between 'high' and 'low' neurotic healthy participants following sad mood induction (relative to neutral mood induction) using a script-rehearsal paradigm. The authors reported that while 'low' neurotic subjects showed increases in medial frontal regions, 'high' neurotic subjects (like depressed subjects) showed decreases in medial frontal regions. In addition, both groups displayed increases in subgenual cingulate (Cg25) and decreases in ventral and dorsal PFC (as seen in non-selected controls).

As indicated in the Introduction to this thesis, functional neuroimaging reports on amygdala activation have been contradictory. For example, some studies that have examined the neural mechanisms underlying emotional experience have completely failed to detect amygdala activation (e.g., Damasio et al., 2000; Geday et al., 2003; Mayberg et al., 1999; Teasdale et al., 1999). The results reported by Canli and colleagues suggest that null results in amygdala activation may be due to the presence of highly extraverted participants in the sample. Furthermore, this work suggests that the contradictory findings reported in the literature regarding amygdala activation during the processing of positively valent stimuli may have been due to studies not controlling for personality. The results reported by Canli and colleagues
as well as Keightley and colleagues (Keightley, Bagby et al., 2003) also suggest that personality factors may modulate activity displayed within frontal cortex. Future studies will need to take note of these issues when investigating emotional processing. Furthermore, well-designed longitudinal studies employing technologies such as SSPT and fMRI are necessary to bridge the gap between immediate neural responsiveness to emotional stimuli and affective phenomena characterised on longer temporal scales such as mood and personality. These studies should investigate the consistency with which individuals respond to emotional stimuli and explore the way in which this responsivity is related to personality and mood state. Studies should continue to record from multiple response systems to further examine the nature of an emotional response. For instance, measurement of peripheral physiology such as HR, is critical for the interpretation that stimuli have elicited an emotional response. If studies do not make such measurements researchers cannot be certain as to whether an emotional response has been evoked or not. Although controversies still exist as to the exact role for activation of the autonomic nervous system (ANS) during emotional states, it is generally agreed that sympathetic activation triggers and marks the subjective experience of emotions (see Gainotti, 2001 for discussion). Indeed, the final two chapters included in this thesis confirmed the interpretation that presentation of the images selected from the IAPS, involved a mild emotional stimulation. In addition, studies have recently reported that brain activation during the processing of emotional stimuli covaries with variations in self-report and peripheral physiology covary. For example, Phan and colleagues (2003) reported that consideration of participant’s ratings of emotional arousal (intensity) into the analysis of fMRI data is useful for the detection of more robust activations within MPFC and SLEA. Similarly, Williams and colleagues (2001) reported that amygdala activation to fearful facial expressions is displayed only when electrodermal skin conductance responses (indexing arousal) are produced. Examination of multiple response components therefore will enable researchers to further understand the nature of central emotional responsiveness.

Clearly one of the most exciting (and most recent) developments in neuroimaging research has been the examination of genetically driven variation in the response of brain regions underlying human emotional behaviour (eg. Hariri et al., 2002). Human emotional behaviour exhibits considerable individual variability and the process of grouping subjects on the basis of genetic variability allows brain function to be explored at the root of individuality. On the basis that functional polymorphisms of the
human 5-HT transporter (5-HTT) gene (SLC6A4) have been associated with several dimensions of neuroticism and psychopathology including increased fear and anxiety-related behaviours, Hariri and colleagues investigated differences in brain activation between individuals with either the short or long allele of the 5-HTT promoter polymorphism. The authors reported that individuals with the short SLC6A4 allele exhibited greater neuronal activity to fearful facial expressions when compared with individuals homozygous for the long allele, as measured by fMRI. Importantly, these results demonstrate that although the relationship between this genotype and behavioural and personality variables has been weak and inconsistent, the functional impact of genetic variability on brain activations are much more sensitive (see Hariri et al., 2002 for discussion). Future studies will undoubtedly explore such genetic variation in more detail.

The effects of SRI antidepressants on such genetic variability should also be investigated. Very few studies however have examined the effects of antidepressants on emotional processing. The current thesis provides initial electrophysiological data on the way in which emotional processing is modulated by a single dose of an SRI. Future studies should further investigate emotional processing following chronic serotonergic augmentation. A fruitful area for future study is the examination of emotional processing in individuals whom emotional processing is known to be dysfunctional. Recently, Davidson and colleagues (2003) used fMRI to examine emotional processing in patients with major depressive disorder before treatment and after 2 and 8 weeks of treatment with venlafaxine (an SNRI). The authors concluded that components of the neural circuitry activated by negative affect are able to be changed within 2 weeks of treatment with antidepressant medication. These studies are critical to our wider understanding of the relationship between the monoaminergic systems and the neurophysiological mechanisms underlying emotional processing.

In addition to focusing on patients diagnosed with disorders of emotion, future studies could employ affective challenge paradigms in sub-clinical populations thought to be ‘at risk’ of developing such disorders. For example, abusers of the recreational drug, “Ecstasy” (2,4- methylenedioxymethamphetamine, MDMA), an indirect monoaminergic agonist that stimulates release and inhibits reuptake of 5-HT, are at risk of serotonergic axonal loss particularly within frontal and temporal lobes and hippocampus and a range of psychiatric disorders including depression (Parrott, 2001). A recent study which explored the acute and chronic effects of MDMA on cortical 5-HT_{2A} receptors in the rat and human brain concluded that 5-HT_{2A} receptors
within the OC of ex-MDMA users upregulate due to low synaptic 5-HT levels (Reneman et al., 2002). These results are particularly interesting in light of the data presented in this thesis on pleasant valence and the model’s tentative conclusions that these findings may relate to activation of 5-HT$_{2A}$ excitatory receptors. Exploring the impact of MDMA-abuse on emotional processing is likely to have profound implications especially given that some see this drug as having therapeutic usefulness in the treatment of emotional disorders such as post-traumatic stress disorder (Doblin, 2002).

7.7 CONCLUSIONS

This thesis has explored a number of crucial issues in the neurobiology of emotion and reports a number of key findings. These include transient, widespread and bilateral frontal SSVEP latency and occipital amplitude reductions during the processing of pleasant and unpleasant valence; more persistent frontal latency reductions in males during the processing of pleasant valence; widespread frontal latency reductions (predominantly right sided) in females during the processing of unpleasant valence; elimination of the HR changes associated with emotional stimuli following administration of 5-HT; potentiated and diminished cortical responses to pleasant and unpleasant valence respectively following administration of 5-HT.

Although technologies such as SSPT do not currently provide high spatial and 3-dimensional information on brain activation during emotional processing, it is important to highlight the utility of the SSPT method to track associated rapid and continuous changes in brain electrocortical activity. Importantly, SSPT provides the opportunity to determine whether the underlying electrocortical activity associated with emotional processing is associated with inhibitory or excitatory processes, an ability in which the hemodynamic imaging techniques do not have.

Results presented in the current thesis support previous studies demonstrating there to be substantial overlap in frontal neural circuitry when the brain processes images with pleasant and unpleasant content. However, results also emphasise the need to take gender differences into account when exploring brain activation associated with emotional processing. Such gender differences may, in part, underlie some of the inconsistencies reported in the literature on frontal hemispheric asymmetries. Results also suggest that responsiveness to pleasant and unpleasant stimuli following neurochemical modulation may vary across different response systems (ie. self-report, HR and SSVEP). In addition, findings reported in this thesis may have
important implications for the pathophysiology of mood disorders such as depression. It is recommended that future studies examine the relationship between neural emotional processing and measures of self-report, personality, mood, physiological reactivity, neurochemical modulation, individual and genetic variability. These studies are important, in order to more fully understand the way in which different individuals process emotional stimuli and consciously regulate their emotional responses. Finally, the functional consequences of neurochemical modulation on networks involved in emotional processing will be better understood by focusing on a more systems-based approach to the study of antidepressant action, through examination of the neurophysiological responses to visual emotional stimuli.
8 REFERENCES


to pleasant, unpleasant, and neutral visual stimuli in a PET study of normal subjects. 


_Human Psychopharmacology, 16_, 557-577.


9 APPENDICES
9.1 PERSONAL INFORMATION QUESTIONNAIRE
9.2 IAPS Task Instructions
9.3 Images Used From IAPS: Positive Category
9.4 **IMAGES USED FROM IAPS: NEUTRAL CATEGORY**
9.5 Images Used From IAPS: Negative Category
9.6  POSTER PRESENTED AT RIKEN BRAIN SCIENCE INSTITUTE SUMMER PROGRAM,
TOKYO, JAPAN, JULY 2001
9.7 Poster Presented at Emotions & The Brain, Toronto, Canada, March 2002
9.8 Poster Presented at the Australasian Society of Psychophysiology Conference, Hobart, Australia, December 2003
9.10 Kemp et al., 2003 Brain and Cognition Article
9.11 Kemp et al., 2003 NeuroImage Article
**NOTE:**

1. **Personal details will remain confidential**

2. For questions requiring a YES / NO response, please **CIRCLE** the correct response

<table>
<thead>
<tr>
<th>Question</th>
<th>Response</th>
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<tr>
<td>Today’s Date</td>
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<td>Date of birth</td>
<td>Age</td>
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<td>Sex</td>
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<td>Have you been a subject for any type of study at BSI before?</td>
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<td>Academic qualifications (Year 11, VCE, B.App.Sci., etc)</td>
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<td>Do you presently suffer or have you ever suffered from epilepsy?</td>
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<td>If yes, specify</td>
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<td>Do you have a colour deficiency?</td>
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<td>Do you have any other visual defects (short sightedness, lazy eye, etc)?</td>
<td>Y / N</td>
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<td>If yes, specify</td>
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<td>Have you ever sustained a serious head injury?</td>
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<td>Do you presently suffer or have you ever suffered from any neurological or psychiatric disorders?</td>
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<td>If yes, specify</td>
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<td>Are you a smoker?</td>
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<td>If yes, indicate the last time you smoked</td>
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<td>Have you consumed tea or coffee today?</td>
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<td>If yes, indicate time of last consumption</td>
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<td>Have you had any recent illness?</td>
<td>Y / N</td>
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<td>If yes, specify</td>
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<tr>
<td>Do you currently take any prescription drugs?</td>
<td>Y / N</td>
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<td>If yes, specify</td>
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</table>
IAPS Recording

We are now interested in how people respond to pictures that represent a lot of different events that occur in life. For approximately 20 minutes you will be looking at different pictures projected on the screen in front of you and rating each picture in terms of how it made you feel. There are no right or wrong answers, so simply respond as honestly as you can.

You will be shown a number of pictures, each of which will be followed by two rating scales. The first scale represents valence, or the emotional quality of the image. The second scale represents arousal, or how strongly your reaction is to the image. Each scale employs a set of icons to help quantify values along the two dimensions of valence and arousal.

Show Examples – remove from plastic sleeve

For instance, the scale for valence shows icons ranging from a happy, smiling figure at Level 9 to an unhappy, frowning figure at Level 1. Thus, the neutral position on this scale would be at Level 5, which shows a figure with no detectable expression. A rating of level 1 would correspond to you feeling: unhappy, annoyed, unsatisfied, melancholic, and despaired. However, a rating of level 9 would indicate, that you felt happy, pleased, satisfied, contented and hopeful.

For the arousal dimension, the icons range from an excited, wide-eyed and agitated figure at Level 9 to a bored or relaxed figure at Level 1. Thus the neutral position on this scale would be at Level 1, which indicates no arousal response. A rating of level 1 would correspond to you feeling: completely relaxed, calm, sluggish, dull, sleepy and unaroused. However, a rating of level 9 would indicate, that you felt stimulated, excited, frenzied, jittery, wide awake and aroused.

Just to emphasize a couple of points: the baseline ratings are 5 for valence and 1 for arousal. Please rate all pictures using these ratings as a baseline. If you find a particular picture does not affect you emotionally then rate it using these values: that is 5 for valence and 1 for arousal. Depending on how the picture affects you rate it accordingly on each of the two scales. Secondly, arousal is completely separate from pleasant or unpleasant valence – you can consider “arousal” as the intensity or your “strength of reaction” to the image.

Each image will be on screen for 6 seconds followed by valence, which is then followed by arousal – always in that order. View the picture for the entire time it is on; focus on the emotional content and then make your valence and arousal ratings immediately after the picture is removed. Some of the pictures may prompt emotional experiences; others may seem relatively neutral. It is important that you do not inhibit any emotion which may be experienced when viewing each of the images. Your rating of each picture should reflect your immediate personal experience and no more. Please rate each one as you actually felt while you watched the picture.
Introduction

Examination of how the brain mediates emotional experience is one of the most active areas of research interest (Le Douarec, 2000). Emotions are expressed in terms of two motivational systems: appetitive and aversive (valence), which vary in terms of activation (Lang, et al., 1998).

Researchers using brain-imaging techniques to examine emotional processing in healthy adult participants have reported varied and contradictory findings. For example, studies using fMRI and PET have reported both frontal lability (Damasio, et al., 1999; Sutton, et al., 1997) as well as specific areas of the limbic system for the processing of pleasant and unpleasant stimuli (Lane, et al., 1999; Teasdale, et al., 1999). While many studies have confirmed the neurophysiological nature of emotional processing, the importance of the temporal course of cortical activation has largely been overlooked.

The International Affective Picture System (IAPS) allows the capacity for high temporal resolution and allows the dynamic brain activity changes associated with extended cognitive tasks to be examined (Koizumi, et al., 1994).

Objective

To determine the spatial-temporal characteristics of the Steady State Visual Evoked Potential (SSVEP) during the processing of unpleasant and pleasant images.

Methods

Subjects

22 healthy subjects; 13 males (M = 24.00, SD = 4.90) and 9 females (M = 27.71, SD = 7.76); right-handed, drug free, with no history of neurological or psychiatric disorder.

Procedure

A set of 13 visual stimuli presented the SSVEP while participants viewed 70 IAPS images. Each subject was tested once for each of the three conditions: pleasant, neutral and unpleasant images selected from the IAPS.

