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Event-Related Potential Evidence of Consciousness
during Wakefulness, Sleep Onset, and Sleep

by

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A thesis submitted to
the School of Graduate Studies and Research
in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

School of Psychology, University of Ottawa

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Abstract

Event-related potentials (ERPs) were recorded during sleep onset and various stages of sleep. A late component of the ERP, P300, is elicited when subjects detect a rare "target" stimulus. It is usually not elicited when subjects fail to detect the stimulus. The presence of P300 has therefore been used to index the extent to which the sleeper is aware of their external environment. During the transition to sleep, subjects were asked to detect a rare 2000 Hz target occurring among a train of 1000 Hz standards. A parietal maximum P300 was apparent in wakefulness, and remained large to detected targets in stage 1 sleep. It was however attenuated at frontal sites in stage 1. There were few detections in stage 2 and P300 was not evident.

ERPs were then recorded within sleep. Very loud stimuli were employed since loud deviants will elicit an obligatory P300 response in waking-ignore conditions. In Experiment 2, 90 dB SPL tone pips were delivered on 5% of trials and 70 dB SPL tones on remaining trials. A large parieto-central positive wave was recorded in REM sleep. In non-REM sleep, a later and more occipital positivity was observed. It remained unclear whether the REM P300 was due to the rareness or the loudness of the deviant. In Experiment 3, various intensities (0, 60, 80, 100 dB SPL) were therefore delivered at equal probability ($p=.25$). A parietal maximum P300 was again recorded in REM following the 100 dB tone, but was not apparent following the lower intensities. A frontal P300 was not apparent following the loud stimulus.

In Experiment 4, pitch- and intensity-deviants were investigated during sleep and wakefulness (attend and ignore conditions). In three separate groups, the rare stimulus was delivered on either 20%, 10% or 5% of trials. The pitch-deviant did not elicit P300 in any

condition. In the waking-ignore condition, the intensity-deviant elicited a parietal maximum P300 that extended into the frontal region. A large REM-specific P300 was apparent at parietal sites following the intensity-deviant when stimuli were delivered on 5% of trials, but was not apparent at frontal sites.

These studies illustrate that P300 can be recorded during sleep onset and during REM sleep. Only stimuli which are sufficiently intrusive and rare will elicit the parietal P300 in REM. While subjects may be able to detect stimulus deviance in stage 1 and REM, the frontal contribution to consciousness may be absent.

Acknowledgements

This section of the Ph.D. dissertation seemed an ideal way to explain how I came to be interested in the field of sleep research. In the summer of 1989, I had just graduated from high school and had accepted an offer of admission to the University of Windsor, when I sat down to read the local newspaper. I very rarely took the time to read the newspaper, but that morning a particular article persuaded my attention. The topic of the article was "Lucid Dreaming". In this article, Dr. Robert Ogilvie, president of the Canadian Sleep Society (at that time), was cited. I noted that he was a professor at Brock University in St. Catharines, Ontario. So, I decided to write him a letter to inquire how I might learn more about sleep and dreams. I sent the letter without a postal code, but assumed that Brock was a big enough place that the post office would find it. Looking back, I find it humorous that I had the chutzpah to write this letter -- it was certainly out of character for me (at that time) to make such an active pursuit. Fortunately for me, Bob Ogilvie was the kind of scientist and teacher who wrote back an enthusiastic reply, in which he suggested that I consider transferring to Brock University to study sleep at the undergraduate level. So, I did -- and everything since then falls from that decision. My continued interest in sleep research began with three simple (but not so small) things: 1. the serendipitous luck of reading that newspaper article in the London, Ontario Free Press; 2. my gumption to write that letter to Bob Ogilvie; and 3. Bob's reply.

Thus, it is with tremendous gratitude that I dedicate this doctoral thesis to my first mentor, Dr. Robert Ogilvie. His kindness and scientific integrity will continue to be an inspiration to me every day.

Dr. Kenneth Campbell adopted the role of mentor during my doctoral studies. At the 2nd World Federation of Sleep Research Societies meeting in The Bahamas, just prior to beginning my PhD studies, Dr. Alistair MacLean told me that he thought Ken Campbell was "well-suited to my personality". This was most certainly the truth! Ken was an ideal mentor for me because he was even-tempered and down-to-earth. He allowed me the freedom to express my ideas, and run as far as my imagination would take me -- all the while steering me as needed. During my studies with Ken, I learned not only about psychophysiological methods, but also a great deal about science. I feel better prepared to face the challenges of an academic career having had the fortunate opportunity to have studied with Ken Campbell. Thank you Ken!

My thesis committee was a truly remarkable group of scientists! The most enjoyable aspect of this committee (and also most terrifying at the same time) was that each of them brought a unique perspective to the same research questions and methodological dilemmas. It was an incredible learning experience. The thesis committee members were: Dr. Roger Broughton, Dr. Joseph De Koninck, Dr. Terrence Pivik, and Dr. Robert Stelmack. Thank you to each of you for your time, commitment, and contributions to this scientific pursuit.

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I have been privileged to meet many friends and colleagues over the years at various scientific meetings. We have had the unique fortune of growing up together in this relatively new discipline, which promises exciting discoveries that will improve the quality of human life and offer insight into understanding the human brain -- the last scientific frontier!

I would sincerely like to thank my friends and family for sustaining this adventure with me. It's been a long road since I read that silly newspaper article back in 1989. Thank you to my best friend Sheila who always forced me to maintain a social life. Thank you to my dog Lightning for walking me everyday. Thank you to Kevin who made the last year of graduate school my best. Thank you to my Dad for the example he set to me in reaching the pinnacle of his own career. His actions taught me that there is absolutely nothing that can't be achieved, if you just believe you can do it, then make it happen. Thank you to my Mom...for everything. I would have accomplished nothing without her positive support, her friendship and her love.

Finally, thank you to all the scientists whose works inspired and provoked me. I only hope I can do the same some day...

Curriculum Studiorum

Kimberly Cote was born in Sarnia, Ontario, Canada on the 19th day of June, 1971. She pursued a Bachelor of Arts (Honors) degree at Brock University and graduated with first class honors in Psychology in 1993. She then completed her Master of Science degree at the University of Toronto, Institute of Medical Science, graduating with distinction in 1995. Her graduate studies were supported by awards from the Natural Science and Engineering Research Council (NSERC) of Canada, and from the National Institute of Mental Health (NIMH) of the United States of America. During her academic training, she published a series of journal articles and abstracts, and presented papers at various scientific meetings.

Awards

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Professional Honours

1992	Canadian Sleep Society (CSS)	Student Abstract Prize
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1998	European Sleep Research Society (ESRS) / University of Ottawa	Madrid, Spain
1998	Sleep Research Society (SRS)	New Orleans, Louisiana
1997	Sleep Research Society (SRS)	San Francisco, California
1997	UCLA Multi-site Training Program For Basic Sleep Research	Lake Arrowhead, California
1996	Sleep Research Society (SRS)	Washington, DC
1995	World Federation of Sleep Research Societies (WFSRS)	Nassau, The Bahamas
1994	Sleep Research Society (SRS)	Boston, Massachusetts
1993	Sleep Research Society (SRS)	Los Angeles, California
1992	Sleep Research Society (SRS)	Phoenix, Arizona

Publications

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Cote, K.A., de Lugt, D.R., Langley, S. and Campbell, K.B. Scalp topography of the auditory evoked K-complex in stage 2 and slow wave sleep. Journal of Sleep Research, in press.

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Organizational Note

This dissertation consists of six chapters. Chapter 1 is an introduction to and review of the relevant literature and presents the rationale for the series of experiments which follow. Chapters two through five represent the four experiments conducted for the doctoral dissertation. They are presented in journal article format and as such the style conforms to the publication requirements of the journal to which it has been (or will be) sent. Each chapter contains a separate Introduction, Methods, Results, and Discussion section. A page precedes each chapter which describes the rationale of the experiment and summarizes the results. A complete reference list is provided at the end of the thesis. Chapter four is followed by a separate reference list. This list is provided because the references in text are identified using a number system, rather than by author's name and year of publication. The sixth and final chapter provides a general discussion which integrates the four experiments, summarizes findings, and discusses their implications. Appendices A and B represent articles published during the doctoral program which are outside the focus of the thesis. Both of these papers are "in press" in the *Journal of Sleep Research*.

Chapters 2 through 5 are manuscripts prepared for publication. K. Cote is senior author on all publications. She was responsible for the collection, scoring and analysis of all data. The design and write-up of the various experiments was discussed with the thesis supervisor and committee. Experiment 1 was carried out in collaboration with D. deLugt, a graduate student in the laboratory. Experiment 2 has been published in *Clinical Neurophysiology*, 1999, 110, 1345-1350. Experiment 3 has been published in the journal *NeuroReport*, 1999, 10, 2313-2318.

Chapter 1

Introduction

The nature of sleep has been pondered since the early philosophers. In a review of the "History of Sleep and Man", Thorpy outlines four major views on sleep which were prevalent throughout the ages: vascular, chemical, neural and behavioural theories (Thorpy, 1991).

Vascular theories of sleep were proposed in 5th Century B.C. by Alcamaeon who believed that sleep resulted from blood flowing to the brain, and by Hippocrates who held the opposite tenet that sleep occurred as a result of blood flowing toward the center of the body. These vascular theories prevailed into the early 19th Century when sleep was thought to result from either congestion (i.e., pressure on the brain) or anemia (i.e., insufficient blood flow to the brain).

Chemical theories of sleep were first put forth by Aristotle, who maintained that ingestion of food caused a build up of "fumes" in the blood vessels resulting in the need for sleep. The notion that sleep resulted from lack of oxygen or a build up of toxic substance continued until the end of the 19th Century. Neural theories of sleep emerged in the mid-19th Century along with an enhanced understanding of the central nervous system. Santiago Ramon y Cajal proposed that sleep could be explained by "neuroglia", small cells which were responsible for the inhibition and facilitation of communication between cells. Finally, behavioural theories of sleep were promoted by the experiments of Heubel, who proposed that sleep was the result of loss of sensory stimulation to the peripheral nervous system. In the 20th Century, behavioural theories were supported by the conditioning experiments of Pavlov who held that sleep resulted from

widespread inhibition that protected the sleeper from overstimulation. The basis of these behavioural theories has carried forth into modern definitions of sleep.

Although most people could readily give a description of what it is to be asleep, a comprehensive definition which includes all facets of the complex behaviour is not so simple. Recently, two encyclopedic volumes have provided extensive listings of definitions and concepts related to sleep (Thorpy & Yager, 1991; Carskadon, 1995). These works are testimony to the complexity of defining sleep. The *Encyclopedia of Sleep and Dreaming* contains over 400 definitions written by experts in the field. Paradoxically, there is no single definition for the term "sleep" itself (Carskadon, 1995). In the *Encyclopedia of Sleep and Sleep Disorders*, the definition for sleep reads, "A leading scholarly journal published bimonthly by..." (Thorpy & Yager, 1991). Although sleep is difficult to define, it has often been described on the basis of observable behaviours in the sleeper. These behaviours include: reduced movement, diminished response to external stimuli, a stereotypical sleep posture, and the ability to awaken (i.e., reversibility) (Anch, Browman, Mitler, & Walsh, 1988; Brain Information Services, 1997). Anch et al. described sleep as:

...a recurring state of existence characterized by 1. reductions in awareness of and interaction with the environment, 2. lowered motility and muscular activity, and 3. partial or complete abeyance of voluntary behavior and consciousness (p.2).

Similarly, Carskadon and Dement (1994) begin an overview of normal human sleep by stating:

According to simple behavioral definition, sleep is a reversible behavioral state of perceptual disengagement from and unresponsiveness to the environment (p. 16).

In the *Basics of Sleep Behavior* syllabus (Brain Information Services, 1997), again sleep is largely defined according to the extent to which information is processed:

The fundamental essence of sleep seems to be disengagement from the outer world, and to some extent an engagement with the inner world, the dream world. This disengagement from the real world is an active process in which sensory input is blocked or modified to a level that results in perceptual blindness and deafness (From: Introduction, p. 3 of 5).

A common feature of the aforementioned definitions of sleep is reference to suppression of processing stimuli from the external environment, suggesting that there is little or no consciousness during sleep. This belief perhaps stems from reflection upon subjective experience, in which memory for external events during sleep is quite impaired upon subsequent waking recall. Broughton proposed that this "retrograde amnesia" upon awakening prohibits accurate recall of internal mental events (e.g., dreams) (Broughton, 1973). As this dissertation will demonstrate, when appropriate methods are employed which allow the experimenter to access the mental state of the sleeper, a very different picture of awareness during sleep emerges.

Measurement of Sleep

Historically, sleep was measured on the basis of behavioural observations, which were used to infer sleep quantity and quality. Kohlschütter, a student of Gustav Fechner, published the first report on measuring sleep depth in 1862 (see Swan, 1929 for review). Kohlschütter used a sound pendulum, which elicited sounds of increasing intensity, to probe the depth of sleep across the

night. The auditory arousal threshold (AAT) was defined by the intensity at which an awakening ensued. Kohlschütter reported that AATs increased after sleep onset then decreased across the night (i.e., easier to awaken the sleeper in the later portion of the night). Some of the earliest experimental investigations of sleep were conducted by Kleitman in the 1920's at the University of Chicago. Kleitman investigated behavioural correlates of sleep such as body movement, eye movement, and respiration (Kleitman, 1963). He believed that there was a relationship between the slow eye movements at sleep onset and depth of sleep.

The electrical recording of human brain wave activity (Berger, 1929) and its application to the study of sleep, (Loomis, Harvey, & Hobart, 1935a; Loomis, Harvey, & Hobart, 1935b; Loomis, Harvey, & Hobart, 1936; Davis, Davis, Loomis, Harvey & Hobart, 1937; Loomis, Harvey, & Hobart, 1937; Davis, Davis, Loomis, Harvey, & Hobart, 1938; Davis, Davis, Loomis, Harvey, & Hobart, 1939; Loomis, Harvey, & Hobart, 1939), greatly advanced the field of sleep research and sleep disorders medicine. In 1953, Aserinsky and Kleitman's discovery of rapid eye movements during sleep (thus labelled REM sleep stage) further stimulated interest in the science of sleep (1953, 1955). The other stages of sleep, stages 1 through 4, became collectively referred to as "non-REM" sleep.

Rechtschaffen and Kales edited a standardized manual for sleep scoring methods which remains the primary guide for stage classification today (1968). Polysomnography (PSG), the physiological recording of sleep, is performed by assessing changes in brain wave activity (electroencephalography, EEG), eye movements (electro-oculography, EOG), and muscle activity (electromyography, EMG). A number of other physiological signals may also be recorded, such as respiration, heart rate, and limb movement. Stage 1 is characterized by reduction in the relative

amount of alpha frequencies (8-12 Hz) and the appearance of slower theta waves (4-7 Hz) compared to wakefulness. The vertex sharp wave, a sharp negative deflection in the EEG maximum at midline central recording sites, is a typical EEG feature of stage 1. This light stage of sleep is usually accompanied by slow rolling eye movements which distinguish it from wakefulness and REM sleep. The duration of stage 1 is quite variable, lasting up to about 15 minutes at the beginning of the night, and reappearing briefly following arousals throughout the night. Some investigators have further subdivided Stage 1 (e.g. "1a" and "1b") in order to more precisely describe the moment-to-moment changes in electrophysiology during the sleep onset period (Valley & Broughton, 1983; Harsh, Voss, Hull, Schrepfer, & Badia, 1994). Hori has described nine distinct categories of the sleep onset period, six of which characterize stage 1 (Hori, Hayashi, & Morikawa, 1994).

Many researchers consider stage 1 to be a transition period between the waking and the sleeping states, rather than a stage of sleep per se (Johnson, Hanson, & Bickford, 1976; Ogilvie, Simons, Kuderian, MacDonald, & Rustenburg, 1991; Cote & Ogilvie, 1994). Johnson et al. (1976) initially proposed that sleep was more closely tied to stage 2 sleep and the appearance of phasic events – K-Complexes and spindles. They observed that subjects were able to detect external signals within stage 1 sleep. In stage 2, subjects were no longer able to respond to these stimuli, and K-Complexes and spindles occupied the EEG tracing. In this definition, sleep occurs when subjects are no longer conscious of the external environment. These phasic events are considered the hallmark of stage 2 non-REM sleep (Rechtschaffen & Kales, 1968). They occur in the background of low amplitude and mixed frequency EEG. Eye movements and muscle activity contribute little to the identification of stage 2 sleep. Loomis et al. (1939) first described K-

Complex activity that was evoked to external stimulation during sleep. That same year, researchers reported that K-Complexes also occurred spontaneously in the absence of any apparent stimulus event (Davis et al., 1939). The types of stimuli that were most likely to elicit the K-Complex were initially described by Roth and colleagues (Roth, Shaw, & Green, 1956). The K-Complex consists of a series of negative and positive waves. The most prominent of these waves is a large amplitude negative deflection peaking at about 550 ms after the stimulus (hence the label "N550"). The functional significance of the K-Complex remains a controversy (Wauquier, Aloe, & Declerck, 1995). Some researchers claim that it serves to protect the sleeper from awakenings (i.e., inhibitory function); while others maintain the opposite belief, that it represents an arousal response (for review, see Campbell, Bell, & Bastien, 1992). The generators of the K-Complex are also subject to much controversy. Steriade and colleagues have identified a slow cortical oscillation in cats and in humans that they associate with the K-Complex (Amzica & Steriade, 1997). The extent to which this slow wave is similar to the human K-Complex remains unknown. In stage 2 sleep, the human K-Complex is maximum over symmetrical fronto-central areas of the scalp. The scalp topography of the N550 wave is independent of the modality of stimulus presentation (Colrain, Webster, Hirst, & Campbell, in press - a). Cote, de Lugt, Langley, and Campbell (in press - a) recently reported that the scalp topography of the N550 varies between stage 2 and Slow-Wave Sleep (SWS). The N550 has a more fronto-central focus in stage 2 and is more widespread in SWS (See Appendix A for details). The scalp generators of the evoked K-Complex must therefore be different between stage 2 and SWS.

Spindle oscillations were also first described by Loomis et al. (1935a, 1935b) as rhythmic 12-14 Hz periodic events lasting from 1 to 1.5 seconds. Spindle activity is thought to be

generated in the thalamus as a result of a network of synaptic interactions involving inhibitory neurons of the reticular thalamic nucleus, thalamocortical cells and cortical pyramidal neurons (Steriade & Llinás, 1988; Steriade, McCormick, & Sejnowski, 1993). The role of the spindle is much better understood. It appears to gate transmission through the thalamus, thereby allowing sleep maintenance through inhibition of sensory processing. Thus, the spindle serves to prevent consciousness of the external environment. Elton and colleagues provided event-related potential evidence in humans to suggest that spindles inhibit processing of relatively moderate intensity stimuli during sleep (Elton, Winter, Heslenfeld, Loewy, Campbell, & Kok, 1997). Cote, Epps, and Campbell (in press - b) subsequently provided ERP evidence that this gating in the presence of spindle activity was effective for even louder and more intrusive stimuli (See Appendix B for details).

Stages 3 and 4 of sleep are collectively referred to as Slow-Wave Sleep (SWS) because of the predominance of large amplitude delta (0.25-3 Hz, $> 75 \mu V$) waves in the EEG. According to Rechtschaffen and Kales (1968) scoring criteria, if 20 to 50 percent of the epoch consists of delta activity, then it is scored as stage 3 sleep, whereas if greater than 50 percent of the epoch consists of delta activity, it is scored as stage 4 sleep. However, it may be reduced when scoring the sleep of the elderly to compensate for changes in the architecture of sleep that are related to aging (Webb & Dreblow, 1982). SWS is often also referred to as "deep" sleep because arousal thresholds are lower than in any other stage of sleep (Rechtschaffen, Hauri, & Zeitlin, 1966). Whereas stage 2 non-REM sleep may constitute about 50-60% of the night, there is substantially less SWS and it occurs mostly in the first half of the night. There may however be an additional episode of SWS later in the sleep period, particularly in circumstances of sleep extended beyond

12 hours (Gagnon & De Koninck, 1984).

There have been numerous functions of sleep proposed. In general, it is thought that SWS may have a restorative function. A restorative theory of sleep is an intuitive one which holds that sleep is a period of recovery to allow restoration of both body and brain functions (Druker-Colin, 1995). Support for this comes from studies of sleep deprivation. Extended sleep loss will result in a "rebound" effect during recovery sleep, where the sleeper will sleep longer and spend more time in deep sleep (Bonnet, 1994). In humans, attempts to delay sleep are marked by increased sleep pressure (Carskadon & Dement, 1981) and cognitive deficits (see Koslowsky & Babkoff, 1992 for review). Selective disruption of SWS using a noise stimulus will result in symptoms of pain and fatigue (Moldofsky & Scarisbrick, 1976).

Based on physiologic criteria, Carskadon and Dement (1994) summarize non-REM sleep as "a relatively inactive yet actively regulating brain in a moveable body", and describe REM sleep as "a highly activated brain in a paralyzed body". The transition from non-REM to REM sleep is marked by reduction in EMG activity and by the presence of binocularly synchronous rapid eye movements. REM sleep appears approximately every 90 minutes throughout the night. The sleeper can thus be expected to experience three to five REM episodes per night. The first REM period is typically very brief, lasting less than 10 minutes, while the final episode may continue for more than an hour. The electrophysiologic characteristics of REM sleep are more similar to wakefulness and stage 1 than non-REM stages of sleep (Kahn, Pace-Schott, & Hobson, 1997). During REM, wakefulness, and stage 1, the EEG consists of high-frequency and low-amplitude activity. Despite this similarity, a unique feature of REM is the presence of muscle atonia. REM sleep is alternatively referred to as "paradoxical sleep" because although the EEG

appears like wakefulness, it is extremely difficult to awaken the sleeper.

Early investigators proposed that the eye movements in REM sleep were associated with dreaming (Aserinsky & Kleitman, 1953; 1955). Dement and Kleitman (1957a; 1957b) speculated that the eye movements were directly related to the dream content (i.e., eyes following direction of activity in the dream), however, this was not found to be the case. Dement and Kleitman (1957a; 1957b) also reported that the most vivid dream reports were obtained after awakenings from REM compared to non-REM sleep. Dreams will however be reliably reported upon awakening from all stages of sleep (Foulkes, 1962). Although dreams are not exclusive to REM sleep, researchers have substantiated that they are indeed more vivid and bizarre in REM sleep (Porte & Hobson, 1986). This enhanced mental activity is consistent with the highly active state of brain wave activity in REM sleep.

REM sleep may be described in terms of phasic or tonic events. Tonic REM is characterized by continuous, stable activity throughout the REM period, such as EMG atonia or low-voltage EEG. Phasic REM is represented by occasional bursts of activity, such as eye movements, middle ear muscle activity (MEMA), EMG twitches, or ponto-geniculo-occipital (PGO) spikes. MEMAs may be evoked by loud auditory stimuli (e.g., greater than 85 dB) or may occur spontaneously in REM sleep (Pessah & Roffwarg, 1972). MEMA and other phasic events such as eye movement are thought to be controlled by another phasic event, PGO waves. PGO waves originate from the pons of the brainstem, ascending through the lateral geniculate nucleus of the thalamus to the occipital cortex (McCarley, Nelson, & Hobson, 1978). Since PGO waves are recorded from electrodes implanted within the brain, they have only been recorded from non-human subjects. They are however assumed to be characteristic of all mammalian REM sleep. In

addition to occurring spontaneously in REM sleep, PGO activity may be evoked by auditory stimuli. The amplitude of PGO activity increases with increasing stimulus intensity (Ball, Hunt, Sanford, Ross, & Morrison, 1991). The functional role of PGO spikes is not known. Researchers have speculated that the role of PGO activity may be related to memory consolidation, facilitation of learning, and/or perception during sleep, perhaps of dreaming (Siegel, 1994).

In examining the many possible functions of sleep, particular attention has been paid to investigating the role of REM sleep. It has been proposed that REM sleep plays a role in memory and learning. It has been demonstrated, for example, that the amount of REM sleep will increase during periods of intensive learning (De Koninck, Lorrain, Christ, Proulx, & Coulombe, 1989). Crick proposed a controversial hypothesis that REM sleep and dreaming served to clear the memory of irrelevant events from the preceding day (Crick & Mitchison, 1983). Other researchers have proposed that REM sleep plays a role in brain development. This is because REM sleep is most prominent in the early years of life and decreases in amount with advancing age in all mammalian species (Roffwarg, Muzio, & Dement, 1966). Of course the prominence of REM sleep in the young may be due to the amount they learn on a daily basis. Other roles of REM sleep have however been proposed. Of particular interest to this thesis, the sentinel theory maintains that REM exists to periodically prepare the brain for arousal in the event that the sleeper should have to respond to threatening stimuli (e.g., predators) in the environment (Snyder, 1966). A sentinel hypothesis would be supported by evidence indicating that the sleeper may become conscious of external stimuli during the night. The extent to which the sleeper processes external stimuli during the night is examined in the following sections of the thesis.

Cognition and Consciousness during Sleep

The study of consciousness has seen a major revival over the past decade. For example, the journal *Consciousness and Cognition* was inaugurated in 1992. It is still however highly controversial. One of the most serious problems with the study of consciousness is definitional. How can "consciousness" be defined? How many types of consciousness exist? Many argue that certain aspects of consciousness, such as knowledge of oneself (i.e., self-consciousness) and understanding the subjectivity of experience are beyond the realm of science. While all of us may be able to detect and be aware of a red rose, it is perhaps indeterminable whether or not we "experience" it in the same way. Most theorists do agree that awareness of the external environment and internal mental state form an essential part of any definition of consciousness. Nevertheless, philosophers have pointed out that detection and awareness is not sufficient. After all, a computer can detect the external world and signal to the user that such detections have been made. Few would argue that computers are conscious. This type of consciousness has been described as automaton awareness. Others use the analogy of voodoo zombie. In this case, an individual is administered a drug which puts them into a catatonic state. They are assumed to be dead and are thus buried. The voodoo priest(ess) then retrieves the body and steals their soul. The body then reawakens from its catatonic state. The "zombie" shows an unusual form of consciousness -- it is conscious enough to be aware of where it is going and to recognize former friends and family (much to their astonishment, believing the individual has returned from the dead). The zombie does not however show any emotional experience. It has lost its "soul". A type of zombie consciousness has been demonstrated to exist in patients who have sustained damage to their frontal lobes (Knight, 1984).

One method to investigate consciousness is to examine differences between conscious and unconscious states. In this thesis, emphasis will be placed on the waking, conscious, and the sleeping, unconscious state. The study of differences between sleep and wakefulness, as well as variations within sleep, is referred to as "state-dependent" aspects of consciousness (Kahn et al., 1997), and is considered to be within the domain of cognitive neuroscience. In the waking state, a method commonly used to study consciousness involves the manipulation of a subject's selective attention. The purpose of selective attention is to permit the observer to be conscious of that which is relevant, and at the same time to prevent that which is irrelevant from attaining consciousness. If one attempts to attend to too many sensory "channels" at the same time, the result is information overload and confusion. This is because the brain is assumed to have a limited capacity to process information. It must select and choose among the many inputs -- those that will be processed and those that will not. In a selective attention task, subjects are asked to attend to and signal their awareness of the relevant event and to ignore events that are irrelevant (Näätänen, 1990). The subjective reports of their overt response to the relevant stimulus is used as empirical proof of their consciousness.

For many cognitive psychologists the interest is in the fate of the unattended, irrelevant channel. To what extent are the irrelevant, to-be-ignored stimuli processed? To what extent is processing inhibited? These questions are much more difficult to answer. For logical positivist philosophers of science, it may be an inappropriate question because it involves testing a null hypothesis. What evidence is the scientist willing to accept that one is *not* processing information? How can the scientist prove a subject is unconscious? The fact that the subject fails to respond to the unattended channel does not necessarily indicate that the stimulus was not

processed. After all, instructions to the subject are usually to withhold responding to the irrelevant stimulus. Many psychologists have also noted that the irrelevant, unattended channel must be processed to some extent in the nervous system to allow for the stimulus to be classified as relevant or irrelevant (Näätänen, 1990). Once the decision has been made that a stimulus channel is irrelevant, the nervous system might then inhibit further processing. In the waking state, the selective attentional system appears to function in a relative, rather than an absolute manner (Alho, Paavilainen, Reinikainen, Sams, & Näätänen, 1986). Attended, relevant channels are processed comparatively more than unattended, irrelevant channels. But, irrelevant channels do receive a considerable amount of processing. During sleep, it is thought that the extent of information processing is much more limited. The function of sleep is obviously not to inhibit information processing or to inhibit consciousness of the external environment. Nevertheless, it does appear that in order for sleep to occur, information processing must be almost completely inhibited or "gated". Perhaps only the most relevant stimuli are processed to the extent that they will awaken the sleeper. Note however that the sleeper need not become conscious of the stimulus in order for it to awaken them. Indeed most subjects who are awoken by an external stimulus generally appear to be initially in a confused state.

If the study of consciousness has proven to be difficult in the waking state, it becomes even more so in the sleeping state. A major problem with the study of information processing during sleep is that the experimenter does not have access to the mental state of the sleeping subject. During waking states, the researcher can ask subjects to make overt responses by providing subjective reports of their mental activity in order to determine the extent of cognitive processing. Subjective reports cannot easily be communicated in sleep. The experimenter could

ask the subject to signal awareness, for example, by pressing a button. Again, this would be difficult during sleep. Muscle tonus may be so low that the sleeper is essentially paralysed. If the sleeper fails to overtly respond to the external stimulus, does this mean they are not conscious of the stimulus or rather that they are conscious but cannot execute the motor response? Or, perhaps the sleeper has detected the stimulus but has forgotten the instruction to respond which was given in the waking state. Some researchers awaken the sleeper and ask them to report prior mental activity. The inability to report awareness of prior stimulus presentation may be due to a failure of memory storage or retrieval. Again, as in the waking state, the interpretation of a failure to respond is equivocal.

In spite of these difficulties, evidence has accumulated to suggest that some form of awareness or consciousness may persist during sleep (for review, see Broughton, 1982a). In non-REM sleep, the so-called "disorders of arousal" such as sleepwalking and sleep terrors provide evidence of a type of consciousness within sleep. During sleepwalking, there is obviously some degree of awareness of the external environment since the person typically navigates through their home successfully. However, there is complete amnesia of the experience upon awakening. The behaviours carried out during sleepwalking can be quite astounding, such as eating, driving a car, violence, and even homicide (Broughton & Shimizu, 1995; Guilleminault, Kushida, & Leger, 1995). Once again, amnesia upon awakening does not provide evidence that the sleepwalker was unaware or unconscious of their mental activity or physical actions.

There is some evidence for consciousness during REM sleep. Support for this comes from studies of incorporation of stimuli into dream content, lucid dreaming, arousal thresholds, and the brain's response to external, relevant stimuli (evoked potentials). The incorporation of

external stimuli into dream content during REM sleep suggests that consciousness may be possible in sleep. Dement and Wolpert (1958) provided early reports that externally presented stimuli could be reliably incorporated into dreams during EEG-defined REM sleep.

Incorporations were determined by awakening subjects five to 20 minutes into each REM period (i.e., four to five times per night) using a doorbell stimulus. Three types of external stimuli were delivered: a five second tone, a flash of light, and a spray of water. Incorporations were most likely following the water spray stimulus and least likely following the tone stimulus. Many subsequent investigations have validated this report (for review, see Arkin & Antrobus 1978; Burton, Harsh & Badia, 1988). Such reports have indicated that incorporation is more favourable during REM compared to non-REM sleep. Of course, the sleeper need not necessarily be conscious of the external stimulus in order to incorporate it into a dream.

It is not easy to dismiss evidence of consciousness provided by lucid dreamers. A lucid dream is one in which the sleeper becomes aware of the fact that they are dreaming (LaBerge & Levitan, 1995). Lucid dreams have typically been recorded during REM sleep. In the laboratory, it has been shown that sleepers are able to communicate their "lucidity" to the experimenter. As mentioned, the muscles are largely inhibited during REM sleep. An exception is the muscle controlling eye movements. In order to communicate their lucidity, the subject employs a pre-established pattern of eye movement responses which are recorded with EOG electrodes. Thus, sleepers are able to not only become conscious of the fact that they are dreaming, but also to consciously signal to the experimenter that they are dreaming. It is possible that the lucid dream is triggered because of a brief arousal from REM to wakefulness. In support of this, Ogilvie, Hunt, Tyson, Lucescu, and Jenkins (1982) reported increased alpha EEG activity associated with

lucid dreaming, a neurophysiological marker typically associated with relaxed wakefulness. Even if one accepts that lucid dreamers are indeed conscious of their mental activity within sleep, only a small proportion of the population are in fact lucid dreamers. LaBerge however claims that all people can be trained to lucid dream.