IAPS

Valence was rated (P = 7.52 to 5.84, N = 4.46 to 5.49, U = 1.8 to 3.47) and arousal was rated (P = 7.52 to 5.84, N = 4.46 to 5.49, U = 1.8 to 3.47). Arousal was rated on a scale with a 5-point scale for categories of images for brightness, F(3,82) = 0.58, p = 0.55, and contrast, F(3,82) = 0.18, p = 0.95. Image selection was based upon standardized American valence and arousal ratings (Lang, et al., 1997).

SSVEP Analysis

Procedure for SSVEP analysis for the IAPS task is included in Box 1. An average of the 76 time series for each condition will be calculated and displayed as topographic maps using a statistical non-parametric procedure (Nunez, et al., 1994). This represents the averaged (N = 76) difference between emotions (unpleasant or pleasant) and neutral conditions. Raw colours in these maps represent reduced amplitude and latency for the affective condition (unpleasant or pleasant) images compared to neutral images. An electrode from the frontal region is the selected (max difference between F7 and F8) and the corresponding time series presented.

Further analysis will be then be conducted and topographic maps presented for the time point that displays the maximum difference between categories (as determined from the 1.5).

Conclusions

Although the SSVEP is expressed in terms of amplitude and latency, for brevity only latency findings will be discussed in the conclusion.

Statistical

The statistical analysis of the differences between the emotional conditions (unpleasant and pleasant) and the neutral condition will be examined using the Kruskal-Wallis statistic. The statistic is calculated using both amplitude and latency parameters to account for multiple comparisons. A critical significance level of 0.05 (0.05) was set.

Results

The unpleasant images displaying the activity for the whole 4.5 s epoch were associated with an SSVEP latency reduction, as well as an amplitude increase in front and left temporal regions (p < 0.025) (Spearman, et al., 1997). Pleasant images were associated with an amplitude decrease and a latency reduction in the left occipital region as well as a smaller but global, frontally distributed latency reduction and amplitude increase (p < 0.005) (see Fig. 1). Further analysis was conducted at the point of maximum latency difference between conditions (approx. 1.5 s epoch) (Spearman, et al., 1997) and displayed in Fig. 2, which indicate a latency reduction in the right posterior frontal region (p < 0.005) as well as a latency increase in the left posterior frontal region (p < 0.005) (see Fig. 2).

References

Damasio, et al. (1996) "IAPS Unpleasant PLE Anterior Frontal SSVEP.
Le Douarec, et al. (2000) "IAPS Unpleasant PLE Anterior Frontal SSVEP.
Lane, et al. (1997) "IAPS Unpleasant PLE Anterior Frontal SSVEP.
Lang, et al. (1997) "IAPS Unpleasant PLE Anterior Frontal SSVEP.
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Teasdale, et al. (1997) "IAPS Unpleasant PLE Anterior Frontal SSVEP.
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Preliminary Electrophysiological Evidence For Modulation of Negative Affect By Serotonin.
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Introduction

Emotional processing of negative affect in healthy subjects is associated with increased brain activity in the ventral frontal and decreased activity in dorsal frontal areas in studies using PET and fMRI.

Increased ventral frontal and decreased dorsal brain activity is consistent with findings in patients with major depressive disorder (MDD).

Electrophysiological studies using steady-state visual evoked potential (SSVEP) have found steady-state visually evoked potential (SSVEP) latency reductions in depressed subjects (Kemp et al., 2001).

Clinical studies suggest that antidepressant drugs lead to normalization of pretreatment brain activity (decreased ventral and increased dorsal frontal activity) in depressed subjects (Brodsky et al., 2001).

Although the relationship between serotonin function and depression is well established, the role of serotonin in modulating emotional processing in healthy individuals is poorly understood.

The aim of the current study was to use SSVEP to investigate the acute effects of a selective serotonin reuptake inhibitor (SSRI) on the electrophysiological indices associated with the processing of negative affect using pictures from the International Affective Picture System (IAPS).

Methods

1. Healthy subjects (M = 23.90, SD = 5.96), Males (n = 24.5), 6 females (n = 24.5), right-handed, non-smoking, daily waking, with history of neurologic or psychiatric disorder.

2. Double-blind placebo-controlled design in which subjects were tested under two conditions: placebo & clomipramine. 20mg each separated by a week washout period.

3. A diffuse 13Hz LED stimulus elicited the SSVEP from the occipital scalp (Fz & C5 respectively). The stimulus was displayed continuously for 30s.

4. Data were acquired using an EyeLink II from SR Research.

5. A repeated measures ANOVA was performed for each parameter, with Group (placebo vs clomipramine) as the between-subjects factor and Time (baseline vs end of treatment) as the within-subjects factor.

6. The results were then compared to the international norms for the IAPS images.

Results

Acute increase in serotonin (with clomipramine) will reduce the magnitude of the frontal SSVEP latency reductions.

Discussion

Frontal SSVEP latency reductions were found during the processing of negative affect in the placebo condition. This finding supports previous findings that have shown frontal latency reductions during the processing of negative emotional valence in healthy adults (Kemp et al., 2001).

The latency reductions during the processing of negative affect may be interpreted as an increase in excitatory processes within ventral posterior cell networks (Stern et al., 2001).

We suggest that the facilitation of latency reductions by clomipramine during processing of negative affect may be mediated by increases in extracellular 5-HT in the frontal cortex, left amygdala and right orbitofrontal regions leading to increases in frontal cell activity, either directly by action of 5-HT1A receptors on inferior 5 pyramidal cells or indirectly through an increase in activity of GABAergic neurons. Increased extracellular 5-HT have been shown to mediate the effects of clomipramine on SSVEP (see Fig. 3).

In summary, preliminary findings suggest that enhanced processing of negative affect is mediated by changes in serotonin particularly in the frontal cortex, as well as regions implicated in prefrontal-accumbal and prefrontal-parietal processing.

The findings support recent electrophysiological studies that indicate modulatory influence of brain activity by antidepressants (Mennini et al., 2001) and the notion that greater levels of serotonin are associated with increased inhibition, control of executive functions (Stern et al., 1997).

References


Acute Augmentation of Serotonin Enhances Pleasant and Suppresses Unpleasant Cortical Electrophysiological Responses to Visual Emotional Stimuli in Humans.
Neuropsychopharmacology Laboratory, Brain Sciences Institute, Swinburne University of Technology, Melbourne, Victoria, Australia.

Abstract
Little is known about how neurophysiological mechanisms underlying the effects of serotonin (5-HT) on emotional processing. The aim of the current study was to investigate how 5-HT activity modulates visually-evoked potentials (SVSEP) and visual ratings associated with the viewing of differently valenced emotional images, in a randomised, double-blind, placebo-controlled design. 17 healthy females underwent four experimental conditions: placebo and sham treatment (STP); 5-HTP augmentation with placebo; 5-HTP augmentation with clonipramine (CLP); and sham augmentation with clonipramine (SCLP). SVSEP augmentation did not alter subject's subjective serotonin to emotional images. Clonipramine relative to placebo suppressed cortical activity to pleasant valence (as indicated by diminished amplitude and latency reductions) within anterior-frontal and occipital regions. The findings suggest a possible neurophysiological mechanism underlying antidepressant drug action on emotion.

Introduction
The serotonergic system is one of the major neurochemical systems involved in the regulation of emotion and is a key target in the treatment of a wide range of mood disorders including depression.

While the behavioral and clinical effects of serotonin antidepressants have been established, the neurophysiological and neurochemical mechanisms underlying the effects of serotonin (5-HT) on emotional processing are relatively unknown.

Acute 5-HTP augmentation with the selective serotonin-reuptake inhibitor (SSRI), clonipramine, has been reported to improve recognition of and reduce response times to happy facial expressions (Harmer et al., 2001), while chronic enhancement has been shown to reduce recognition of negative facial expressions, pain and disgust (Harmer et al., 2002). While the cortical regions affected by serotonin manipulation has been shown to overlap with regions associated with emotion (Semple et al., 2002), the cortical neurophysiological mechanisms involved in the neurophysiological effects of serotonin to emotional processing are yet to be examined.

The aim of the current study was to investigate how augmenting 5-HTP, with SSRI modulators selectively activates visual evoked potentials (550x500) and 5-HTP-related responses associated with the viewing of differentially valenced emotional images.

Methods
Subjects
17 healthy female, non-smoking, drug-free subjects with no history of neurological or psychiatric disorders participated in the current study (mean age, 22.68; SD, 4.81; mean education, 13.69; SD, 1.50).

Procedure
Randomized, double-blind, placebo-controlled design in which subjects were tested under two acute treatment conditions: placebo & clonipramine (CLP) vs. 5-HTP, spaced by a 1 week washout period. A diffuse 13Hz visual flicker elicited the SVSEP while participants viewed 78 images, categorised as pleasant, neutral or unpleasant.

IAPS
Valence was rated as (P): 7.62 to 3.34, N: 4.64 to 2.46, U: 1.8 to 3.74 and arousal was held constant (P: 2.20 to 4.22, N: 1.04 to 1.97, U: 1.8 to 3.74). Significant differences were found between categories of images for both valence and arousal (P, F(2) = 27.1, p < 0.001) and arousal (F(2) = 18.3, p < 0.001). Image selection was based upon published American valence and arousal ratings (Lang et al., 1999).

SVSEP Analysis
The procedure for the SVSEP analysis of the IAPS task is presented in Box 1.

Discussion
Acute serotonin augmentation with clonipramine was found to modulate cortical electrophysiological processing of emotionally valent stimuli such that response to pleasant valence was reduced and response to unpleasant valence was enhanced. These results were observed despite no changes in subjective response to emotional images.

The findings are consistent with earlier neurophysiological and functional MRI imaging data that have shown enhanced responsiveness to pleasant and decreased responsiveness to unpleasant stimuli following enhancement of serotonin function (Harmer et al., 2002, Harmer et al., 2003, Nitz et al., 1997).

Gender differences were also observed such that clonipramine potentiated males' responsiveness to pleasant stimuli, whilst suppressing females' responsiveness to unpleasant stimuli.

Serotonergic augmentation of cortical activity to unpleasant valence is consistent with a reversal of pre-treatment brain activity following antidepressant administration in depressed patients (Grady, Busatto, Bole & Santesso, 2001; Divac, Bogers & Plaisier, 2002; Meyer et al., 1993)

Serotonergic potentiation of cortical activity to pleasant valence in male subjects may reflect enhanced perception to or orienting towards pleasant stimuli (Harmer et al., 2003, Krystal et al., 1998, Majewicz et al., 2001, Porner, 1994, Porner & Diamond, 1994).

Previous models have proposed that serotonin may exert a general controlling influence on behavior (e.g. Spiegel, 1992) for a review. Our findings suggest that while processing of unpleasant stimuli may be constrained, initial processing of pleasant stimuli may be enhanced.

The current findings provide a possible neurophysiological mechanism for the effects of serotonin enhancing antidepressants on emotion, which may underlie a shift in attentional bias from negative to positive stimuli.

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References
Steady-State Visually Evoked Potential Topography during Processing of Emotional Valence in Healthy Subjects

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The International Affective Picture System (IAPS) is increasingly used in brain imaging studies to examine emotional processes. This task allows valence and arousal content to be systematically investigated; however, previous studies have generally failed to select images that vary in one dimension as well as hold constant the variability on the other dimension. In addition, no studies have investigated the temporal structure associated with the conscious, ongoing processing of emotional stimuli following systematic selection of IAPS images. The aim of the present study was therefore to use steady-state probe topography (SSPT) to examine the steady-state visually evoked potentials (SSVEPs) associated with the processing of pleasant and unpleasant images low in arousal content. Seventy-five IAPS images, categorized as unpleasant, neutral, or pleasant, were presented to 16 healthy subjects while brain activity was recorded from 64 scalp sites. Analysis subtracted the activity associated with the presentation of neutral images from the activity associated with the presentation of pleasant as well as unpleasant images. Results demonstrate that both pleasant and unpleasant valence is associated with transient, widespread, and bilateral frontal SSVEP latency reductions. Unpleasant images were also associated with a transient bilateral anterior frontal amplitude decrease. Latency reductions are interpreted as increases in neural information processing speed, while amplitude reductions are interpreted in the current paper as analogous to an event-related desynchronisation commonly associated with the alpha bandwidth. These key findings support previous literature in terms of there being substantial overlap in frontal neural circuitry when the brain processes pleasant and unpleasant valence relative to neutral valence.

Key Words: emotional processing; emotion; unpleasant; pleasant; International Affective Picture System; IAPS; valence; arousal; electrophysiology; SSPT: amplitude; latency.

INTRODUCTION

The investigation of emotional processing and examination of how the brain mediates emotional experience is once more an area of significant research interest (LeDoux, 2000). The brain mechanisms involved in emotional processing, however, are no longer explained solely by reference to the limbic brain (MacLean, 1949, 1952; Papez, 1995), but by a number of regions and their interconnections. These include the dorsolateral prefrontal cortex (DLPFC), ventromedial prefrontal cortex (vmPFC), orbitofrontal cortex (OFC), amygdala, hippocampus, anterior cingulate cortex (ACC), and insular cortex (Davidson, 2000).

A number of studies have reported anterior lateralization during discrete emotional states. This has generally involved increases in right-sided activation during unpleasant affect (e.g., disgust) and left-sided activation during pleasant affect (e.g., happiness). Cortical lateralization has been demonstrated in EEG studies using video clips (e.g., Davidson et al., 1990; Jones et al., 1992) as well as images from the International Affective Picture System (IAPS) (Aftanas et al., 2001) and these findings are supported in studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (Sutton et al., 1997a; Canli et al., 1998). Following presentation of images selected from the IAPS, researchers report hemispheric lateralization when arousal is similar between differently valenced conditions (Canli et al., 1998). However, Canli et al. (1998) also indicate that valence-related lateralization was only partially evident and that hemispheric laterality is a fragile phenomenon.

Other studies have reported activation in overlapping areas and no frontal hemispheric laterality (Pardo et al., 1993; George et al., 1995; Baker et al., 1997; Lane et al., 1997a,b,c; Teasdale et al., 1999). For example, a study using PET reported increased activation within the thalamus and medial prefrontal cortex (Brodmann’s area 9) for happiness, sadness, and disgust emotions induced by film as well as recall (Lane et al., 1997b). Similarly, another PET study using the IAPS distinguished pleasant and unpleasant emotions from neu-
central emotion by increased activation within the medial prefrontal cortex, thalamus, hypothalamus, and midbrain (Lane et al., 1997). These findings have been supported by an fMRI study, using picture–caption pairs (Teasdale et al., 1999).

Findings in terms of frontal hemispheric laterality have been varied and contradictory and could in part be accounted for by failing to control for the levels of arousal within and between valenced categories. Although it has been suggested that arousal is associated with the activation of the right parietotemporal region (Heller et al., 1998; Aftanas et al., 2001), frontal regions including the orbitofrontal cortex (Barbas, 2000), the ventromedial PFC, the dorsolateral PFC, and the anterior cingulate have also been associated with the modulation of emotional autonomic responses such as skin conductance (Damasio et al., 1990; Tanel and Damasio, 1994; Zahn et al., 1999). It should be highlighted that this measure of emotional responsivity has been reported to covary positively with judged emotional arousal (Lang et al., 1998a).

It is recognized that research must focus on well-defined aspects of emotion (LeDoux, 2000) in order to properly investigate emotional processing. The IAPS is an increasingly popular means to investigate emotional processing in neuroimaging studies. This task has been developed to selectively activate appetitive and defensive motivational systems and is believed to be able to evoke a broad range of emotions experienced outside the laboratory (Lang et al., 1997).

This IAPS contains emotional visual stimuli that have been rated on valence and arousal dimensions, which allows research to systematically manipulate these variables to determine the associated processing. However, many imaging studies using this task have not attempted to control for the effects of one dimension while varying the other. For example, pleasant content has previously ranged from sexual content to ice cream and smiling babies, while unpleasant image content has ranged from mutilations to a gun aimed at the viewer (Mintei et al., 1996; Lane et al., 1997a,c). Such examples confound emotional valence with emotional arousal which may lead to differences in brain activation.

A number of studies have systematically varied either valence or arousal (e.g., Canli et al., 1998, using fMRI and Taylor et al., 2000, using PET, respectively); however, due to the nature of the techniques used, these studies focused on the regions involved rather than temporal dynamics. The varied and contradictory findings reported in the literature may also be due in part to not investigating the temporal processes associated with the processing of emotional stimuli. The phasic nature of emotional processing has long been understood (Ekman, 1984); however, studies are only beginning to investigate the temporal processes. It has been suggested that hemispheric asymmetry may be more prominent early on in image processing (Roschmann and Wittling 1992) and that differential hemispheric laterality for pleasant and unpleasant conditions cannot be distinguished when data is averaged across the entire film period (Davidson et al., 1990). Studies that have focused upon the temporal dynamics have largely focused on event-related potential (ERP) activity associated with the initial processing of emotional stimuli (e.g., Junghofer et al., 2001; Kawasaki et al., 2001). Such studies have also been characterized by failing to control for arousal when varying valence (e.g., Mini et al., 1996).

Steady-state probe topography (SSPT) is a technique that is able to track rapid changes occurring in brain electrical activity during the ongoing processing of stimuli (Silberstein et al., 1990, 1995, 1996, 1998, 2000, 2001). SSPT examines changes to the 13-Hz steady-state visually evoked potentials (SSVEPs) and offers not only relatively high temporal resolution compared with PET and fMRI, but also temporal continuity. The SSVEP is characterized by two components. These are SSVEP amplitude, which may be compared to alpha activity in association with cognitive tasks (Silberstein, 1995a,b), and SSVEP latency, which has been proposed to index changes in the neural information processing speed (Silberstein et al., 1996, 2000).

The aim of the present study therefore was to use SSPT to examine the spatiotemporal characteristics of the SSVEP associated with the processing of pleasant and unpleasant images low in arousal content. It was hypothesized therefore that the effects of emotional stimuli on the SSVEP would be most evident when observing the time series data and that the valence-related effects on the SSPT would be predominantly frontally distributed. It was also hypothesized that emotional images, after subtraction of the processing associated with the neutral images, would induce valence-specific lateralization within the frontal regions.

METHODS

Participants

Twenty healthy subjects participated in the current study, though only 16 could be used for technical reasons. The subjects included in the analysis consisted of 11 males (M, 24.18 years; SD, 5.51) and 5 females (M, 25.40; SD, 6.07). All participants were right handed as assessed by the Edinburgh Inventory (Oldfield, 1971), drug free, and had no history of epilepsy, head injury, stroke, psychiatric disorders, neurological illness, or alcoholism.

The International Affective Picture System

Seventy-five images were selected from the IAPS instruction manual and affective ratings (Lang et al., 1999). Images were chosen based upon the standardized valence and arousal ratings provided in the manual and were selected so that valence was varied (unpleasant, neutral, and pleasant), while arousal remained relatively low for all categories.

Chosen images were not rated greater than 6 on the arousal scale, which ranged up to a score of 9. The unpleasant, neutral, and pleasant categories were characterized with valence (V) ratings ranging between 1.8 and 3.47; 4.46 and 5.46; 7.02 and 8.34, respectively, and arousal (A) ratings ranging between 3.52 and 5.5; 1.55 and 4.27; 2.67 and 5.94, respectively. A one-way ANOVA was conducted on SPSS software (SPSS Inc., Chicago) to assess for any differences in brightness and contrast (as determined by luminosity histogram plots in Adobe Photoshop) between pleasant, unpleasant, and neutral picture categories. No significant differences were found between any of the categories of images for brightness.
\[ F(2,72) = 0.67, P = 0.52, \text{ and contrast, } F(2,72) = 0.83, P = 0.44, \text{ respectively.} \]

**Procedure**

The SSPT recording technique was explained and the recording only took place when participants were completely relaxed and comfortable and when they had a complete understanding of how to rate each image. IAPS images were presented to participants in three blocks (25 unpleasant, 25 neutral, and 25 pleasant). Each image was presented once only (6 s duration) and was followed by the Self-Assessment Manikin (SAM) rating scale. SAM enables participants to rate each image for valence and arousal (Lang et al., 1999). For the purposes of the present study, SAM was modified to display the valence and arousal scales only. An additional modification was the exclusion of the "preparation slide," as our experimental procedure did not require participants to take their attention away from the computer screen.

Participants were randomly allocated to a group that had either the unpleasant or the pleasant category presented first. All participants were presented each of the three categories of images, and the neutral category was always presented between the pleasant and unpleasant categories. Participants were asked to focus on emotional content, to refrain from emotive inhibition, and to rate each image (using SAM) as they actually felt while viewing it.

Brain electrical activity was recorded by 64 monopolar leads using a lycra electrode cap with chin strap, with linked ears as the reference and a nose electrode used for ground. The sites were located in International 10/20 positions as well as sites midway between these positions. Electrode impedance generally remained lower than 5 kOhm. The band-pass filter was set at 0.74 and 74 Hz prior to digitization to 16-bit accuracy at a rate of 500 Hz. A diffuse 13 Hz sinusoidal white flicker superimposed on the visual field by a pair of goggles elicited the SSVEP and subtended a horizontal angle of 160° and a vertical angle of 90°.

**Signal Processing and Analysis**

Previous studies have described the major features of the signal processing procedures within our institute (Silberstein et al., 1990, 1995, 1998, 2000, 2001; Line et al., 1998; Thompson et al., 2000); thus, for the purposes of brevity, only procedures specific to the present study's analysis will be discussed. Box 1 summarizes the major methodological steps taken to analyze the IAPS SSVEP data and to present the topographic maps.

SSVEPs were produced for all electrodes from the 13-Hz Fourier coefficients (FC) and were then evaluated using a 10-unit window. For each stimulus cycle, this evaluation period averaged overlapping blocks of 10 FCs and the coefficients recalculated for this overlapping period. This yields a time series with 13 points/second corresponding to a temporal resolution of 0.77 s. This procedure was conducted for the entire recording period for all three image categories. A target averaging technique was then used to select the SSVEP epochs that corresponded with the presentation of an image and all selected epochs for each of the three categories were then averaged.

**Box 1.** Methodological steps taken to analyze the recorded data for the IAPS task.

Both components of the SSVEP (amplitude and phase difference between the sinusoidal visual stimulus and the SSVEP) were normalized. Amplitude was normalized by first calculating a mean value for each of the 64 electrodes for the neutral category across the length of the time series data and then averaging these 64 values to yield a single normalization factor (NF). The amplitude from all categories from each electrode for each individual was then divided by NF. In addition, SSVEP phase was normalized by first calculating a mean value for each of the 64 electrodes in the SSVEP time series for the neutral condition (reference task). These values (one for each of the 64 electrodes) were then subtracted from the phase for all categories for each of the corresponding electrodes. Changes in phase (expressed as radians) are expressed in terms of latency (milliseconds) using the formula: 

\[
\text{change in phase/2}\pi \times \left(\frac{1000}{13}\right)
\]

The SSVEP epochs for each individual were then averaged together to form averages of pleasant, neutral, and unpleasant images. The SSVEP epoch corresponding to the neutral images was then subtracted from both emotional categories to yield the activity associated with either pleasant or unpleasant valence. The neutral condition was used as a reference task for both emotional conditions in order to remove aspects of processing unrelated to the processing of either pleasant or unpleasant valence.
Topographic Mapping and Statistical Analysis

The complex SSVEP time series was then displayed in topographic maps produced using a spherical spline interpolation procedure (Nunez et al., 1994). The topographic maps express the SSVEP in terms of amplitude and the latency differences following subtraction of the neutral reference condition. The statistical strength of the differences between the emotional conditions and the neutral condition were examined using the Hotellings $T^2$ parameter and each presented topographic map was corrected for five multiple comparisons as discussed previously (Silberstein et al., 1995).

Topographic maps representing unpleasant and pleasant valence are presented for the average of the 6-s data (Fig. 1). For these reasons a strict alpha level of 0.005 was chosen to counter for the multiple comparisons being made in terms of both the number of electrodes used and the two types of emotional activation. In addition, a time point was chosen for display of temporal characteristics (Fig. 2). The literature suggests that it is the anterior frontal locations that are most important in the emotional processing of valence; therefore, a frontal electrode was chosen. A time point at which unpleasant and pleasant categories are maximally discriminated from the neutral condition was then selected from the time series (of the selected electrode) (see Fig. 1) and then represented in additional topographic maps (Fig. 2).

RESULTS

Behavioral Results

All participants were required to rate each image on valence and arousal scales. The means and standard deviations for the ratings of valence and arousal made by our Australian participants are detailed in Table 1.

A univariate repeated measures ANOVA was conducted for both the valence and arousal dimensions separately. Multivariate results are reported for the valence dimension as Mauchly's Test of Sphericity (M.T.S.) was violated for this dimension. (Visit: http://www.statsoft.com/textbook/stanman.html#assumptions for a discussion on M.T.S.).

FIG. 1. Topographic maps are presented which represent the 6-s averaged 13-Hz steady-state visually evoked potential (SSVEP) data (amplitude and latency) for affective images (unpleasant and pleasant) relative to neutral images. Both amplitude and latency SSVEP time series plots for electrode 0 are displayed (middle column).

FIG. 2. Topographic maps are presented which represent the 13-Hz amplitude and latency SSVEP for affective (unpleasant and pleasant) relative to neutral images at 1462 ms (temporal resolution, 0.77 s).
TABLE 1
Means and Standard Deviations for Valence and Arousal Behavioral Rating Scores

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>Valence M (SD)</th>
<th>Arousal M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpleasant</td>
<td>16</td>
<td>3.50 (0.17)</td>
<td>3.81 (0.45)</td>
</tr>
<tr>
<td>Neutral</td>
<td>16</td>
<td>5.08 (0.08)</td>
<td>1.96 (0.19)</td>
</tr>
<tr>
<td>Pleasant</td>
<td>16</td>
<td>6.14 (0.14)</td>
<td>3.20 (0.35)</td>
</tr>
</tbody>
</table>

Significant category effects were found for both valence [Pillai's, 0.91, F(2,13) = 63.18, P < 0.001, partial eta squared, 0.907] and arousal [F(2,13) = 19.60, P < 0.001, partial eta squared, 0.724]. For the valence rating scale, planned comparisons revealed significant differences between the unpleasant and neutral categories [F(1,14) = 116.73, P < 0.001, partial eta squared, 0.893] as well as pleasant and neutral categories [F(1,14) = 36.68, P < 0.001, partial eta squared, 0.724]. For the arousal rating scale, significant differences between the unpleasant and neutral categories [F(1,14) = 32.79, P < 0.001, partial eta squared, 0.701] and pleasant and neutral categories [F(1,14) = 27.50, P < 0.001, partial eta squared, 0.663] were also revealed, but not for pleasant and unpleasant categories [F(1,14) = 3.65, P = 0.077].

SSPT Results

The amplitude and latency SSVEPs for affective (unpleasant and pleasant) relative to neutral images are displayed in Fig. 1. These maps reflect the SSVEP activation that corresponds to the mean of the 6-s time series (78 13-Hz cycles). Warmer colors in these maps indicate reduced amplitude and reduced latency during the presentation of affective images.

Figure 1 demonstrates that pleasant valence is associated with a frontal amplitude increase and latency decrease bilaterally as well as an amplitude decrease and latency increase within the occipital region (Hotellings T(15) > 3.29, P < 0.005). SSVEP during the processing of unpleasant valence displayed an amplitude increase and latency reduction in the left temporoparietal, posterior frontal, and right anterior temporal regions (Hotellings T(15) > 3.29, P < 0.005).