Arousal thresholds provide an indirect measure of consciousness. In the usual arousal threshold study, subjects are presented with auditory stimuli at different times of the night. Stimulus intensity is gradually increased until the subject awakens. This arousal threshold is used to infer depth of sleep across the night. Auditory arousal thresholds have been shown to vary according to sleep stage, time of night, salience of the stimulus, and stimulus intensity (Williams, Hammack, Daly, Dement, & Lubin, 1964; Rechtschaffen et al., 1966; Busby, Mercier, & Pivik, 1994). Arousal thresholds are highest in SWS relative to both stage 2 and REM. As the night progresses, there is a general decrease in arousal thresholds, indicating that sleepers can be more easily awakened by stimuli in the early morning hours (Williams et al., 1964). There is however a confound between time-of-night and stage effects. Stage 2 and REM sleep occur more frequently in the second-half of the night, whereas SWS (for which the arousal threshold is high) occurs mainly in the first-half of the night. Meaningful stimuli such as the sleeper's name or a baby's cry apparently require less intensity to evoke an arousal from any stage of sleep (Oswald, Taylor, & Treisman, 1960). These elicited awakenings provide some evidence that information processing is maintained during sleep. The system that detects intrusive or psychologically relevant stimuli that then arouses the subject from sleep may well do so independently of consciousness. Arousal from sleep might rely on a reflexive/automatic system, rather than on conscious detection. Once again, such a null hypothesis would be difficult to test.

Another way to investigate consciousness during sleep is through the use of event-related potentials (ERPs). ERPs offer a method to probe the extent of information processing in sleep independently of the subject's overt response (Campbell et al., 1992). They are changes in electrical activity of the nervous system that are "evoked" by an external physical stimulus or "emitted" by an internal psychological event (Picton, Lins, & Scherg, 1995). These psychological events include alteration of the subject's level of attention or arousal, decision-making, memory comparison, or relevance of the stimulus. ERPs will be used in this thesis to probe the extent of information processing during sleep onset and within sleep itself. For this reason, ERP methodology will be reviewed in detail in the following section.

Event-Related Potentials (ERPs)

ERPs have been recorded in sleeping subjects since the early 1960s. This experimental method typically requires that different categories of stimuli be presented. For example, some stimuli might be relevant and others irrelevant. The computer recording the ERPs must be able to sort trials according to the category of the stimulus that was presented. Moreover, the EEG needs to be stored continuously so that trials may be sorted according to stage of sleep. This requires a relatively fast computer with a large hard disk. While even the most inexpensive of computers will now meet these requirements, such technological advances are quite recent. Most of the research in this field has thus been carried out in the last 10 years. ERPs provide a non-invasive tool to monitor information processing capabilities of the brain. They are particularly useful to investigate cognitive processes, such as attention and speed of processing, which are associated with changing levels of arousal and consciousness.

Recording and Analysis of ERPs

ERPs can be elicited by stimuli in all sensory modalities. A problem with recording evoked potentials during sleep is that constancy of stimulus input cannot always be assured. The experimenter must be able to determine that changes observed in the evoked potential are due to sleep itself and not to variation in stimulus input to the receptor. Constancy of presentation of visual stimuli to the receptor, the retina, is not easily managed since eyes are closed during sleep. Moreover, the eye position will change during sleep due to changes in body position. Somatosensory stimuli (usually brief electric pulses) have been employed. Again, limb position and muscle tension will vary during sleep. Moreover, the intensity of the somatosensory stimulus varies over time in an unpredictable manner due to changes in skin potential resulting from sweat or evaporation of the electrolyte medium. Most researchers thus employ auditory stimuli. Constancy of auditory input to the inner ear can be assured in spite of variation of head position and body movement during the night, if the acoustic stimuli are delivered through earphone inserts. Auditory stimuli were thus employed in all studies reported in this dissertation.

Auditory evoked potentials are typically recorded from scalp electrodes and consist of a series of negative and positive "components". There are two different schools of thought concerning the definition of a component. Donchin, Ritter, and McCallum (1978) initially defined a component as being "a source of controlled observable variability". Thus, the independence of two or more components was determined by experimental manipulation. Experimental manipulation must affect one component, but not another. Näätänen and Picton (1987) subsequently provided the following definition: a component is ... "the contribution to the recorded waveform of a particular generator process, such as the activation of a localized area of

cerebral cortex by a specific pattern of input". Thus, Näätänen and Picton (1987) assume that different components must have different intra-cranial dipole sources. This is usually determined by comparing the voltage distribution of different components over the scalp. Components that have different scalp distributions must have different intra-cranial generators. In this thesis, Näätänen and Picton's definition is used (1987).

The components of the auditory ERP occur within a few to several hundred milliseconds following the stimulus event. Many of the early-latency components are affected by manipulation of the physical parameters of the stimulus, such as intensity, duration, rate of presentation, and abruptness of rise-fall time. Since these components are affected by factors outside the subject (i.e., in the external environment), they are called "exogenous" ("exo" = outside). On the other hand, many longer-latency components are primarily affected by internal, psychological factors, such as the relevance of the stimulus, or the level of attention/arousal. They are thus said to be "endogenous" ("endo" = inside). Exogenous evoked potentials are unaffected by psychological factors. Thus, the subject's level of attention will not affect exogenous evoked potentials. Manipulation of physical characteristics of the stimulus will not affect endogenous evoked potentials. However, there can be seemingly contradictory effects. Manipulation of certain stimulus parameters can apparently interact with the endogenous, psychological state of the subject. For example, as a stimulus is made increasingly more intense (an exogenous manipulation), it may become impossible to ignore and thus "intrude" into consciousness (an endogenous effect). Parsing the exogenous and endogenous effects can be difficult, requiring elaborate experimental design.

ERP components are also often grouped into three rather arbitrary sub-divisions: "short-"

(1-12 ms); "mid-" (12-50 ms); and "long-latency" (50-800 ms) (Davis, 1976). The relatively early components of the short-latency ERPs reflect neuronal activity in low levels of the nervous system, such as the peripheral auditory nerve and the brain stem (Jewett & Williston, 1971). They are thus referred to as brainstem auditory evoked potentials (BAEPs). These short-latency EPs are markedly affected by physical parameters of the stimulus, but are minimally affected by selective attention processes (Picton, Stapells, & Campbell, 1981). Furthermore, most reports have indicated that the BAEPs are not altered by sleep (Amadeo & Shagass, 1973; Osterhammel, Shallop, & Terkildsen, 1985; Campbell & Bartoli, 1986; Deacon-Elliott, Bell, & Campbell, 1987; Bastuji, García-Larrea, Bertrand, & Mauguière, 1988), or are only minimally altered (Stelmack, Campbell, & Bell, 1993). The BAEP remains present during deep anesthesia and during coma (Thornton, 1991; Jones, 1994). For this reason, the BAEP is a very poor measure of consciousness. Manipulation of the subject's level of attention or consciousness therefore appears to have little effect on processing of acoustic stimuli through the periphery and the brainstem of the auditory pathway. As the acoustic information reaches the thalamus, such manipulations begin to show an effect. The thalamus plays a crucial role in attention and consciousness. The thalamus acts as the junction at which all sensory modalities (except olfactory) first merge (Steriade & Amzica, 1998). It appears to act as a filter or "gate" for the inhibition of unattended information, preventing the irrelevant and redundant information from interrupting the limited capacity processing carried out by the cortex. The cortex thus becomes conscious of only the most relevant information. This likely represents only a very small proportion of the sensory input that bombards the receptors. The mid-latency components thus appear to offer the transition between the largely exogenous influences of the short-latency

components and the largely endogenous influences of the longer latency components.

The mid-latency ERPs, occurring between 12 and 50 ms, are thought to originate from the thalamus and auditory primary cortex (Picton, Hink, Perez-Abalo, Linden, & Wiens, 1984). Like the early ERP, the mid-latency components are also affected by the physical properties of the stimulus (Picton & Hillyard, 1974; Picton et al., 1984). The mid-latency evoked potentials are not easily recorded from the scalp. This is because the auditory stimulus will also elicit a middle ear muscle reflex. This reflex is recorded from the scalp as muscle "artifact" occurring from 15 to 30 ms after stimulus onset. This coincides with the latency of the middle components of the evoked potential response. Thus, the scalp-recorded ERPs may consist of a summation of a true brain response and muscle artifact. Differentiating between the two has proven to be methodologically difficult. The mid-latency components have therefore received only limited study. Studies of selective attention are contradictory. Similarly, even manipulation of the subject's level of consciousness during sleep (Campbell et al., 1992) and general anesthesia (Plourde & Picton, 1991; Plourde, Joffe, Villemure, & Trahen, 1993; van Hoof, De Beer, Brunia, Cluitmans, Korsten, Tavilla, & Grouls, 1995) have produced equivocal results. In addition to the problem of overlapping muscle artifact, the different studies have employed varying stimulus parameters and experimental designs. In their review, Campbell et al. (1992), noted that sleep will attenuate the amplitude of the mid-latency components, but only if stimuli are presented very rapidly. When stimuli are presented slowly, sleep does not alter the mid-latency components. This is perhaps because stimuli that occur infrequently are more psychologically relevant and thus their processing is difficult to inhibit.

The late components of the auditory ERP are particularly relevant to this thesis. They are

often labelled according to their polarity and peak latency. "P1" is the first large positive wave recorded at the scalp having a peak latency of approximately 50-75 ms. It is followed by "N1", a negative peak at approximately 80-100 ms, then by "P2", a positive peak at approximately 175-225 ms. A later "N2", a negative peak at approximately 250-350 ms, can sometimes be observed depending on the experimental paradigm. P3 is a late positive wave which is elicited (depending on the complexity of the stimulus), when a rare stimulus is detected in a series of frequently occurring stimuli (Sutton, Braren, Zubin, & John, 1965; Donchin, 1981; Pritchard, 1981; Picton, 1992; Verleger, 1997). When the stimulus is easy to detect (depending on its complexity and ease of stimulus classification), P3 typically peaks at about 300 ms.

The process of recording ERP data involves several steps: selection of recording montage, amplification, analog filtering, analog-digital (A-D) conversion, artifact rejection, averaging, and digital filtering (Picton et al., 1995). ERPs are obtained by recording on-going EEG activity during stimulus presentation. They are typically recorded from scalp electrodes. The different components of the ERP have different scalp distributions. For this reason, almost all modern studies record the EEG from at least three midline anterior-posterior electrodes (usually Fz, Cz, Pz). Multiple channel recordings (e.g., 16-32 sites) are used for more extensive display and "mapping" of the scalp distribution of the ERP. Some laboratories have employed as many as 128 channels (Tucker, 1993). Such high density spatial recording is almost always done using some form of electrode cap in which the electrodes are attached to an elastic cap or net. The use of very large electrode arrays may not be practical during sleep studies since the cap may move during the all-night sleep session. The use of multiple electrode placements has however been reported (Niiyama, Fujiwara, Satoh, & Hishikawa, 1994; Bastuji, García-Larrea, Franc, &

Mauguière, 1995; Colrain et al., in press - a; Cote et al., in press - a). Another factor mitigating against the use of high density arrays is that the tight-fitting cap is uncomfortable, especially after being worn for several hours. It may disturb the quality of sleep.

The ERP signal is quite small relative to the large amplitude background EEG noise. Signal averaging techniques are thus applied to circumvent this signal-to-noise problem (Picton et al., 1995). The EEG signal is assumed to be random, characterized by both positive and negative deflections. The average of an infinitely large number of negative and positive potentials will tend toward zero. The amplitude of the ERP however is assumed to be constant and time-locked to the stimulus event. The average of a constant is of course that constant. Through averaging a large number of sweeps or trials, the background EEG noise is reduced, and the constant ERP remains. The number of trials required to discern a given ERP signal is dependent on the relative amplitude of the signal of interest and the amplitude of the background EEG. This is called the signal / noise (S/N) ratio. The evoked potential signal can be exceedingly small. During sleep, the amplitude of the background EEG may become very large relative to that in the waking state. Therefore, many more trials may have to be presented in sleep than in wakefulness in order to discern the evoked potential in the background EEG. During stage 2, this is not particularly problematic because subjects may spend as much as four hours in this stage of sleep and the amplitude of the EEG signal is relatively low. SWS is more problematic. The background delta waves are very high in amplitude (perhaps 400 μ V). Many more trials will have to be presented in SWS than in stage 2 or REM in order to extract the evoked potential signal. Since SWS may last only one hour in some subjects, there may be insufficient time to do so.

Once the EEG activity has been acquired and averaged, off-line procedures may be

applied to the data in order to improve the quality of the signal. The EEG is often contaminated by artifact. Artifact is defined as any electrical activity that is not generated in the brain.

Electrodes are connected to the amplifier by a "lead" (an electrical wire). This lead can act as an antenna and pick-up electrical noise. If the frequency spectra of this noise artifact is outside of the ERP frequencies of interest, then it can be much reduced by analogue and/or digital filters. A particularly problematic artifact is eye movement and blinking contamination. Eye movement artifact can generate a potential measuring more than 100 μV at frontal electrode sites. Since the frequency spectra of eye movements overlap those of the EEG, they cannot be removed by filtering. The averaging process can be used to reduce the amplitude of the eye movement artifact. This is the case however only if the eye movements are random and not time-locked to the stimulus. Such an assumption appears to be appropriate with the slow rolling eye movements observed in stage 1 sleep and with the rapid eye movements in REM sleep. During the waking state, eye movements and blinks may however be time-locked to the stimulus (i.e., the artifact occurs at a constant time after the stimulus). This may be particularly true for loud intrusive auditory stimuli which are known to elicit an eye blink or a "startle" response (Blumenthal, 1988; Putnam & Roth, 1990). Averaging procedures will not remove a constant latency artifact. A commonly used method to circumvent this problem is to remove (or "reject") any trial containing EOG artifact. If there is a great deal of artifact, there may be too few non-artifact trials remaining to permit reliable averaging. EOG correction routines have now been developed to allow for statistical prediction of how the EEG would have appeared had there been no artifact. Nevertheless, such correction routines are still considered by many to be a compromise. They represent, after all, a statistical prediction of how the EEG would have appeared without artifact.

Attention-Related ERPs

N1 Component of the Auditory ERP

N1 is a negative wave peaking at about 100 ms after stimulus onset. It is maximum over central areas of the scalp (Näätänen & Picton, 1987). The N1 component is elicited by abrupt changes in the level of physical energy at the level of the sensory receptors. It is affected by manipulations to physical properties of the stimulus, such as intensity, pitch, inter-stimulus interval and duration of the stimulus. It is also affected by manipulations to the subject's level of attention. Early studies of selective attention indicated that when subjects attend to an auditory stimulus, the amplitude of N1 was larger compared to when it was ignored. Hansen and Hillyard (1980) and Näätänen (1982) have indicated that it is not N1 per se that is affected by attention. Rather, a long-lasting attentional-related negative wave, that Näätänen termed "Processing Negativity" (PN), overlaps P1, N1, and P2. PN reflects the additional processing that an attended channel receives. It may result in negativity being added to P1, N1, and P2. As such, while N1 becomes larger (more negative), P1 and P2 may appear to become smaller in amplitude (less positive or more negative) as a result of attention. Alho et al. (1986) have noted that all stimuli will receive some PN. In order for an irrelevant stimulus to-be-ignored, it must be processed to a certain extent. A relatively large N1 is still apparent to the ignored auditory stimulus. This could however be due to the exogenous influences of the physical stimulus on N1 or due to at least some endogenous PN.

P300 Component of the Auditory ERP

A late positive wave, "P3", is particularly relevant to the study of consciousness and sleep. The

P3 component of the ERP is most often elicited in the "odd-ball" task. In this paradigm, the subject is presented with a train of frequently occurring "standard" stimuli. At odd and random times, the stimulus is changed. The subject may be asked to detect this "target" usually by keeping a running mental count of its occurrence or by button pressing upon its presentation. If the infrequently occurring target is detected, P3 is elicited (Sutton et al., 1965). When the target is easy to detect (i.e., the physical deviance between the standard and the target is large), P3 peaks at about 300 ms (thus the label "P300"). P300 is maximum over parietal regions of the scalp. Its amplitude is inversely proportional to the probability of the occurrence of the target (Duncan-Johnson & Donchin, 1977; Campbell, Courchesne, Picton, & Squires, 1979). If the subject ignores the stimuli while engaged in a secondary task, P300 is not elicited upon presentation of the rare stimulus. Moreover, even if the subject is actively engaged in the signal detection task, but fails to detect the rare target, P300 is not elicited. For these reasons, the presence of a P300 has been claimed by many researchers to reflect conscious detection or awareness of the rare stimulus (Picton, 1992). There is some evidence that P300 might more accurately reflect pre-consciousness. In the case of a neurological patient with cortical blindness (i.e., damage to the occipital cortex leading to a so-called "blindsight"), while engaged in a visual odd-ball task, the patient will fail to signal detection of a rare stimulus, yet a P300 will still be elicited (Shefrin, Goodin, & Aminoff, 1988). Thus, although evidence of consciousness is not provided by the behavioural response, P300 was still apparent.

The absence of a P300 is more ambiguous. Subjects can make conscious detections yet a P300 will not always be elicited. For example, if subjects are asked to push one button upon detection of the frequently presented "standard" stimulus and another button upon detection of

the infrequently presented target, as mentioned a P300 will be elicited following the target. No P300 (or only a very small amplitude P300) will be visible following the standard, even though the subject clearly signalled their awareness of it. Thus, while the presence of a P300 can be used as positive evidence of consciousness (or at least pre-consciousness), its absence cannot be used to infer unconsciousness. Although P300 is generally not elicited in inattentive subjects, there is an exception to this rule. If the rare stimulus is sufficiently loud as to “intrude” into consciousness, a P300 may be elicited (Roth, Blowes, Doyle, & Kopell, 1982; Roth, Dorato, & Kopell, 1984; Polich, 1989; Putnam & Roth, 1990).

The amplitude and latency measures of the P300 waveform may be manipulated by a number of experimental variables, such as task difficulty, task relevance, probability, and expectancy. In healthy young adults, the latency of the P300 is delayed with increasing difficulty to discriminate stimulus deviance, whereas, its amplitude is attenuated (Picton, 1992). In addition, P300 may be influenced by psychological variables such as attention and arousal. The arousal state of the subject is an important determinant of P300 measures, as indicated by the effects of circadian rhythms, sleep deprivation, fatigue, exercise, and drugs (Polich & Kok, 1995).

Since the first report of this late positive component (Sutton et al., 1965), the P300 has been widely studied and the conditions required to elicit it extensively outlined (Donchin, 1981; Pritchard, 1981; Picton, 1992; Verleger, 1997). Vaughan and Ritter (1970) first described the scalp distribution of the P300 as maximum in amplitude at mid-parietal regions. The scalp distribution of the P300 has been shown to be somewhat variable depending on a number of factors, including modality of presentation and task demands (see Johnson, 1993). In light of this

widespread variability, the source generators of the P300 have been speculated to be multi-focal (Picton, 1992), perhaps dependent on the specific task and modality of stimulus presentation. The functional significance of the P300 wave remains disputed (see for example, the review by Verleger, 1988, and the ensuing commentary). The most commonly cited function of P300 is that of contextual updating (Donchin, 1981, Donchin & Coles, 1988). The memory for the frequently occurring stimulus is well-formed. The memory for the rare stimulus is relatively less well-formed. Upon presentation of the rare stimulus, its memory representation needs to be revised or updated.

A number of late positivities have been reported in the literature. According to Donchin and Coles (1988), a true P300 however must meet certain criteria: maximum over parietal-central areas of the scalp; peak at approximately 300 ms (but this is dependent on the ease of target detection); and vary inversely in amplitude as a function of stimulus probability. As mentioned, a number of other late positivities can also be recorded to rare stimuli. These may be distinguished by their response to experimental manipulation and scalp topography. An earlier P3 peaking at approximately 250 ms has a prominent frontal distribution (Squires, Squires, & Hillyard, 1975). It can be elicited by highly novel and unexpected stimuli even if the subject is not actively attending to the stimulus. This “P3a” is thus considered to reflect pre-conscious detection of novelty. The P300 (also called P3b to distinguish from P3a), peaking later at approximately 350 ms, has the more typical parieto-central distribution. Unlike P3a, the amplitude of P3b is highly dependent on the level of attention of the subject. Fabiani and Friedman (1995) and Spencer, Dien, and Donchin (1999), have also described a P3-like wave that is elicited by novel environmental sounds (animal sounds, musical instruments, etc.). It is similar to the P3a in that it

has a more anterior scalp topography than the P3b. However, its latency (350 ms) is much too late to be considered a P3b. Fabiani and Friedman (1995) and Spencer et al. (1999) thus label this the "novel-P3". Interestingly, in patients with frontal lobe dysfunction, the novel stimulus will still elicit the parietal P3 (peaking at about 350 ms) but the anterior P3 is absent (Knight, 1984).

Event-Related Potentials During Sleep Onset and Sleep

P1-N1-P2-N2

As discussed previously, experimental manipulation of the subject's level of attention has profound effects on the late components of the auditory ERP. Research has also shown that ERPs vary according to the subject's level of daytime sleepiness (Broughton 1982b; Broughton & Aguirre, 1987), and under conditions of total sleep deprivation (Harsh & Badia, 1989). ERPs during sleep onset and in the various stages of sleep have been extensively studied (see Campbell et al., 1992 for review). The late components of the ERP, P1-N1-P2-N2, are markedly affected by sleep. Studies which report changes to the positive components (P1, P2) at sleep onset have yielded inconsistent findings. The P1 component has been reported to either increase (Williams, Tepas, & Morlock, 1962; Weitzman & Kremen, 1965), decrease (Osterhammel et al., 1985; Erwin & Buchwald, 1986), or remain unaltered (Weitzman & Kremen, 1965; Fruhstorfer & Bergström, 1969). Similarly, the P2 component has been described as increasing (Noldy, McGarry, & Campbell, 1988; Campbell, McGarry, & Bell, 1988; Ogilvie et al., 1991; Harsh et al., 1994; de Lugt, Loewy, & Campbell, 1996; Elton et al., 1997; Cote et al., in press - b), decreasing (Williams et al., 1962; Williams et al., 1964; Fruhstorfer & Bergström, 1969), or remaining unchanged during sleep (Weitzman & Kremen, 1965). These equivocal results are

most likely due to variable recording and analysis procedures (Campbell et al., 1992).

Differences in amplifier filter settings can account for some of the variance in these studies. In wakefulness, the slow overlapping negative wave, "processing negativity" (PN), which is associated with attention, overlaps the P1-N1-P2 ERP waveform (Näätänen, 1990). This slow wave is markedly attenuated during non-REM sleep. If the time constant setting is too short, low frequencies will be filtered, effectively removing the long-lasting negativity and hence its summing effects. Furthermore, Campbell et al. (1992) have noted that the differences in measurement of P2 could also account for the variable results. In recent studies, peak amplitude of a component is measured relative to a pre-stimulus baseline. However, in older studies, the distance between adjacent negative and positive peaks was often measured. There are problems with such peak-to-peak measurement. Peak-to-peak measurement is insensitive to the summing effects of a long-lasting slow wave. Since peak-to-peak measurement involves the subtraction of the amplitude of one peak from another, the constant overlapping PN slow-wave (that summates to both N1 and P2) will be removed by the subtraction process. In summary, it appears that when a sufficiently long time constant is employed, and when baseline-to-peak measurements are appropriately used, both P1 and P2 increase in amplitude at sleep onset.

Changes to the negative components of the ERP at sleep onset are more consistent in the literature. Numerous investigations have shown that baseline-to-N1 amplitude is attenuated in non-REM sleep compared to wakefulness (Williams et al., 1962; Weitzman & Kremen, 1965; Fruhstorfer & Bergström, 1969; Noldy et al., 1988; Campbell, et al., 1988; Ogilvie et al., 1991; Campbell et al., 1992; Salisbury, Squires, Ibel, & Maloney, 1992; Harsh et al., 1994; de Lugt et al., 1996; Loewy, Campbell, & Bastien, 1996; Elton et al., 1997; Cote et al., in press - b). This

decrease in N1 amplitude is not altered by changes in stimulus intensity (Campbell, et al., 1992) nor by changes to the inter-stimulus interval (Armitage, Bell, Campbell, & Stelmack, 1990). During the transition from an alert, conscious waking state, to drowsiness and finally to sleep, N1 gradually decreases in amplitude. In actual fact, it is not so much N1 that is affected by the loss of consciousness, but rather the long-lasting slow overlapping negative wave that summates to both earlier and later positive waves as well as to N1 (Campbell et al., 1992; Näätänen, 1990). During REM sleep, N1-P2 may return to 25-50% of their waking amplitude.

The N2 component typically increases at sleep onset (Williams et al., 1962; Weitzman & Kremen, 1965; Noldy et al., 1988; Ogilvie et al., 1991; Harsh et al., 1994; Voss & Harsh, 1998). Although not all labs report this, these effects may be due to the incorporation of evoked K-complex activity into the ERP average (Campbell et al., 1992). Within sleep, a late negative wave sometimes labelled the "sleep N2" or the "N350" can be recorded in non-REM sleep (Ornitz, Ritvo, Carr, La Franchi, & Walter, 1967; Näätänen & Picton, 1987; Harsh et al., 1994). Colrain, Webster, Hirst, and Campbell (in press - b) have recently indicated that the sleep-N2 and the vertex sharp wave have the same central maximum scalp topography and occur at the same latency. This sleep N2 occurs much later and its scalp topography is more central than the anterior N2 seen in the waking state.

Mismatch Negativity (MMN)

Many of the early ERP studies of sleep employed a single train of repetitive stimuli (Weitzman & Kremen, 1965; Ornitz, et al., 1967; Fruhstorfer & Bergström, 1969). More recently the trend has been to employ odd-ball paradigms in which the frequently occurring "standard" stimulus at

random and at odd times is replaced by a "deviant" ("deviant" is used to label the odd stimulus rather than "target" since subjects are not always asked to detect the odd stimulus). In addition to the P1-N1-P2-N2 waveforms, the deviant may also elicit a Mismatch Negativity (MMN) and/or a P300. When stimuli are presented very rapidly, the deviant stimulus will elicit an additional long-lasting negative wave peaking between 100 and 200 ms (Näätänen, 1990). The MMN is best observed as a difference wave, obtained by subtracting the standard from the deviant ERP. Virtually any change in the physical features of the standard — its frequency, intensity, duration, or location — will elicit the MMN. The MMN is thought to reflect a very simple sensory or echoic memory. The sensory memory for the standard stimulus is well-formed since it occurs frequently. The presentation of the deviant fails to match this representation in memory and the MMN is elicited because of the additional processing that is required. The MMN appears to occur automatically, independent of the subject's level of attention. The MMN occurs in both "attend" and "ignore" conditions (Näätänen, 1982), although there is some dispute over the extent of the attentional influence (Woldorff & Hillyard, 1991; Näätänen, Paavilainen, Tiitinen, Jiang, & Alho, 1993; Trejo, Ryan-Jones, & Kramer, 1995). The MMN is thus claimed to provide a means for the pre-conscious detection of deviance.

There has been much interest in the influence of unconscious states on the MMN. The MMN cannot be elicited in deep coma but begins to emerge as the depth of coma lessens. Indeed, it has come to act as a predictor of the emergence from coma (Kane, Curry, Butler, & Cummins, 1993; Kane, Curry, Rowlands, Manara, Lewis, Moss, Cummins, & Butler, 1996). Loewy et al. (1996) have provided convincing evidence of the occurrence of MMN during REM sleep. They employed either small or large pitch deviants and presented stimuli rapidly (every 0.6 s). Atienza,

Cantero, and Gómez (1997) have replicated the finding of a pitch MMN in REM sleep. Loewy, Campbell, de Lugt, Elton, and Kok (in press), have however been unable to elicit a MMN in REM sleep when the deviant stimulus was either a slight increase or decrease in the intensity of the standard. The MMN cannot be elicited during SWS to any type of deviant stimulus. Loewy et al. (1996) point out that this might be because the amplitude of the MMN is very small (less 1 μ V) in sleep and the amplitude of the background EEG is exceedingly large in SWS.

Alternatively, sensory memory may fade very rapidly in SWS. Stimulus presentations will therefore have to be exceedingly fast in order to capture the MMN. There is some controversy about the effects of stage 2 sleep. Most labs have failed to elicit an MMN in stage 2. However, Sallinen, Kaartinen, and Lyytinen (1994) report that an MMN-like wave can be elicited if the deviant also elicits a K-Complex. It is however possible that this small amplitude MMN is an artifact of the overlapping and summing effects of the much larger amplitude N350 and N550 waveforms.

P300 in Sleep?

If P300 cannot be elicited in awake but inattentive subjects, it is unlikely that it can be elicited in sleeping subjects. In separate reviews, Campbell et al. (1992) and Harsh (1994) both claimed that there was little evidence that P300 could be elicited during sleep. Some early studies have employed odd-ball paradigms during non-REM sleep and did record a late positive wave to the deviant stimulus (Wesensten & Badia, 1988; Nielsen-Bohlman, Knight, Woods, & Woodward, 1991; Winter, Kok, Kenemans, & Elton, 1995). These positive waves peaked much too late to be considered a true P300. It is of course possible that P300 was simply delayed in sleep. This is

unlikely since when subjects are awake but inattentive, P300 is not delayed when it can be elicited. This late positivity is more consistent with what has been termed the "P900" component of the evoked K-complex (Bastien & Campbell, 1992). Rare deviant stimuli will also elicit the K-complex in non-REM sleep (Sallinen et al., 1994; Niiyama, Fushimi, Sekine, & Hishikawa, 1995; Sallinen, Kaartinen, & Lyytinen, 1997; Colrain et al., in press - a).

Salisbury et al. (1992) reasoned that an intrusive stimulus would be most likely to elicit a P300 in sleep. A P300 was not elicited in either wake or sleep states when a rare low intensity stimulus (40 dB nHL) was employed; however, a P300 was elicited in stage 2 sleep when a louder deviant (60 dB nHL) was employed. There were a number of methodological and interpretive problems with this study. In the modified odd-ball paradigm used by Salisbury et al. (1992), the deviant stimulus changed in both intensity and pitch from the standard. Thus, the effects cannot be said to be due to the intensity of the rare stimulus. Furthermore, the small amplitude P300 (approximately 2 μ V) peaked at about 400 ms, but could only be detected when employing peak-to-peak (N2-P3) measurement because of the overlapping "N3" (i.e., negative wave from 450 ms to 800 ms, also referred to as the "N550") of the K-complex. To what extent this positive wave was related to the actual K-Complex was not stated. To demonstrate that it is independent of the K-Complex, trials in which a K-Complex is elicited need to be averaged separately from those in which it is not. If the positive wave is independent of the K-Complex, it should still occur in the non-K-Complex trials.

In a subsequent investigation, Salisbury and Squires (1992) conducted separate experiments to investigate the effects of pitch and intensity deviance on P300 in non-REM sleep. Once again, peak-to-peak measurement methods were employed, and K-Complexes were not

removed. In the first experiment, the standard 1000 Hz, 50 dB nHL tone pip occurred on 90% of trials. In separate conditions, the pitch-deviants (1500, 2000, 2500, 3000, and 3500 Hz) were delivered on the remaining 10% of trials. A total of 200 trials were presented in each condition. Since only 20 deviants would be presented within a block, the signal-to-noise ratio of the smaller amplitude ERPs relative to the much larger amplitude background EEG of sleep would be poor. A late positive peak that authors labelled as "P3" was nevertheless identified. This positive wave peaked at 404 ms and did not vary in latency or amplitude across the different pitch conditions. The candidate P300 had an unusual occipital scalp distribution. In the second experiment, the standard was a 2000 Hz, 50 dB nHL tone pip delivered on 90% of trials. Four different intensity-deviants (55, 60, 65, 70 dB nHL) were delivered in separate conditions. The latency of the P3, (stated as 388 ms in their text but appearing closer to 450 ms in their Figure 5), did not differ between stages 2 and SWS, nor did it differ across the four intensity-deviant conditions. The amplitude of the very small positive-going wave was again unusual, being measured as a negative potential at all electrode sites. It was maximum (less negative) at Oz. Thus, while there may be a small amplitude positive-going wave recorded to rare, deviant stimuli in non-REM sleep, it is very different from the P300 recorded during wakefulness. Its latency is unusually long. Most importantly, its scalp distribution does not conform to the parietal-central maximum of the usual P300.