The time series for electrode 0 (located in the left anterior prefrontal region) is displayed in Fig. 1. Latency was best able to differentiate between emotional and neutral images at frontal sites. In particular it was electrode 0 (left prefrontal) which displayed the maximal latency difference. A time point was selected which corresponded to the point at which emotional images were maximally discriminated from the neutral condition and this time point is displayed topographically in Fig. 2. This difference was significant at an alpha level of 0.005 at the 1462-ms time point for both emotional conditions relative to the neutral condition.

Figure 2 indicates that unpleasant valence is associated with a bilateral anterior frontal amplitude decrease (particularly pronounced within the left anterior frontal), an amplitude increase within the centrotemporal region, an amplitude decrease within the occipital region, as well as latency reductions within the frontal, left temporal, and right occipitotemporal regions. These effects are statistically significant within the left anterior frontal, right posterior frontal, left anterior temporoparietal, and right occipitotemporal regions (Hotellings T(15) = 3.29, P < 0.005). Pleasant valence produced a reduction in amplitude within the occipital region and frontal latency reductions. These effects were significant in the left anterior frontal, right lateral posterior frontal, and occipital regions (Hotellings T(15) = 3.29, P < 0.005).

DISCUSSION

The current study investigated the statisiternal characteristics of the SSVEP associated with processing of low arousal, unpleasant and pleasant emotionally valenced images.

Behavioral results for valence confirm that the allocation of images to pleasant, neutral, or unpleasant categories based upon IAPS standardized values was appropriate for the current Australian sample. In addition, behavioral results indicate that although pleasant and unpleasant categories do not differ from each other on the arousal dimension, both emotional conditions differ from the neutral condition. As illustrated in a recent paper (Bradley et al., 2001), when IAPS images are distributed on a two-dimensional plot according to valence and arousal ratings, the resulting plot forms a boomerang shape in which the arms reach toward the high arousal quadrants. This indicates that it is difficult to select either positively or negatively valenced images which are equivalent to neutral images on the arousal dimension. It is important to note, however, that all categories of images presented to subjects in the current study did not contain content rated high on the arousal dimension (e.g., violent death and erotic), unlike in previous studies (e.g., Mini et al., 1986; Lane et al., 1997a,c).

A number of key findings are reported which demonstrate the spatiotemporal characteristics of the SSVEP during emotional processing (Fig. 2). First, latency reductions were displayed within the frontal regions for both unpleasant and pleasant valence. In addition, latency reductions were also displayed within the left temporal and right occipitotemporal regions for unpleasant valence. Second, a number of amplitude effects were identified. Both pleasant and unpleasant valence was associated with an amplitude reduction within the occipital region. In addition, an amplitude reduction within the bilateral anterior frontal and an amplitude increase within centrotemporal regions were displayed for unpleasant valence. Results also demonstrate differences between averaged and transient activity (discussed below) emphasizing the importance of focusing on transient activity when investigating the nature of emotional processing.

The SSVEP is believed to reflect neuronal activity primarily within the pyramidal cells of the neocortex and is characterized by two components. The first component is amplitude, which has been previously interpreted as analogos to the amplitude of regional activity within the alpha frequency range (Silberstein, 1995a,b). In this framework, an amplitude reduction may be regarded as an electrophysiological correlate of activated cortical areas and is known as event-related desynchronization (ERD) (see Pfurtscheller and Lopes da Silva, 1999, for a review). Previous findings, which
have demonstrated reductions in SSVEP amplitude within occipito/parietal and centro/parietal regions during a visual vigilance task (Silberstein et al., 1990) and also within prefrontal sites during the set change of the Wisconsin Card Sort Task (Silberstein et al., 1995), support this interpretation. Latency, the second component of the SSVEP, may be interpreted as reflecting neural information processing speed. This interpretation is again supported by previous studies, which have demonstrated that reaction time in a visual vigilance task (continuous performance task, CPT A-X) correlates with frontal SSVEP latency (Silberstein et al., 1996, 2000). Increases in neural information processing speed may be related to increased levels of cortical excitability, as indicated by animal studies which have shown reductions in thalamocortical transmission time following the release of the excitatory neurotransmitter ACh (e.g., Methrath and Ashe, 1993). In addition, latency reductions may also be related to cortico-cortical processing, as proposed in a recent model (Silberstein et al., 2001).

Before discussing the significance of our findings, it is important to emphasize that the time point (1462 ms) discussed in the present paper (and presented topographically in Fig. 2) corresponds to the point in the time series in which the emotional conditions are maximally discriminated from the neutral condition.

Previous studies have reported much earlier emotional discrimination. These have used, for example, scalp-recorded event-related potentials (150–260 ms) (Junghäuser et al., 2001) and in-dwelling electrodes (120–160 ms), to record from single neurons (Kawasaki et al., 2001). It is indicated by the authors that these effects are related to initial conceptual or encoding processing of the images, rather than conscious processing of stimuli, likely to be reflected in the SSVEP (Silberstein et al., 1990). The present study does not investigate the temporal aspects of early emotional discrimination processes, but rather the spatiotemporal characteristics associated with ongoing emotional processing with specific regard to the point of maximal difference in SSVEP between the emotional and neutral categories.

All methodologies have their strengths and weaknesses and while event-related potential (ERP) techniques are useful for investigating the processing immediately following stimulus presentation, they do not allow for investigation of time-extended processes (Silberstein et al., 1990). SSPT, by contrast, does not have as good a temporal resolution as ERPs; however, it allows for an examination of the temporal continuity of cortical activations and, as a result, may be useful for investigating the conscious, ongoing processing of emotion. Interestingly, our findings support the proposition that the prefrontal cortex will be important for guiding the processing of emotional stimuli at not only preconscious but additional temporal scales (Kawasaki et al., 2001).

The current paper reports a number of interesting SSPT findings, and these are discussed in the following paragraphs. Spatiotemporal characteristics for the latency data indicate increases in excitatory processes within frontal regions during processing of both unpleasant and pleasant valence, suggesting that processing within these regions is not specific to valence type. This finding supports those of previous studies (Lane et al., 1997b,c; Relman et al., 1997) that have shown substantial overlap in emotional activation irrespective of valence. Our findings indicate, temporally at least, that anterior neural mechanisms for pleasant and unpleasant affect are closely linked.

Both unpleasant and pleasant valence also displayed transient amplitude reductions within the occipital regions. In addition, unpleasant valence displayed transient latency reductions within the right occipitotemporal region. While the literature suggests that emotional processing involves areas within the prefrontal cortex (for a comprehensive review, see Davidson and Irwin, 1999), the role of posterior regions in emotional processing are yet to be clarified. As cortical and subcortical structures are involved in emotional processing, the interpretation of the data within regions posterior to frontal locations is tentative because the SSPT technique does not allow investigation of subcortical activation.

However, our findings support those of previous studies which demonstrate activation within visual cortical areas in response to emotional images of both unpleasant and pleasant valence. For example, Lang and colleagues (1998b) have reported an fMRI study that compared emotional to neutral images and found activation within the occipital gyrus bilaterally, the right fusiform gyrus, and the right inferior and superior parietal lobules and this was reported not to be due to eye movement artifact. More recent studies have confirmed visual cortical activation in response to emotional stimuli (Lane et al., 1999; Hamman et al., 2002), and suggest that this activity may be modulated by the amygdala (Cahill and McGaugh, 1998; Morris et al., 1998, 1999). Studies are beginning to suggest that pleasant valence may modulate the amygdala and visual cortical activity in addition to unpleasant valence, though this still appears more extensive with unpleasant valenced images (Hamman et al., 2002). This may account for our finding that in addition to amplitude reductions, unpleasant valence was also associated with a transient latency reduction in the right occipitotemporal region. Interestingly, a common pattern of increased activation within the right extrastriate visual cortex has been reported in association with valence, arousal, and attention (Lane et al., 1999).

A number of spatiotemporal differences were also apparent between unpleasant and pleasant valence. In comparison to neutral images, unpleasant images are associated with a transient bilateral anterior frontal amplitude reduction. These effects were not evident for pleasant images relative to a neutral reference. While our findings demonstrate that positively and negatively valenced images compared with neutral images are associated with increases in neural information speed (reductions in latency), unpleasant images compared with neutral images are also associated with increased activation (reductions in amplitude) within anterior frontal regions. This latter finding is consistent with the findings reported in a previous study which demonstrate that positive emotional processing is associated with weaker activation (compared with negative emotional processing) within the lateral orbitofrontal/prefrontal cortex (Northoff et al., 2000).
Unpleasant images also displayed significant activation within the left anterior temporoparietal cortex. Again, it is important to be cautious regarding the interpretation of activation posterior to the frontal lobes; however, left temporoparietal activation during unpleasant valence may be related to increased left amygdala activation, previously associated with sad mood induction (Grodzinski et al., 1995; Schneider et al., 1997). Significant activation within this same region remains when averaging the entire image viewing period for unpleasant images compared to the average for neutral images (Fig. 1). In addition, significant frontal activation was not displayed during the average of the picture viewing period for unpleasant valence, suggesting that frontal activation may be more of a transient phenomenon when processing unpleasant images low on arousal. By contrast, the averaged SSVEP for the pleasant valence over this same period (Fig. 1), however, is associated with a bilateral frontal activation. These findings emphasize the importance of focusing on transient, phasic activity associated with the processing of emotional valence.

The use of the IAPS task in brain imaging studies is becoming established as a useful way to probe the brain during the processing of emotional stimuli. However, it is important that valence and arousal are carefully controlled in the design of activation studies. Our data demonstrate that both pleasant and unpleasant pictures low in arousal content are associated with bilateral frontal latency reductions, supporting previous studies that have shown substantial overlap in processing of unpleasant and pleasant valence. Furthermore, our results support previous research which, after subtracting activation associated with neutral images from that associated with both pleasant and unpleasant images, reported an increase in cerebral blood flow within the medial prefrontal cortex (Lane et al., 1997c). Activation within this area in the context of viewing emotional images may be associated with increased attention (Lane et al., 1999).

The current findings imply that overlapping frontal activations for unpleasant and pleasant valence are not due to the failure of some studies to control for the levels of arousal within and between valenced categories. Although frontal regions (such as the OFC) have been implicated in arousal functions, a body of evidence suggests that the frontal lobes may mediate expressive functions, whilst the posterior regions mediate perceptual (Ley and Bryden, 1981; Ahern and Schwartz, 1985) and arousal functions (Heller et al., 1998; Aftanas et al., 2001). Therefore it is possible that contradictions, in terms of frontal laterality specifically, may be due to other factors, such as the degree with which stimuli are able to elicit approach or withdrawal behaviors. For example, anger, despite its negative valence, is regarded as an approach-related emotion and has been associated with left frontal activation (Harmon-Jones and Allen, 1998). In addition, adult emotions such as sadness have been described as complex blends of approach and withdrawal emotions (Jones and Fox, 1992). The current results support the notion that anterior laterality is not related to differences in valence after possible effects of arousal have been controlled for. It is possible, however, that the selected time point reflects conscious and voluntary regulatory processes in addition to affective elicitation.

While the current study investigated brain activation in response to images differing on emotional valence, there are other factors that influence emotional processing which include emotional arousal and gender differences. In order to fully understand the neurophysiology of emotional processing, future studies will need to address the specificity of these issues to the processing of affective images. Given the relative ease with which IAPS images can be systematically selected, it would be particularly interesting for future studies to investigate the effects of images varying in terms of arousal on regions believed to mediate the processing of emotional arousal, primarily the right parietotemporal region (Heller et al., 1998) but also frontal regions which may modulate the SCR in specific situations having emotional significance (Zahn et al., 1999). With respect to gender, very few neuroimaging studies have investigated differences in emotional processing. This may in part be due to the perception of women's increased responsiveness to emotional stimuli (e.g., Lane et al., 1997b; Canli et al., 2001). It is important to note, however, that although men and women may differ in terms of global, memory-based measures of emotion, they do not differ when documenting their emotional reactions on a moment-to-moment basis (Barrett et al., 1998).

In summary, the current study suggests that bilateral frontal latency reductions are associated with both pleasant and unpleasant valence (in terms of transient activation for images rated low on arousal) and supports previous literature that suggests substantial overlap in frontal emotional activation irrespective of emotional valence. In addition, unpleasant images were associated with a transient bilateral anterior frontal amplitude decrease, which may reflect differences in degree of activation. Finally, regional posterior differences were noted for unpleasant and pleasant valence and these may be related to connections with subcortical areas.

ACKNOWLEDGMENTS

The authors thank Peter Line, Geoff Niel, and Andrew Pippings for their technical expertise, as well as Stuart Armstrong and Jim Thompson for their comments on the paper.

REFERENCES


Binding of contextual information to target information is essential if a source discrimination is to be made. In normal subjects, emotion seems also to perturb this binding process (cf. Laroi et al., 2001). Results from the present study reveal that subjects predisposed towards hallucinations are particularly sensitive to the effects of emotion on the contextualisation of internal information.

References


21. Preliminary electrophysiological evidence for modulation of the processing of negative affect by serotonin

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Despite a well-known relationship between serotonergic function and depression, little is known on how modulation of 5-HT affects the processing of negative affect. The current study was double-blind-placebo-controlled and investigated the neurophysiological changes associated with the processing of negative affect using pictures from the International Affective Picture System (IAPS).

**Hypotheses**

Emotional processing of negative affect (negative images) relative to neutral affect (neutral images) will be associated with frontal SSVEP latency reductions, whilst increases in serotonin (with citalopram) will reduce the magnitude of these frontal SSVEP latency reductions.

**Method**

**Subjects**

Thirteen healthy subjects, consisting of 8 males (M = 23.90, SD = 3.60) and 6 females (M = 24.50, SD = 4.00), participated in the current study. Subjects were right-handed, non-smoking, drug-free, and had no history of neurological or psychiatric disorders.