In contrast to the above studies, many investigators have found no evidence of a P300 in sleep. Paavilainen, Cammann, Alho, Reinikainen, Sams, and Näätänen (1987) failed to show evidence of P300 activity in non-REM sleep, even though they employed an odd-ball task in which a deviant varying in pitch was used. They suggested that the negative results may have

been due to the small magnitude of difference (only 50 Hz) in stimulus deviance. Loewy et al. (1996) used a similar pitch oddball task. A 1000 Hz standard tone pip was presented on 80% of trials. In different conditions, a “small” (1200 Hz) or “large” (2000 Hz) deviant was presented on the remaining 20% of trials. There was still no evidence of a late positive wave.

Since the initial reviews by Campbell et al. (1992) and Harsh (1994), reports of more convincing P300-like waves have been made. Niiyama et al. (1994) and Bastuji et al. (1995) have independently reported P300 activity in REM sleep. Both of these studies varied stimulus pitch using an odd-ball paradigm. A 1000 Hz standard was presented on 80% of trials in the Niiyama et al. (1994) study, and on 90% of trials in the Bastuji et al. (1995) study. On the remaining trials a 2000 Hz deviant was presented. These studies reported a small amplitude P300 (less than 4 uV) during REM sleep. Neither investigation observed P300 activity in non-REM sleep. In addition to recording ERPs during REM sleep, these studies had two further strengths: topographic recordings and waking data collection for appropriate comparison. When subjects were awake, they were asked to detect the rare, deviant stimulus. Unfortunately, neither study included a waking condition in which subjects ignored the auditory stimulus. An ignore condition might provide a more appropriate comparison to sleep since subjects also do not respond while asleep (and presumably also ignore the stimulus). The REM P300 was delayed in latency and attenuated in amplitude, relative to its waking comparison. Niiyama et al. (1994) included only trials without K-Complex activity in non-REM averages, and included only trials without eye movement in REM averages (i.e., tonic REM). They present their ERP data using a difference wave method, which is obtained by subtracting the frequent from the rare stimuli. The studies by Niiyama et al. (1994) and Bastuji et al. (1995) report a P300 which is similar to the waking-P300

in polarity, latency, duration and scalp distribution. This REM-P300 is however much attenuated in amplitude compared to the waking P300.

Specific Aims of the Thesis

The studies in this dissertation investigate stimulus processing during wakefulness, the sleep onset period, and during non-REM (stage 2) and REM sleep. Probing the extent of information processing and consciousness in the sleeper is not an easy task. The experimenter does not have direct access to the subjective mental state of the apparently unconscious sleeper. ERPs do offer a method to probe the extent of information processing in the behaviourally unresponsive sleeper. Information processing capabilities at sleep onset and throughout sleep have been previously studied using ERP methodology. The purpose of this thesis was to examine possible consciousness awareness during sleep.

The presence of a MMN during REM sleep provides evidence that stimulus deviance can be at least detected during sleep. The possibility of a P300 elicited in REM, as reported by Niiyama et al. (1994) and the Bastuji et al. (1995), suggests that processing stimulus deviance may be even more extensive -- enough to attain consciousness. The evidence provided by these prior studies is however weak. The P300 is barely discernable in the background noise, and its latency is prolonged. Several other manipulations need to be carried out to make a convincing case. Most studies to date have relied upon an odd-ball paradigm in which the pitch of the odd, rare stimulus is changed. In subjects who are awake but inattentive, a P300 will not be elicited to pitch deviants. A large amplitude P300 can however be elicited to very loud and very rare deviant stimuli even when subjects are engaged in a secondary task. This is perhaps because the loud and

infrequently presented deviant stimulus intrudes into consciousness. The effects of loud deviant stimuli will thus be explored in this thesis.

The thesis consists of four experiments. Each of the experiments is written in article format appropriate for the journal to which it has been, or will be, submitted. Particular focus will be placed on the P300 component of the auditory ERP as a probe of consciousness. As emphasized in this review, the occurrence of a P300 provides evidence of at least some form of consciousness. Many ERP researchers will argue that it is an index of conscious detection and awareness of the rare, deviant stimulus. On the other hand, a failure to elicit a P300 cannot be used as evidence that the sleeper is not conscious.

The first experiment investigated changes in ERPs and behavioural responsiveness during the transition to sleep employing a pitch odd-ball paradigm. The purpose of this study was to illustrate that when there is little evidence of consciousness (based on EEG-criteria and the subjects' behavioural response), P300 cannot be elicited. On the other hand, when subjects signal their detection of the rare, deviant stimulus, P300 can be elicited, even if the subject is apparently asleep (and by this definition, "unconscious").

The second, third, and fourth experiments examined the extent to which P300 can be elicited during actual sleep. ERPs were recorded in both non-REM and REM sleep. In experiment 2, ERPs were elicited by loud, intrusive deviants. The EEG was recorded from multiple channels in order to map the scalp topography of a possible P300. In Experiment 3, stimulus intensity was manipulated in order to determine the effect on P300 amplitude during sleep.

The final experiment was a large study in which the probability of deviant occurrence was

manipulated in three different groups of subjects. A "true" P300 varies inversely with the probability of deviant occurrence. The effects of pitch and intensity deviants were also examined. Pitch deviants will not elicit P300 in awake and inattentive subjects but may elicit a small amplitude P300 in REM sleep. Intensity deviants will elicit a P300 in inattentive subjects. It is not known whether they will also elicit a P300 in sleep.

Chapter 2

Experiment 1: Concordance between Event-Related Potentials and Reaction Time during the Sleep Onset Period

This chapter is in preparation for submission to the journal *Psychophysiology*. The Introduction is however extended for the purpose of the thesis. This is so that the rationale for subsequent experiments in the thesis can be well-established. In the actual journal submission, the Introduction will be shortened. This study investigated event-related potentials (ERPs) and behavioural responsiveness during the transition from wakefulness to sleep. Ten subjects were presented with an auditory oddball task during repeated sleep onset periods. A 1500 Hz tone pip was presented infrequently ($p=.04$) within a series of lower pitch 1000 Hz “standard” stimuli ($p=.96$). Subjects were required to button press upon detection of the rare “target” stimulus. Trials were sorted by sleep stage and by reaction time (RT), in which bins 1-3 represented increasing time to respond and bin 4 represented a failure to respond. During wakefulness, almost all targets were detected (97%). A large amplitude P300 was observed to these detected targets. This P300 was maximum over parietal areas of the scalp. During stage 1 sleep, subjects continued to respond on 47% of trials. The parietal P300 amplitude remained large to these detected targets. It was however much attenuated at frontal sites. When the subject failed to detect the target, no P300 was visible. P300 is thus associated with behavioural detection of the target stimulus, whether in wakefulness or stage 1 “sleep”.

Introduction

The sleep onset period is characterized by continuous changes in a host of measures, such as "...behavioural responsiveness, level of consciousness, brain wave or electroencephalographic (EEG) activity, the firing patterns of many neuronal systems, and most physiological measures" (Ogilvie, 1995, p. 566). The topic has been extensively reviewed in an edited book by Ogilvie and Harsh (1994). Several methods have been employed to examine the transition between a fully conscious state to one of sleep and unconsciousness. A method often used to monitor the subject's level of consciousness is to record the brain's response to external stimuli. Event-related potentials (ERPs) are changes in the electrical potential of the brain which may be elicited by an external physical stimulus or an internal psychological event (Picton, Lins, & Scherg, 1995). They consist of a series of positive and negative deflections or "components" following the onset of the stimulus. These components are often labelled according to their polarity and their sequential order in the series of peaks. The "vertex complex" consists of a negative component ("N1"), peaking at approximately 80 to 100 ms following the stimulus, and is followed by a positive component ("P2"), peaking at about 175 to 225 ms. These late components of the auditory ERP are markedly altered by sleep (Campbell, Bell, & Bastien, 1992). Recent studies concur that during non-REM sleep, N1 decreases while P2 increases in amplitude relative to wakefulness (Noldy, McGarry, & Campbell, 1988; Campbell, McGarry, & Bell, 1988; Harsh, Voss, Hull, Schrepfer, & Badia, 1994; de Lugt, Loewy, & Campbell, 1996; Elton, Winter, Heslenfeld, Loewy, Campbell, & Kok, 1997; Cote, Epps, & Campbell, in press - b). During REM sleep, N1-P2 may return to 25-50% of their waking amplitude (Campbell, et al.,

1992). In wakefulness, N1 amplitude will vary with changes in the subject's level of attention. Such changes in N1 during sleep are consistent with its role as a type of "general" conscious processing (Näätänen, 1990).

The "P3" component of the ERP (i.e., third positive peak in the waveform) is most often elicited in the "odd-ball" task. In this paradigm, the subject is presented with a train of frequently occurring "standard" stimuli. At odd and random times, the stimulus is changed. This could involve a change in one of many different parameters (e.g., pitch, intensity, duration, location). The subject is asked to detect this "target" stimulus perhaps by keeping a running mental count of the number of target occurrences or by button pressing upon its detection. If the rare target is detected, P3 is elicited (Sutton, Braren, Zubin, & John, 1965). When the target is easy to detect (i.e., the physical deviance between the standard and the target is large), P3 peaks at about 300 ms following the stimulus (thus the alternate label "P300").

The latency of P300 is used by many cognitive neuroscientists as a complement to the behavioural reaction time (RT) measure of the timing and duration of various cognitive "stages" of information processing (Campbell, 1985; Donchin & Coles, 1988). The overall RT reflects the duration of what cognitive psychologists refer to as stimulus classification and response production/execution processes. Stimulus classification includes those cognitive processes that involve encoding, identification, and classification/categorization of the stimulus (Campbell, 1985). In the odd-ball task, the subject needs to classify the incoming stimulus as either a standard or a target and then initiate the appropriate response. Response production/execution refer to those processes involved in the subsequent selection and execution of the response. Processes that affect stimulus classification will alter both P300 latency and RT. However, later

response-related processes (including response bias-confidence, production and execution) will affect RT, but not P300 latency (Campbell, 1985; Donchin & Coles, 1988).

The P300 component of the auditory ERP is considered to be an attention-dependent phenomena. If the subject ignores the train of auditory stimuli or fails to detect the target stimulus, P300 is not elicited. For this reason, P300 has been claimed by many researchers to reflect some aspect of conscious detection or "awareness" of the target stimulus (Picton, 1992). An earlier positive peak, "P3a" peaking at approximately 250-280 ms, has a prominent frontal or fronto-central distribution (Squires, Squires, & Hillyard, 1975). It can be elicited by highly novel and unexpected stimuli even if the subject is not actively attending to the stimulus. The P3a is thus considered to reflect pre-consciousness. The P3a may well be apparent when subjects are no longer in a conscious state (e.g., during sleep or anesthesia). The longer latency P300 (also called P3b to distinguish it from P3a), has a parieto-central distribution (Picton, 1992). As mentioned, unlike P3a, the amplitude of P300 is highly dependent on the subject's level of attention. Recently, researchers have identified another fronto-central P3 elicited by novel stimuli in attentive subjects (Fabiani & Friedman, 1995; Spencer, Dien, & Donchin, 1999). This P3 occurs much later than P3a, at about the same time as the parietal P3b. It has thus been labelled the "novel-P3" to distinguish it from the earlier frontal P3a and the parietal maximum P3b.

Auditory ERP paradigms have recently been employed to investigate memory comparison /stimulus classification capabilities during the transition to sleep and throughout the various stages of sleep (Ogilvie, Simons, Kuderian, MacDonald, & Rustenburg, 1991; Harsh et al., 1994; Niiyama, Fujiwara, Satoh, & Hishikawa, 1994). Ogilvie and colleagues (1991) were among the first to investigate the convergence of EEG, ERP and behavioural measures during the loss of

consciousness associated with the transition from wakefulness to sleep. They did not employ an odd-ball task. Rather, a single low intensity tone was presented at a very slow rate, every 30 seconds. A relatively long five second duration stimulus was employed. Such long duration stimuli may be intrusive and therefore disrupt the sleep onset process (Campbell et al., 1992; de Lugt et al., 1996). The study by Ogilvie et al. (1991) was unique in that they employed RT latency to the stimulus to define arousal, rather than the usual sleep scoring methods which apply electrophysiologic criteria. Data were sorted into different RT "bins", in which bins 1-4 represented fastest to slowest RTs, and a fifth bin included responses failures. Another unique feature of this study was that subjects were repeatedly awakened. Data were collected over five sleep onset periods. This repeated sleep onset paradigm increased the number of trials available for analysis since the duration of stage 1 is quite short. The small amplitude ERPs require the averaging of a large number of trials in order to reduce the much higher amplitude of the random background EEG. The results of this study indicated that changes in ERP amplitude were related to decreasing behavioural responsiveness. More specifically, as RT lengthened, both N1 and P300 amplitudes decreased. P2 amplitude increased as RT slowed, but the difference was not significant. These data provide evidence to support a loss of consciousness associated with behaviourally-defined sleep onset. No ERP latency changes were reported. In spite of the fact that an odd-ball paradigm was not employed in this study, the authors suggest that P300 may have been elicited because of the unpredictable nature of the stimulus presentation (i.e., 30 seconds between tones). Fitzgerald and Picton and (1981) have indicated the amplitude of P300 may be more dependent on the probability of the rare stimulus in time, rather than its probability relative to the standard stimulus (temporal versus sequential probability respectively). Thus, a

single, repetitive stimulus can elicit a P300 if it occurs very infrequently in time.

Harsh et al. (1994) did employ an odd-ball stimulus presentation paradigm to investigate the effects of task relevance on ERPs during sleep. They sorted trials on the basis of electrophysiologic sleep stages, rather than the behavioural speed of responding. Sleep periods were categorized into stage 1A and B, and stage 2A and B on the basis of the EEG. Subjects were separated into either "attend" or "ignore" groups. In the attend group, subjects responded upon detection of the rare ($p = .20$), target stimulus using a "finger-lift" response method. In the ignore group, subjects were simply instructed to ignore all tones. Stimuli were presented during a single sleep transition period until 10 minutes of stage 2 sleep had been reached. There was thus a limited amount of stage 1 data for ERP analysis. Harsh endeavoured to investigate the ERP data through the binning of RTs, similar to the method employed by Ogilvie et al. (1991). This analysis was however carried out in only one subject because of insufficient trials. Across all subjects, the percentage of detected target stimuli diminished from wakefulness, to stage 1A and stage 1B (83.4%, 64.7%, and 29.9% respectively). A large amplitude parietal maximum P300 was recorded in wakefulness and stage 1A to detected target stimuli in the attend condition. It was substantially smaller in stage 1B. P300 latency was significantly prolonged in stage 1A (405 ms) compared to wakefulness (369 ms). RT was also significantly delayed. The authors interpret the delay in both P300 latency and RT as reflecting a delay in the time required for stimulus classification (the time required to classify the present stimulus as standard or target). In the ignore condition, a much smaller and later parietal positivity was apparent. This may have been because subjects were unable to ignore the stimuli. Stage 2 sleep was characterized by the presence of a large P2 and a sleep-related set of waveforms, the N350-P450. The target-specific

P300 was not apparent in either stage 2A or 2B.

Both Ogilvie et al. (1991) and Harsh et al. (1994) employed a limited electrode array. Ogilvie et al. (1991) reported ERP data recorded from a single site (C4), whereas Harsh et al. (1994) recorded from midline sites (Fz, Pz, Cz). A "true" P300 should have a parieto-central scalp distribution, being reduced in amplitude over anterior and lateral sites. Recently, Spencer et al. (1999) employed a dense 128 electrode array to investigate the effects of novel stimuli on ERPs in wakefulness. They argued that such a dense electrode array may reveal differences not visible with only a limited number of electrodes. The topographic characteristics of ERPs during sleep onset have been investigated (Niiyama et al., 1994). However, the duration of recording was limited to a single sleep onset period. Niiyama et al. (1994) recorded the EEG from 21 scalp sites employing an odd-ball paradigm in which target stimuli were delivered on 20% of trials. Although the EEG was recorded from a relatively large number of sites, the authors relied on a descriptive level of analysis of the P300 scalp distributions in wakefulness compared to stage 1. Actual statistical analyses were not carried out. Subjects were instructed to respond to stimuli as they fell asleep by pressing a hand-held button switch. The waking P300 peaked parietally at 351 ms. Subjects continued to detect most (93.4%) of the targets in early stage 1 (stage 1a). The P300 in stage 1a was delayed in latency (405 ms) compared to the waking state, but had the same parieto-occipital distribution. In deeper stage 1 sleep (stage 1b), responses were made on only 1.5% of trials. A low amplitude P300 (at 356 ms) was apparent in 6 of 8 subjects. The P300 in stage 1b sleep was apparent in spite of the lack of behavioural response. The response rates in stage 1 were nevertheless substantially different from those reported by Harsh et al. (1994). This may be explained by the different methods employed for classification of stage 1a and stage 1b.

Harsh et al. (1994) marked stage 1a by the presence of 50-80% alpha waves in the epoch, and stage 1b by the disappearance of alpha (i.e., consistent with Rechtschaffen and Kales' original definition of stage 1). Niiyama et al. (1994) classified stage 1A on the basis of the disappearance of alpha waves, and stage 1B on the appearance of vertex sharp waves (2-4 Hz).

Bastuji, García-Larrea, Franc, and Mauguière, (1995) also examined scalp topography (19 sites) using an odd-ball paradigm (deviant = .10) in sleep. Again, only a description rather than a statistical analysis of the differences in the scalp distribution was provided. The actual transition from wakefulness to sleep was not systematically examined although ERPs in stage 1 were reported. In wakefulness prior to sleep, subjects were asked to keep a running mental count of target stimuli. However during stage 1 and subsequent stages of sleep, no behavioural task was required. The disadvantage of this is that the experimenter cannot determine precisely when targets are detected. There is thus no independent criteria for consciousness. Moreover, since the experimenter does not know when the subject actually detects the target, the resulting ERP will be an average of both detected and undetected targets. Previous studies have indicated that hit rates can be quite variable in stage 1 (Ogilvie & Wilkinson, 1984, 1988; Cote & Ogilvie, 1994). In stage 1 sleep, P300 was attenuated in amplitude and prolonged in latency compared to the waking state (438 and 344 ms respectively). It is of course possible that the delay in P300 could be due to the averaging of detected and undetected targets. P300 in stage 1 was maximum at Pz. Its scalp distribution was however less widespread (i.e., not visible at frontal sites). It is not clear however whether the absence of a frontal positivity was unique to stage 1 or was again due to the averaging of detected and undetected targets. There was no evidence of a P300 in non-REM sleep.

In the present study, ERPs were sorted by both RT and sleep stage. Subjects were asked to button press throughout the sleep onset period. To increase the amount of stage 1 data available for analysis, the Ogilvie et al. (1991) method of repeated sleep onset periods was employed. In addition, a multiple channel electrode array was employed, as in the Niiyama et al. (1994) and Bastuji, et al. (1995) studies, in order to examine possible changes in the topographic distribution of the P300 recorded in stage 1 and wakefulness. Unlike previous studies, a statistical analysis of the scalp distribution was performed. Thus, both the signal-to-noise ratio of the ERP and the spatial resolution has been improved from previous studies. In addition, the use of a behavioural response measure allowed for a comparison of stimulus classification and response production/execution processes. ERPs were averaged according to RT bins to further investigate the effects of the slowing of apparent decision-making on P300.

Methods

Subjects

Ten self-reported "good sleepers" (6 female, mean age = 23.7, $SD = 5.8$) spent a single night in the sleep laboratory. They had no reported history of neurologic or psychiatric disorders. Hearing thresholds were verified to be below 15 dB ISO at 500, 1000, 1500, and 2000 Hz in both ears. Subjects were asked to refrain from alcohol and caffeine use on the evening of the study. All participants signed informed consent prior to screening and data collection procedures. Volunteers were provided a \$25 honorarium for their participation in the study. The study was conducted in accordance with ethical guidelines of the Medical Research Council of Canada.

Procedure

Subjects arrived at the sleep laboratory two hours prior to their regularly scheduled bedtime. Following the electrode application procedure, subjects participated in a practice session. A behavioural response device was secured to the subject's preferred hand using a soft elastic bandage. Button responses were made using the thumb. Subjects were instructed to lie supine with eyes-closed and respond to "target" tones by pressing the hand-held button. Following the practice session, the lights were closed and participants were permitted to fall asleep. Stimuli were presented throughout the sleep onset period and continued into stage 2 until a maximum of 600 trials was reached. Subjects were immediately woken if they moved into slow-wave sleep. In order to increase the number of trials available for signal averaging, subjects were repeatedly woken (10-15 times) and then permitted to return to sleep. This procedure yielded 6000-9000 trials per subject. The number of repetitions varied from subject-to-subject since not all subjects were actually able to sleep within each block of trials. This assured a relatively constant number of sleep onsets for each subject.

Physiological Recording

Electroencephalogram (EEG) and electro-oculogram (EOG) were recorded using tin electrodes. The EEG electrodes were mounted in an elastic electrode cap, which was secured to the subject's head using a chin strap. The EEG was recorded from 29 scalp sites according to the American Electroencephalographic Society's modified 10-20 system of electrode placement (see Pivik, Broughton, Coppola, Davidson, Fox, & Nuwer, 1993). Electrodes were placed over pre-frontal (Fp1, Fp2), frontal (Fz, F3, F4, F7, F8, F9, F10), pre-central (FC3, FC4), central-temporal (Cz,

C3, C4, T7, T8), pre-parietal (CP3, CP4), parietal (Pz, P3, P4, P7, P8, P9, P10), and occipital (O1, O2) sites. In addition, the left and right mastoids (M1 and M2) were recorded as active sites. All EEG electrodes were referenced to the tip of nose. Vertical eye movements were recorded from the supra- and infra-orbital ridges of the left eye. Horizontal eye movements were recorded from the outer canthus of each eye. Electrophysiological signals were digitized continuously at a sampling rate of 128 Hz using an InstEP systems 12-bit A/D converter and stored on hard disk. The EEG and EOG signals were recorded using a high frequency filter set at 30 Hz. The time constant was 2 s.

Auditory Stimuli

Auditory stimuli were delivered binaurally via insert earphones. Stimuli were synthesized using an InstEP Systems 16-bit waveform generator card. An odd-ball paradigm was employed.

"Standard" 70 dB SPL 1000 Hz tone pips having a total duration of 55 ms and a rise-fall time of 5 ms were presented on 96 % of trials. On the remaining 4 % of trials, a 1500 Hz "target" was presented. The order of presentation was pseudo-random, such that there were between 20 and 30 standard tones between target presentations. This probability of stimulus occurrence was chosen to permit sorting and averaging of a large number of standard stimuli occurring prior to detected and undetected target stimuli. Subjects were instructed to attempt to button press upon detection of the rare target stimulus during the sleep onset period. The stimuli were presented in repeated blocks of 600 trials.

Data and Statistical Analysis

Sleep staging was carried out using central and occipital EEG leads. ERPs were averaged for all 29 scalp sites. Sleep scoring was conducted by two independent raters using standard scoring procedures (Rechtschaffen & Kales, 1968). Inter-scorer agreement was above 90%. In the case of stage scoring ambiguity, trials were excluded from analysis. The continuous EEG was classified as being either stage wake, 1 or 2. A 15 second scoring epoch was used for scoring purposes.

The EEG data were later reconstructed off-line into discrete trials or "sweeps". The sweep began 100 ms prior to stimulus presentation and continued for 900 ms after it. Data were rejected if either the EEG or EOG exceeded $\pm 100 \mu\text{V}$, effectively removing artifact which results from large eye blinks and/or movement. Subjects frequently move and change body position during the sleep onset period. For this reason, a number of trials were lost due to artifact (as many as 40% in some subjects). ERPs were digitally filtered in the frequency domain using a low-pass filter set at 15 Hz. Trials were subsequently sorted and averaged by sleep stage and by reaction time to the target stimuli. RTs to the target were sorted into 3 different bins representing fast, medium and slow RTs. A fourth bin was employed for response failures. These RT bins were calculated separately for each subject to account for individual differences in response times. The three response bins were divided such that there was a relatively equal number of trials per bin. RTs are not normally distributed. RTs may be skewed since there are many more fast and medium than slow RTs. For this reason, the following algorithm was applied: (1) Fast RTs (bin1) included responses that were less than -0.5 SD from the mean; (2) medium RTs (bin 2) included responses that were -0.5 to + 0.25 SD about the mean; (3) slow RTs included responses greater than 0.25 SDs above the mean.

For the purpose of this thesis, only the ERPs to the target stimuli were quantified since only these will contain a possible P300. The peaks of N1 and P2 were identified in the grand average ERP waveform at Cz, where they were maximum. The amplitudes of N1 and P2 were measured for individual subjects relative to pre-stimulus baseline (the average of all data points in the 100 ms period before stimulus onset). N1 was measured as the maximum negative-going peak between 75 and 125 ms following stimulus onset. P2 was measured as the maximum positive-going peak between 175 and 250 ms following stimulus onset. One way repeated ANOVAs were run on the N1 and P2 amplitude and latency data to compare differences between the waking and stage 1 states when subjects detected the targets. A second one-way ANOVA was run on the N1 and P2 data when subjects failed to detect the target in stage 1 and stage 2.

A primary purpose of this experiment was to compare the changes in the amplitude of P300 during the sleep onset period. There are a number of methodological problems in determining if changes in P300 are statistically significant. Previous research has indicated that P300 is not elicited when subjects fail to detect the target stimulus. Since overt target detection is rare in stage 2, it was expected that no P300 would be discernible. In stage 1, a P300 was expected to be observed when subjects signalled their detection of the target. It is also possible that a P300 will be elicited even if subjects fail to actually respond. This of course assumes that subjects can discriminate the target from the standard, but for whatever reason, fail to button press. The hit rates were expected to be near 1.0 in the waking state.

P300 was initially identified in the Pz grand average waveform. Its amplitude was then quantified for each subject using a time interval that extended ± 100 ms around the peak latency of the grand average. When no P300 was apparent in the ERP waveform (in stages 1 and 2), the

latency was selected on the basis of the grand average latency of P300 during stage 1 for trials on which the target was detected. This arbitrary measurement of perhaps background noise was necessary so that the P300 following the detected and undetected targets could be compared statistically. Differences in the amplitude of "P3" between detected and undetected targets in stage 1 were determined using a one-way repeated measures ANOVA. Differences between stage 1 and 2 were also determined using the ANOVA procedure.

Comparing the P300 in the waking and stage 1 states is more difficult. This is because the scalp distribution of P300 also needs to be taken into consideration. Comparison of scalp distribution data is fraught with statistical problems (Picton et al., 1995). In the present study, the scalp distribution data were initially displayed as "maps". The scalp voltage distribution data were graphically illustrated as two-dimensional spherical spline potential maps using the Perrin, Pernier, Bertrand, and Echallier (1989) computational method. All data points within ± 10 ms of the peak amplitude of P300 (measured at Pz) were averaged at all 29 electrode sites. Inter-electrode space was filled with derived (or "interpolated") values. The choice of the reference site has important consequences on voltage distribution maps. An inactive reference site provides the best representation of the EEG signal. No reference is, however, truly inactive. The nose was used as a reference in the present study. It is nevertheless still somewhat active, particularly for possible frontal lobe source dipoles. Although other reference sites have been employed in the literature, none can be demonstrated to be truly neutral. Scalp distribution maps may vary simply because of the choice of the reference. A so-called "averaged" reference is often used to overcome this confound. The averaged reference is computed, as its name implies, by averaging the voltage across all electrode sites (and this should approximate zero voltage). This averaged

voltage is then subtracted from each of the active electrode P3 values. Since the resultant values will be identical regardless of the actual reference, this technique is also called "reference-free" recording (Lehmann & Skrandies, 1980; Bertrand, Perrin, & Pernier, 1985).

Maps provide an efficient means to illustrate similarities and differences in scalp distribution data. However, the maps provide only a description of the data and as such are not a statistical analysis. Although "statistical mapping" procedures have been employed in the clinical literature to compare different maps, there are serious limitations. Perhaps the most notable is the fact that most of the data in a particular map are based on derived (or predicted) rather than actual measured data. It is inappropriate to attempt to carry out a statistical analysis on nonexistent data. A method gaining popularity is to statistically compare actual data and then to derive topographical maps of the probability of significance on the inter-electrode space (Hassainia, Petit, & Montplaisir, 1994).

In this study, a somewhat different approach was taken. The usual approach to compare scalp differences between wakefulness and stage 1 would be to run a two-way ANOVA (electrode site x stage). Changes in scalp distribution would be evaluated as an interactive effect. One of the assumptions of the ANOVA model is that of additivity -- experimental effects add a constant to a baseline condition. Thus in this study, the ANOVA model assumes that stage 1 "effects" result in a constant being added to (or subtracted from) the waking P300 at all electrode sites. With EEG data, experimental effects often result in a constant being multiplied (rather than added) to the scalp data. McCarthy and Wood (1985) have proposed a method to circumvent this problem by scaling and normalizing scalp distribution data in different conditions when an initial significant condition x electrode interaction is found. The maximum peak amplitudes of P300

were scaled to eliminate differences between wakefulness and stage 1. The data were then normalized in each stage by computing the maximum and minimum values of each peak over the scalp for each subject. The minimum values were then subtracted from the amplitude at each electrode site and then divided by the difference between the maximum and minimum. All amplitude values were thus scaled to be between 0 and 1.

The McCarthy-Wood correction method is employed if a significant electrode site x experimental effect interaction is found. In the present study to maximize the likelihood of finding significance, separate one-way repeated measures ANOVAs were run on the Fz and Pz P300 amplitude data. The alternative would have been to run a two-way electrode site x stage ANOVA using all electrode sites. The single two-way ANOVA procedure is much more conservative than separate one-way ANOVAs. Since there is evidence in the literature of anterior-posterior scalp differences at sleep onset (Bastuji, et al., 1995), the use of more liberal procedures is justified. If one of the one-way ANOVAs was significant but the other not, this would be considered to be equivalent to a significant interaction. An overall two-way ANOVA with repeated measures on the 29 sites and stage (wake, stage 1) was then run on the *normalized* data. Thus the result of the liberal use of the one-way ANOVA procedure was that a further corrected/normalized study of possible significant scalp differences would be investigated. An assumption of the use of repeated measures ANOVA is that of sphericity. Greenhouse-Geisser and Hyunth-Feldt correction procedures may be applied to compensate for violations of this assumption. The Hyunth-Feldt procedure was employed in this study since it is more appropriate for small sample sizes.

Results

The transition from wakefulness to stage 1 occurred on average within 3.5 minutes ($SD = 3.1$) of stimulus presentation. There was no significant trend toward shorter sleep onset latencies across the repeated sleep onset attempts. The data from all sessions were therefore collapsed for subsequent analyses in order to increase the number of trials available for signal averaging.

Behavioural Data

Subjects detected almost all targets while awake ($M = 94.4\%$). During stage 1, subjects continued to detect 47.0% of the targets, although there was high inter-subject variability in detection rate, ranging from .04 to .78. There were few overt detections in stage 2 (fewer than 3%). The mean RT for behavioural responses was significantly faster in the waking state ($M = 746$, $SD = 236$ ms), compared to stage 1 ($M = 895$, $SD = 239$ ms), $t = -3.68$, $df = 9$, $p < .01$.

Event-Related Potential Data

Detected Targets in Wakefulness and Stage 1 Sleep

Only six subjects were included in this analysis due to an insufficient number of response trials in stage 1 for some subjects. Figure 1 illustrates the grand average waveforms for detected targets (bins 1-3 collapsed) in wakefulness and stage 1. Table 1 presents the amplitude and latency values of N1, P2, P3 at Fz, and P3 at Pz in wakefulness and stage 1 sleep.

————— Insert Figure 1 and Table 1 about here —————

The amplitudes of N1 and P2 did not significantly vary between wakefulness and stage 1 when subjects detected the target ($F_s \leq 1.83$). P2 latency was significantly delayed in stage 1 compared to the waking state, $F(1,5) = 9.49$, $p < .05$. A large amplitude P300 was apparent to the detected target tones in wakefulness, and remained large to the *detected* targets in stage 1 "sleep". At Pz, the amplitude of P300 did not differ significantly, $F(1,5) = 1.41$. The amplitude of P3 at Fz ("P3a") however was significantly larger in wakefulness compared to stage 1, $F(1,5) = 8.44$, $p < .05$. Although the latency of P300 was somewhat delayed at Pz in stage 1 compared to wakefulness, the difference was not significant ($F < 1$).