**Procedure**

The study design was double-blind-placebo-controlled, in which subjects were tested under two conditions (placebo and citalopram 20 mg), each separated by a one-week washout period. Testing was conducted 2h following drug administration to coincide with the approximate peak plasma levels of citalopram. A diffuse 13 Hz visual flicker elicited the SSVEP while participants viewed 50 IAPS images, categorized as either neutral or negative. These images were presented in 2 blocks (neutral and negative), which contained 25 images in each block.
IAPS

Image selection was based upon standardized American valence and arousal ratings (Lang, Bradley, & Cuthbert, 1999). Valence ranged between 4.46 and 5.46 (neutral images) and 1.8 and 3.47 (negative images), whilst arousal ranged between 1.55 and 4.27 (neutral images) and 3.52 and 5.5 (negative images).

A one way ANOVA was conducted to assess for any differences in brightness and contrast between neutral and negative picture-categories. No significant differences were found between categories of images for brightness, \( F(1,48) = 1.04, p = .31 \), and contrast, \( F(1,48) = 1.26, p = .27 \).

SSVEP analysis

The SSVEP was produced for all 64 electrodes from the 13 Hz Fourier coefficients evaluated over 10 stimulus cycles at the stimulus frequency of 13 Hz using a cosine window, thus yielding a temporal resolution of 0.77 s. This evaluation period was shifted 1 stimulus cycle and the coefficients recalculated for this overlapping period. This procedure was continued for the entire recording period for both neutral and negative categories.

A target averaging technique was then used to select the SSVEP epochs that corresponded with the presentation of an image and all selected epochs for each category averaged. The SSVEP for each individual were then averaged together to form a group average for both the placebo and citalopram conditions. The SSVEP corresponding to the neutral category was then subtracted from the negative category to yield the activity associated with negative valence for each condition.

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To investigate the spatio-temporal processing of valence after administration of placebo and citalopram one time point was selected that corresponded to the most negative deflection in the placebo condition for the negative images relative to the mean of the 6-s image presentation for the neutral images. To aid time-point selection electrodes, Fp1 and Fp2 were chosen as these were regions at which activity was hypothesized. The time series for these electrodes are displayed in Fig. 1.

For the purposes of brevity, only the latency results for the negative versus the neutral images will be displayed. Lighter regions in these maps represent reduced latency for the negative images compared to neutral images. Results displayed below are preliminary and general trends are discussed. Statistical analysis was not conducted as the minimum required subject numbers for in-house multiple permutations testing have not, as yet been reached.

Results

Results display the SSVEP latency change at the 2.4 s time point for the negative images after the latency of the neutral images has been subtracted.

Reductions in SSVEP latency predominantly in bilateral frontal but also in the left temporal and right parieto-temporal regions were found in the placebo condition during processing of negative affect (Fig. 1a), whilst the magnitude of this latency change was globally reduced by citalopram (Fig. 1b).

Conclusions

Frontal SSVEP latency reductions were found during the processing of negative affect in the placebo condition. This finding supports previous findings that have shown strong frontal latency reductions during the processing of negative emotional valence in healthy subjects (Kemp et al., 2001).

The latency reductions during the processing of negative affect may be interpreted as an increase in excitatory processes within pyramidal cell networks (Silberstein et al., 2001). In this model, latency reductions in the activation task (negative images) compared to the control task (neutral images) are interpreted as a stronger activation within local networks during the processing of negative affect.

Acute administration of citalopram was associated with reductions in these effects (reduced excitatory processes). A reduction in such processes within pyramidal cell networks following citalopram administration may be explained by increases in extracellular 5-HT in the frontal cortex, left temporal and right parieto-temporal regions leading to inhibition of pyramidal cell activity, either by direct activation of 5-HT_1A receptors on layer 5 pyramidal cells or indirectly through activation of GABA interneurons innervating pyramidal cells.

In summary, preliminary findings suggest that emotional processing of negative affect is modulated by acute changes in serotonin particularly within the frontal cortex, as well as regions that have been implicated in physiological arousal (the right parieto-temporal region) and danger recognition (left temporal region). The findings support metabolic blood flow studies that also indicate frontal modulation of brain activity by antidepressants (e.g., Kennedy et al., 2001).

Acknowledgment

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22. Effects of catecholamine depletion on D2 receptor binding and mood in healthy humans

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The effects of catecholamine depletion, achieved by per-oral administration of 4.5–5.25 g -methyl-para-tyrosine (AMPT) over 25–29 h, were studied on measures of dopamine (DA) release and mood in twelve healthy subjects. Neostriatal DA levels in vivo were estimated by comparing the D2 receptor-binding potential (D2RBP) before and after catecholamine depletion using [11C]raclopride positron emission tomography. The AMPT treatment increased D2RBP significantly by 14.7 ± 5.4% and decreased plasma levels of the DA metabolite homovanillic acid by 66 ± 14% and of the norepinephrine metabolite 3-methoxy-4-hydroxyphenethyleneglycol by 64 ± 11%. Catecholamine depletion resulted in decreases in happiness, talkativeness, feeling high, vigor, attentiveness, and openness to feelings, and in increases in drowsiness, sleepiness, sedation, and persistence. These changes were not correlated with the D2RBP increments.

Report

Neostriatal dopamine (DA) levels have been estimated in humans in vivo by comparing radiotracer binding at baseline and after rapid catecholamine depletion induced by the tyrosine hydroxylase inhibitor a-methyl-para-tyrosine (AMPT) using [11C]raclopride positron emission tomography (PET) and observed significant AMPT-induced changes in subjective happiness and sleepiness (Verhoeff et al. 2001). However, because of the small sample size in our original study, we believed that an imperative step in establishing the validity of these effects was to test them in a larger subject group.

Materials and methods

Human subjects

The study was approved by the Human-Subjects-Review-Committee of the University of Toronto and has been carried out in accordance with the Declaration of Helsinki (1975). Five men and 7 women, age 27 ± 7 years (all values in this article are expressed as means ± standard deviation) and all right-handed, completed the study. In- and exclusion criteria were the same as for our original study (Verhoeff et al., 2001).

Depletion regimen and mood monitoring

Each subject was scanned twice, at baseline (PET1-day 1) and after DA depletion (PET2-day 3). DA depletion was induced by oral AMPT administration in total 4500 mg over 25 h (6 subjects) or 5250 mg over 29 h (6 subjects). AMPT was administered in doses of 750 mg each at the following times: at 10 AM, 1.30 PM, 6 PM, and 10 PM on day 2, and at 7 AM and 11 AM plus 3 PM (6 subjects) on day 3. During AMPT administration, subjects remained under direct observation at the PET Centre during the day and on a psychiatric inpatient unit during the night. To prevent AMPT urine crystal formation, subjects were instructed to drink ≥4L/24h of fluids starting on day 2. In addition, to alkalize the urine, which increased AMPT solubility, sodium bicarbonate 1.2 g orally was given at 10 PM on day 1 and at 7 AM on day 2. Urine samples were collected at 3 PM on day 2 and at 7 AM on day 3 to examine the presence of AMPT crystals.

Subjects were evaluated five times using rating scales for mood states pre-AMPT (on day 1 and on day 2) and post-AMPT (cumulative oral doses of 750 mg on day 2, and 3750 and 4500 mg on day 3). The subjects rated 19 subjective feelings on a continuous visual analog scale (VAS) ranging from 0% (‘‘not at all’’) to 100% (‘‘most ever’’). Subjective feelings were also rated using the ordinal Profile Of Mood States (POMS) developed by McNair et al. In addition, subjects rated depressive symptoms using the Beck Depression Inventory, Short Form (BDI).

Plasma analyses

Plasma homovanillic acid (HVA), 3-methoxy-4-hydroxyphenethyl-enedi glycol (MHPG) and prolactin samples were collected at 10 AM (day 1), at 10 AM (day 2 pre-AMPT), at 3 PM (day 2 post-1500 mg AMPT), at 1 PM (day 3 post-4500 mg AMPT), and at 3 PM (day 3 post-5250 mg AMPT for 6 subjects). Plasma AMPT samples were collected at 3 PM (day 2 post-1500 mg AMPT), at 1 PM (day 3 post-4500 mg AMPT), and at 3 PM (day 3 post-5250 mg AMPT for 6 subjects). HVA, MHPG, and AMPT levels were measured as the methylated then acetylated derivative, as the 4-acetyl-di-trifluoro-acetyl derivative, and as the pentafluorobenzoyl derivative, respectively, using gas chromatography-mass spectrometry with selected ion monitoring. Prolactin levels were measured using microparticle enzyme immunoassay technology (Abbott Laboratories, Abbott Park, Illinois).

[11C]raclopride PET data acquisition

PET images were obtained with a GEMS PC2048-15B camera (General Electric Medical Systems, Milwaukee, Wisconsin) in five 1-min frames followed by twenty 2-min frames and three 5-min frames after [11C]raclopride bolus injection (pre-AMPT: 376 ± 28 MBq, specific activity: 61.7 ± 15.215 GBq/mmol; post-AMPT: 376 ± 28 MBq, specific activity 43.76 ± 20.764 GBq/mmol). There was no significant difference in the pre- and post-AMPT injected radioactivity and specific activity. The images were attenuation corrected via 68Ge trans-
Gender differences in the cortical electrophysiological processing of visual emotional stimuli

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The processing of visual emotional stimuli has been investigated previously; however, gender differences in the processing of emotional stimuli remain to be clarified. The aim of the current study was to use steady-state probe topography (SSPT) to examine steady-state visually evoked potentials (SSVEPs) during the processing of pleasant and unpleasant images relative to neutral images, and to determine whether this processing differs between males and females. Thirty participants (15 males and 15 females) viewed 75 images low on the arousal dimension (categorised as pleasant, neutral or unpleasant) selected from the International Affective Picture System (IAPS), whilst a 13-Hz sinusoidal white visual flicker was superimposed over the visual field and brain electrical activity was recorded from 64 electrode sites.

Results suggest that pleasant and unpleasant images relative to neutral images are associated with reductions in frontal latency and occipital amplitude. In addition, electrophysiological gender differences were observed despite there being no differences found between males and females on subjective mood or behavioural ratings of presented images (valence and arousal dimensions). The main gender difference reported in the current study related to the processing of unpleasant images (relative to neutral images) which is associated with widespread frontal latency reductions (predominantly right sided) in females but not in males. Our results suggest that gender differences do exist in the processing of visual emotional stimuli, and illustrate the importance of taking these differences into account during investigations of emotional processing. Finally, these gender differences may have implications for the pathophysiology of mood disorders such as depression.

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Keywords: Gender; Sex; Emotional processing; Emotion; Unpleasant; Pleasant; IAPS; Valence; Arousal; Electrophysiology; SSPT; Imaging

Introduction

Gender differences in the brain have been well characterised in animals and to a lesser extent, in humans (Cooke et al., 1998; Rabinowicz et al., 1999, 2002; Supprian and Kalus, 1996). Although the functional significance of these differences are unclear (Rabinowicz et al., 1999; Supprian and Kalus, 1996), research is now beginning to examine the gender differences in emotion. This is an important endeavour considering that emotion has been described as the key component in personality and vulnerability factors governing risk for psychopathology (Davidson, 2002). With regards to the disorders of emotion, it is known that the lifetime risk for depression is 10–25% for women but only 5–12% for men (American Psychiatric Association, 1994). A more recent survey based on the ICD-10 classification found 6% of adults to suffer from depressive disorders and that twice as many females as males experience depression (Australian Bureau of Statistics, 1997). Differences in biology as well as gender-related environmental experiences are regarded as the key to understanding these gender differences in depression (Kessler, 2003).

‘Emotion’ may be defined as a relatively brief episode of synchronised response involving multiple components including cognitive processes, physiological responses, motivation changes, motor expression and subjective feeling (Borod, 1993; Ekman, 1984, 1992; Lang, 1968, 1984; Scherer and Peper, 2001). By contrast, ‘mood’ is generally considered to be a more diffuse state, characterised by low intensity but relatively long duration (lasting hours to days) (Ekman, 1992; Ketter et al., 2003; Scherer and Peper, 2001). Although, these terms relate to different behavioural constructs, it is possible that chronic or repeated activation of certain underlying neurophysiological mechanisms may be the connection between emotional experience and mood disorders such as depression and anxiety. For example, theories have been proposed which describe certain neurophysiological processes as they relate to longer-lasting affective phenomena such as depressed and anxious mood (e.g. Heller, 1993). Furthermore, brain activation resulting from the use of certain mood induction techniques in healthy participants is regarded as similar to that seen in mood disorders such as depression (see Lawrence and Grasby, 2001, for discussion).

A limited but growing number of studies have investigated whether gender differences in brain activation exist on tasks designed to assess a broad range of emotional processes. These studies are important to enable researchers to move beyond employment of behavioural methodologies which have been criticised for their inability to illuminate processes inaccessible to consciousness (e.g. Davidson et al., 2003). Studies that have investigated emotional perception have reported either no gender differences (Meyers and Smith, 1986), subtle differences between males and females (Morita et al., 2001; Wildgruber et al., 2002), or...
sex-specific areas of brain activation (Kilgore et al., 2001; Lee et al., 2002). Tasks that involve more the experience of emotion (e.g. Del Parigi et al., 2002; George et al., 1996; Pardo et al., 1993; Pendergrass et al., 2003; Schneider et al., 2000) have reported more consistent gender differences. Healthy women have been shown to display more activity (i.e. larger number and more widespread significant differences between transient induced negative mood states and baseline) than healthy men in anterior limbic structures such as the inferior frontal, orbital and prefrontal cortices, during transient induced sadness (e.g. George et al., 1996 and Pardo et al., 1993). These studies have also demonstrated that women show more bilateral activation without asymmetries during induced sadness. For example, in a female-only study, increases in activity were reported within the thalamus and medial prefrontal using both film as well as recall-induced emotional states (Lane et al., 1997a). Pardo et al. (1993) demonstrated left-sided activation of inferior frontal and orbitofrontal cortices in males, whilst bilateral activation of these areas was reported in females. In addition, sadness has been associated with amygdala activation in males but not females (Schneider et al., 2000). The authors suggested that females produce less concentrated and less lateralised brain activation than males. Bilateral findings in females are consistent with a widely held neuropsychological theory on the organisation of the brain, which posits that females are more bilateralised than men (Iaccino, 1993; Levy and Heller, 1992; McGlone, 1986). Whilst these studies examining emotional experience have shown gender differences, inconsistent differences have been reported. For example, George et al. (1996) report that women activate a greater portion of their limbic system than men during transient sadness, whilst Schneider et al. (2000) report that processing of sadness is more focal and subcortical in men. The literature focusing on transient happiness has also been inconsistent. For example, decreases as well as increases in activity have been reported for happiness (see George et al., 1995 and Lane et al., 1997a, respectively). Gender differences in transient happiness however may be more subtle. This is supported by previous studies which report either slight or no differences for happiness (George et al., 1996 and Schneider et al., 2000, respectively).