Because of the frontal-parietal differences in wakefulness and stage 1, the scalp distribution of P300 was examined further. Figure 2 illustrates iso-contour maps of P300 to detected target stimuli in wakefulness and stage 1 sleep. Both maps show a parietal maximum peak in the latency range of the P300. The waking positivity extended much more anterior than that observed in stage 1. While the maximum of P3 was focussed around Pz in the waking state, in stage 1 the maximum voltage extended over lateral parietal and occipital regions. Since a significant interaction was found between scalp site and stage (i.e., P300 did not vary at Pz but was significantly attenuated at Fz in stage 1 compared to wakefulness), the scalp data were therefore scaled using the McCarthy-Wood correction procedure. A two-way ANOVA with repeated measures on electrode site (29 channels) and stage (wake and 1) was run on the normalized data. Hyunth-Feldt correction procedures were employed on the interaction term. The electrode site by stage interaction was significant, $F(11,56) = 2.17$, $p < .05$. During stage 1, P300 was significantly attenuated at Fz, F3, and F4 compared to the waking state. Differences were not significant elsewhere.

----- Insert Figure 2 about here -----

Detected Versus Undetected Targets in Stage 1 Sleep

A failure to respond to the target is considered to reflect a behavioural measure of unconsciousness and sleep onset. ERPs for detected and undetected targets within stage 1 were therefore compared. ERPs to detected targets were illustrated in Figure 1, while those to undetected targets are illustrated in Figure 3.

----- Insert Figure 3 about here -----

The amplitude and latency values for N1, P2, P3 at Fz, and P3 at Pz for detected and undetected targets in stage 1 are presented in Table 1. There was no significant change in N1 or P2 latency between detected and undetected targets ($F_s \leq 1.21$). Although N1 was attenuated following undetected targets, the difference was not significant, $F(1,5) = 2.69$, $p > .05$. The amplitude of P2 was significantly larger for the undetected targets, $F(1,5) = 14.04$, $p < .01$. No P300 was evident in the ERP waveforms when the targets were undetected (see Figure 3). It was significantly attenuated in amplitude when subjects failed to respond, $F(1,5) = 27.65$, $p < .01$.

Undetected Targets in Stage 1 and Stage 2 Sleep

All 10 subjects were included in this analysis because there was ample non-response data in stages 1 and 2 sleep. Figure 3 illustrates the ERP waveforms following targets in both stages 1 and 2. The ERPs to undetected targets are essentially identical at all scalp sites in stages 1 and 2.

Table 2 presents the means and standard deviations of latency and amplitude values. There were no significant differences in N1 or P2 latency or amplitude following undetected targets in stage 1 versus stage 2 sleep ($F_s \leq 3.71$). As may be observed, the mean amplitude values of the P300 are quite small, some being negative in polarity.

----- Insert Table 2 about here -----

Wakefulness by RT Bin

Eight subjects were included in the analysis of ERPs in wakefulness sorted by RT bin. The ERPs are illustrated in Figure 4 and the amplitude and latency values are presented in Table 3.

----- Insert Figure 4 and Table 3 about here -----

N1 amplitude decreased while P2 amplitude increased from the fastest to the slowest RT bins, but the differences were not significant ($F_s \leq 2.46$). P300 amplitude remained large in all three RT bins and did not significantly vary from bin 1 to 3 ($F < 1$). P300 latency was significantly delayed as RT lengthened, at both Pz, $F(2,14) = 5.11$, $p < .05$, and at Fz, $F(2,14) = 3.68$, $p < .05$. P300 morphology was somewhat different in the slowest RT bin. It appeared to be double peaked compared to the single peak in bins 1 and 2. The peak latency values at Pz were 362, 371, and 387 ms for bins 1 through 3 respectively (i.e., fast to slow RTs). Similarly, the latency values at Fz were 331, 338, and 359 respectively. The differences in mean RT for each bin were much larger -- 572, 752, and 1084 ms respectively.

Stage 1 by RT Bin

There was an insufficient number of trials in stage 1 across all RT bins to permit reliable averaging within each subject. Descriptive data based on the grand averages are therefore provided in Table 4 for five subjects in bin1, seven subjects in bin2, and nine subjects in bin3. Statistical comparisons between these groups were not performed. P300 amplitude at Pz remained large within stage 1 sleep, despite variation in RT (see Figure 4). The frontal P3 was however much more variable. It became increasingly attenuated as RT slowed. Moreover, at Pz, P300 morphology was variable within each of the RT bins. P300 peak latency was relatively invariant across RT bins within stage 1 sleep. The mean RT in stage 1 was 604, 750, and 1033 ms for bins 1 through 3 respectively.

----- Insert Table 4 about here -----

Discussion

When subjects were awake, hit rates were high and overall mean RT was rapid. In stage 1, subjects' hit rate fell slightly below 0.50 while RTs were prolonged. In stage 2, subjects detected very few of the targets. Thus in terms of consciousness, subjects were almost always able to signal their awareness of the target in the waking state, and almost never in stage 2 sleep. The few detections made in stage 2 may have represented sleep stage mis-classification (recall that staging was based on the average EEG in a 15 second epoch, during which time 10 stimuli might be presented).

When subjects detected the target stimuli, there was no difference in P300 amplitude over posterior areas of the scalp between wakefulness and stage 1. In stage 1 “sleep”, the parietal P300 remained large to detected targets. This is in agreement with Ogilvie et al. (1991), Harsh et al. (1994), and Niiyama et al. (1994), who also reported that subjects were able to maintain detection of target stimuli during stage 1, even though hit rates typically declined. Subjects are thus at least partially able to maintain consciousness in stage 1. Although there was no difference in P300 amplitude over posterior sites in wakefulness and stage 1, significant differences were apparent at anterior sites. P3 (at Fz) was significantly larger in wakefulness compared to stage 1. This was mainly due to attenuated P3s when RT slowed (bins 2 and 3). ERP differences across the various bins in stage 1 do however need to be interpreted with caution since there was an insufficient amount of data to permit statistical comparison. An attenuated P300 at frontal sites was also reported by Bastuji et al. (1995). This may be due to frontal lobe deactivation associated with the cortical slowing of stage 1 “sleep” (Broughton & Hasan, 1995; Wright, Badia, & Wauquier, 1995). Perhaps the large parietal P300 in stage 1 indicates that subjects are able to detect the target stimulus. The attenuation of the frontal P3 may mean that any contribution to consciousness from the frontal region wanes during the transition to sleep. The frontal lobes may provide the “subjective experience” of the detected stimulus.

A failure to detect target stimuli marks “behavioural sleep onset”. Since subjects continued to respond on almost half of the trials within stage 1, it appears that behavioural sleep onset occurs at a different time than EEG measures have traditionally indicated. This is one of the reasons why many authors now subdivide stage 1 sleep. ERPs permit comparison between behaviourally-defined wakefulness (i.e., responding) and behaviourally-defined sleep (i.e.,

response failures) within a single stage of sleep. In stage 1, N1 amplitude was smaller and P2 amplitude was larger for the undetected targets compared to the detected targets. This is consistent with ERP studies which indicate that P2 increases in amplitude in non-REM sleep compared to wakefulness (Noldy et al., 1988; Campbell et al., 1988; Harsh et al., 1994; de Lugt et al., 1996; Elton et al., 1997; Cote et al., in press - b). Campbell et al. (1992) suggest that this increase in amplitude of the positive wave, P2, and decrease in the amplitude of the negative wave, N1, is due to the removal of a long-lasting negative wave during sleep. This slow negative wave, labelled "Processing Negativity" (Näätänen, 1982) overlaps and summates to the N1 and P2 components during wakefulness. Processing Negativity is thought to reflect the additional processing that is received by attended stimuli. The changes in the N1-P2 vertex complex in stages 1 and 2 therefore might reflect inhibition of information processing.

Also within stage 1, P300 was not visible when subjects failed to detect targets compared to when they actively signalled detection of the stimulus. Furthermore, ERPs did not differ between stage 1 when subjects failed to detect targets and stage 2 when there were very few detections. Importantly, for the purpose of this thesis, when there was evidence of awareness of the rare target stimulus (i.e., subjects signalled their detections), a large P300 was apparent over parietal regions in both the waking state and in stage 1. When there was no evidence of awareness (i.e., subjects failed to signal their awareness of stimuli), no P300 was elicited by the rare target. A failure to observe P300s to undetected targets has also been reported during general anaesthesia (Plourde & Picton, 1991; Plourde, Joffe, Villemure, & Trahen, 1993; van Hoof, De Beer, Brunia, Cluitmans, Korsten, Tavilla, & Grouls, 1995). Thus, whether unconsciousness occurs naturally through sleep processes or is induced through anesthetics, P300 is not elicited by

the rare target stimulus.

Ogilvie and colleagues (1991) demonstrated that RT becomes progressively prolonged during the sleep onset period. The source of the variation in RT was however not explained. Harsh et al. (1994) reported RTs that were prolonged by 250 ms in stage 1a compared to wakefulness. This is quite similar to the RT delay noted in the present study. Harsh et al. (1994), Niiyama et al. (1994) and Bastuji et al. (1995) reported significant latency shifts in P300 from wakefulness to stage 1 (36 ms, 94 ms and 54 ms delays respectively). In the present study, the P300 latency shift was less than 15 ms. Thus, the largest source of RT variance must occur after stimulus classification processes have been completed. Delays in RT therefore appear to be largely due to response bias, production and / or execution processes. Motivational factors could be considered to be a response bias – the subject's willingness to respond rapidly. Fatigue could, for example, affect the subject's motivation to respond. For example, in the Harsh et al. study (1994), one subject reported an unwillingness to respond early in wakefulness in order to fall asleep. This type of response bias would affect RT but have minimal effect on P300 latency. Another possible explanation for prolonged RT is that subjects might "double check" their decisions before responding. There is some evidence of this in the P300 waveforms. During stage 1, a double-peaked P300 was apparent in the grand average, possibly reflecting additional decisions. It is possible that subjects may make an initial decision about the detection of the target and a secondary decision about how to respond. A double-peaked P300 might also be explained by latency jitter – stimulus classification occurring at various times within and across subjects. This is unlikely to be the case since latency jitter results in an overall reduction in amplitude and broadening of the waveform. P300 amplitude was not reduced at Pz in stage 1.

There was no evidence for the P300 potential in the early stage 2 sleep recorded in the present study. This is consistent with many previous investigations of ERPs during sleep (Paavilainen, Cammann, Alho, Reinikainen, Sams, & Näätänen, 1987; Loewy, Campbell, & Bastien, 1996; Nordby, Hugdahl, Stickgold, Bronnick, & Hobson, 1996). It is possible that processing stimulus deviants which vary in tonal pitch will not elicit the P300 in sleep. Roth, Dorato, and Kopell (1984) report that sufficiently loud stimuli will elicit an obligatory P300 under waking ignore conditions. Subsequent studies in this dissertation will investigate the effects of loud intrusive stimuli on eliciting the P300 in sleep across the entire night.

Table 2.1. Mean amplitude and latency values (SDs in parentheses) of detected targets (Bins 1-3 collapsed) in wakefulness and stage 1, and undetected targets in stage 1.

Stage		LATENCY ¹	AMPLITUDE
Wake - Detected	N1 at Cz	95 (5)	-3.32 (2.08)
	P2 at Cz	154 (7)	1.70 (1.66)
	P3 at Fz	371 (38)	9.70 (5.82)
	P3 at Pz	391 (30)	24.55 (6.83)
Stage 1 - Detected	N1 at Cz	98 (14)	-3.06 (3.07)
	P2 at Cz	171 (13)	4.71 (6.54)
	P3 at Fz	357 (15)	3.23 (7.89)
	P3 at Pz	403 (21)	21.01 (7.05)
Stage 1 - Undetected	N1 at Cz	93 (13)	-0.57 (1.47)
	P2 at Cz	172 (8)	8.54 (6.27)
	P3 at Fz	N/A ²	0.76 (6.29)
	P3 at Pz	N/A	4.03 (9.77)

Note: the grand average waveforms (n=6) contained 415 trials in wakefulness to detected targets, 109 trials in stage 1 to detected targets, and 78 trials in stage 1 to undetected targets.

¹ The sampling rate of the A/D converter was 128 Hz. The maximum precision of latency values is therefore 1/128 s (7.8 ms). The latency values presented here and throughout the thesis however represent the mean of all subjects, rounded to the nearest millisecond.

² In stage 1, when targets were not detected, P3 was not discernable in the background EEG. Its amplitude was arbitrarily measured at maximum peak latency in the grand average for all subjects.

Table 2.2. Mean amplitude and latency values (SDs in parentheses) of undetected targets in stage 1 and 2 sleep.

		LATENCY	AMPLITUDE
Stage 1	N1 at Cz	98 (12)	-2.05 (3.84)
	P2 at Cz	181 (17)	8.03 (4.83)
	P3 at Fz	N/A	-1.66 (6.36)
	P3 at Pz	N/A	2.56 (8.58)
Stage 2	N1 at Cz	104 (6)	-1.08 (2.84)
	P2 at Cz	189 (29)	7.67 (4.96)
	P3 at Fz	N/A	-3.19 (5.54)
	P3 at Pz	N/A	-1.61 (4.99)

Note: the grand average waveforms (n=10) contained 128 trials in stage 1, and 851 trials in stage 2.

Table 2.3. Mean amplitude and latency values (SDs in parentheses) by RT bin in wakefulness.

RT Bin		LATENCY	AMPLITUDE
Bin 1 - Fast RTs	N1 at Cz	91 (16)	-5.42 (3.64)
	P2 at Cz	147 (17)	1.72 (4.09)
	P3 at Fz	331 (25)	8.30 (11.22)
	P3 at Pz	362 (21)	20.69 (12.50)
Bin 2 - Medium RTs	N1 at Cz	100 (14)	-5.28 (3.46)
	P2 at Cz	159 (17)	2.89 (3.80)
	P3 at Fz	338 (21)	5.28 (6.59)
	P3 at Pz	371 (16)	18.56 (10.88)
Bin 3 - Slow RTs	N1 at Cz	98 (16)	-0.57 (4.15)
	P2 at Cz	155 (14)	7.12 (5.62)
	P3 at Fz	359 (24)	10.16 (6.51)
	P3 at Pz	387 (15)	26.02 (11.55)

Note: the grand average waveforms (n=8) contained 225 trials in Bin1, 199 trials in Bin2, and 144 trials in Bin3.

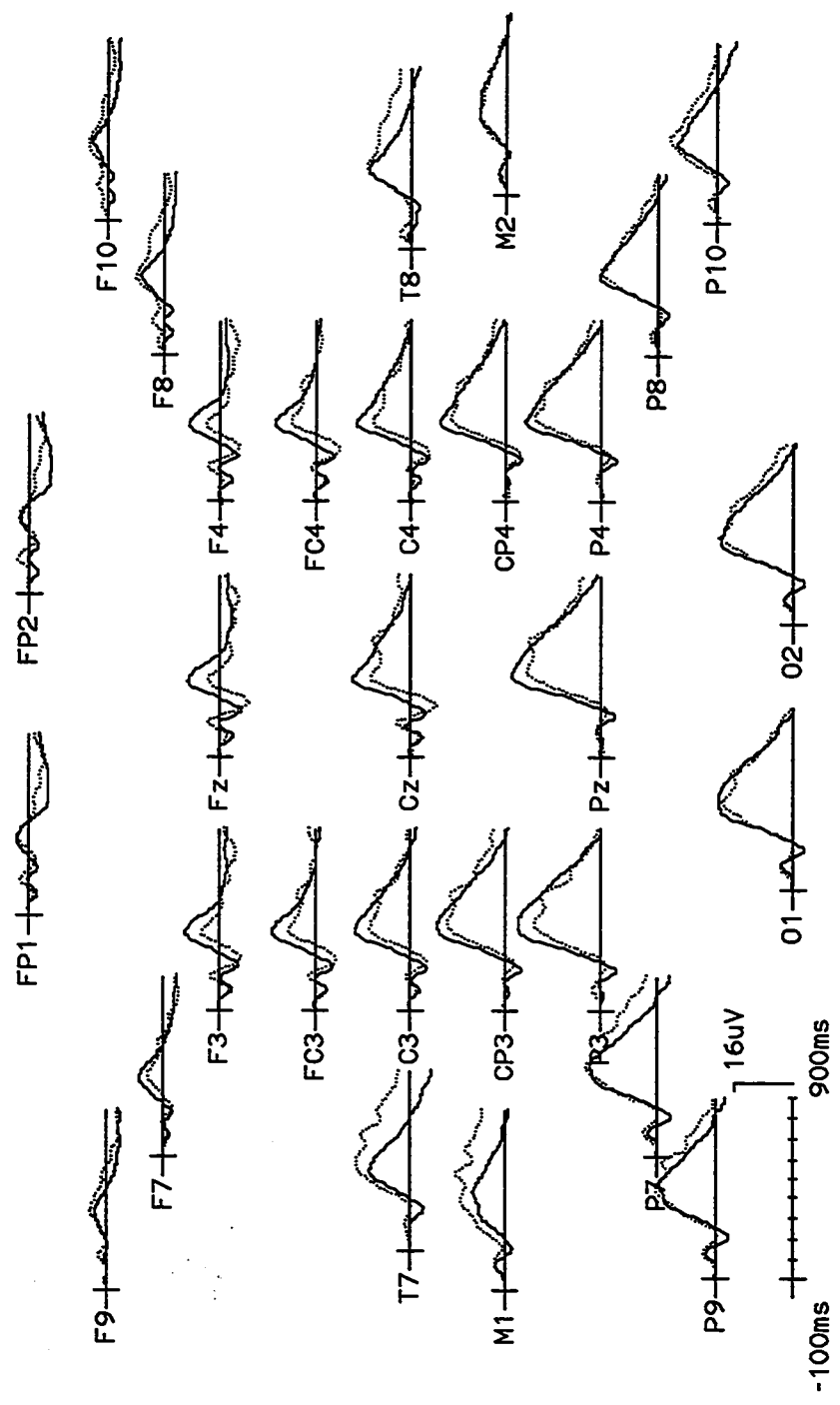
Table 2.4. Mean amplitude and latency values (SDs in parentheses) by RT bin in stage 1.

RT Bin		LATENCY	AMPLITUDE
Bin 1 - Fast RTs	N1	103 (15)	-9.03 (5.86)
	P2	155 (16)	0.77 (10.56)
	P3 at Fz	372 (16)	12.50 (9.77)
	P3 at Pz	400 (49)	29.28 (8.36)
Bin 2 - Medium RTs	N1	98 (13)	-6.62 (9.68)
	P2	166 (21)	10.81 (6.65)
	P3 at Fz	353 (14)	7.76 (8.28)
	P3 at Pz	400 (68)	25.63 (11.33)
Bin 3 - Slow RTs	N1	92 (16)	-6.79 (8.78)
	P2	158 (25)	7.00 (7.76)
	P3 at Fz	363 (44)	4.69 (9.28)
	P3 at Pz	401 (22)	24.69 (22.97)

Note: the grand average waveforms contained 28 trials in Bin1 (n=5), 26 trials in Bin2 (n=7), and 62 trials in Bin3 (n=9).

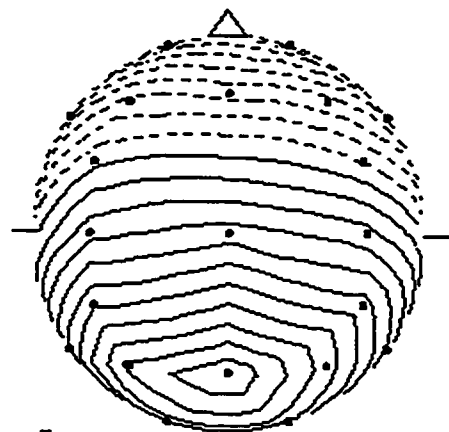
Figure Legends

- Figure 2.1. ERPs to detected targets in wakefulness and stage 1 sleep. The grand average ERPs from 29 sites are illustrated. Positivity of the scalp relative to the reference is shown as an upward deflection in this and all figures. Note that a large amplitude P300 is apparent at Pz in both wakefulness (391 ms) and stage 1 (403ms). P300 is much attenuated at frontal sites in stage 1.
- Figure 2.2. Scalp distribution spherical spline iso-contour maps of P300 following detected targets in wakefulness and stage 1. The maps were computed using an average reference and projected onto 4 different 2-dimensional views of the scalp, from the top, back, left, and right sides. The solid lines represent contours for positive voltage and the dashed lines represent contours for negative voltage. The interval between contours is $1 \mu\text{V}$. The maps were computed over an average of 10 ms surrounding the peak amplitude of P300. In wakefulness, P300 is maximum at Pz decreasing in amplitude at anterior, posterior and lateral sites. In stage 1, the maximum is more widespread.
- Figure 2.3. ERPs following failed detections in stage 1 and stage 2 sleep. The grand average ERPs from 29 sites are illustrated. No P300 is apparent in either stage 1 or stage 2 when the subject failed to signal detection of the target stimulus.
- Figure 2.4. ERPs by RT bin in wakefulness and stage 1 sleep. Bins 1 to 3 (i.e., fastest to slowest response times) are illustrated for midline sites (i.e., Fz, Cz, Pz).

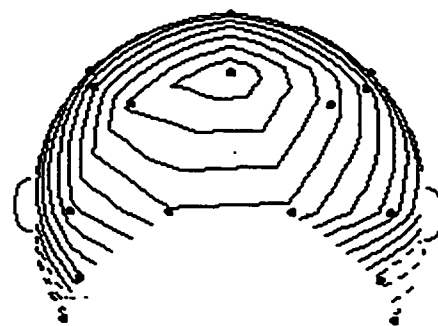


— Stage W
 Stage 1

Stage W



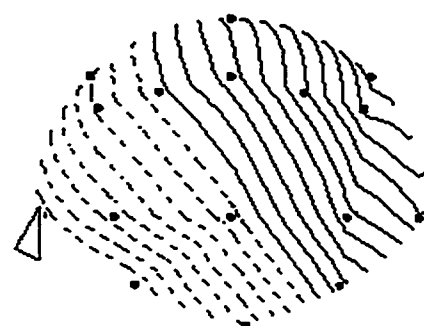
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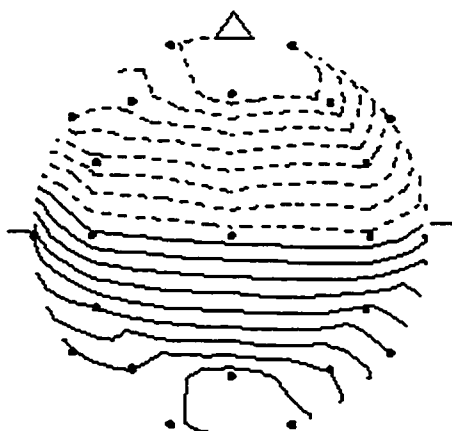


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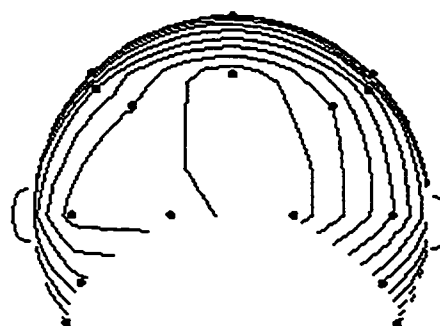


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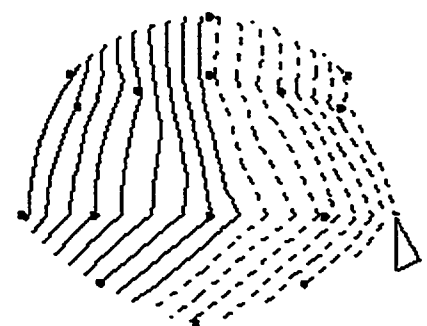
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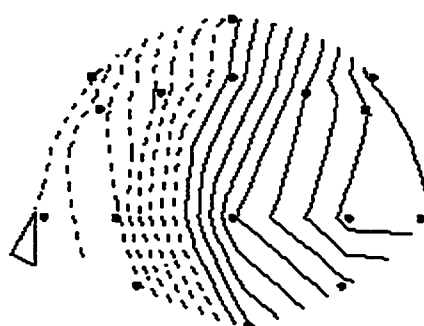
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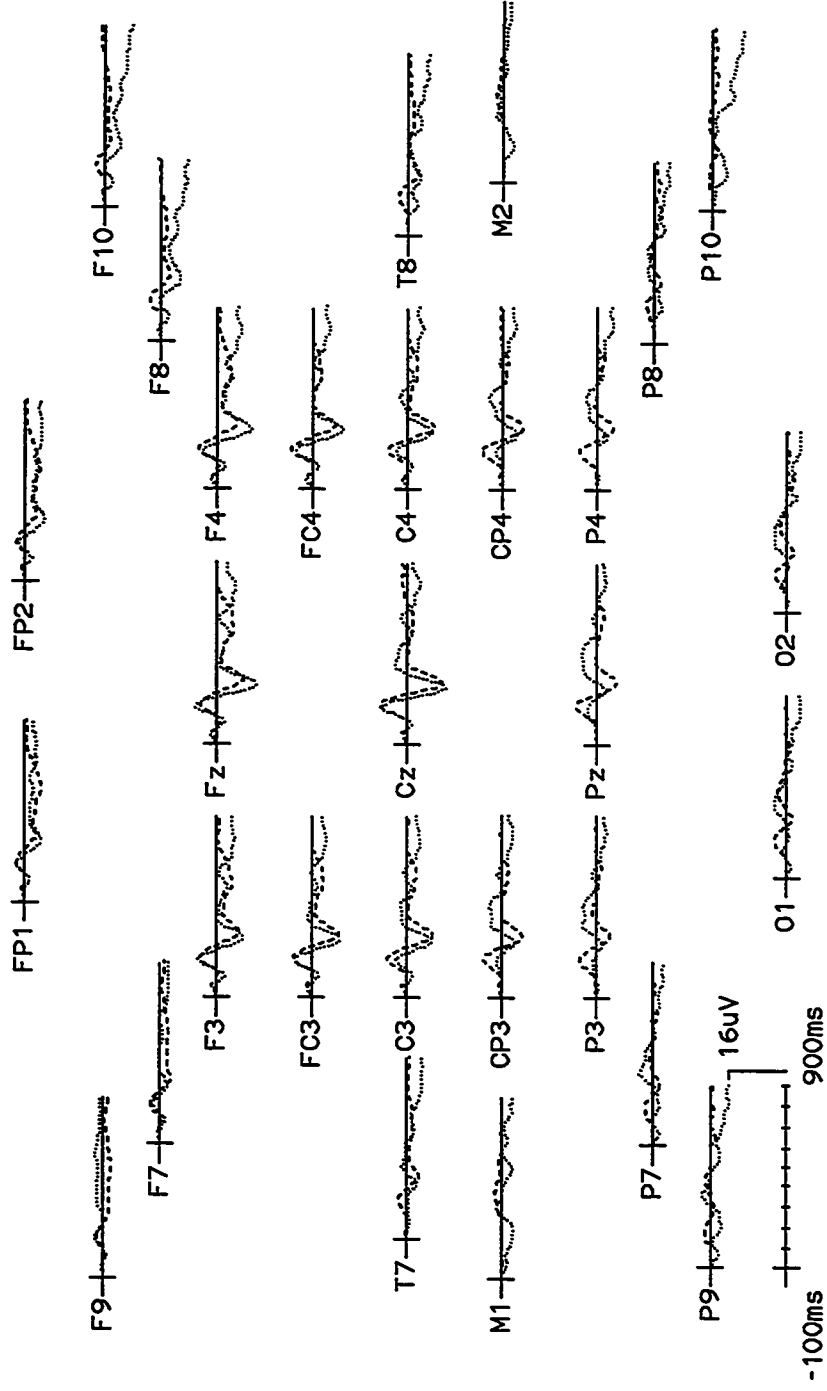
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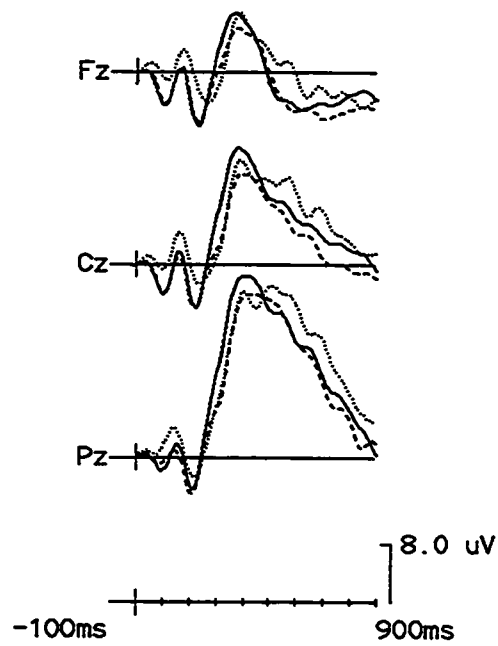


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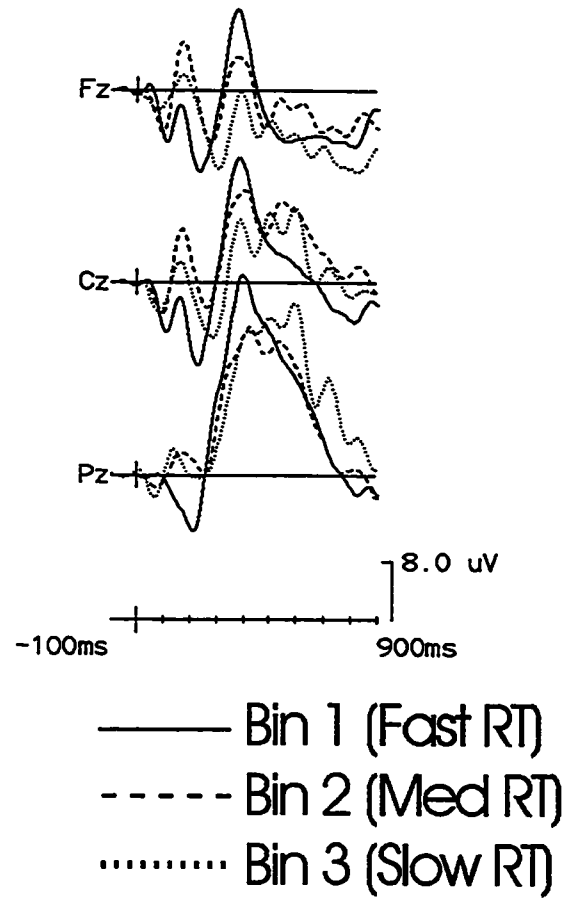


..... Stage 1
 ----- Stage 2

Stage W



Stage 1



Chapter 3**Experiment 2: P300 to High Intensity Stimuli During REM Sleep**

The following manuscript has been published in *Clinical Neurophysiology*, 1999, 110, 1345-1350. The present study endeavored to investigate whether or not a P300 could be recorded in definitive sleep. Although P300 is typically only evoked when the subject attends to the stimulus, it may also be elicited in an awake, inattentive subject if the stimulus is sufficiently intrusive. We therefore employed an oddball task to determine if high intensity stimuli would elicit the P300 during sleep. A loud 90 dB SPL tone pip was presented infrequently ($p = .05$) in a train of lower intensity 70 dB SPL standard stimuli. A multiple channel EEG was recorded in order to map the scalp topography of a possible P300. A large amplitude parieto-central P300, peaking at 321 ms, was apparent in REM sleep following loud deviant stimuli. In stage 2 non-REM sleep, a later positive wave, peaking at 446 ms, was apparent when K-Complexes were absent. This non-REM P450 was however maximum over occipito-parietal areas of the scalp. The presence of a P300 in REM sleep indicates that sensory discrimination capabilities remain intact during this state. This may be associated with either pre- or conscious processing of relevant stimuli.

Introduction

The extent of information processing during sleep remains an issue of considerable debate. A major methodological barrier is that researchers cannot rely on methods such as overt behavioural responses or subjective report to access mental activity in the sleeping subject. Event-related potentials (ERPs) may be employed as a probe of such mental activity. The late components of the auditory ERP, peaking between 50 and 500 ms, are markedly affected by sleep onset and sleep (see Campbell et al., 1992 for review). In general, when baseline-to-peak measurements are employed, a late negative wave "N1" (peaking at about 100 ms) decreases in amplitude, while a later positive wave "P2" (peaking at about 180 ms) increases in amplitude during non-REM sleep relative to wakefulness. During REM sleep, N1 and P2 may return to 25-50% of their waking amplitude.