It should be noted however that many problems arise when attempting to compare studies that have investigated gender differences in emotion. First, brain imaging studies have used a variety of different paradigms. These paradigms have included recollection of sad events (Pardo et al., 1993), perception and experience of human emotional nonverbal sounds (Meyers and Smith, 1986; Smith et al., 1995), recollection of affect-specific events (George et al., 1996), viewing of faces with either happy or sad facial expressions to aid mood induction (Schneider et al., 2000), the recognition of facial affect (Kesler-West et al., 2001; Kilgore et al., 2001; Lee et al., 2002; Morita et al., 2001), the processing of emotionally evocative images (Canli et al., 2002; Pendergrass et al., 2003), the detection of emotional intonation (Wildgruber et al., 2002), and the experience of hunger and satiation (Del Parigi et al., 2002). Second, there are a large number of different neuroimaging techniques used, which range from PET (Del Parigi et al., 2002; George et al., 1996; Pardo et al., 1993), fMRI (Canli et al., 2002; Kesler-West et al., 2001; Lee et al., 2002; Kilgore et al., 2001; Pendergrass et al., 2003; Schneider et al., 2000; Wildgruber et al., 2002) and different electroencephalographic techniques (Meyers and Smith, 1986; Morita et al., 2001; Smith et al., 1995).

The International Affective Picture System (IAPS) has become increasingly used amongst brain imaging studies to investigate emotional processes as it allows for systematic selection of images that range in emotional content. Specifically, these images are associated with standardised ratings for valence and arousal which allows researchers to easily replicate published findings for a specific selection of images and also aid interpretation of and allow conclusions to be drawn from multiple studies using this task. Previous studies using the IAPS have investigated emotional processing with haemodynamic imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) (e.g. Canli et al., 1998, 2002; Lane et al., 1997b,c; Lang et al., 1998; Paradiso et al., 1999; Pendergrass et al., 2003; Taylor et al., 1998; Wrase et al., 2003), electroencephalographic-based techniques (e.g. Ahnans et al., 2001a,b, 2002; Junghofer et al., 2001; Kawasaki et al., 2001; Kemp et al., 2002; Mini et al., 1996; Palomba et al., 1997; Schupp et al., 2000, 2003) as well as magnetoencephalography (Northoff et al., 2000, 2002).

However, few of these studies have examined gender differences (Canli et al., 2002; Pendergrass et al., 2003; Wrase et al., 2003). These studies suggest gender differences in several neural structures including the insular, prefrontal and parietal cortices, bilateral visual processing areas, thalamic nuclei, amygdala, caudate, putamen and pons regions, and the postcentral and parahippocampal gyri during the processing of visual emotional stimuli. By contrast, a recent behavioural study that examined gender differences in IAPS images in terms of valence and arousal ratings, facial EMG, skin response and heart rate suggests that “remarkable congruence” was displayed in the physiological profile between the two genders when viewing images having less arousing appetitive and defensive contexts (Bradley et al., 2001).

In addition, Wrase et al. (2003) have recently reported that although no significant differences were found between males or females in valence, arousal, skin conductance response and startle modulation, men displayed stronger brain activity for positive visual stimuli in the inferior and medial frontal gyri, whilst women displayed stronger brain activity for negative visual stimuli in the anterior and medial cingulate gyri. These findings suggest that although males and females may not differ in terms of behavioural and peripheral physiological measures of emotional responsivity, the two genders may well differ in neurophysiology.

The present study focuses on aspects of emotion that relate to the processing of emotional stimuli or emotional processing. Emotional processing may be defined as the perception and evaluation of emotional stimuli which may or may not involve emotional experience. For example, emotional processing may involve recognition of emotional facial expressions (emotional perception), recollection of an emotional event (emotional experience), or the viewing of emotional film or images (emotional perception and experience). Note however, that such categorisations are a simplification as studies have clearly demonstrated the presence of autonomic arousal during tasks involving recognition of emotional facial expressions (e.g. Williams et al., 2001). Although the present study involves presentation of images rated low on the dimension of arousal, these pictures do appear to involve aspects of emotional experience, such as alterations in heart rate (Kemp and Nathan, in press).

It has been suggested that techniques with a superior temporal resolution may better address gender differences in emotional processing (e.g. Schneider et al., 2000). Event-related potential techniques however are unable to elucidate time-extended processes following stimulus presentation (Silberstein et al., 1990). Two other techniques, magnetoencephalography (MEG) and steady-state
probe topography (SSPT) provide such information; however, relatively few studies using these techniques have investigated how the brain differentially responds to emotional stimuli over time. In the current study, SSPT was used to investigate gender differences during the viewing of emotional stimuli selected from the IAPS. SSPT may be characterised by three features which include (1) the presentation of a rapid and repetitive visual flicker distinct from and irrelevant to the cognitive task undertaken by the participants, (2) the recording of brain electrical activity from 64 scalp-electrode sites within the area defined by the International 10-20 system and (3) a relatively short integration period which enables the rapid changes in brain electrical activity as well as the time-extended processes following stimulus presentation to be tracked (Gray et al., in press; Kemp et al., 2002; Silberstein et al., 1990). We have previously shown that transient widespread and bilateral frontal SSVEP latency and occipital amplitude reductions are associated with the cortical processing of pleasant and unpleasant emotional stimuli in a mixed-gender sample (Kemp et al., 2002). The aim of the current study was to investigate how cortical steady-state visually evoked potentials (SSVEPs) recorded using the SSPT technique are modulated by pleasant and unpleasant images (relative to neutral images) in a larger sample size, and to investigate whether this processing differs between males and females. Based on the extant literature reviewed above, we hypothesise (1) that males and females will not differ in subjective verbal report, (2) that females will display bilateral frontal latency reductions during the processing of both unpleasant and pleasant images relative to neutral images, and (3) that males will display more focal changes.

### Methods

### Participants

Thirty healthy participants (mean age 23.00 ± 2.30 years), were included in the current study. All participants were right handed as assessed by the Edinburgh Inventory (Oldfield, 1971), nonsmokers, drug-free, and had no history of epilepsy, head injury, stroke, psychiatric disorders, neurological conditions or alcoholism. A medical examination was conducted by a physician who, from physical- and questionnaire-based assessment, screened and excluded potential participants according to these exclusion criteria. Participants were recruited by advertising on noticeboards and word of mouth, were generally from a university population and gave informed consent to participate in the current study.

### Procedure

All participants were informed that they should not drink alcoholic or caffeinated beverages in the 12 h before the experiment being conducted. Participants then arrived for testing in the morning at approximately 8 am after which a standard breakfast was provided. Participants were then brought to the testing room and the recording procedure explained. Participants completed a short in-house questionnaire relating to standard demographics such as age, gender, years of education and exclusion criteria, the Oldfield handedness inventory (Oldfield, 1971), and the Profile of Mood States (POMS; McNair et al., 1988) before the SSPT recording (see Table 1). Participants indicated how they felt “RIGHT NOW” on the POMS questionnaire to determine current mood state. As outlined by Kemp et al. (2002), 75 images selected from the International Affective Picture System (IAPS) were then presented to participants in three blocks (categorised as pleasant, neutral or unpleasant) of 25 images. The images were carefully selected so that images were relatively low on the dimension of arousal and did not contain high arousal content such as violent death and erotica. Pleasant (P) stimuli included kittens, puppies, babies, flowers, sailing, etc; unpleasant (U) stimuli included cemeteries, smoke, garbage, dead cows, handicapped individuals, etc.; neutral (N) stimuli included mushrooms, animals, abstract art, buildings, kitchen objects, etc. Neutral images were always presented between pleasant and unpleasant image categories, and the presentation order of the categories was counterbalanced (i.e. P, N, U or U, N, P). There were no statistical differences in brightness and contrast between any of the image categories. Following each image, the Self Assessment Maniken (SAM) valence rating scale, ranging from 1 (maximally unpleasant) to 9 (maximally pleasant), and the SAM arousal rating scale, ranging from 1 (low arousal) to

### Table

<table>
<thead>
<tr>
<th>Category</th>
<th>Males and females (n = 30)</th>
<th>Males (n = 15)</th>
<th>Females (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unpleasant: valence</td>
<td>3.38 ± 0.65</td>
<td>3.57 ± 0.51</td>
<td>3.18 ± 0.72</td>
</tr>
<tr>
<td>Unpleasant: arousal</td>
<td>3.94 ± 1.40</td>
<td>3.97 ± 1.49</td>
<td>3.90 ± 1.35</td>
</tr>
<tr>
<td>Neutral: valence</td>
<td>5.15 ± 0.28</td>
<td>5.15 ± 0.32</td>
<td>5.16 ± 0.26</td>
</tr>
<tr>
<td>Neutral: arousal</td>
<td>1.75 ± 0.62</td>
<td>1.87 ± 0.58</td>
<td>1.63 ± 0.66</td>
</tr>
<tr>
<td>Pleasant: valence</td>
<td>6.33 ± 0.64</td>
<td>6.32 ± 0.64</td>
<td>6.35 ± 0.66</td>
</tr>
<tr>
<td>Pleasant: arousal</td>
<td>3.23 ± 1.43</td>
<td>3.10 ± 1.45</td>
<td>3.36 ± 1.44</td>
</tr>
</tbody>
</table>

*Missing data for one female.*
The major methodological steps taken to analyse and present the SSVEP data are presented in Box 1. Signal processing involved calculation of the 13-Hz Fourier coefficients (FC) for each stimulus cycle, smoothing the subsequent time series by averaging overlapping blocks of 10 FCs, extracting the 6-s SSVEP associated with each image, averaging across categories, averaging across subjects and then subtracting the averaged SSVEP associated with the neutral category from that associated with the emotional categories to produce activity interpreted as reflecting emotional valence.

Data are presented firstly to efficiently summarise the activity associated with the electrophysiological processing of unpleasant and pleasant valence. Rather than selecting a specific time point through identification of the maximal difference between the emotional categories and the neutral category as described in our previous study (Kemp et al., 2002), we now present time-series plots (including both amplitude and latency SSVEP components) and statistical cluster plots (Hotellings T), which display all 64 electrodes and all seventy-eight 13-Hz time points. These time-series and statistical cluster plots reflect the SSVEP associated with the processing of emotional valence for the entire 6-s image presentation. In all plots and topographic maps, warmer colours reflect reduced amplitude and latency during the presentation of emotional images relative to neutral images as well as larger t values in the Hotellings maps. The time series as well as the statistical cluster plots present electrodes on the y-axis and time points on the x-axis. Amplitude is always in the top row, latency on the second row and the Hotellings statistical cluster plots on the third row. Electrode numbers have been demarcated as having either frontal (including electrodes Fp1, Fp2, F7, F3, Fz, F4 and F8), centro-parieto-temporal (including electrodes T3, C3, Cz, C4, T4, T5, P3, Pz, P4 and T6) and occipital locations (including electrodes O1, O2 and O2) to aid in interpretation of these plots.

Statistical clusters of surrounding electrodes and consecutive time points were identified and used as a guide for determining epochs of interest within the larger 6-s epoch. Consecutive time points within these epochs were then averaged and represented in the form of topographic maps using a spherical spline interpolation procedure (Nunez et al., 1994). These epochs were normalised using normalisation factors (NFs) which were identical to those used for the amplitude and latency components in the 6-s epoch in order for the newly epoched data to be directly comparable to the 6-s epoched data. (For description of normalisation procedures, see Kemp et al., 2002.) The topographic maps display amplitude and latency components of the SSVEP for emotional images relative to neutral images (emotional valence), as well as the statistical strength of these differences.

Results are presented in the following way. Firstly, Fig. 1 displays the time series and statistical cluster plots for the entire sample (n = 30), whilst Fig. 2 displays the epochs of interest in the form of topographic maps. Fig. 3 presents time series and statistical cluster plots for males (n = 15) and females (n = 15) separately, whilst Fig. 4 presents male and female epochs of interest in the form of topographic maps. Results summarise all significant effects into the following regions: (1) left frontal region, (2) right frontal region, (3) left temporal, central and parietal regions, (4) right temporal, central and parietal regions and the (5) occipital region.
In summarising the results displayed in the topographic maps, if an SSVEP component (amplitude or latency) did not appear to be either increased or decreased in the emotional condition relative to the neutral condition (as indicated by topographic difference maps) within the statistically significant region (as indicated by the Hotellings maps), then the other SSVEP component is reported only and it is this component that is interpreted as being responsible for this effect.