Some investigators have applied stimulus deviance paradigms to determine whether a late positive wave, "P3", can be recorded during any stage of sleep. The occurrence of this endogenous ERP component in sleep would suggest that selective information processing of the external environment remains possible. In wakefulness, P3 is usually elicited in the so-called "oddball" task, in which subjects are asked to detect an infrequently occurring, odd "target" stimulus occurring among a train of more frequently occurring "standard" stimuli (Sutton et al., 1965). When the target is easily distinguished from the standard, P3, maximum over parieto-central regions of the scalp, occurs at a peak latency of about 300 ms (thus the alternative label "P300"). If the subject fails to detect the target or ignores the stimulus, P300 is usually not elicited. Since P300 is elicited only when the subject actually signals detection of the rare

stimulus, many associate P300 with consciousness (Picton, 1992; van Hooff et al., 1995).

To date, studies investigating the processing of stimulus deviance during sleep have reported inconsistent findings. Some researchers have recorded late positivities in non-REM sleep peaking after 400 ms to rare stimuli that are clearly distinguishable from the standards (Nielsen-Bohlman, Knight, Woods, & Woodward, 1991; Salisbury et al., 1992; Winter, Kok, Kenemans, & Elton, 1995). These waveforms possibly peak too late to be considered a definitive "P300". Also, scalp distribution data have often not been provided. Moreover, both Harsh et al. (1994) and Bastuji et al. (1995) report positive peaks around 450 ms in non-REM sleep following *both* the standard and rare stimuli. Other investigations have not observed P300s to similar pitch deviants during oddball tasks (Paavilainen et al., 1987; Loewy et al., 1996; Nordby et al., 1996). Niiyama et al. (1994) and Bastuji et al. (1995) reported that a P300 could be recorded in REM sleep. In both studies, the P300 was elicited by a rare pitch deviant in an odd-ball paradigm. In the Niiyama et al. (1994) study, the waking and REM P300s had similar latencies (351 and 361 ms respectively) and parieto-central scalp distributions. The amplitude of this positive waveform was nevertheless much attenuated in REM sleep ($3.0 \mu\text{V}$) compared to the waking state ($9.9 \mu\text{V}$). The positive wave in REM reported by Bastuji et al. (1995) was attenuated (again by approximately $7 \mu\text{V}$) but also delayed in latency compared to the waking state (445 versus 344 ms respectively). The late positive wave did however have the usual P300 parieto-central scalp topography.

In awake but inattentive subjects, rare deviants do not typically elicit the parietal P300 during states of inattention (Näätänen, 1990). There is however an exception to this. If the rare stimulus is sufficiently loud, it may intrude into consciousness, and an obligatory P300 will be

elicited (Roth et al., 1982). The present study therefore employed an oddball task in which the rare auditory stimulus deviated in intensity rather than pitch.

Methods

Subjects

Eight young adults (3 female) between the ages 18 and 28 (mean age = 22.5, SD = 3.8) volunteered to participate in this study. Inclusion criteria stipulated that participants be self-reported good sleepers, right-handed, nonsmokers and free from medication. Hearing levels were verified to be within 15 dB at 500, 1000, 1500 and 2000 Hz. Subjects were instructed to abstain from alcohol and caffeine on the evening of the study. Participants signed a consent form and received an honorarium for their participation. This study was conducted according to the guidelines for ethical principles of the Medical Research Council of Canada.

Physiological Recording

The EEG was recorded from 19 tin electrodes mounted in an electrode cap. The EEG was referenced to the tip of the nose. A vertical EOG was recorded between the supra- and infra-orbital ridge of the right eye. A horizontal EOG was recorded from the outer canthi of each eye. Subjects were grounded with an electrode placed in the cap between Fz and Cz (i.e., FCz). Electrode impedances were below 2 kOhms. The amplifier high filter was set at 35 Hz. The time constant was set at 2 seconds. The data were sampled continuously at 256 Hz using a 12-bit A-D

converter. Off-line, a high 15 Hz digital filter operating in the frequency domain (employing an inverse FFT algorithm) was applied to the data.

Procedure

Subjects spent a single night in the laboratory. Auditory presentation began after consolidated sleep onset (i.e., 5 minutes uninterrupted stage 2 sleep) and continued throughout the night. A "standard" 70 dB SPL 1000 Hz tone pip, having a total duration of 52 ms and a rise-and-fall time of 2 ms was presented on 95% of trials. At random, on the remaining 5% of trials, a 90 dB SPL tone pip was presented. These stimuli were delivered in an oddball paradigm, in which an infrequently occurring "target" stimulus occurs among a train of more frequently occurring "standard" stimuli. Stimuli were delivered at a fixed inter-stimulus interval of 1500 ms in blocks of 600 trials. The auditory stimuli were synthesized using an InstEP Systems 16-bit waveform generator card. All auditory stimuli were presented monaurally to the right ear via a modified hearing-aid device. The hearing-aid system assured constancy of auditory input in spite of possible head movements. A Bruel and Kjaer 2209 sound-level meter equipped with a 2 cm³ coupler was used to calibrate the auditory intensities. A minimum of two blocks was presented in each stage of sleep. If the subject showed signs of movement or stage change, the data were rejected from further analysis.

Analyses

Sleep staging was done by an experienced rater using standard Rechtschaffen and Kales (1968) criteria. In cases of stage ambiguity, the epochs were excluded from further analysis. The data

were reconstructed into discrete epochs ("trials") off-line. An epoch began 100 ms prior to stimulus presentation and continued for 1900 ms following it. Trials were sorted and averaged by sleep stage and stimulus intensity. There were an insufficient number of trials to permit reliable averaging of ERPs in slow-wave sleep. Stage 2 data were further sorted by halves of night (early versus late) and on the basis of the presence of K-Complexes (KC+ trials) or their absence (KC- trials). Rare, deviant stimuli will also elicit a K-Complex on as many as 50% of trials. The major component of the K-Complex consists of a large negative waveform peaking around 550 ms. This N550 will overlap and summate with earlier positive waves such as the P300.

The P300 was initially identified in the grand averaged waveform. Its amplitude was then quantified for each subject using a time interval that extended ± 100 ms around the peak latency of the grand average. The peak amplitude of P300 in REM sleep was measured both frontally and parietally. The frontal P3 (also labeled as "P3a") occurs earlier and is more anteriorly distributed than the later and more posterior, parietal P3 (also called "P3b" or the "classic P300"). One-way repeated measures ANOVAs were run for both REM and stage 2 sleep to compare amplitude values between the rare and standard stimuli.

Results

Six subjects provided a sufficiently long period of REM to permit analysis. As Figure 1 illustrates, in REM sleep, a large amplitude positive wave was observed following the rare deviant but not following the standard. This P300 waveform peaked at 321 ms ($SD = 42$ ms) at

Pz and was apparent in all 6 subjects (amplitude ranging from 6.1 to 17.6 μV ; $\underline{M} = 11.1$, $\underline{SD} = 3.9$ μV). The parietal peak was significantly larger following the rare compared to the frequent stimuli, ($\underline{F} = 26.19$, $\underline{df} = 1, 5$, $p < .01$). At Fz, P300 peaked somewhat earlier ($\underline{M} = 273$, $\underline{SD} = 31$ ms). This frontal P300 was also significantly larger in amplitude to the rare compared to the frequent stimuli ($\underline{F} = 28.14$, $\underline{df} = 1, 5$, $p < .01$). The lower portion of Figure 1 illustrates voltage distribution maps of the P300 in REM sleep. As may be observed, P300 is maximum over parieto-central areas of the scalp and declines in amplitude at anterior and lateral sites. The P300 was followed by a large negative slow wave peaking from 600 to 700 ms following stimulus onset. This slow wave was maximum over occipito-parietal regions of the scalp.

----- Insert Figure 1 about here -----

During stage 2 of sleep, the rare loud stimulus elicited a large amplitude K-Complex on 54% of trials. Since the major components of the evoked K-Complex overlap and summate with a possible late positive wave, trials containing K-Complexes (KC+ trials) were analyzed separately from those not containing a K-Complex (KC- trials). The grand average of KC+ and KC- trials following the loud rare stimulus is illustrated in Figure 2. A large amplitude negative wave peaking at 550 ms is apparent on KC+ trials. It was maximum over anterior sites. This is the K-Complex. The N550 component is much attenuated on KC- trials, although it is still apparent. This may be because some small amplitude K-Complexes (i.e., < 75 μV) were included in the averaged data. A late positivity was apparent at occipital sites. At O1 and O2, the amplitude of the late positive wave did not significantly differ between KC+ and KC- trials ($\underline{F} <$

1). In KC+ trials, the initial portion of the N550 wave overlapped with the late positivity and may have obscured its distribution over anterior regions. In KC- trials, the late positive wave was dispersed over wide areas of the scalp. The KC- data were therefore analyzed further.

----- Insert Figure 2 about here -----

Data for KC- trials in stage 2 sleep were available from all 8 subjects. Five of the 8 subjects showed a positivity that exceeded 5 μ V at Pz (ranging in amplitude from 5.0 to 34 μ V; $\underline{M}$ = 18.2, $\underline{SD}$ = 13.8 μ V). The late positive wave was larger during the first half compared to the second half of the night but this difference was not significant. Trials were therefore collapsed across the entire night. Figure 3 compares the ERPs following the standard and deviant stimuli in stage 2. At Pz, the late positivity peaked at 446 ms ($\underline{SD}$ = 30 ms). At Pz, O1 and O2, it was significantly larger following the rare than frequent stimuli ($p < .01$ in all cases). The difference did not reach significance at Fz. The lower portion of Figure 3 illustrates the voltage distribution maps of this late positivity. As may be observed, it was maximum over occipital regions of the scalp and progressively declines in amplitude over more anterior areas.

----- Insert Figure 3 about here -----

Discussion

During REM sleep, a large positive wave peaking at 321 ms was elicited following the rare stimulus. This REM positivity is similar to the classic waking P300 since it was largest following the rare stimulus, was maximum over parieto-central sites, and peaked in the expected latency range. P300s have also been observed in REM by Niiyama et al. (1994) and Bastuji et al. (1995) to stimuli that deviated in tonal pitch. These REM-P300s were smaller in amplitude than the one reported in the present study, and the latency reported by Bastuji et al. (1995) was considerably delayed. In the present study, when a loud, intense deviant was employed, the REM-P300 was larger than that reported in previous studies and occurred in all 6 subjects. In the waking state, loud deviants will also elicit a large, obligatory P300 in inattentive subjects (Roth et al., 1982). On the other hand, in awake but inattentive subjects, pitch deviants will elicit only a small amplitude P300 if it can be detected at all (Duncan-Johnson & Donchin, 1977). Differences between the Niiyama et al. (1994) and the present study might be explained by variation in the probability of deviance occurrence (.20 versus .05 respectively). Such an explanation is however unlikely to account for differences with the Bastuji et al. (1995) findings since their deviant also had a very low probability of occurrence (.10).

P300 peaked earlier at frontal sites during REM sleep. A frontal "P3a" has been described as a measure of the automatic orienting towards a novel intrusive stimulus (Picton, 1992). Näätänen (1990) has suggested that this frontal P3a may be related to preconscious detection of the novel stimulus.

High stimulus intensity per se will not necessarily elicit a P300 in REM sleep. Campbell

et al. (1988) presented tone pips that ranged in intensity from 60 to 100 dB SPL. Each intensity was presented in a separate block. The loudest, 100 dB stimulus did not elicit a late positive wave in either REM or NREM of sleep. High stimulus intensity and rarity therefore appear to be required for the eliciting of the P300.

P300 was followed by a large amplitude negative "Slow Wave" (SW). In the waking state, the classic P300 is also often followed by a negative SW. However, this waking SW is maximum over anterior regions of the scalp. During REM sleep, SW was maximum over occipito-parietal regions. A late negative slow wave has also been reported by Nordby et al. (1996) and Pratt, Berlad, and Lavie (1999). Nordby et al. suggest it may be related to the Mismatch Negativity (MMN) since the SW is larger to the infrequent deviant stimulus than the standard. This is however unlikely since the MMN peaks much earlier, even within REM sleep (Loewy et al., 1996). It is possible that the SW is related to pontine-geniculate-occipital (PGO) spikes. In the waking state, intense auditory stimuli will elicit PGO spikes (Wu, Mallick, & Siegel, 1989). PGO waves accompany eye movements and other sensory and motor events in REM sleep. They are generated in the pons and project to the cortex via thalamic nuclei. The occipital SW might thus represent the source activity of either thalamic and/or occipital dipoles.

A late positivity was also recorded in NREM stage 2 sleep in trials which no K-Complex was elicited to the rare stimulus. This P450 wave is similar to that previously reported by Nielsen-Bohlman et al. (1991), Salisbury et al. (1992), and Winter et al. (1995) using pitch deviants. On the other hand, Niiyama et al. (1994) reported no discernable waveforms in stage 2 of sleep. Similarly, a late positive wave is not visible in the study by Loewy et al. (1996) or Nordby et al. (1996) in which pitch deviants were employed. In the present study, the late stage 2

positivity was visible in only 5 of 8 subjects. There is thus considerable inter-subject and inter-laboratory variability in the P450 wave. This late positivity was maximum over occipital regions of the scalp where it was apparent in both KC+ and KC- trials. In KC+ trials, a large negativity peaking at about 500 ms overlapped and summated to this positive wave in more anterior regions. Bastien and Campbell (1992) have noted that intense stimuli will elicit a K-Complex more often than less intense stimuli. In KC- trials, a much smaller amplitude negativity also occurs over fronto-central regions. This is also in agreement with the findings reported by Bastien and Campbell (1992). The overlapping activity from different sources makes the true scalp distribution of this late positive wave difficult to determine. Nevertheless, its delayed latency and possibly more posterior scalp distribution suggest that it is not equivalent to the P300 recorded in the waking and REM states.

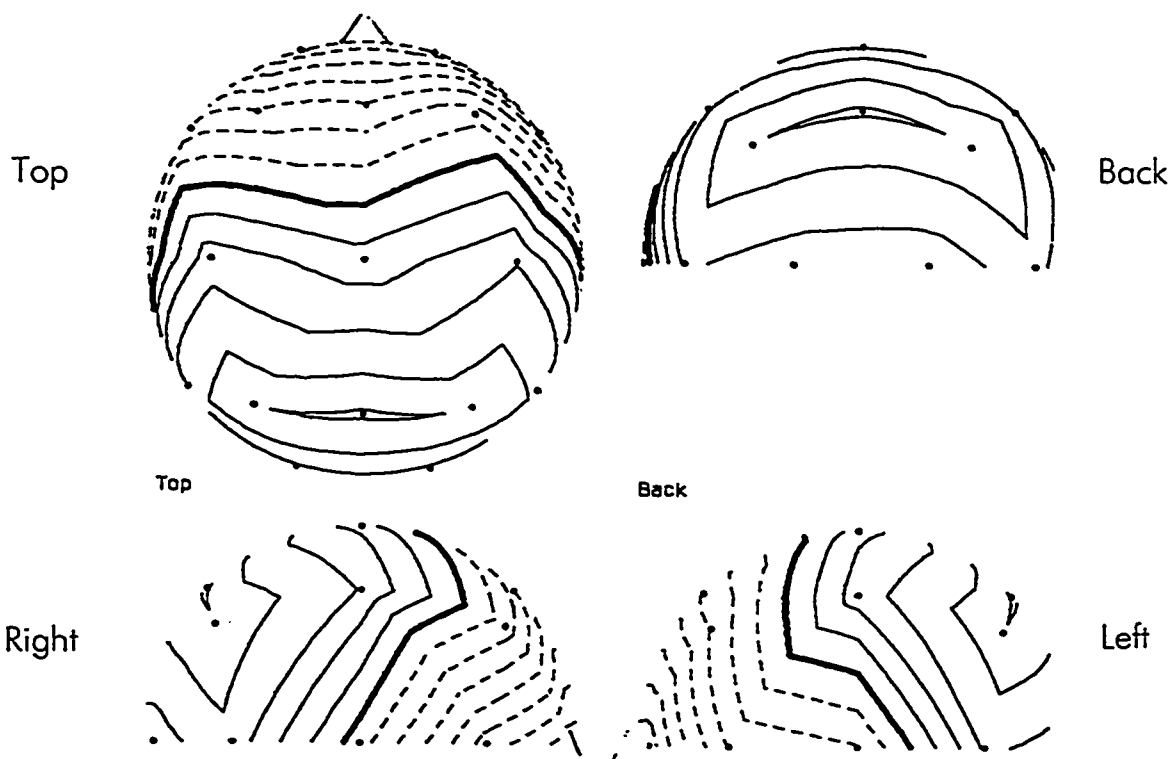
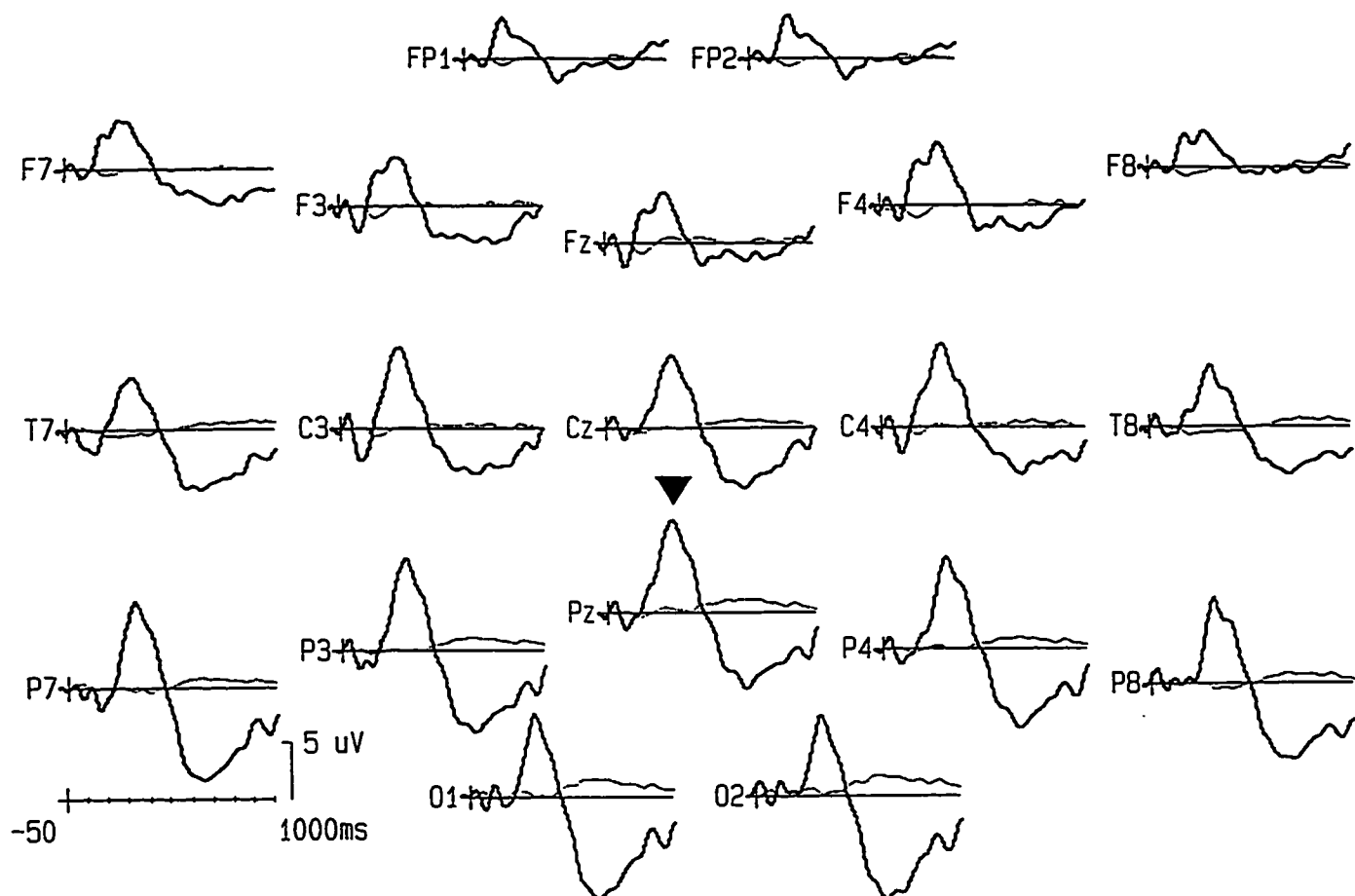
The data provide support for the claim that processing stimulus deviance does occur in REM sleep. Moreover, if a P300 can be elicited in sleep, it is perhaps not surprising that it will occur in REM. The electrophysiological characteristics of REM sleep are more similar to wakefulness than non-REM stages of sleep (see Kahn et al., 1997 for review). The amplitude of N1 and P2 are closer to waking levels in REM than in NREM. In addition, a MMN can be elicited in REM but not non-REM sleep (Loewy et al., 1996; Atienza et al., 1997). In the odd-ball task, the deviant stimulus occurring in a train of rapidly presented standard stimuli, will elicit the MMN (Näätänen, 1990). The MMN is a negative wave typically beginning around 100 ms and lasting up to 300 ms following stimulus onset. It is thought to occur independently of conscious "awareness". The MMN has however been related to "general" consciousness since it has been shown to gradually decline in amplitude at sleep onset (Sallinen et al., 1997). It also

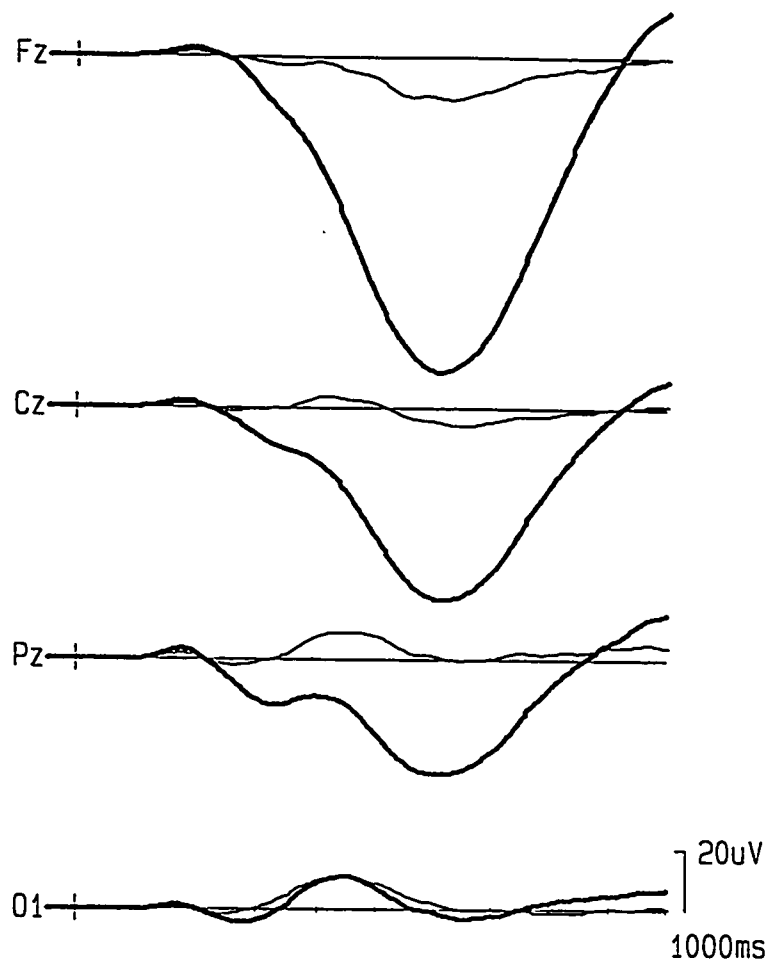
appears to signal the recovery from coma (Kane et al., 1996). Some authors maintain that P300 can serve as an index of consciousness of the eliciting stimulus. This claim must be tempered by the fact that patients with cortical blindness cannot overtly signal their detection of a rare visual target stimulus even though it will elicit a P300 (Picton, 1992; Shefrin et al., 1988). Nevertheless, the presence of the frontal P3a and parietal P300 during REM sleep does provide strong evidence of at least pre-conscious if not consciousness of relevant aspects of the external environment. Such results would however contradict claims that there is active exclusion of external input during REM sleep (Kahn et al., 1997).

Figure Legends

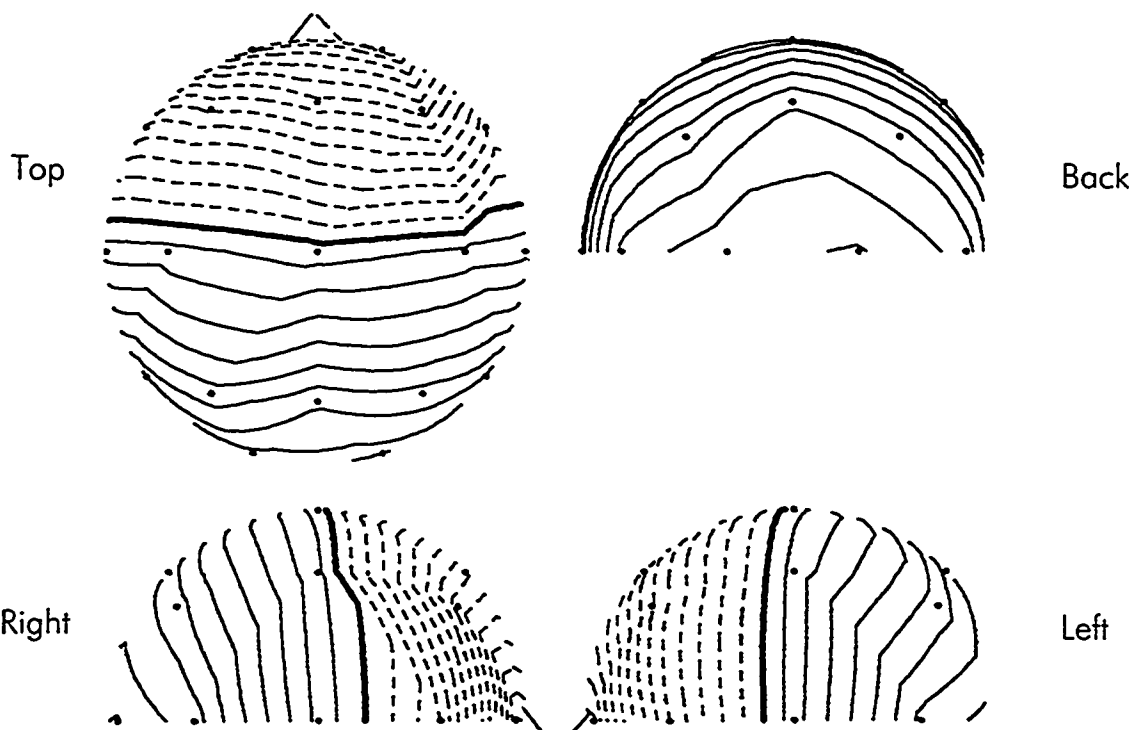
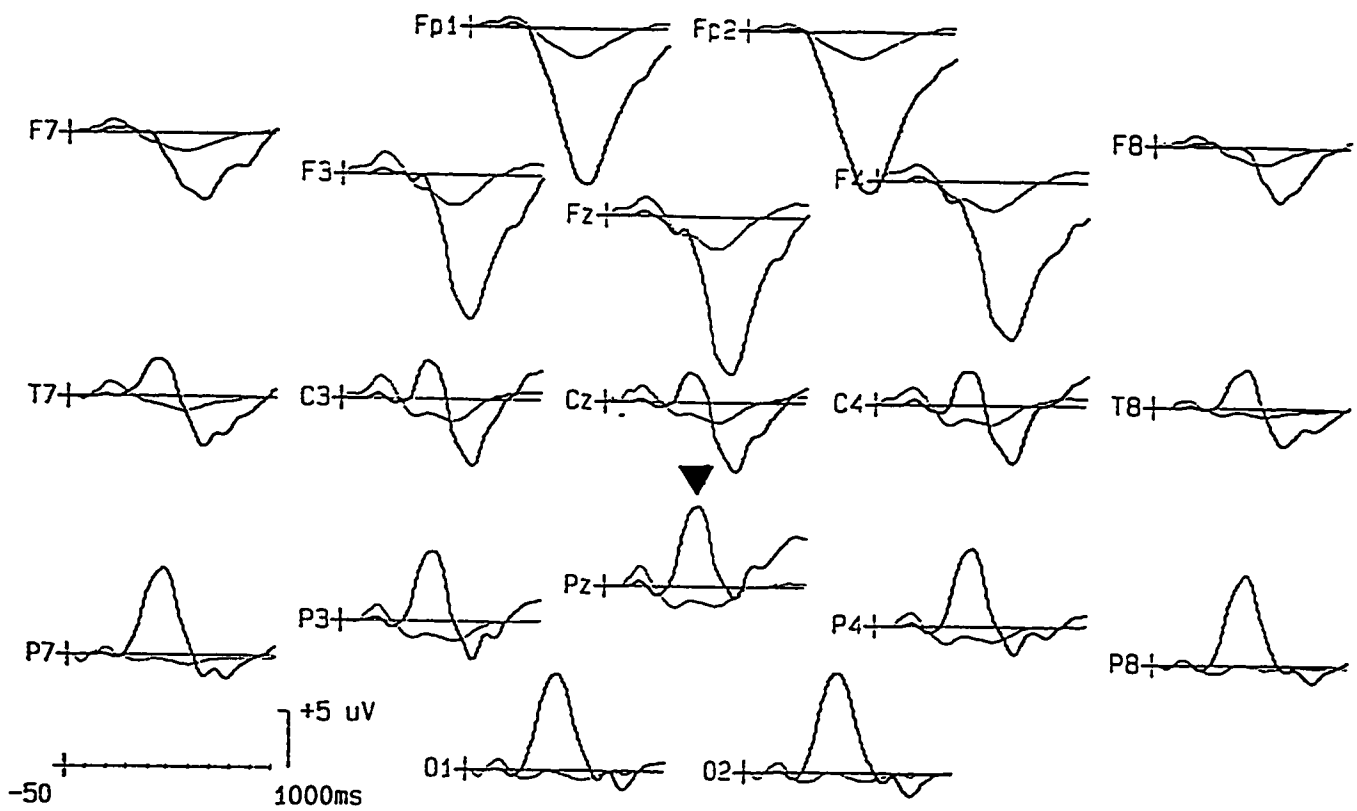
- Figure 3.1.** Grand averaged ERPs following frequent (thin line) and rare (thick line) stimuli during REM sleep. Note that a late positive wave (indicated by arrow) is much larger following the rare stimulus. The voltage distribution isocontour maps were computed on the average of all data points between 300 and 325 ms, using an averaged reference. The thick solid line in the map represents zero voltage. The thin solid lines represent contours for positive voltages, and the dashed lines represent contours for negative voltages. The isocontour lines are separated by $2 \mu\text{V}$. The P300 is maximum at parietal regions decreasing in amplitude over anterior regions.
- Figure 3.2.** ERPs following the rare stimulus when K-Complexes were elicited (thick line) and when were not elicited (thin line) during non-REM sleep. These are labeled as KC+ and KC- trials respectively. A large amplitude N550 can be observed on KC+ trials. A much smaller N550 is also visible on KC- trials. A late positivity (peaking at 450 ms) can be observed at central, parietal and occipital sites on KC- trials. The P450 can also be seen over occipital sites on KC+ trials. However, the overlapping effects of the large amplitude N550 obscure the much smaller amplitude P450. For this reason, only KC- trials were analyzed further.
- Figure 3.3.** Grand averaged ERPs following frequent (thin line) and rare (thick line) stimuli on KC- trials during non-REM sleep. Note that the amplitude scale has been changed from Figure 2. A late positive wave (indicated by arrow) is much larger following the deviant. The voltage distribution isocontour maps were computed on the average of all data points between 425 and 450 ms, using an averaged reference. The thick solid line in the map represents zero voltage. The thin solid lines represent contours for positive voltages, and the dashed lines represent contours for negative voltages. The isocontour lines are separated by $4 \mu\text{V}$. The P450 is maximum at occipital regions decreasing in amplitude over anterior regions.

REM SLEEP





STAGE 2 SLEEP



Chapter 4**Experiment 3: The Effects of Varying Stimulus Intensity on P300 during REM Sleep**

The following manuscript is "in press" in the journal *NeuroReport*, 1999, 10. The present study examined the role of stimulus intensity in eliciting a P300 during REM sleep. Eight subjects were presented with auditory tone pips having an intensity of either 0, 60, 80 or 100 dB SPL. Stimuli were delivered at random with equal probability. Trials were sorted by stage of sleep, stimulus intensity, and presence or absence of rapid eye movements in REM sleep. During the waking state, when subjects read a book, the loud 100 dB stimulus elicited short (P3a) and long latency (P300) positive waves (peaking at 293 and 373 ms respectively). In stage 2 of non-REM sleep, N1 decreased to baseline level while P2 increased in amplitude compared to the waking state. A P300 could not be observed in stage 2 sleep regardless of the level of stimulus intensity. During REM sleep, a late P300 (latency 363 ms) was elicited by the 100 dB stimulus. The earlier positive peak (i.e., P3a) was not apparent. The P300 was reduced in amplitude compared to the waking state. Its amplitude did not differ between phasic and tonic states of REM sleep. These data suggest that stimuli which are sufficiently intrusive to elicit a P300 in the waking state continue to do so in REM sleep.

Introduction

Event-related potentials (ERPs) are changes in electrical activity of the nervous system which may be elicited by an external physical stimulus or an internal psychological event ¹. ERPs are typically recorded from scalp electrodes and consist of a series of negative and positive deflections or "components". These components may be affected by changes to the physical properties of the stimulus (e.g., intensity) and by variation in endogenous cognitive processes, such as attention and level of arousal. The late "vertex" complex of the auditory ERP consists of an "N1" (negative wave peaking between 80-100 ms) and a "P2" component (positive wave peaking between 175-225 ms). During non-REM sleep, N1 decreases to near baseline level while P2 may increase in amplitude relative to wakefulness. In REM sleep, N1 may return to one-half of its waking amplitude ². In the so-called "odd-ball" paradigm, awake and alert subjects are asked to detect a rare "target" stimulus occurring among a train of more frequently occurring "standard" stimuli ³. If the target is detected, a late parieto-central positive wave, peaking at about 300-350 ms (thus labelled "P300"), will be elicited. If the stimuli are ignored or if the target is not detected, a P300 will not be elicited. The presence of a P300 has thus been claimed by many researchers to reflect pre-conscious or conscious detection ("awareness") of the rare stimulus ⁴.

There have been reports of positive potentials to rare deviant stimuli in non-REM sleep. The latency of these positive peaks is nevertheless much longer than that of the usual waking P300 ⁵⁻⁷. There is some evidence suggesting that a "true" P300 can be recorded in REM sleep. Positive potentials, peaking at 344 and 445 ms, have been identified in REM sleep to rare stimuli

that deviate in tonal pitch from a frequently occurring standard ⁸⁻⁹. These positive waves did peak at parieto-occipital sites, but were much attenuated in amplitude ⁸⁻⁹ and delayed in latency ⁹ compared to the waking P300. Other studies have however failed to observe the pitch-elicited P300 in either REM or non-REM sleep ¹⁰⁻¹¹. In a recent study, a very small amplitude late positive wave was recorded during REM sleep when complex word stimuli were employed ¹². This late positive wave was larger to the rare stimuli, but there was no effect of stimulus relevance (subject's own name versus irrelevant word stimuli). Again, the latency of this positive wave was very prolonged.

Although P300 is generally not elicited in inattentive subjects, there is an exception to this rule. If the rare stimulus is sufficiently loud to “intrude” into consciousness, an “obligatory” P300 may be elicited ¹³. We recently employed an odd-ball paradigm during sleep in which a rare ($p=.05$) auditory stimulus deviated in intensity rather than pitch ¹⁴. A late positive wave was elicited in stage 2 sleep following this rare, loud deviant (90 dB SPL). Its peak latency (446 ms) and its scalp topography (occipito-parietal) were not typical of the waking P300. In stage REM, a large amplitude parieto-central positive wave, peaking at 321 ms, was observed following the loud deviant. It was unclear whether the P300 recorded in REM sleep was due to the high intensity or the rareness of the deviant. In the present study, this confound was controlled by presenting stimuli of various intensities with equal probability during the waking state and in the different stages of sleep.

Materials and Methods

Subjects

Eight young adults (four female; mean age = 22.3; $SD = 4.0$) who were “self-reported” good sleepers spent a single night in the sleep laboratory. All subjects were right-handed, nonsmokers, and were free from medication at the time of study. They were verified to have hearing thresholds below 15 dB ISO at 500, 1000, 1500 and 2000 Hz. This study was conducted under the ethical guidelines of the Medical Research Council of Canada. All participants signed informed consent prior to screening and data collection procedures.

Procedure and stimuli

EEG was recorded and stimuli were delivered during relaxed wakefulness while subjects read a book (i.e., instructed to ignore stimuli and divert attention to reading; see Footnote 1). Subjects were then permitted to fall asleep. Presentation of auditory stimuli began after consolidated sleep onset latency (i.e., five minutes uninterrupted stage 2 sleep) and continued throughout the night. EEG was recorded using gold electrodes affixed at midline frontal, central, and parietal sites (i.e., Fz, Cz, Pz) and referenced to the nose. A limited electrode montage was employed in order to reduce discomfort in the sleeping subject. A vertical EOG was recorded from electrodes placed at the supra- and infra-orbital ridges of the right eye. Horizontal EOG was recorded from electrodes placed at the outer canthi of each eye. Physiologic signals were amplified using a Nihon Kohden model 4314B polygraph. The high frequency filter was set at 35 Hz and the time constant was set at one second. EEG and EOG were sampled continuously at a rate of 256 Hz using a 12-bit analogue-to-digital (A/D) converter. Auditory stimuli were delivered to the subject’s right ear via

a hearing-aid device. A 1000 Hz tone pip having a total duration of 55 ms and a rise-and-fall time of 5 ms was presented every 2000 ms. Stimulus intensity was set at either at 0, 60, 80 or 100 dB SPL and presented at random with equal probability (i.e., $p = 0.25$) in blocks of 480 trials. A minimum of two blocks of 480 trials was recorded in both non-REM and REM sleep. During sleep, stimulus presentation was halted if the subject showed signs of arousal or movement.

Data Analysis

Data were later reconstructed off-line into discrete trials or "sweeps". A sweep consisted of 256 data points beginning 100 ms prior to stimulus presentation and continued for 900 ms following it. Single trials were sorted and averaged on the basis of stage of sleep and stimulus intensity (0, 60, 80, 100 dB SPL). During the waking state, trials in which the EOG exceeded $\pm 100 \mu\text{V}$ were rejected from further analysis. Sleep staging was performed by an experienced rater using standard procedures¹⁵. REM sleep was further divided into "tonic" and "phasic" epochs on the basis of eye movements. An ERP trial was binned into the phasic REM category if there was any eye movement activity in the 2000 ms window following stimulus onset. If no eye movements were present in the window, the trial was binned into the tonic REM category. Stage 2 was divided into first and second halves of the night in order to determine time-of-night differences. The ERP waveforms were later digitally filtered (operating in the frequency domain using an inverse FFT algorithm) using a 15 Hz low pass filter.

The P300 component was scored for each individual subject by measuring its amplitude relative to the pre-stimulus "baseline". The P300 was only apparent in wakefulness and REM sleep following the 100 dB stimulus. P300 was initially measured at Pz as the maximum positive

peak between 250 and 400 ms following the 100 dB stimulus. This individual subject P300 latency was then used for the scoring of the ERP waveforms for other REM intensity conditions and the non-REM data. A late negative slow wave (SW) was apparent in REM sleep following both the 80 and 100 dB intensities. This long-lasting SW was measured using a data-point averaging method. SW was defined as the average of all data points from 600 to 800 ms following stimulus onset.

Results

There was insufficient time to permit replication of the complete procedure in all subjects during slow-wave sleep. Analyses are therefore based on waking, stage 2 and stage REM data. During wakefulness, the ERPs following the 0 dB tone pip did not deviate from baseline. The usual N1-P2 deflection was observed following the 60, 80, and 100 dB stimuli. Figure 1 illustrates ERPs in wakefulness, stage 2 and REM sleep following 60, 80 and 100 dB stimuli.

----- Insert Figure 1 about here -----

In wakefulness, the amplitude of N1 and P2 varied directly as a function of stimulus intensity. Two late positive waves were apparent following the 100 dB stimulus. An early positivity ("P3a") peaked at 293 ms ($SD = 14$ ms), while a later positivity ("P300") peaked at 373 ms ($SD = 31$ ms). The P3a was noted in only six of eight subjects, whereas the P300 was apparent in all eight subjects. P3a measured $11.1 \mu V$ ($SD = 6.0 \mu V$) at Pz, decreasing by 22%

and 50% at Cz and Fz respectively. P300 measured $7.9 \mu\text{V}$ ($\text{SD} = 4.1 \mu\text{V}$) at Pz, decreasing by 40% at Cz. It was not visible at the frontal site. Neither P3a nor P300 were visible following the 80 or 60 dB intensities.

There were no significant differences in ERPs found between the first and second halves of the night for stage 2 sleep. Data were therefore collapsed over these time periods to increase the number of trials available for averaging. During stage 2, N1 was at or near baseline level for all intensities. P2 increased in amplitude during stage 2 sleep relative to wakefulness, but the difference was not significant ($F < 1$). There was no evidence of a late positive wave in the 250-400 ms interval in stage 2. Rather, the morphology of the potentials was characterized by a late negative wave peaking at 330 ms, followed by a later 550 ms negativity.

During REM sleep, a single positive peak was apparent following the loud 100 dB stimulus. It was maximum over parieto-central areas of the scalp and peaked at 363 ms ($\text{SD} = 30.5$ ms). This REM P300 was reduced in amplitude ($\text{M} = 4.4$, $\text{SD} = 4.2 \mu\text{V}$) compared to that in the waking state. A positive-going waveform in the 300-400 ms latency range, was observed for all subjects during REM. It was however less than $2 \mu\text{V}$ for two subjects. These two subjects also had small P300s in the waking state. No P300 was visible following either the 60 or the 80 dB stimuli. A Stage (wake, REM) by Intensity (60 vs 80 vs 100 dB) ANOVA was performed on P300 amplitude. It was significantly larger following the 100 dB compared to both the 60 dB and the 80 dB stimuli ($F(2,14)=20.39$, $p < 0001$). It was also significantly larger in wakefulness compared to the REM state ($F(1,7)=6.45$, $p < .05$).

Figure 2 illustrates the averaged ERPs after the REM data were sorted and averaged according to the presence and absence of eye movements within two seconds of stimulus

presentation. There was no evidence of a P3a in either phasic or tonic REM. Moreover, there was no significant difference in P300 amplitude between the two states.

----- Insert Figure 2 about here -----

A late negative slow wave (SW) was also apparent in REM sleep. It was maximum at Pz, declining in amplitude at Cz and Fz. A State (phasic vs tonic) X Intensity (60 vs 80 vs 100 dB) X Site (Fz vs Cz vs Pz) repeated measures ANOVA was performed to isolate significant effects. Although the SW was larger on phasic trials, the main effect was not significant ($p > .05$). A significant Site X Intensity interaction was observed, with the SW being larger for the 100 dB stimulus at Pz ($F(4,28)=7.31, p < .0001$).

Discussion

A long-latency positive wave was apparent to the loudest stimuli during wakefulness. This positive wave consisted of two peaks, an early P3a that extended into anterior areas of the scalp, and a later parieto-central P300 (or P3b). This is consistent with reports that loud stimuli will elicit a P3a in waking-ignore conditions¹³. The P3a has been described as reflecting an automatic detection of novelty⁴. The P300 appears to be more closely associated with classification of the eliciting stimulus resulting in either a preconscious detection or conscious perception of it. Neither the P3a nor the P300 could be observed in stage 2 sleep. In REM sleep,

the late P300 wave peaking at about 363 ms was observed following the 100 dB stimulus. It was not apparent following any of the lower intensity stimuli. The early latency anterior P3a was not apparent for any stimuli in REM sleep. This might be explained by “functional deafferentation” of the forebrain during REM sleep ¹⁶. A recent investigation in our laboratory indicated that very rare ($p=.05$) high intensity deviants will elicit a P300 in REM sleep ¹⁴. It was not clear however whether this P300 was due to its extreme rareness or the extreme loudness of the stimulus. The results of the present study indicate that when various stimulus intensities are presented with equal probability, only the loudest stimuli will elicit a P300. A number of authors have noted that P300 is elicited by either task or biologically relevant stimuli ⁴. In the present study, both the waking and the REM P300 were elicited by biologically relevant, loud stimuli. It does not appear that P300 can be elicited by personally relevant stimuli, such as the subject’s own name, during REM sleep although this stimulus will elicit a P300 in wakefulness ¹². Not all loud stimuli will elicit a P300 during REM. Campbell et al presented stimuli ranging from 60 to 100 dB in 10 dB steps ¹⁷. Each intensity was presented in *separate* conditions whereas in the present study they were presented in a random order in a single condition. P300 was not elicited by the 100 dB stimulus in the Campbell et al. study. Relative loudness and rareness of stimulus presentation may both be important requirements for P300.

The late positive waves could not be observed in stage 2 of sleep. This is consistent with many other studies ^{2,7-12}. The loud stimuli did elicit large negative waves peaking at about 330 and 550 ms. These probably reflect components of the evoked K-Complex. The K-Complex is elicited more often by high than low intensity stimuli ². Within REM sleep, P300 was not influenced by phasic eye movements. Sallinen and colleagues employed an odd-ball task and

noted that the REM P300 was larger in tonic REM¹⁸. The authors interpreted this as reflecting that the brain was more "open" to input from the external environment during tonic REM, and more focussed on internal mental activity during phasic REM. There are important methodological differences that could account for the discrepancies between the two studies. In the Sallinen study, rare stimuli were presented on 1.5% of trials in an odd-ball paradigm. The time between rare stimulus presentations was thus very long. As such, they were able to sort trials on the basis of eye movements occurring prior to stimulus onset. They used long 15 second epochs to make these discriminations. In the present study, trials could not be sorted on the basis of prior eye movements since these would have occurred during or following presentation of other equally probable stimuli. The phasic/tonic sorting was thus done on the basis of eye movement activity within the 2000 ms sweep time following stimulus onset.

The late SW reported in the present study was larger on phasic trials, although the difference was not significant. The SW was maximum at parietal sites and was evident following both 80 and 100 dB intensities. This negative SW has been reported previously^{11, 12, 14}, although the significance of it is not known. Cote and Campbell recorded the SW following rare loud 90 dB stimuli and Pratt et al reported it following meaningful word stimuli^{12, 14}. The SW may therefore be related to the biological or psychological salience of the stimulus. Nordby et al suggested that the SW may be related to Mismatch Negativity (MMN) since the SW was larger following the rare stimulus. Since the MMN occurs at about 100-300 ms following the stimulus, it is however unlikely that the late negative SW (around 600-800 ms) is related to the earlier MMN. An alternative explanation is that the SW is related to ponto-geniculo-occipital (PGO) spikes¹⁴. PGO spikes, as their name implies, appear in the occipital region of the cortex. PGO

spikes have been reported to occur in REM sleep during periods of phasic excitability¹⁹, and may be elicited by intense auditory stimuli in the waking state²⁰. In the present study, the posterior topography of the SW and its association with stimulus intensity suggest that it may be related to PGO activity.

Conclusion

The present study demonstrates that stimulus intensity plays a role in eliciting a P300 potential in REM sleep. A loud 100 dB tone pip elicited a short latency, anteriorly-distributed P3a and a longer latency parietal-maximum P300 while subjects were awake but ignoring the auditory stimuli. Lower intensity stimuli did not elicit the late positive waves. The loud stimulus also elicited a P300 during REM sleep but the P3a was not observed. Neither a P3a nor a P300 were observed in stage 2 of sleep. A loud stimulus may intrude into consciousness, either in waking ignore conditions or during REM sleep. A late posterior-distributed Slow Wave (SW) was also observed to the loud stimuli in REM sleep. This may be related to PGO spike activity.

Footnote

During the waking state, subjects were asked to read a book while physiologic signals were recorded. This is a common procedure in many ERP studies. Nevertheless, reading will result in large horizontal eye movements. Trials in which these eye movements exceeded ± 100 μV were rejected. Lower amplitude eye movements would not have been excluded from the ERP average. However, since they are not time-locked to the stimulus, the averaging procedure would attenuate their influence on the scalp ERPs. Nevertheless, horizontal eye movements would mainly affect lateral EEG electrode sites. Since the EEG was recorded from midline sites, the amount of artifact caused by horizontal eye movements would be minimal.

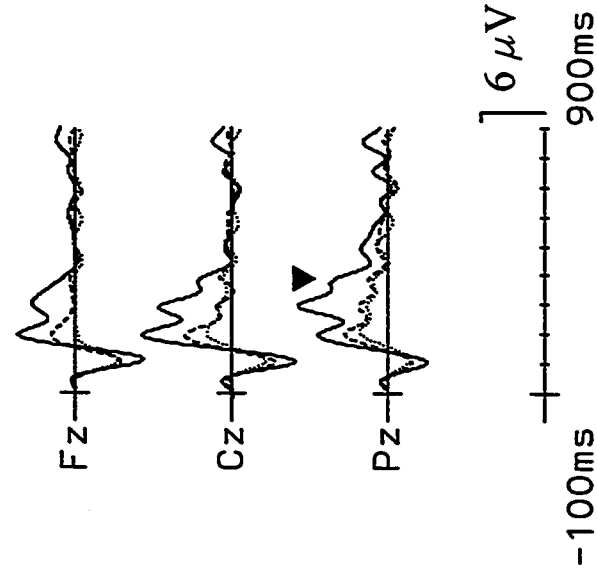
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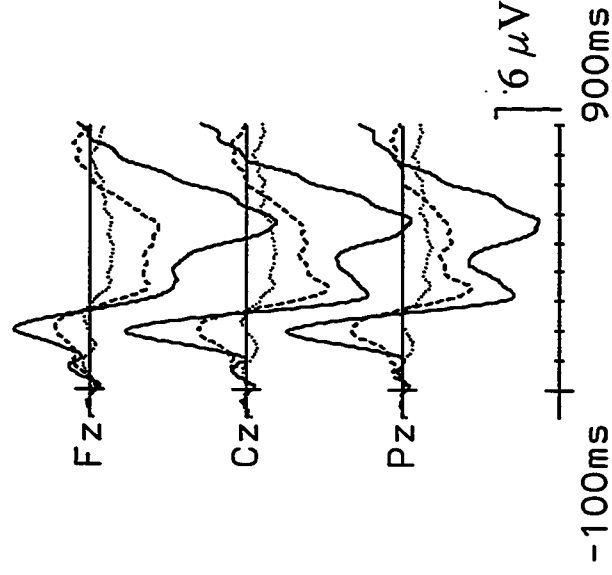
Figure Legends

- Figure 4.1. Event-related potentials (ERPs) to stimuli varying in intensity in wakefulness, stage 2 and REM sleep. In wakefulness, an N1-P2 vertex complex can be observed for all intensities. A P300 is apparent to the 100 dB SPL tone pip (positive polarity is indicated as an upward deflection in this and all other figures). Late positive peaks are apparent at 293 and 373 ms. The late positive wave (P300) is indicated by a triangle. In stage 2, N1 is at or near baseline. P2 amplitude increases with each increase in stimulus intensity. In REM sleep, a positive peak at 373 ms remains prominent at parietal sites following the loudest (100 dB) stimulus.
- Figure 4.2. Event-related potentials (ERPs) in tonic and phasic REM sleep. A P300 may be observed for the loudest 100 dB SPL stimulus. There is no difference between tonic versus phasic REM for the positive wave. However, there are tonic/phasic differences for the later negative slow wave (SW) after both the 80 dB and 100 dB intensities, although these differences did not reach statistical significance.

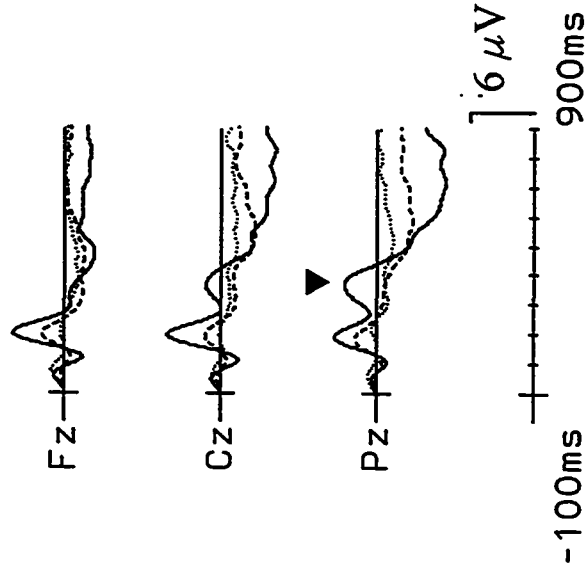
Stage Wake



Stage 2 Sleep

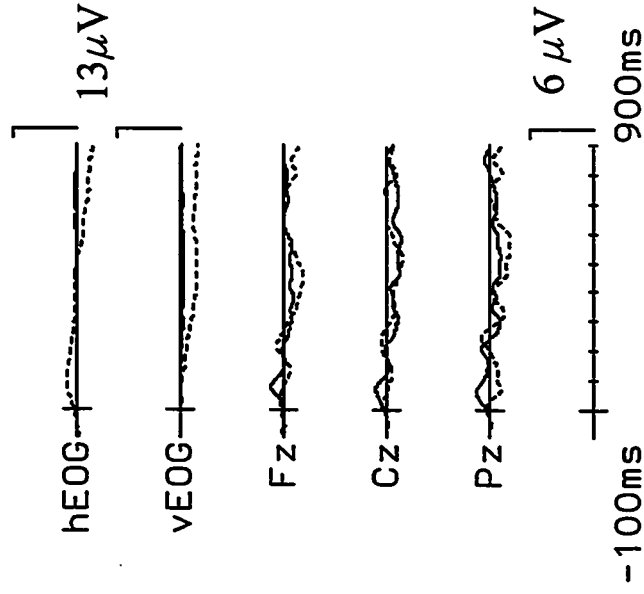


REM Sleep

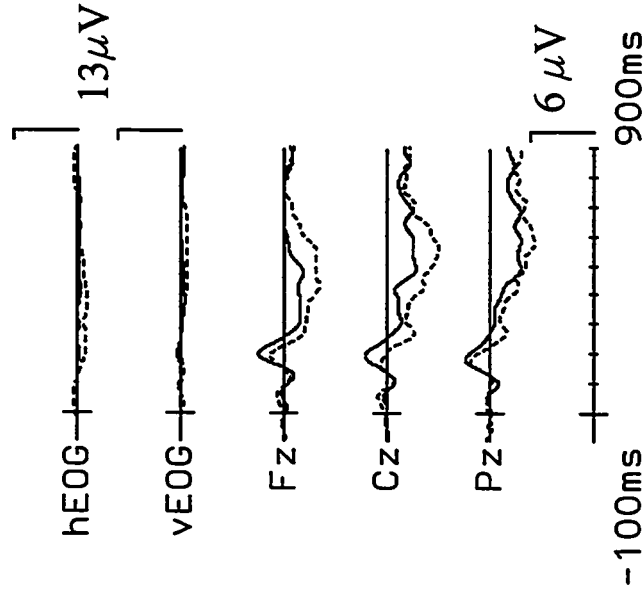


Intensity	(dB SPL)
.....	60
-----	80
————	100

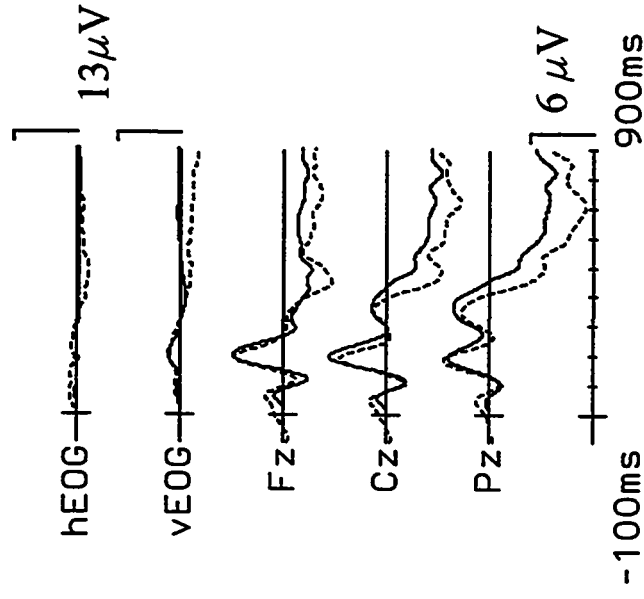
60 dB SPL



80 dB SPL



100 dB SPL



Chapter 5

Experiment 4: The Effects of Pitch and Intensity Deviance and Varying Stimulus Probability on Information Processing during Sleep

This chapter is in preparation for submission to the journal *Psychophysiology*. For the purpose of the thesis, the text is however extended to include reference to the previous experiments in the thesis when necessary. Research in Japan and France has indicated that rare pitch deviants may elicit a small amplitude P300 in REM sleep. Two experiments in this thesis indicated that loud stimuli will elicit a large amplitude P300 in REM. The purpose of the present study was to compare the effects of pitch and intensity deviants on P300 amplitude. Probability of deviant occurrence was also manipulated since it is known to affect the amplitude of a true P300. Subjects were randomly assigned to one of three deviant probability groups, in which the deviant occurred on 20%, 10%, or 5% of trials. During wakefulness, subjects were asked to either attend to (count the deviants), or ignore (read a book) the stimuli. A large amplitude P300 was apparent following both pitch and intensity deviants when subjects were asked to count the number of deviant stimuli. It was maximum at parietal sites but was also apparent at fronto-central sites. Its amplitude decreased with increasing probability of deviant presentation. In the waking ignore condition, a slightly smaller P300 was evident to the intensity deviant. No P300 was evident to any of the pitch deviants in the waking-ignore condition. During stage 2 of sleep, a small amplitude positive wave was visible following pitch and intensity deviants. Its latency was however much more prolonged than the usual P300 and its scalp distribution was parieto-

occipital maximum. During REM sleep, the pitch deviant failed to elicit a late positivity. An attenuated positivity having the usual P300 latency and parieto-central scalp distribution was visible to the very rare intensity deviant (i.e., .05 condition). However, the frontal dispersion of P300 that was observed in the waking state was absent during REM sleep.

Introduction

One of the great puzzles of sleep is the extent to which the apparently unconscious sleeper is aware of the events in their external environment. A major difficulty encountered in the study of awareness during sleep is that the experimenter does not have direct access to the sleeper's mental state. The sleeper is usually unable to provide either a verbal or behavioural response. Thus, even if the sleeper is conscious of the external environment, they may be unable to signal their awareness to the experimenter. It is of course possible to awaken the subject and ask them to recall their mental activity. However, the reliability of such subjective recall is questionable. A failure to recall events immediately prior to awakening may reflect a failure of memory storage or retrieval rather than a lack of consciousness of the external stimulus (Broughton, 1973). Event-related potentials (ERPs) offer a means to probe the extent of information processing during sleep, independently of subjective reports or behavioural responses. The long-latency auditory ERPs are particularly affected by manipulation of the subject's level in attention, arousal and consciousness (Näätänen, 1990; Picton, 1992; Campbell, Bell, & Bastien, 1992).

The vertex complex of the ERP consists of an "N1" (negative wave peaking between 80-100 ms) and a "P2" component (positive wave peaking between 175-225 ms). Näätänen (1990) has suggested that N1 may act as a transient-detector system that triggers an internal attentional system. N1 may subserve "conscious perception of auditory stimuli in general... without indicating what the stimulus is or what its precise features are" (p.212). As might be expected by a measure of "general consciousness", N1 is attenuated to near baseline level in non-REM sleep

compared to wakefulness. During REM sleep, N1 returns to about 25-50% of its waking amplitude. (Campbell et al., 1992). There is thus some evidence that a general type of conscious perception may remain possible in REM sleep.

In the odd-ball task, subjects are presented with a train of frequently occurring standard stimuli. At rare and unpredictable times, the standard is changed to a deviant. When subjects are asked to actively detect the rare deviant stimulus, a late positive potential is elicited (Sutton, Braren, Zubin, & John, 1965). When the deviant is easy to detect, it peaks at about 300 ms. It has thus been labelled "P300". P300 is maximum over parieto-central areas of the scalp. It only occurs when the deviant stimulus is actually detected. It will not be elicited if the subject ignores the stimulus or fails to detect it. The P300 is therefore thought by many authors to reflect consciousness of the deviant stimulus (Picton, 1992).

Campbell et al. (1992) and Harsh (1994) claimed that there was no convincing evidence that a P300 could be recorded in sleep. More recent studies have provided evidence that P300 can perhaps be elicited in REM sleep. Niiyama, Fujiwara, Satoh, and Hishikawa (1994) and Bastuji, García-Larrea, Franc, and Mauguière (1995) both employed oddball tasks in which the pitch of a frequently occurring standard stimulus was changed on 20% and 10% of trials respectively. Both reported a small amplitude parietal maximum P300 during REM sleep. Cote and Campbell (1999b) also employed an odd-ball task in sleep. Their deviant stimulus was very loud (90 dB SPL) compared to the standard (70 dB) and occurred very infrequently, on only 5% of trials. They recorded a large amplitude parietal P300 in REM sleep. During non-REM, a longer latency positive wave was observed. However it peaked too late (446 ms) and its scalp distribution was too posterior (parieto-occipital maximum) to be considered a true P300. Cote and Campbell

(1999a) subsequently investigated whether this REM P300 was a result of the loud, intrusive nature of the stimulus or its extreme infrequency of occurrence. They presented 0, 60, 80 or 100 dB SPL tone pips each having an equal probability of occurrence. Only the loudest stimulus elicited a P300 in REM sleep.

The purpose of present study was to examine the effects of both pitch and intensity deviants in the same subjects on P300 in sleep. A classic manipulation known to affect a true P300 is to change the probability of deviant occurrence. P300 amplitude increases in amplitude as the probability of deviant occurrence decreases. (Duncan-Johnson & Donchin, 1977; Campbell, Courchesne, Picton, & Squires, 1979). The probability of deviant occurrence was therefore manipulated. P300 can usually only be elicited when subjects actively detect the rare deviant stimulus. Thus, a pitch deviant will only elicit a P300 if subjects attend to and detect it. An exception to this rule is for very loud stimuli. They will elicit an obligatory P300 in waking and alert subjects even if the subjects are not attending to the stimuli (Roth, Blowes, Doyle, & Kopell, 1982; Roth, Dorato, & Kopell, 1984; Polich, 1989; Putnam & Roth 1990). Waking data were therefore also collected when subjects were attentive to the auditory stimuli and when they ignored them.

Methods

Subjects

Twenty-four subjects (16 female), aged 18 to 30 years ($M = 23.3$, $SD = 3.8$), volunteered to

participate in the study. Participants signed a consent form and received an honorarium for their participation. All subjects completed a series of sleep/wake questionnaires and had a hearing test prior to study. Hearing levels were verified to be within 15 dB ISO at 500, 1000, 1500 and 2000 Hz. Subjects were excluded from participation if they reported a history or current evidence of poor sleep. All subjects were right-handed, non-smokers, and free from medication at the time of study. None had a history of psychiatric or neurological disorders. Subjects were instructed to abstain from alcohol and caffeine for a 12 hour period prior to the start of the study.

Physiologic Recording

The EEG was recorded from midline frontal, central, parietal, and occipital scalp sites (Fz, Cz, Pz, and Oz) and referenced to the left mastoid. In previous studies in this thesis, a nose reference was used. This is because a midline reference is more appropriate when recording from multiple channels that include active electrodes over left and right hemispheres. In the present study, EEG activity was recorded from only a limited number of active midline sites. Since hemispheric differences were not the interest of the present study, a mastoid reference was chosen since it is more commonly used in P300 studies. A vertical EOG was recorded between the supra- and infra-orbital ridge of the right eye. A horizontal EOG was recorded from the outer canthus of each eye. EMG was recorded from submental electrodes. In previous studies, EMG was not recorded. EMG can be used to increase the reliability of scoring REM sleep. Moreover, it is possible that the loud stimulus could produce transient arousals from sleep that are not visible in the EEG. Such arousals might however be observed as an increase in the amplitude of the EMG. A ground was placed on the forehead. Inter-electrode impedance was below 5 kOhm. The high

filter for the EEG and EOG channels was set at 35 Hz, while the EMG was set to 120 Hz. The time constant was set at 1 s for the EEG and EOG channels, and at 0.03 s for the EMG. All physiological data were sampled continuously at 256 Hz using a 12-bit A-D converter and stored on hard disk.