Statistics

Separate independent-sample $t$ tests were firstly conducted on behavioural variables including age, education and each of the POMS subscales to determine whether differences existed between males and females. In addition, between-groups repeated-measures ANOVAs were conducted on participant’s valence and arousal ratings to determine whether gender modified the main effects of these two variables. The statistical strength of the SSVEP differences between the emotional images (unpleasant, pleasant) and the neutral images were examined using the Hotellings $T^2$ parameter and presented in both statistical cluster plots and topographic maps. Due to the exploratory nature of this study, an alpha criterion for the Hotellings $T^2$ was arbitrarily set at $P = 0.01$ (uncorrected for multiple comparisons) for the SSVEP data. It is assumed that real effects will have some degree of statistical continuity both in terms of surrounding electrodes and consecutive time points, and that if one particular electrode or point in time is statistically significant, then adjacent electrodes and consecutive

Fig. 2. Topographic maps associated with the difference between emotional categories (pleasant and unpleasant) and the neutral category are presented for amplitude and latency as well as Hotellings $T$ values. Contours are plotted on the Hotellings $T$ maps at three levels of probability ($P = 0.01$, $P = 0.005$ and $P = 0.001$) uncorrected for multiple comparisons. Three time periods are presented which relate to early (0–2 s), middle (2–4 s) and late (4–6 s) components of image viewing. Warmer colours represent reductions in amplitude and latency during presentation of emotional images relative to neutral images and larger $t$ values in the Hotellings $T$ maps.
time points will also be significant. The rationale that clusters of statistical significance are likely to reflect real effects has been used previously (e.g. Gray et al., in press; Guthrie and Buchwald, 1991; Murray et al., 2002).

The Hotellings $T$ statistic illustrates statistical differences of within-subject effects. Although topographic mapping of within-subject statistical differences allow for a potentially useful, visual comparison between different groups (e.g. Silberstein et al., 1998, 2000), the Hotellings $T$ statistic does not directly compare differences between males and females. Therefore, in addition to the Hotellings $T$ statistics, the SSVEP was also analysed using six, 2 (male, female genders) × 3 (neutral, pleasant, unpleasant categories) × 2 (left, right hemispheres) × 2 (frontal, posterior regions), mixed-between and within-subject repeated-measures ANOVAs (RMANOVAs) for early, middle and late time points separately. Separate tests were conducted for each time-period to complement the effects displayed in the topographic maps and provide some means of comparison between the two statistical tests (and topographic maps) conducted on the SSVEP. To reduce the number of within-subject dependent variables (i.e. electrodes) entered into these analyses, three standard electrodes within each quadrant were selected and averaged, yielding four SSVEP (amplitude and latency) values per quadrant (i.e. left frontal: Fp1, F7, F3; right frontal: Fp2, F8, F4; left posterior: O1, P3, T5; right posterior: O2, P4, T6). Although Hotellings $T$ statistics were conducted on complex numbers (combination of amplitude and phase) using in-house software, no in-house software is available at present for conducting repeated-measures statistics on such data. Therefore, RMANOVA statistics were performed on normalised amplitude and latency data (separately), which enabled the tests to be run using the Statistical Package for Social Sciences (SPSS) V.10 (SPSS Inc., 1999). In total, six RMANOVAs were conducted.

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Fig. 3. Amplitude (row 1) and latency (row 2) time series plots and Hotellings $T$ statistical cluster plots (row 3) for emotional valence in 15 males and 15 females. These maps display SSVEP activation across time ($x$-axis) for each of the 64 electrode positions ($y$-axis), in which warmer colours represent reduced amplitude and latency during presentation of emotional images, as well as larger $t$ values in the Hotellings maps.
Fig. 4. Topographic maps associated with pleasant and unpleasant valence (pleasant and unpleasant image categories (−) neutral category) in males and females are presented which display the 13-Hz SSVEP data (amplitude and latency) as well as Hotellings $T$ values which demonstrate the statistical significance for the difference data. Contours are plotted on the Hotellings $T$ maps at three levels of probability ($P = 0.01$, $P = 0.005$ and $P = 0.001$) uncorrected for multiple comparisons. Three time periods are presented which relate to early (0–2 s), middle (2–4 s) and late (4–6 s) components of image viewing. Warmer colours represent reductions in amplitude and latency during presentation of emotional images relative to neutral images and larger $t$ values in the Hotellings $T$ maps.
to examine effects of gender in amplitude and latency data at each of the three time periods. As these tests were run to examine effects of gender and to (partially) minimise the impact of experiment-wise type I error resulting from the running of multiple statistical tests, only gender-associated effects and their interactions are reported in this paper. An alpha level of 0.05 uncorrected for multiple comparisons was arbitrarily set for the repeated-measures ANOVAs conducted.

Results

Behavioural results

A series of independent-samples t tests were conducted on age, education and each of the POMS subscales separately to determine whether any differences exist between males and females. None of these variables were found to significantly differ between males and females (P > 0.05). Means (M), standard deviations (SD) and range are provided for age, years of education and all six POMS subscales for males and females (grouped as well as separated by gender) in Table 1.

Participants rated each image on valence and arousal scales. These ratings were averaged for each subject (25 images per category) and then averaged across subjects. Means (M), standard deviations (SD) and ranges for these ratings are provided below in Table 2 for the group averaged data, as well as males and females separately.

To determine whether gender modified the main effects for either valence or arousal, a between-groups repeated-measures ANOVA was conducted for both valence and arousal (separately). The tests revealed no significantvalence×Gender interaction [F(1,19,33.31) = 1.51, P = 0.20] (Greenhouse–Geisser adjusted), or arousal×Gender interaction [F(1,58,44.24) = 0.41, P = 0.62] (Greenhouse–Geisser adjusted), indicating that gender did not modify the significant main effect of valence [F(1,19,33.31) = 175.18, P < 0.001] (Greenhouse–Geisser adjusted) or arousal [F(1,58,44.24) = 30.52, P < 0.001] (Greenhouse–Geisser adjusted). For the valence rating scale, planned comparisons revealed differences between pleasant and neutral categories, [F(1,28) = 119.86, P < 0.001, partial eta squared = 0.81] as well as unpleasant and neutral categories [F(1,28) = 170.72, P < 0.001, partial eta squared = 0.71]. For the arousal rating scale, significant differences between pleasant and neutral categories [F(1,28) = 41.42, P < 0.001, partial eta squared = 0.60], as well as unpleasant and neutral categories [F(1,28) = 68.92, P < 0.001, partial eta squared = 0.71] were also revealed, but not for pleasant and unpleasant categories [F(1,28) = 4.08, P = 0.053].

SSPT results

On examination of the amplitude, latency and Hotellings plots in Fig. 1, distinct significant clusters of activation differ between pleasant and unpleasant valence. To better examine these activations in terms of spatial scalp location, the data presented in the time-series plots below were averaged into three 2-s time periods [specified as early (0–2 s), middle, (2–4 s) and late components (4–6 s)], and examined topographically (Fig. 2). These maps reflect the SSVEP that corresponds with the mean difference [emotional category (−C0)] of early, middle and late components (4–6 s), and examined topographically (Fig. 2). These maps map the SSVEP that corresponds with the mean difference [emotional category (−C0)] of early, middle and late components (each map containing an average of the twenty-six 13-Hz cycles) as demarcated in the time-series plots in Fig. 1. The statistically significant findings displayed in these topographic maps are summarised in Table 3 and discussed below.

Pleasing and unpleasant valence display similarities as well as differences across the 6-s epoch, suggesting that activation of both unique and common cortical areas occurs during the processing of pleasant and unpleasant stimuli (see Fig. 2 and Table 3). During both pleasant and unpleasant valence, reductions in amplitude and latency are observed within the left frontal region (latency only) and the occipital region. A number of differences are also observed and these indicate that pleasant valence unlike unpleasant valence is associated with increases in amplitude within left frontal and decreases in amplitude within right frontal regions; more persistent and widespread frontal latency decreases; and amplitude and latency changes within both left and right temporal,
Table 3

Summary of the statistically significant SSVEP findings (amplitude and latency) as indicated by the Hotellings $T$ ($P < 0.01$, uncorrected for multiple comparisons) for pleasant and unpleasant valence for 30 participants, where $Amp =$ amplitude and $Lat =$ latency.

<table>
<thead>
<tr>
<th>Valence</th>
<th>Region</th>
<th>Time periods</th>
<th>Early component (0–2 s)</th>
<th>Middle component (2–4 s)</th>
<th>Late component (4–6 s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleasant</td>
<td>left frontal region</td>
<td>$Amp \neq ?$</td>
<td>$Lat = ?$</td>
<td>$Lat = ?$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>right frontal region</td>
<td>$Amp \neq ?$</td>
<td>$Lat \neq ?$</td>
<td>$Lat = ?$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>$Amp \neq ?$</td>
<td>$Lat \neq ?$</td>
<td>$Lat = ?$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>right temporal, central and parietal regions</td>
<td>$Amp \neq ?$</td>
<td>$Lat \neq ?$</td>
<td>$Lat = ?$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>occipital region</td>
<td>$Amp \neq ?$</td>
<td>$Amp \neq ?$</td>
<td>$Amp \neq ?$</td>
<td></td>
</tr>
<tr>
<td>Unpleasant</td>
<td>left frontal region</td>
<td>$Lat \neq ?$</td>
<td>$Lat \neq ?$</td>
<td>$Lat \neq ?$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>right frontal region</td>
<td>$Amp \neq ?$</td>
<td>$Amp \neq ?$</td>
<td>$Amp \neq ?$</td>
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<tr>
<td></td>
<td>left temporal, central and parietal regions</td>
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<td>$Lat \neq ?$</td>
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<td></td>
<td>right temporal, central and parietal regions</td>
<td>$Amp \neq ?$</td>
<td>$Lat \neq ?$</td>
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<tr>
<td></td>
<td>occipital region</td>
<td>$Amp \neq ?$</td>
<td>$Amp \neq ?$</td>
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</table>

485 central and parietal regions. Finally, latency reductions during unpleasant valence occur during the early time period only, thus appearing as more of a transient phenomenon compared to pleasant valence in which latency reductions occur throughout all time periods.

488 To examine potential gender differences, amplitude and latency time series and statistical cluster plots are displayed in Fig. 3 for males and females for both pleasant and unpleasant valence. Inspection of the Hotellings $T$ plots, particularly for unpleasant valence, suggests that the number and size of statistically significant clusters of activation differ between males and females.

490 Again, to allow a better spatial scalp profile of these findings, early, middle and late epoch components are presented in the form of topographic maps (Fig. 4). Statistically significant results displayed in these maps are summarised in Table 4 and discussed below.

491 Male and females display both similarities as well as differences in the processing of emotional valence, suggesting that unique as well as common cortical areas are activated when males and females process emotional stimuli. During the processing of pleasant valence, both males and females display reductions in latency within the right frontal and left temporal regions. However, males display left frontal increases in amplitude and reductions in latency as well as reductions in occipital amplitude, whilst females display reductions in latency within left and right temporal, central and parietal regions and increases in occipital latency.

492 During the processing of unpleasant valence, both males and females display amplitude increases within the right temporal region during the middle epoch, although males display latency increases and females display latency decreases. A number of differences are also observed indicating that females display frontal SSVEP changes (predominantly latency reductions and distributed primarily in the right hemisphere), whilst males do not display frontal SSVEP changes. In addition, females display reductions in both amplitude and latency in left temporal, central and parietal regions, whilst males display occipital reductions in both amplitude and latency. In summary, the processing of pleasant valence was associated with latency reductions within right as well as left frontal regions in males, and latency reductions within right frontal regions (during the late time period only) in females. By contrast, the processing of unpleasant valence was associated with latency reductions within right frontal and temporal regions in females only.

493 A series of mixed-, between-(gender) and within-subject (category, hemisphere, region) repeated-measures ANOVAs were conducted for amplitude and latency data at early, middle and late time points to directly compare between males and females. These RMANOVAs revealed a significant Category $\times$ Hemisphere $\times$ Gender interaction ($F(2,56) = 3.172, P = 0.050$, partial eta squared = 0.102) for amplitude during the middle time period, a significant Category $\times$ Region $\times$ Gender interaction ($F(2,56) = 3.307, P = 0.044$, partial eta squared = 0.106) for latency during the middle time period and a significant Category $\times$ Hemisphere $\times$ Gender interaction ($F(2,56) = 3.899, P = 0.026$, partial eta squared = 0.122) for amplitude during the late time period. No other gender-associated effects or any overall between-subject effects (main effects of gender) were found to be significant.

494 The Category $\times$ Hemisphere $\times$ Gender interaction for amplitude during the middle time period indicates that Category modified a Hemisphere $\times$ Gender interaction. Tests of within-subject contrasts indicated that Category modified the Hemisphere $\times$ Gender interaction during presentation of unpleasant images relative to neutral images ($F(1,28) = 4.324, P = 0.047$, partial eta squared = 0.134), but not during pleasant images relative to neutral images ($F(1,28) = 0.402, P = 0.531$). To investigate this effect further, two Hemisphere $\times$ Gender RMANOVAs were conducted on neutral ($F(1,28) = 0.262, P = 0.613$) and unpleasant ($F(1,28) = 6.830, P = 0.014$, partial eta squared = 0.196) images separately. These findings indicate that amplitude within the right hemisphere (anterior and posterior) in females is greater than the left hemisphere whilst viewing unpleasant images. This effect however, was not present in the male sample.

495 The Category $\times$ Region $\times$ Gender interaction for latency during the middle time period indicates that Category modified a Region $\times$ Gender interaction. Tests of within-subject contrasts indicated that Category modified the Region $\times$ Gender interaction during presentation of unpleasant images relative to neutral images ($F(1,28) = 6.000, P = 0.021$, partial eta squared = 0.176), but not during pleasant images relative to neutral images ($F(1,28) = 0.073, P = 0.788$, partial eta squared = 0.003). To investigate this effect further, two Region $\times$ Gender RMANOVAs were conducted on neutral ($F(1,28) = 2.753, P = 0.108$) and unpleasant images ($F(1,28) = 3.144, P = 0.087$, partial eta squared = 0.101) separately. The results for the latter ANOVA indicate a trend for a Region $\times$ Gender interaction during viewing of unpleasant images. As the initial ANOVA was significant however, further examination of the associated SPSS profile plot (not displayed) was warranted. This plot indicated that females may...
Table 4
Summary of the statistically significant SSVEP findings (amplitude and latency) for 15 males and 15 females as indicated by the Hotellings T (P < 0.01, uncorrected for multiple comparisons) for both pleasant and unpleasant valence, where Amp = amplitude and Lat = latency

<table>
<thead>
<tr>
<th>Valence</th>
<th>Gender</th>
<th>Region</th>
<th>Time periods</th>
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<tbody>
<tr>
<td></td>
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<td>Early component (0–2 s)</td>
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<td>Late component (4–6 s)</td>
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<tr>
<td>Pleasant</td>
<td>Males</td>
<td>left frontal region</td>
<td>Amp ?; Lat ?</td>
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<tr>
<td></td>
<td></td>
<td>right frontal region</td>
<td>Lat ?</td>
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<tr>
<td></td>
<td></td>
<td>left temporal, central and parietal regions</td>
<td>Lat ?</td>
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<td>right temporal, central and parietal regions</td>
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<td></td>
<td>Females</td>
<td>occipital region</td>
<td>Amp ?</td>
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<tr>
<td>Unpleasant</td>
<td>Males</td>
<td>left frontal region</td>
<td>Amp ?; Lat ?</td>
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<td>right frontal region</td>
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<td>left temporal, central and parietal regions</td>
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Discussion

The current study investigated the spatiotemporal characteristics of the SSVEP associated with the processing of low arousal, unpleasant and pleasant images relative to neutral images in males and females using the SSPT technique. SSVEP results support our previous study (Kemp et al., 2002) which reports that processing of emotional valence (pleasant and unpleasant) is associated with frontal SSVEP latency and occipital amplitude reductions; however, substantial gender differences exist particularly within the frontal regions during the processing of unpleasant valence and these are discussed below. Key findings demonstrate (1) that electrophysiological differences between males and females exist despite there being no differences in subjective mood (POMS questionnaire) or behavioural ratings (valence and arousal dimensions), (2) that the processing of pleasant valence is associated with left and right frontal latency reductions in males, but not in females, (3) that the processing of unpleasant valence is associated with widespread frontal latency reductions (predominantly right sided and most apparent during the middle time period) in females, but not in males, and (4) that only females display increased amplitude within the right hemisphere relative to the left hemisphere during unpleasant images in the middle and late time periods.
The SSVEP amplitude and latency components have been interpreted previously as being analogous to the amplitude of regional cortical activity within the alpha frequency range and as reflecting neural information processing speed, respectively (Kemp et al., 2002; Silberstein et al., 1990, 1995, 1996, 2000). In this framework, reductions in SSVEP amplitude may be considered comparable to the transient reduction in alpha activity, known as event-related desynchronisation (see Pfurtscheller and Lopes da Silva, 1999, for review), whilst reductions in SSVEP latency may be considered as increased neural information processing speed or more generally as either increased excitation or reduced inhibition. Supporting this interpretation, increases in visual attention are associated with decreases in SSVEP amplitude at occipital and prefrontal sites (Silberstein et al., 1990, 1995) and variations in reaction time in a visual vigilance task correlates with frontal SSVEP latency changes (Silberstein et al., 1996, 2000).

The literature implicates anterior frontal locations more in emotional valence and emotional experience, whilst posterior regions are implicated more in perceptual and arousal components of emotion (Davidson, 1992, 1998; Davidson and Irwin, 1999; Heller, 1990, 1993). This background in terms of the neural structures underlying experiential and perceptual components of emotion is important for the interpretation of the gender differences presented in the results, particularly within frontal regions. Right frontal activation during unpleasant emotion (e.g. disgust) and left frontal activation during pleasant emotion (e.g. happiness) has been reported extensively in the EEG literature, and activation of these regions may be specialised for withdrawal and approach behaviours, respectively (for review, see Davidson, 1998). These EEG findings have more recently been supported by PET, fMRI and electrophysiological studies (Canli et al., 1998; Sutton et al., 1997; Aftanas et al., 2001a,b, respectively), though others have reported overlapping activation and no hemispheric asymmetries (Baker et al., 1997; George et al., 1995; Kemp et al., 2002; Lane et al., 1997a,b,c; Pardo et al., 1993; Teasdall et al., 1999).

Regardless, the prefrontal region is strongly implicated in emotional processing and the experience of emotion (Davidson and Irwin, 1999).

The processing of unpleasant valence is associated with widespread frontal latency reductions (predominantly right sided) in females but not in males. It is possible that males were less responsive to unpleasant stimuli, supporting previous studies which have reported greater activations particularly in frontal regions, in females relative to males. For example, Pardo et al. (1993) reported that whilst women displayed bilateral inferior and orbitofrontal activation in response to recalled sad mood, males displayed left-sided activation only. In addition, George et al. (1996) reported that whilst women displayed increased activity in the bilateral anterior cingulate, left medial prefrontal cortex, left insula and thalamus during the transient sadness, males activated only the left insula and right caudate, completely failing to activate the prefrontal cortex. Furthermore, women in this latter study displayed a greater number of significant changes from neutral to sadness tasks. It is important to note that SSPT is unable to acquire functional information from subcortical structures and it is possible therefore that the processing of unpleasant valence in males may be more subcortical than in females. This interpretation has been made previously in an fMRI study (Schneider et al., 2000) in which the authors speculated that men may have displayed increased amygdala involvement because they exerted greater effort to experience sadness.

These results are particularly interesting given that females are more susceptible to lowered mood as demonstrated in tryptophan depletion studies (e.g. Ellenbogen et al., 1996; Smith et al., 1997; Booji et al., 2002) and, more generally, that females are more likely to experience depression and anxiety (see Darlington, 2002, for review). Nishizawa et al. (1997) reported that serotonin synthesis in normal females was 52% lower than normal males, indicating that females may be less able to maintain adequate stores of the serotonin neurotransmitter, particularly under stressful situations. In addition, it has been argued from differing social and gender roles that females are more likely to experience feelings of sadness, hurt and disappointment, which is likely to lead to excessive rumination and clinical depression (Brody, 2001; Brody and Hall, 1993; Hankin and Abramson, 2001; Nolen-Hoeksema, 1991). Hankin and Abramson (2001) have posited a cognitive vulnerability-transactional stress theory to explain the "big fact" that more girls become depressed than boys after the age of 13 or "middle puberty" and that this difference persists throughout adulthood. On the basis of experimental data, these authors indicate that adolescent girls are more likely than boys to encounter negative life events, experience cognitive vulnerabilities to depression, personality traits such as neuroticism and environmental adversity such as sexual abuse. The model suggests that these experiences will lead to increased negative, anxious and depressive affect, which in turn generates more dependent negative life events, eventually leading to depression. Recent neuroimaging evidence has provided a direct relationship between depressed mood and regions of the frontal cortex following modulation of certain neurochemical systems. For example, PET suggests that both tryptophan and a-methylparatyrosine (AMPT)-induced return of depressive symptoms leads to decreases in metabolism within common brain circuitry including the orbitofrontal, dorsolateral and thalamus (Bremner et al., 1997, 2003). Interestingly, Davidson (2002) has suggested that greater relative right-sided prefrontal metabolism is associated with higher metabolic activity within the amygdala and that such activations have been associated with mood disorders such as anxiety and depression. It is possible therefore that the gender differences observed within right frontal and anterior temporal regions reflect decreased and increased responsiveness in males and females, respectively, to unpleasant images (relative to neutral) despite similar ratings on verbal report.

By contrast, the processing of pleasant valence is associated with left and right frontal latency reductions in males but only the right frontal region (during one time period) in females. The literature has indicated that positive affect is much harder to elicit in the laboratory (Davidson, 2002) and given that the present study presented images previously rated as low on the arousal dimension, it is possible that our sample of females did not engage the cortical areas involved in the experiential components of emotion to the same extent as males. It could be argued that females do not need the same degree of cortical activation to have a subjective feeling comparable to those of males. However, several recent studies suggest that this is not the case. For example, a previous study which investigated sex differences in hunger and satiety concluded that men may have a brain response producing greater hedonic effects from eating and more rewarding feelings associated with satiety (Del Parigi et al., 2002). Whilst not directly comparable, it does suggest that brain responses in males may be more sensitive to pleasant valence than females. Most recently, another study using fMRI to investigate gender differences in the viewing of IAPS images reported that men display a stronger brain activity...
for positive visual stimuli than women within the inferior and
medial frontal gyrus as well as the amygdala (Wrase et al., 2003).
If we consider pleasant and unpleasant valence to lie on a
continuum as previously hypothesised (Feldman-Barrett and Rus-
sell, 1999), it is possible that females are more orientated towards
the unpleasant pole on this continuum further supporting female
susceptibility to lowered mood. Although the Hotellings statistics
provide support for the conclusion that males activate frontal
regions more consistently than females during viewing of pleasant
images, no gender differences were evident in the RMANOVA
statistics for these images. Instead, the gender differences (as
reported in the RMANOVA) in both amplitude and latency were
specific to unpleasant emotional stimuli, suggesting perhaps that
differences in males and females may relate more to unpleasant
than pleasant stimuli. Gender differences therefore, relating to the
neurobiological mechanisms underlying the processing of pleasant
images and pleasant emotion more generally, will need verification
in future studies.

A number of similarities between males and females were also
observed in the processing of emotional valence. This is consistent
with previous reports that there are both similarities and differences
between males and females in resting state (e.g. Gur et al., 1995) as
well as in the processing of emotional stimuli (e.g. Del Parigi et al.,
2002; Pendergrass et al., 2003). During pleasant valence, both
males and females display activations (reduced latency) within
right frontal and left temporal regions. In addition, the left frontal
region in females was close to, but did not reach significance
within the late component of image viewing. Left temporal
activation during the processing of pleasant valence may relate
to findings reported in a previous study in which a more posterior
distribution of activity, in the region of the pre- and post-central
gyri, was associated with an extended-picture presentation to evoke
positive mood (Sutton et al., 1997). However, in the current study,
females did not engage the frontal structures to the same extent as
males during pleasant valence, which may reflect a lower respon-
siveness of neural structures involved in the experiential compo-
nents of emotion in our female subjects. Finally, during unpleasant
valence, both males and females display reduced activation (am-
plitude increases) within right temporal regions.

As discussed above, SSVEP amplitude may be interpreted in
exactly the same way as the amplitude of regional cortical activity
within the alpha frequency range. In this framework, an amplitude
enhancement has been interpreted as a deactivated state in which
the brain region is neither receiving nor processing sensory
information and that this may be important for the introduction
of inhibitory effects (Pluutscheller and Lopes da Silva, 1999).
Consistent with this model, males also display latency increases
(increase in inhibitory processes) within this region, possibly
reflecting a compensatory response to override expressions of
emotion generated by limbic–subcortical structures (as discussed
in Liotti et al., 2000). Unlike males however, the processing of
unpleasant valence in females is associated with widespread
reductions in latency (increases in excitatory processes) which
may reflect increased responsiveness to unpleasant (relative to
neutral) images and possibly an inability to successfully suppress
activation associated with the presentation of unpleasant stimuli.
This interpretation is consistent with the purported role of the right
hemisphere in inhibitory control (Garavan et al., 1999) and its
dense interconnectivity with the paralimbic core (Tucker, 2001).

The finding that only females displayed a more general increase
in amplitude during viewing of unpleasant images within the right
hemisphere deserves some comment as it could be argued that this
finding contradicts traditional theorised patterns in the processing
of emotion. For example, one of the oldest theories of emotions in
the brain is the key role of the right hemisphere in the processing
of emotion (e.g. Levine and Levy, 1986; Ross and Mesulam, 1979;
Sackeim et al., 1978). In terms of brain activation and from the
‘arousal’ model of alpha amplitude, in which reductions in alpha
amplitude are thought to reflect increases in activation (e.g.
Lindsey and Wicke, 1974; Ray and Cole, 1985), it could be argued
that decreases in amplitude within the right hemisphere
should be displayed rather than the increases found in the current
study. However, given that participants were requested to focus on
emotional content and refrain from emotional inhibition, it is
possible that as part of this process, females primed particular
emotional circuits through imagining and remembering similar
e emotional events. Amplitude findings could therefore be consid-
ered to be consistent with previous reports of an increase in alpha
activity associated with mental imagery (Ray and Cole, 1985; Tesche et al., 1995) and also the finding that this alpha increase is
specific to the right hemisphere (Ray and Cole, 1985).

Finally, some limitations of the study are worth noting. Firstly,
participant’s ratings of arousal indicate that pleasant and unpleasant
images significantly differed from neutral images, thereby making
the interpretation that difference maps (emotional versus neutral
images) reflect only ‘valence’ difficult. It is important to mention
however, that pleasant and unpleasant images did not significantly
differ from each other and also that this finding may reflect the
more general difficulty of selecting positively or negatively
valenced images which are equivalent to neutral images on the
arousal dimension. Moreover, the current study did not select
images containing high arousal content such as ‘violent death’ and
‘eroticia’, which would have otherwise confounded emotional
arousal with emotional valence. Secondly, the calculation of
RMANOVA statistics required data reduction procedures, which
limited the potential conclusions able to be drawn from the results
of these tests. Although these statistics confirmed differences
between males and females with respects to the processing of
unpleasant images, the fact that no statistical differences were
displayed for the pleasant stimuli does not rule out that differences
for such stimuli may exist (as reported recently by Wrase et al.,
2003). Thirdly, it is possible that a range of variables including
psychological, cognitive and social variables, could in part, ac-
count for the observed effects. Authors have argued for example,
that women may employ more cognitive strategies and internal
cues, whilst men may focus on the external stimulus to generate
emotion (e.g. Schneider et al., 2000). Future studies should
examine these issues in more detail when investigating gender
differences in emotional processing.

In summary, electrophysiological differences in the processing
of pleasant and unpleasant valence between males and females
were observed despite there being no differences in subjective
mood or ratings of pleasant, neutral or unpleasant images. These
results suggest that gender differences do exist in the processing
of visual emotional stimuli, and illustrate the importance of taking
these differences into account during investigations of emotional
processing. The main gender difference reported in the current
study relates to the processing of unpleasant valence which is
associated with widespread frontal latency reductions (predomi-
nantly right sided) in females but not in males. This finding is
consistent with the interpretation that females rather than males are
more susceptible to negative life experiences and lowered mood,
and may have implications for the pathophysiology of mood disorders such as depression.

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