Procedure and Stimuli

Participants arrived to the laboratory at approximately 18:00 in order to allow time for electrode application procedures and waking data collection. Each subject was fitted with a hearing-aid device in their right ear through which the auditory stimuli were presented. The hearing-aid device assured constancy of auditory input in spite of variations in head movement.

Subjects were presented with auditory stimuli during wakefulness and throughout the night during all stages of sleep. An 80 dB SPL, 1000 Hz stimulus was delivered on average every 1.5 s. The inter-stimulus interval (ISI) varied from 1000 to 2000 ms. In previous studies in this thesis, a constant ISI was used. It may be possible for subjects to predict stimulus onset when a constant ISI is employed, particularly when subjects are awake. This is however unlikely since in Experiment 3, a constant ISI was employed, but neither an N1 nor a P300 was apparent following the 0 dB stimulus. Regardless, the variable ISI should prevent any possible prediction of stimulus occurrence. Total stimulus duration was 52 ms, with an abrupt 2 ms rise-and-fall time. In different conditions the standard was changed at rare and unpredictable times to either a high pitch (2000 Hz), or a loud intensity (100 dB SPL) deviant. Subjects were randomly assigned to one of three probability groups in which the probability of deviant occurrence was either .20, .10, or .05. A between groups design was employed to avoid possible carry over/ habituation effects

that might occur in a within groups, repeated measures design. The number of subjects in the .20, .10, and .05 deviant probability groups was 7, 7, and 10 respectively. Additional subjects were assigned to the .05 group since previous studies indicated that this was the condition most likely to elicit a P300.

During wakefulness, subjects were asked to either attend to (and count the deviant) or ignore (and read a book) the stimuli in different conditions. Stimuli were presented in blocks of 400 trials. The order of presentation of conditions was randomized. All conditions were presented at least two times while awake and in each stage of sleep to ensure reliability of results.

Data Analysis

The continuous EEG was classified by an experienced rater using standard sleep scoring criteria (Rechtschaffen & Kales, 1968) based on 15 s epochs. In the case of stage ambiguity, movement arousals, or the presence of stage 1 sleep, the data were excluded from further analysis. Stages 3 and 4 were combined to form slow wave sleep (SWS). Stage 2 was sub-divided into early and late halves of the night to examine for possible time-of-night differences. REM sleep was divided into tonic and phasic epoches on the basis of eye movements. A method similar to that described by Sallinen et al. (1996) was employed. In the Sallinen et al. (1996) study, REM sleep was characterized as “tonic” if no rapid eye movements occurred during the 10 s interval prior to the onset of the deviant, or the 5 s interval following it. If there was at least one eye movement in the 5 s prior to the onset of the deviant, the trial was classified as “phasic”. In the present study, tonic trials included those with no REMs occurring 6 trials prior to or 4 trials following the deviant. Phasic trials were those with at least one REM in the 4 trials prior to the onset of the deviant. If

an eye movement occurred within 4 trials following the deviant, but not before it, the REM interval was classified as neither phasic nor tonic. These trials were rejected from further analysis.

Data were reconstructed off-line into discrete trials or "sweeps". A sweep consisted of 256 data points beginning 100 ms prior to stimulus presentation and continued for 900 ms following it. During wakefulness, trials in which the EOG or EEG exceeded $\pm 100 \mu\text{V}$ were rejected from further analysis. Within sleep, trials were rejected if the EEG exceeded $\pm 100 \mu\text{V}$. During stage 2, these rejection criteria essentially removed trials in which the stimulus elicited a K-Complex. The K-Complex will be elicited on 25-50% of deviant presentations (Bastien & Campbell, 1992). The large negative component of the K-Complex, peaking at about 500-600 ms overlaps and summates with earlier positive waves, possibly masking a P300 (Cote and Campbell, 1999b). Within REM sleep, the rejection criteria removed trials with abnormally high amplitude EEG. Since the rejection criteria were not applied to the EOG channels, trials in which rapid eye movements occurred were not rejected. The EOG artifact (phasic eye movements) was not however time-locked to the stimulus (i.e., it occurred randomly). The averaging procedure, summing both negative- and positive-going EOG activity, would tend to cancel out the random eye movements.

Trials were sorted and averaged on the basis of sleep stage (stage 2, SWS, REM sleep), stimulus type (pitch, intensity), and deviant probability (.20, .10, .05). They were later digitally filtered (operating in the frequency domain using an inverse FFT algorithm) using a 15 Hz high frequency filter.

Determining if a P300 exists in sleep is fraught with statistical and technical difficulties.

A simple approach would be to compare the waking and sleeping P300s. However, a failure to find a difference does not imply the presence of a sleeping P300. For example, it is quite possible that there may not be a P300 in the ignore condition in the waking state. Similarly, a difference between waking and sleeping amplitudes of P300 does not imply the absence of P300 in sleep.

Previous research has indicated that a pitch-elicited P300 may not be elicited in waking-ignore conditions (Putnam and Roth, 1990), nor within non-REM sleep (Winter, Kok, Kenemans, & Elton, 1995; Loewy, Campbell, & Bastien, 1996). For statistical purposes, a waveform in which no P300 is apparent still needs to be quantified. This was accomplished using a data-point averaging technique. Each standard and deviant waveform sweep in all conditions was divided into five equal time interval windows beginning 250 ms and ending 500 ms following stimulus onset. All data within the following intervals were averaged: interval 1 = 250-300 ms; interval 2 = 300-350 ms; interval 3 = 350-400 ms; interval 4 = 400-450 ms; and interval 5 = 450-500 ms. Initially, the presence of a candidate P300 was determined by comparing the standard and deviant waveforms across each of the 5 intervals using the t-test statistic. Separate t-tests were run for the attend and ignore conditions within the waking state and within each stage of sleep, for the pitch and intensity conditions within each probability group. It was expected of course that a P300 would be larger (i.e., more positive), following the rare deviant than the standard stimulus. Because a positive directionality was predicted (i.e., the polarity of P300), a one-tailed test of significance ($p < .05$) was applied.

Such multiple liberal tests of significance will likely result in chance significance. This was not considered to be problematic since these preliminary analyses were followed up with more precise measurement of the P300 waveform and a conservative statistical analysis among

stages and/or conditions. When the t-test comparing the standard and deviant waveforms was significant, the peak amplitude of P300 was measured at Pz in the deviant ERP as the maximum positive peak between 250 and 500 ms. The amplitude and latency of the deviant ERP were then compared using ANOVA procedures. Because the specific analyses vary within each stage of sleep, they will be described in the Results section.

Results

Waking ERPs

Figures 1, 2, 3 and 4 illustrate the standard and deviant ERPs during wakefulness in the attend and ignore conditions for the pitch and intensity conditions respectively. In wakefulness, across all conditions, the standard N1 peaked at about 75 ms. A double-peaked P2 was apparent, with an initial peak at 140 ms and a later peak at 205 ms. The type of deviant (i.e., pitch, intensity) did not affect the amplitude of N1. Similarly, the amplitude of the standard N1 did not vary among the three probability groups. N1 was slightly reduced in amplitude in the ignore conditions, compared to attend conditions, but this difference was not significant.

————— Insert Figures 1, 2, 3, & 4 here —————

Table 1 presents the results of the 60 t-tests run to compare the standard and the deviant ERPs in the waking state. The p-values are underlined for differences that were significantly more positive. When subjects were asked to count the number of deviant presentations, in both

the intensity and the pitch conditions for all probability groups, the deviant ERP was significantly more positive-going than the standard over all intervals from 250 to 500 ms. The results were quite different when subjects ignored the auditory stimuli. When the pitch deviant was employed, the standard and the deviant waveforms did not significantly vary in any of the five time intervals, among any of the probability groups. When the intensity deviant was employed, significant differences were found among the standard and deviant ERPs when the deviant occurred infrequently (.10 and .05 groups). ANOVA procedures were restricted to those conditions in which the P300 was apparent.

----- Insert Table 1 here -----

A Group (3 probability conditions) by Deviant (pitch versus intensity) by electrode Site (Fz, Cz, Pz, Oz) repeated measures ANOVA was run to compare P300 latency and amplitude data in wakefulness during the attend condition. The latency of P300 was significantly earlier following the intensity ($\bar{M} = 316$ ms) compared to the pitch deviant ($\bar{M} = 355$ ms), at all electrode sites, $F(1,21) = 26.29$, $p < .0001$. A significant main effect of type of deviant was also found for the amplitude of P300, $F(1,21) = 46.65$, $p < .00001$. P300 amplitude was larger following the intensity ($\bar{M} = 22.8 \mu\text{V}$) compared to the pitch deviant ($\bar{M} = 13.05 \mu\text{V}$). There was a trend toward differences among the three probability groups, $F(2,21) = 2.97$, $p = .07$. P300 was largest when probability of deviance occurrence was .05 ($\bar{M} = 23.0 \mu\text{V}$) and declined in amplitude in the .10 and .20 conditions ($\bar{M}$ s = 17.53 and 13.19 μV respectively). There was also a highly significant main effect of electrode site, $F(3,63) = 57.79$, $p < .0000001$. P300 was largest

at Pz ($\underline{M} = 26.89 \mu\text{V}$). Its amplitude was however widely dispersed, remaining large at both Cz ($\underline{M} = 21.34 \mu\text{V}$) and Oz ($\underline{M} = 16.23 \mu\text{V}$). It was much smaller in amplitude at frontal sites ($\underline{M} = 7.20 \mu\text{V}$).

In the ignore condition, as mentioned a significant P300-like wave was not apparent following the pitch deviant regardless of its probability of occurrence. Candidate P300s were identified following the intensity deviant when the probability of occurrence was .10 or .05. To further investigate the effects of attention on the waking P300, an ANOVA was run to compare the attend and ignore conditions for the intensity deviant. The peak latency of P300 in the ignore conditions was 315 ms. It did not significantly differ from the attend conditions ($F < 1$). A main effect of attention on P300 amplitude was observed. P300 amplitude was significantly larger in the attend ($\underline{M} = 26.42 \mu\text{V}$) compared to the ignore condition ($\underline{M} = 11.54 \mu\text{V}$), at all electrode sites and for both the .10 and .05 probability conditions, $F(1,15) = 139.73$, $p < .000001$. Again, although P300 was largest at Pz, its remained apparent at all sites.

Non-REM Sleep

There was insufficient data from SWS to permit reliable averaging. This may have been because noise stimuli, especially loud stimuli, can reduce the amount time spent in SWS. Non-REM data were therefore reported for stage 2 sleep only. There were no time-of-night differences apparent in the grand average waveforms. ERPs were therefore collapsed across the entire night. In stage 2 non-REM sleep across all conditions, the latency of the standard N1 was prolonged to approximately 110 ms, while P2 peaked at approximately 200 ms. The amplitude of N1 was below baseline while P2 increased in amplitude in all conditions relative to wakefulness.

Figure 5 illustrates the pitch and intensity deviant ERPs within stage 2 sleep. At occipital sites, a small amplitude positive wave is apparent around 421 ms, particularly for the rarest deviant condition. The upper portion of Table 2 shows the results of the t-tests comparing standards and deviants for the pitch and intensity conditions at all levels of probability. No significant differences were observed between the standard and the deviant ERPs waveforms in any of the intervals between 300 and 500 ms.

----- Insert Table 2 and Figure 5 here -----

REM Sleep

In REM sleep across all conditions, the standard N1 and P2 peaked at about 110 and 180 ms respectively. N1 was reduced in amplitude by approximately 25 to 33% compared to the waking state. Figure 6 illustrates ERPs following pitch and intensity deviants in REM sleep across the three probability conditions. The P300 was maximum at Pz. The average peak latency of the individual data was 365 ms. In the grand average waveforms, P300 peaked later at 387 ms. The difference between the mean of the individuals' P300 (i.e., the arithmetic mean of the individually measured P300s in each subject), and the grand averaged P300 (i.e., measured in the grand average waveform), can be attributed to possible measurement of noise in some individual subjects. Unlike the case in the waking conditions, its positivity was not dispersed to the Fz site. Rather a very large negativity was observed here.

----- Insert Figure 6 here -----

The lower portion of Table 2 provides the results of the t-tests comparing the standard and deviant ERPs in REM sleep for the pitch and intensity conditions at each level of probability. Again, no significant differences were apparent in the 300 to 500 ms intervals when a pitch deviant was employed, regardless of its probability of occurrence. When the loud, deviant was employed, the deviant waveform was significantly different from the standard in the four intervals ranging from 300 to 500 ms, but only when deviant occurrence was extremely rare (.05).

There was no evidence of a P300 following the loud deviant when the probability of occurrence was either .10 or .20. The P300 following the loud deviant in the .05 probability group was therefore analyzed further. P300 peak latency was not significantly different in REM compared to the waking-ignore condition (365 and 315 ms respectively). A one-way ANOVA run on P300 amplitude at Pz indicated that it was significantly larger in the waking-ignore condition ($\bar{M} = 21.47 \mu V$), than in REM sleep ($\bar{M} = 10.72 \mu V$), $F(1,9) = 45.10$, $p < .0001$.

A late large amplitude negative slow wave (SW) was observed over parieto-occipital sites. It peaked at approximately 600-850 ms. It was larger following the intensity than the pitch deviant at occipital sites across all probability levels.

REM sleep was further investigated by sorting trials into phasic and tonic epochs (see Figures 7 and 8). This sorting was done because it is possible that averaging over the entire REM period could have obscured intra-REM differences. In general, there were more phasic than tonic epochs of REM in the analysis. A Group (3 probability conditions) by Deviant (pitch versus intensity) by REM State (tonic versus phasic) repeated measures ANOVA was run at each electrode site separately.

----- Insert Figures 7 & 8 here -----

At Cz, a main effect of REM state was observed for the amplitudes of N1 and P2. N1 was reduced in tonic REM, but the difference did not reach significance, $F(1,21) = 3.13$, $p = .09$. The amplitude of P2 was significantly larger in tonic REM at Fz and Cz, $F(1,21) = 5.28$ and $F(1,21) = 13.16$, $p < .01$. There were no significant interactions involving N1 and P2.

A P300 was still not apparent in either tonic or phasic REM when a pitch deviant was employed. Similarly, a P300 was not apparent in either tonic or phasic REM when the probability of occurrence of the loud deviant was either .10 or .20. P300 was however visible in both tonic and phasic REM to the very rare intense deviant. The amplitude of this P300 did not significantly vary between tonic and phasic REM states, $F < 1$. The latency of P300 was prolonged during tonic REM, but the difference was not significant, $F(1,9) = 2.41$, $p < .15$.

The late SW did not show consistent differences between tonic and phasic REM. The SW tended to be larger in tonic REM when the probability of deviant occurrence was high (.20) and to be larger in phasic REM (particularly for the intense deviant), when probability of deviant occurrence was low (.05). There was however wide inter-subject variability.

Discussion

The standard stimulus elicited the usual N1-P2 waveform. During the waking state, it was not affected by the type of deviant stimulus nor the probability of occurrence. The physical characteristics of the standard did not vary in either the pitch or the intensity conditions. N1 was slightly reduced in amplitude when subjects were awake and alert but ignoring the auditory stimulus. The attend-ignore difference was however not significant. It has long been known that stimuli that are presented at rates slower than every 1 s are difficult to ignore. As a result, N1 may not be affected by manipulation of the subject's direction of attention (Schwent, Hillyard, & Galambos, 1976). N1 was consistently below baseline while P2 increased in amplitude during stage 2 sleep. This also replicates many previous studies (Campbell et al., 1992; Salisbury, Squires, Ibel, & Maloney, 1992; Harsh, Voss, Hull, Schrepfer, & Badia, 1994; de Lugt Loewy, & Campbell, 1996; Loewy, Campbell, & Bastien, 1996; Elton, Winter, Heslenfeld, Loewy, Campbell, & Kok, 1997; Cote, Epps, & Campbell, in press - b). During stage REM, the amplitude of N1 and P2 occupied an intermediate position between the waking state and stage 2.

In wakefulness, the experimental manipulations affected P300 amplitude as expected. When subjects counted the deviant stimuli, P300 became larger as its probability of occurrence decreased. This is consistent with the classic study by Duncan-Johnson and Donchin (1977). P300 was significantly larger to the loud, intense stimulus than to the pitch deviant. This appears to contradict the well-documented endogenous nature of P300. Many authors report that P300 is unaffected by the physical characteristics of the stimulus. It is quite possible that this apparently exogenous effect might be due to an endogenous confound – the ease of detection of the loud

deviant. Deviants that are easier to detect may elicit larger amplitude P300s than those that are difficult to detect (Picton, 1992).

A parietal maximum P300 was observed whenever subjects were asked to actively detect the deviant stimulus. When subjects were asked to ignore the stimulus, there was no P300 to the pitch deviant, even if it occurred very rarely. When the deviant was very loud and very rare (.10 and .05), a P300 was elicited in the ignore condition. This is consistent with the research by Roth et al. (1982), Roth et al. (1984), Polich (1989) and Putnam and Roth, (1990) who noted that an obligatory P300 may be elicited in waking ignore conditions when stimuli are sufficiently loud. This is presumably because the very intense stimuli intrude into consciousness since subjects are not able to inhibit the processing of them. In both the attend and ignore conditions, it appears that a P300 will be elicited whenever the subject becomes conscious of the deviant stimulus whether this consciousness is internally or externally controlled. The amplitude of this P300 was widespread, remaining large at both Cz and Fz in the waking conditions.

In stage 2 sleep, there were no significant differences between the standard and deviant waveforms in any of the intervals between 300 and 500 ms for either the pitch or intensity deviant at any probability of occurrence. Therefore, even when very liberal multiple one-tailed *t*-tests were employed, a significant P300-like wave could not be found in stage 2 sleep. Despite our efforts to remove trials in which a large amplitude K-Complex was elicited, there was still evidence of a late negativity particularly over frontal sites. A late parieto-occipital positive peak was visible in the grand averaged waveforms following the rare, intense deviant. It peaked at about 421 ms. This late deviant positivity was however not statistically different from the standard waveform data in the 400 to 500 ms interval. The late parieto-occipital positivity is

however consistent with what was observed in the second study in this thesis (Cote & Campbell, 1999b), and with late stage 2 positivities reported by others (Wesensten & Badia, 1988; Nielsen-Bohlman, Knight, Woods, & Woodward, 1991; Salisbury & Squires, 1993; Winter et al., 1995). As noted by Cote and Campbell (1999b), this positivity peaks too late and its scalp distribution is too posterior to be considered to be a true P300.

In REM sleep, there was no evidence of a late positive wave following the pitch deviant regardless of how infrequently it was presented. The failure to find a REM P300 when the pitch deviant was employed is consistent with the often observed failure to elicit it in the waking state when subjects ignore the stimuli. The results are however inconsistent with reports by Niiyama et al. (1994) and Bastuji et al. (1995) who did observe a small amplitude positive wave in REM sleep following pitch deviants. Both Niiyama et al. (1994) and Bastuji et al. (1995), and the present study employed brief duration 1000 Hz standard and 2000 Hz deviant stimuli. The rate of presentation was quite similar. The probability of deviant occurrence was .20 in the Niiyama et al. (1994) study. They could not observe a P300 in the actual deviant ERP. However, when the standard and deviant ERPs were subtracted, a small amplitude parieto-occipital positivity occurring at about 361 ms was visible in the difference wave. This subtraction procedure is equivalent to running a t-test to compare the standard and deviant waveforms. In the present study, no significant differences were apparent in the 300 to 500 ms range when the probability of deviant presentation was .20. Bastuji et al. (1995) delivered pitch deviants on 10% of trials. They noted a late (445 ms), small ($3.6 \mu V$) positivity following the deviant in REM sleep. This is unusually late for a "true" P300. In the present study, a similar late positivity could not be observed in the .10 deviant probability condition. In both the Niiyama et al. (1994) and the

Bastuji et al. (1995) studies, each subject was presented with only a small number of deviant stimuli (50 and 40 respectively). In the present study, at least 160 deviants were presented in the .20 conditions, and 80 deviants were presented in the .10 conditions. The Niiyama et al. (1994) and Bastuji et al. (1995) waveforms thus had more contamination from noise.

The very rare loud deviant did elicit a parietal P300 in REM sleep. This P300 was significantly reduced in amplitude compared to that observed in the waking-ignore condition. The more frequently occurring intensity deviants did not elicit the P300 in REM sleep. It is possible that the failure to observe a significant P300 in the .10 and .20 deviant probability condition was due to the smaller number of subjects in these groups compared to the .05 condition. The absence of statistical power cannot however explain the effects of probability of deviant occurrence. During REM, the ERPs following the loud deviant were actually more *negative* going than following the standard in the 300-500 ms interval for the .10 and .20 groups. Only very loud and very rare stimuli will elicit the REM P300. This is consistent with the second experiment in this thesis but is somewhat at odds with the results of the third. In the third experiment, 0, 60, 80 and 100 dB stimuli were presented with equal probability. The loud 100 dB stimulus did elicit a REM P300 even though it was presented on 25% of trials. In the present study, equally loud stimuli presented on either 10 or 20% of trials failed to elicit a P300.

In present study, P300 was not apparent at the frontal site in REM sleep. This was also the case in Experiment 3 in this thesis. Bastuji et al. (1995) also observed a sharp attenuation in P300 over frontal sites. They noted a negative-going wave at Fz at about 330 ms. In the present study, a rather large amplitude frontal negativity was also visible over frontal sites in the 300 to 400 ms time period in REM sleep. This frontal negativity was not observed in Experiment 2 of

this thesis. In the sleep onset study (Experiment 1), an attenuation in P300 was observed over frontal sites in stage 1. The scalp distribution of P300 in the waking state, and in stage 1 and REM is different. This implies that the intra-cranial generators of P300 must be different in wakefulness and in stage 1 and REM. This conclusion is at odds with those in Experiment 2. A frontal P300 was observed in that study. However, in that study the deviant was not as loud, 90 dB. It is possible that a much more intense stimulus will affect the frontal generation of P300.

The parietal P300 in REM was apparent in all studies of this thesis. P300 might be involved with contextual updating of memory following the detection of the deviant. Frontal lobe patients show a parietal P3 following the presentation of novel stimuli but the frontal P3 to these same stimuli is absent (Knight, 1984). It is possible that the frontal contribution to P300 is also absent in REM sleep. There is evidence of frontal disconnection during REM. For example, neuroimaging studies have shown that there is significant deactivation in the prefrontal cortex during REM sleep (for review, see Hobson, Pace-Schott, Stickgold, & Kahn, 1998). In terms of consciousness, it would appear that subjects may be able to make conscious detections (parietal P300) during REM sleep, but not "experience" this consciousness (absence of frontal P300). This may also explain why subjects do not awaken following apparent detection of the loud, intrusive deviant.

The late negative SW in REM sleep has also been observed previously (Nordby, Hugdahl, Stickgold, Bronnick, & Hobson, 1996; Pratt, Berlad, & Lavie, 1999; Cote & Campbell, 1999a, 1999b). In our previous studies, we suggested that this SW may be related to PGO activity, since it had an occipital dominant scalp distribution, accompanied eye movements in REM, and was sensitive to changes in stimulus intensity. In the present study, the SW was again

larger to more intense stimuli. There were no consistent differences in the amplitude of this SW between phasic and tonic REM trials across the various probability conditions. Cote and Campbell (1999a) observed SW to be larger on phasic trials, but a single intense deviant, whose presentation was very rare, was employed. In the present study, when the intense deviant was presented on only 5% of trials, the SW was again largest on phasic trials. While the SW may be large to very rare intense deviants during phasic REM, there is little consistency with other probabilities or with the pitch deviant.

Sallinen, Kaartinen, and Lyytinen (1996) suggested that there might be more extensive information processing during tonic than phasic REM. This is because subjects' attention may be directed to internal mental activity, dreaming, during phasic REM. There was little support for this theory in the present study or in Experiment 3. N1 was consistently larger and P2 smaller during phasic compared to tonic REM. The increase in N1 and the decrease in P2 can be explained by the effects of an overlapping attentional waveform, Processing Negativity (PN). PN overlaps and summates with both N1 and P2, causing N1 to become larger (i.e., more negative), but P2 to become smaller (i.e., less positive or more negative). PN is thought to reflect the additional processing that an attended channel receives (Näätänen, 1990). This explanation is at odds with that of Sallinen et al. (1996). Subjects appear to be more attentive to the external environment during phasic REM. In support of this, P300 latency was prolonged during tonic REM. Stimulus classification therefore takes longer during tonic REM. The amplitude of P300 was however not affected by the different types of REM. Differences between phasic and tonic REM therefore occur relatively early in information processing (at the time of N1) rather than at the stimulus classification stage of processing.

Table 5.1. One-tailed t-test comparisons of standard versus deviant stimuli in wakefulness

Stimulus Type	Task	Interval	80_20 %				90_10 %				95_05 %			
			Standard Mean	Rare Mean	t	p	Standard Mean	Rare Mean	t	p	Standard Mean	Rare Mean	t	p
Intensity	Attend	250-300	-0.37	14.16	4.53	<u>.0003</u>	0.75	23.22	5.58	<u>.0001</u>	1.08	27.27	7.11	<u>.0000</u>
		300-350	-3.03	21.14	5.94	<u>.0000</u>	-2.79	27.02	7.47	<u>.0000</u>	-1.34	40.31	10.98	<u>.0000</u>
		350-400	-2.53	15.62	5.40	<u>.0001</u>	-1.48	16.75	7.71	<u>.0000</u>	-1.03	36.60	13.18	<u>.0000</u>
		400-450	-1.99	5.38	2.57	<u>.0124</u>	-1.67	5.83	2.43	<u>.0158</u>	-0.96	20.33	6.88	<u>.0000</u>
		450-500	-2.02	4.43	2.44	<u>.0156</u>	-0.30	5.90	2.34	<u>.0188</u>	-0.78	14.30	4.88	<u>.0001</u>
Pitch	Ignore	250-300	-0.54	1.07	0.80	.2206	-0.11	11.31	3.58	<u>.0019</u>	0.24	12.57	3.94	<u>.0005</u>
		300-350	-2.17	0.00	1.66	.0613	-1.81	9.81	2.58	<u>.0121</u>	-1.24	19.15	5.58	<u>.0000</u>
		350-400	-1.49	-1.73	-0.25	.4022	-1.64	1.88	1.80	<u>.0483</u>	-1.02	15.14	7.94	<u>.0000</u>
		400-450	-1.00	-1.83	-0.66	.2595	-0.91	-2.68	-1.07	.1524	-0.79	5.98	2.62	<u>.0087</u>
		450-500	-0.96	-2.61	-1.42	.0901	-0.21	-3.05	-1.85	.0445	-0.57	0.98	0.72	.2400
	Attend	250-300	0.23	9.15	3.08	<u>.0048</u>	0.88	8.08	2.06	<u>.0311</u>	1.62	12.50	4.02	<u>.0004</u>
		300-350	-3.84	13.49	4.13	<u>.0007</u>	-2.63	13.10	3.39	<u>.0027</u>	-1.22	21.74	8.56	<u>.0000</u>
		350-400	-2.41	14.58	3.80	<u>.0013</u>	-1.41	18.21	5.18	<u>.0001</u>	-0.55	26.38	11.72	<u>.0000</u>
		400-450	-2.06	7.59	2.48	<u>.0146</u>	-1.35	10.10	3.42	<u>.0026</u>	-0.90	22.49	10.11	<u>.0000</u>
		450-500	-2.00	6.44	2.95	<u>.0061</u>	-0.44	6.84	1.98	<u>.0353</u>	-0.11	15.61	5.92	<u>.0000</u>
	Ignore	250-300	-0.57	-1.14	-0.5	.3137	0.07	-0.37	-0.18	.4304	-0.10	0.96	0.72	.2396
		300-350	-1.40	-2.94	-1.18	.1325	-1.48	-1.58	-0.03	.4880	-1.49	0.90	1.01	.1628
		350-400	-0.82	-1.58	-0.79	.2230	-1.13	-1.38	-0.13	.4507	-0.98	2.45	1.24	.1159
		400-450	-0.32	-0.90	-0.48	.3215	-0.40	-1.21	-0.47	.3220	-0.58	1.09	0.68	.2527
		450-500	-0.75	-1.56	-0.72	.2426	-0.10	-1.00	-0.65	.2641	-0.74	-1.19	-0.21	.4174

Table 5.2. One-tailed t-test comparisons of standard versus deviant stimuli in sleep

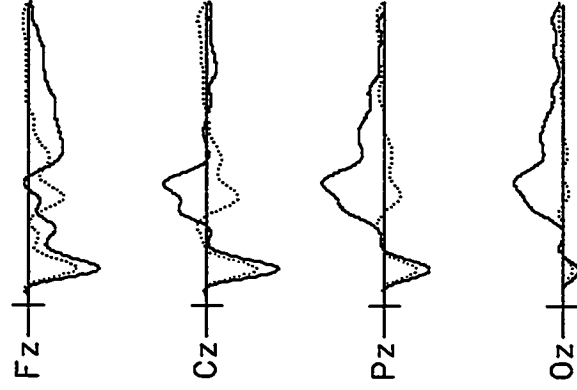
Stimulus Type	Sleep Stage	Interval	80_20 %				90_10 %				95_05 %			
			Standard Mean	Rare Mean	t	p	Standard Mean	Rare Mean	t	p	Standard Mean	Deviant Mean	t	p
Intensity	Stage2	250-300	2.48	7.02	1.42	.0904	2.86	8.54	1.30	.1092	1.38	7.09	2.30	<u>.0167</u>
		300-350	0.09	-6.29	-1.67	.0599	0.81	-2.63	-0.77	.2290	-1.79	-4.49	-0.6	.2777
		350-400	-0.30	-6.40	-3.07	.0049	0.33	-6.72	-1.63	.0644	-2.88	-1.85	0.18	.4291
		400-450	-0.03	-4.51	-2.36	.0181	0.39	-8.46	-2.79	.0082	-1.62	3.67	0.97	.1726
		450-500	0.11	-7.49	-2.72	.0092	0.61	-11.24	-2.67	.0101	-1.29	3.42	0.89	.1913
Pitch		250-300	2.80	8.24	3.16	<u>.0041</u>	5.16	10.60	1.24	.1187	1.79	9.55	3.51	<u>.0012</u>
		300-350	-0.37	-3.39	-1.08	.1509	2.78	4.64	0.40	.3466	-2.04	-0.49	1.04	.1566
		350-400	-1.22	-7.96	-2.91	.0065	1.96	0.21	-0.37	.3572	-3.51	-5.23	-0.83	.2092
		400-450	-0.84	-4.53	-2.13	.0272	2.74	-1.33	-1.04	.1598	-2.16	-1.81	0.17	.4316
		450-500	-0.62	-3.90	-1.71	.0568	3.44	-2.72	-1.87	.0432	-1.80	-1.57	0.11	.4566
Intensity	REM	250-300	-1.33	0.34	0.76	.2299	0.95	-0.08	-0.32	.3784	0.25	5.71	1.32	.1017
		300-350	-1.92	-3.59	-1.19	.1279	-0.57	-3.89	-0.89	.1955	-0.14	4.57	1.77	<u>.0464</u>
		350-400	-2.09	-5.99	-2.89	.0068	-1.14	-5.63	-1.21	.1245	-0.95	7.09	2.51	<u>.0109</u>
		400-450	-2.32	-7.17	-2.2	.0241	-0.60	-8.81	-1.66	.0610	-0.68	8.32	4.12	<u>.0003</u>
		450-500	-3.03	-11.16	-2.76	.0086	-0.54	-11.92	-2.66	.0105	-0.58	4.90	2.33	<u>.0160</u>
Pitch		250-300	-0.58	4.90	2.24	<u>.0224</u>	0.21	4.13	1.60	.0682	0.15	1.71	0.64	.2664
		300-350	-1.33	0.68	0.86	.2029	-0.27	0.81	0.52	.3073	-1.00	-2.05	-0.58	.2852
		350-400	-1.58	-0.90	0.33	.3735	-0.16	-1.63	-0.74	.2364	-2.21	-1.67	0.26	.3997
		400-450	-2.03	0.00	0.71	.2441	-1.03	-2.15	-0.53	.3039	-1.89	-0.11	0.91	.1886
		450-500	-2.63	-2.84	-0.1	.4614	-2.48	-2.26	0.10	.4598	-2.59	0.16	1.26	0.1128

Figure Legends

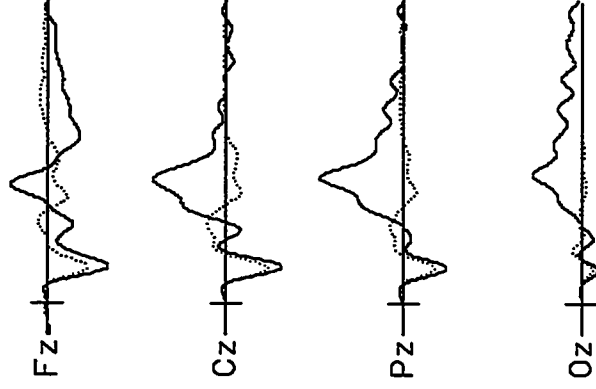
- Figure 5.1. ERPs following pitch deviants and standards in wake - attend condition. In this and all subsequent figures, the ERPs in the .20, .10, and .05 probability conditions are illustrated in the left, centre, and right panels respectively. Positivity in this and all other figures is indicated by an upward deflection. Note the N1-P2 complex following standard and deviant stimuli in all probability conditions. P300 is largest following the deviant in the .05 probability condition, decreasing in amplitude for the .10 and the .20 conditions.
- Figure 5.2. ERPs following intensity deviants and standards in wake - attend condition. Again, the P300 is largest in the rarest probability condition ($p = .05$) and decreases in amplitude with increasing probability of the deviant. P300 amplitude following the intensity-deviant is larger than that following the pitch deviant.
- Figure 5.3. ERPs following pitch deviants and standards in wake - ignore condition. No P300 is apparent in any condition.
- Figure 5.4. ERPs following intensity deviants and standards in wake - ignore condition. P300 is apparent following the loud deviant stimulus. It is largest for the rarest probability condition.
- Figure 5.5. ERPs following intensity and pitch deviants in stage 2 non-REM sleep. No P300 can be observed in any condition. A positive peak is apparent in the 400 - 450 ms range at occipital sites and it is largest for the rarest probability condition. This could be due to a limited number of small amplitude (less than 100 μV) K-Complexes present in the average.
- Figure 5.6. ERPs following intensity and pitch deviants in REM sleep. P300 is visible following the intensity-deviant in the rarest ($p = .05$) probability condition only. It is not visible at fronto-central sites. In the .20 and the .10 probability conditions, the ERP waveform is characterized by large amplitude P1 and P2 peaks, followed by a late slow negative wave. Note that this and all other figures of REM data are displayed at double the amplitude.
- Figure 5.7. ERPs following the pitch deviant in tonic versus phasic REM sleep. Note that N1 tends to be smaller and P2 larger in tonic REM.
- Figure 5.8. ERPs following the intensity deviant in tonic versus phasic REM sleep. Again, note that N1 tends to be smaller and P2 larger in tonic REM.

Wake - Attend Condition

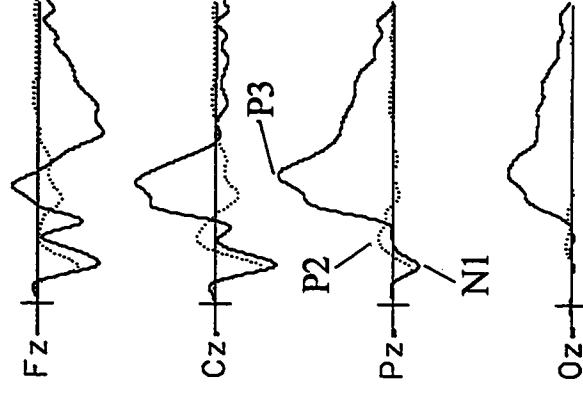
p = .20



p = .10

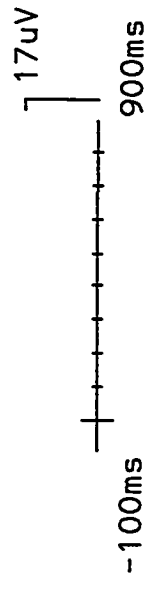


p = .05



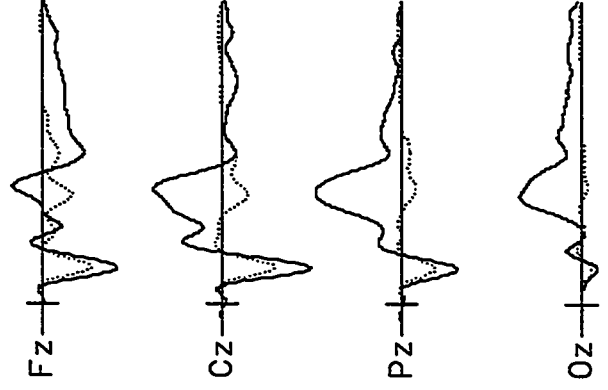
— Deviant (pitch)

..... Standard

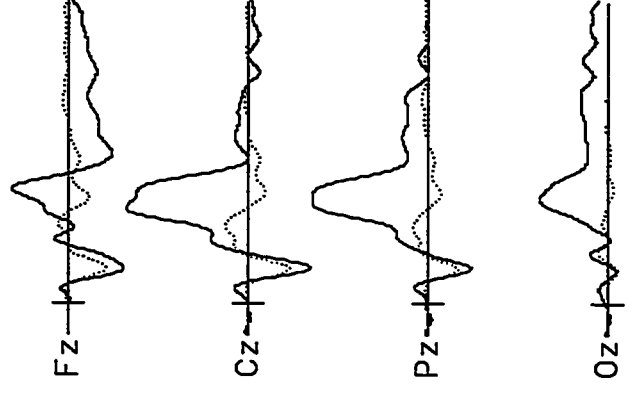


Wake - Attend Condition

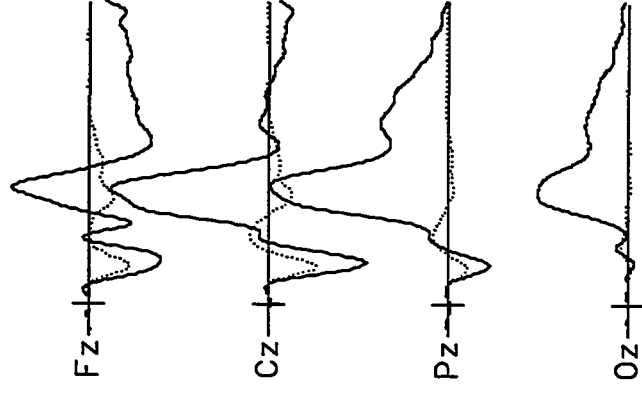
p = .20



p = .10

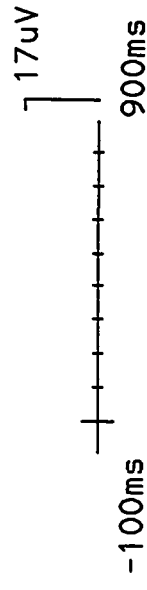


p = .05



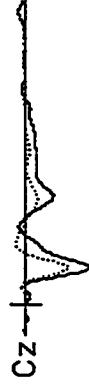
— Deviant (intensity)

..... Standard



Wake - Ignore Condition

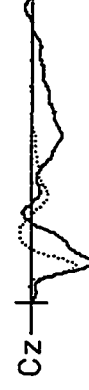
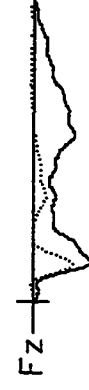
p = .20



p = .10

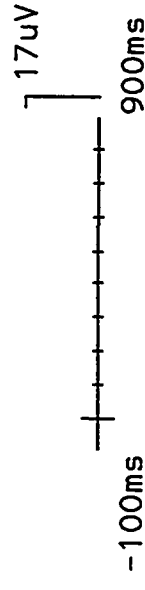


p = .05



— Deviant (pitch)

..... Standard

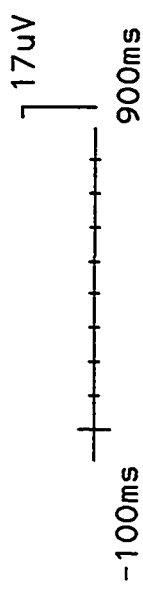
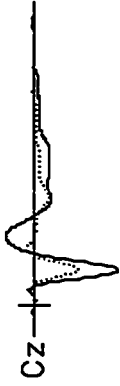


Wake - Ignore Condition

p = .20

p = .10

p = .05

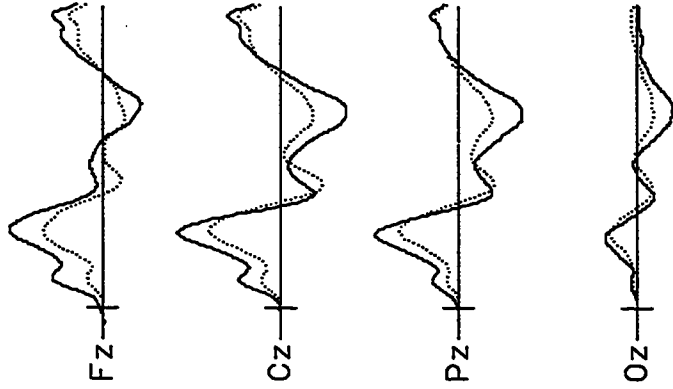


— Deviant (intensity)

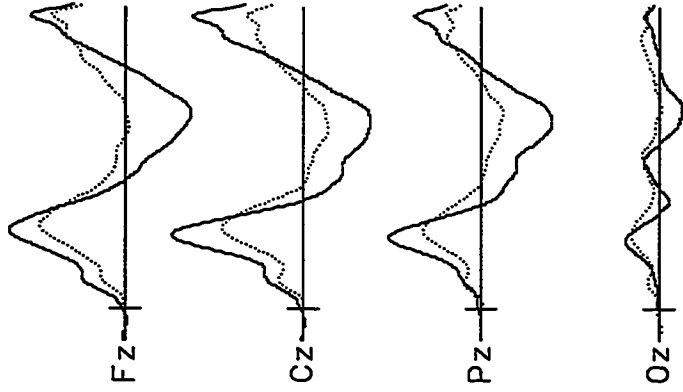
..... Standard

Stage 2 Sleep

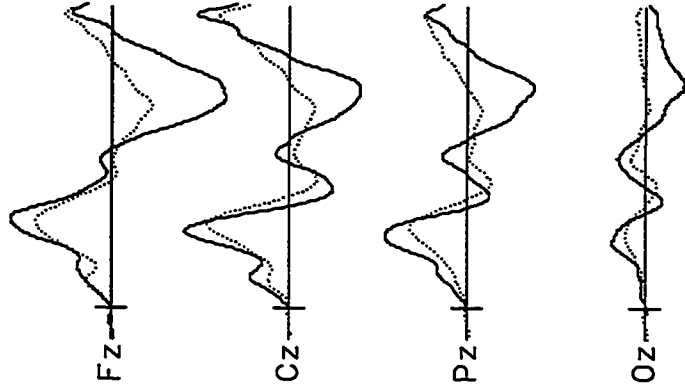
p = .20



p = .10

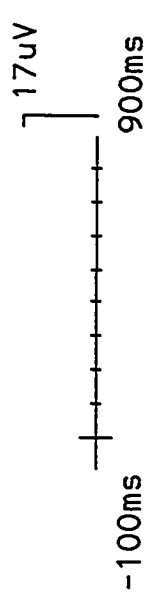


p = .05



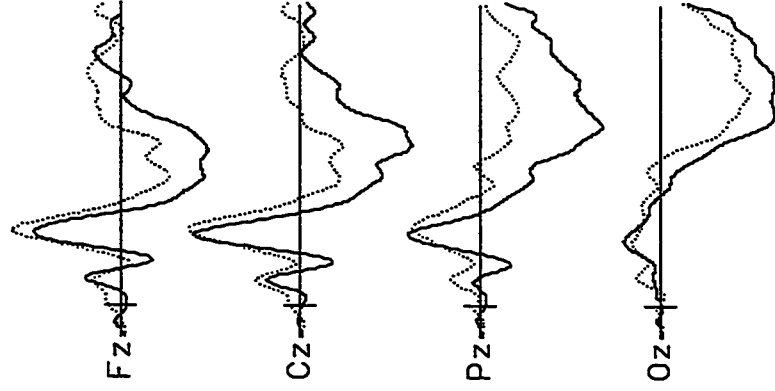
— Intensity

..... Pitch



REM Sleep

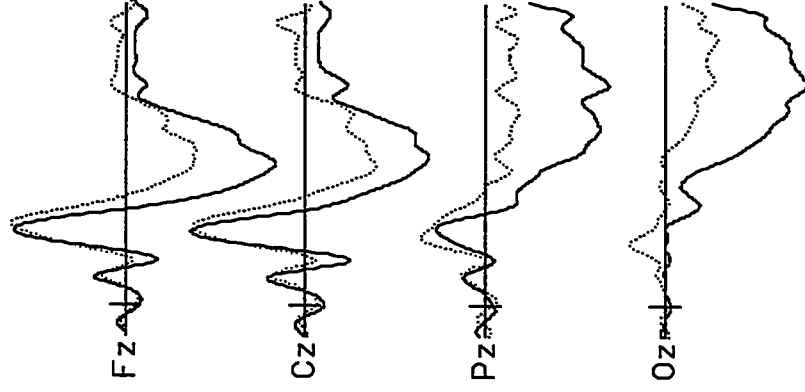
p = .20



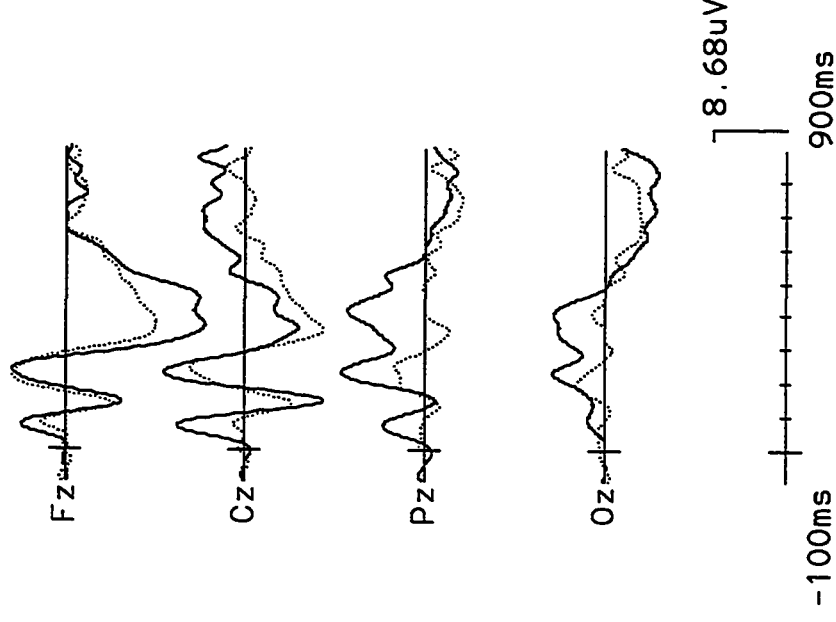
—— Intensity

..... Pitch

p = .10



p = .05

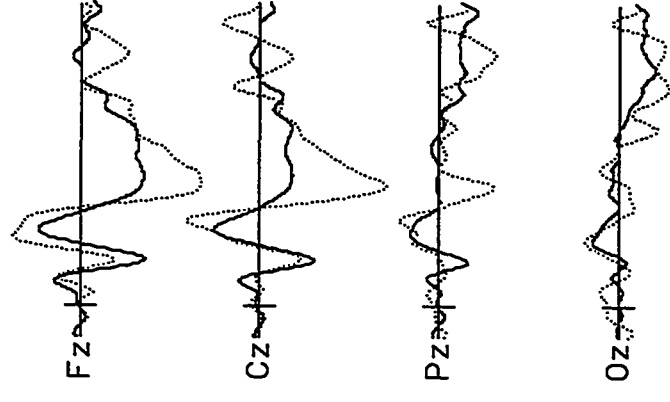
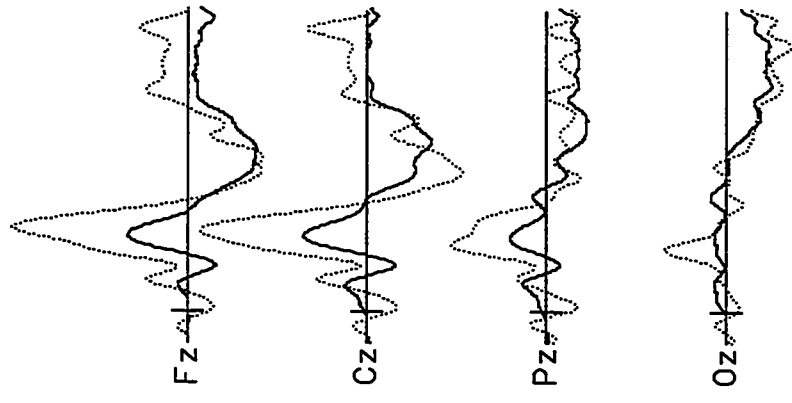
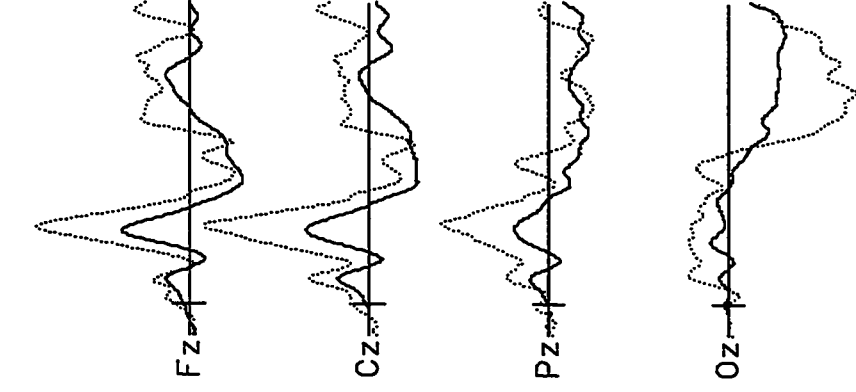


Probability of the Pitch Deviant

p = .20

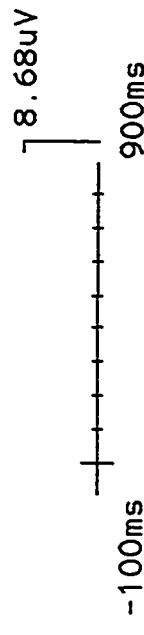
p = .10

p = .05



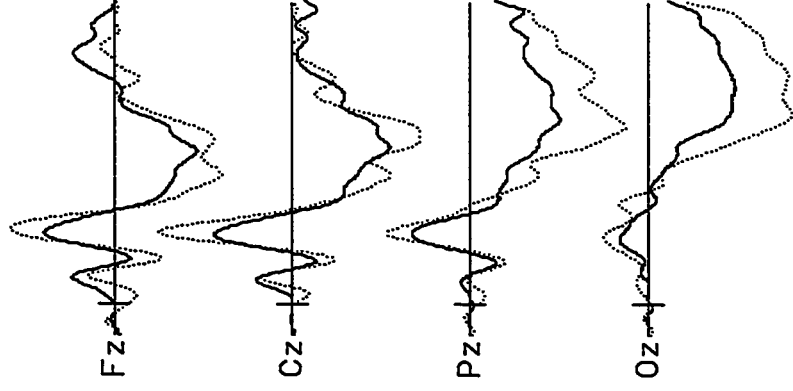
—— Phasic REM

..... Tonic REM

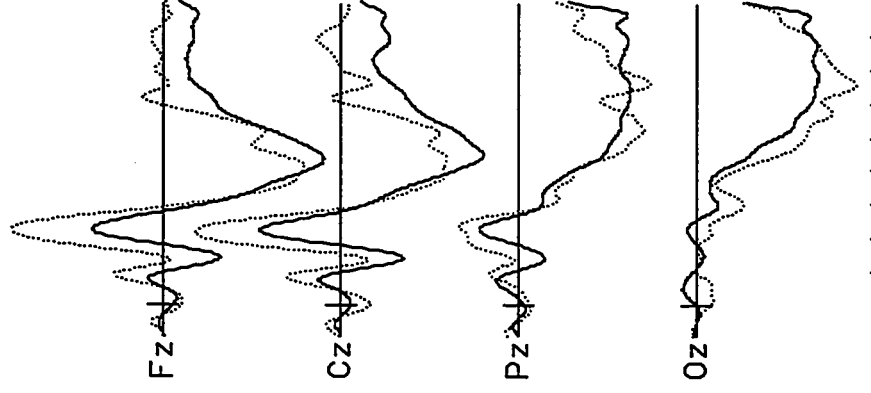


Probability of the Intensity Deviant

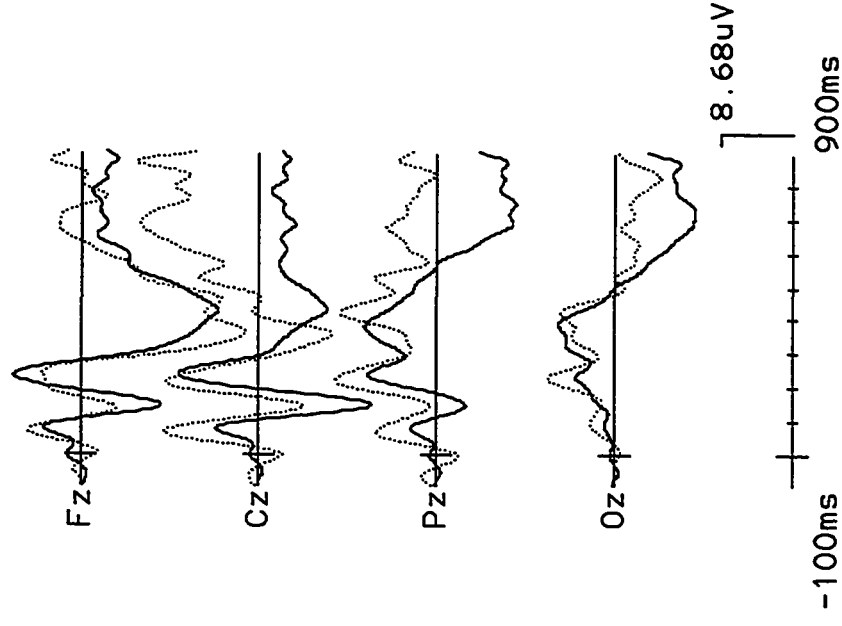
p = .20



p = .10



p = .05



—— Phasic REM

..... Tonic REM

Chapter 6

General Discussion and Conclusions

The various studies in this thesis have investigated information processing in wakefulness, during the sleep onset period, and within non-REM and REM sleep. The purpose of these investigations was to determine the extent to which the sleeper processes auditory stimuli. The extent of information processing and consciousness during sleep has been debated for some time. Hobson and colleagues argue that awareness of external events is not possible, particularly during REM sleep when mental resources are focused toward internal dream activity. On the other hand, LaBerge and colleagues argue that consciousness must be possible in order for the sleeper to be aware of their dreams and to subsequently signal to the experimenter that they are dreaming. A major methodological problem in this area of research is how to define and measure consciousness. In this thesis, consciousness was measured in a number of ways — on the basis of the EEG, subjects could be said to be awake or asleep (conscious or unconscious). On the basis of the subject's response to externally presented targets, they could be said to be consciously aware of the stimuli. Finally, on the basis of P300, subjects could also be said to be aware of the external stimulus, even in the absence of a behavioural response. The P300 was especially critical to defining consciousness within sleep, a period in which behavioural responses are rare.

A number of researchers have endeavored to determine whether or not a P300 can be recorded in any stage of sleep. P300 occurs following presentation of infrequently occurring stimuli that deviant along some physical parameter from more frequently occurring standard

stimuli. When the stimulus is easy to detect, P300 typically peaks around 300-400 ms following the onset of the deviant stimulus. It is maximum over parieto-central areas of the scalp, but is also widespread over anterior and posterior regions. P300 is typically elicited only if the subject is attentive to the stimuli and detects the relevant stimulus. Stimuli that are however very loud will elicit a P300 in subjects whose attention is directed elsewhere, possibly because the loud stimulus intrudes into consciousness.

Early sleep studies considered a late positive wave, peaking at about 700-900 ms, to be a candidate P300. These late positivites were most likely in fact components of the auditory evoked K-Complex. Others have reported small amplitude positive waves around 400-450 ms in non-REM stage 2 sleep, following presentation of a rare, deviant stimulus. The similarity of this non-REM P450 to the attention-dependent P300 of wakefulness remains to be shown through experimental manipulation. Perhaps the most convincing evidence of a P300 in sleep comes from studies that recorded small amplitude positive waves following pitch-deviants during REM. These small amplitude potentials were however suspect as a candidate P300 since one peaked late and the other had an unusual parietal-occipital scalp distribution. The various experiments in this thesis were conducted to determine the conditions necessary for the P300 potential to be recorded during sleep.

Summary of Findings and Conclusions

In Experiment 1, the measures that could be used to define consciousness were established. This study examined the sleep onset period — the transitional period between a waking, conscious state, and a sleeping, unconscious state. The EEG was recorded from 29 different sites in order to

map the distribution of P300. Subjects were repeatedly awakened so that a large amount of data could be obtained from the normally short duration transitional period. As expected, subjects detected almost all targets while awake, and almost none while in stage 2 non-REM sleep. A large amplitude parieto-central P300 was apparent in wakefulness, but it was not discernable in stage 2. During stage 1, subjects detected about half of the deviants, and their reaction time was prolonged. P300 remained large at parietal sites when subjects detected the target in stage 1. It was however attenuated at frontal sites, particularly when RTs were slow.

Conclusions from Experiment 1:

1. Subjects are obviously conscious when awake. They are able to signal their consciousness of an external target stimulus. A large parieto-central P300 was recorded following detection of these targets.
2. In stage 1, consciousness begins to wax and wane. A parietal maximum P300 remains large to detected targets, but the frontal aspects of this P300 are reduced or absent.
3. There is little evidence of consciousness in stage 2 sleep. Subjects are not able to signal their awareness of an external target stimulus. P300 cannot be elicited to the target stimuli.
4. The parietal P300 appears to be an excellent measure of consciousness. It is large when subjects detect the stimuli while awake and in stage 1, and absent when they do not.
5. It is possible that subjects are able to detect significant external events, but the frontal aspects of consciousness begin to diminish. These frontal aspects of consciousness might include rather abstract constructs such as experience and affect. RT might be slowed because of a decrease in the frontal control of motivation (or "will").

In the next three experiments, ERPs were recorded during an entire single night of sleep.

Loud stimuli were employed to investigate the effects of intrusive stimuli on the sleeping ERPs.

It is quite possible that these stimuli altered the architecture of sleep. For example, there may have been less SWS than usual. This could have been verified by recording additional nights without stimulus presentation. The purpose of this series of studies was not however to determine the effects of external stimuli on the quality of sleep. In this regard, very few ERP studies provide details of the percentage of time spent in each stage of sleep.

In wakefulness, when the subject is instructed to ignore the stimuli, a P300 will in general not be elicited. However, if the stimulus is sufficiently loud, a P300 will be elicited in inattentive subjects. This may be because the subject is not be able to completely ignore the loud stimulus. We therefore hypothesized that a loud stimulus would be most likely to elicit the P300 in sleep. In Experiment 2, an intensity odd-ball paradigm was employed. A loud 90 dB SPL tone pip was presented on 5% of trials, and a lower intensity 70 dB SPL tone pip was presented on the remaining trials. A multiple channel EEG was recorded in order to map the scalp topography of the candidate P300. A large amplitude parieto-central P300 (latency = 321 ms) was apparent in REM sleep following the rare, intense deviants. An earlier P3a was also apparent at frontal sites in REM sleep. There was no evidence of a P300 in non-REM stage 2 sleep. A later positive wave having an occipital scalp distribution was apparent at 446 ms when K-Complexes were absent in stage 2.

Experiment 3 was conducted to determine whether the REM recorded in the previous study was due to the loudness or the rareness of the stimulus. Auditory tone pips, having an intensity of either 0, 60, 80 or 100 dB SPL, were delivered at random with equal probability. Stimuli were delivered during wakefulness while subjects read a book. The loud 100 dB stimulus elicited short-latency fronto-central maximum (P3a) and long-latency parieto-central maximum

(P300) positive waves (peaking at 293 and 373 ms respectively). Neither the P3a nor the P300 could be observed in stage 2 sleep, regardless of the level of stimulus intensity. During REM sleep, a late parietal P300 (latency 363 ms) was elicited by the 100 dB stimulus. The earlier positive frontal peak (i.e., P3a) was not apparent. These data suggest that stimuli that are sufficiently intrusive to elicit a parietal P300 under waking-ignore conditions will continue to do so in REM sleep. However, the frontal distribution of this positive wave is not apparent in REM.

Experiments 2 and 3 demonstrated that P300 could be elicited in REM sleep following loud stimuli. There are however reports in the literature that pitch deviants can also elicit the P300 in REM. In Experiment 4, a comparison was therefore made between pitch and intensity deviants. In addition, probability of deviant presentation was manipulated since it is known to affect the amplitude of a true P300. Participants were randomly assigned to one of three probability groups, in which the deviant was presented on either 20%, 10%, or 5% of trials. To investigate the hypothesis that only stimuli sufficiently loud to elicit the P300 in waking-ignore conditions would also elicit the P300 in REM, subjects were delivered stimuli during the waking state. Subjects were asked to either attend to (and count the deviant), or ignore (and read a book) the stimuli during wakefulness. A large amplitude P300 was apparent following both pitch and intensity deviants in the attend (i.e., count) condition. It was maximum at parietal sites but was also apparent at fronto-central sites. As expected, the amplitude of this parietal P300 decreased with increasing probability of deviant presentation. In the waking-ignore condition, a slightly smaller P300 followed the intensity deviants. There was however no evidence of a P300 following the pitch deviant. During stage 2 of sleep, a small amplitude positive wave was visible at 421 ms following both pitch and intensity deviants. Consistent with Experiments 2 and 3, its

latency was prolonged compared to the waking P300 and its scalp distribution was maximum over posterior, parieto-occipital sites. During REM sleep, the pitch deviant failed to elicit the P300. An attenuated positivity having the usual P300 latency (365 ms) and parieto-central scalp distribution was visible following the intensity deviant in the rarest probability condition (i.e., $p = .05$). However, the frontal spread of P300 that was observed in the waking state was absent during REM sleep. This final study demonstrated that the REM P300 can only be elicited following intensity deviants when the stimuli are extremely rare.

There were some inconsistencies among experiments 2, 3, and 4. These included the recording of the frontal P300 and the effects of probability of deviant occurrence.

1. Frontal P300. In the waking state, P300 is maximum over parietal sites but it is also widely dispersed over frontal sites. During stage 1 and within REM sleep, a parietal maximum P300 can still be elicited by infrequently occurring deviant stimuli. However, in general the frontal aspect of P300 is much reduced or absent. In Experiment 2, a large parietal P300 was again recorded, but in this experiment P300 was also dispersed into frontal regions. This was an odd-ball task in which the loud deviant stimulus was delivered on only 5% of trials. This is quite similar to the 5% intensity deviant condition in Experiment 4. There were however differences in these studies. In Experiment 2, the intensities of the standard and the deviant stimuli were 70 and 90 dB, while in Experiment 4, they were 80 and 100 dB respectively. Although in both cases there was a 20 dB difference between the standard and the deviant stimuli, the perceived difference in loudness is much greater for the 80 and 100 dB stimuli, since the intensity scale is logarithmic. Another marked difference between studies is that in Experiment 4 subjects were presented with more blocks of stimuli (across both pitch and intensity conditions). It is possible

that this bombardment from the external environment might have elicited frontal deactivation.

2. Probability of Deviant Occurrence. In Experiment 4, P300 was apparent only when the loud stimuli were delivered on 5% of trials. When it was presented on either 10 or 20% of trials, P300 was not elicited. On the other hand, in Experiment 3, a P300 was elicited by the loud stimulus when its probability of occurrence was 25%. Such an inconsistency is not easy to explain. In Experiment 4, the loud stimulus was always presented less frequently than the standard (i.e., an odd-ball task was employed). In Experiment 3, stimuli were presented with equal probability. Moreover, among the stimuli that were presented was a 60 dB intensity tone. It is possible that a P300 can be elicited to more frequently occurring loud stimuli if they are presented among a train of considerably lower intensity standards.

Although there were some differences across these studies, most results were quite consistent. A number of general conclusions can therefore be made.

Conclusions from Experiments 2, 3 and 4:

1. A parietal P300 is apparent in REM sleep only. There is thus evidence for pre-conscious or conscious processing during REM sleep.
2. The P300 in REM will only be evoked by loud stimuli which are sufficiently intrusive to elicit the P300 in waking-ignore conditions.
3. In general, the P300 following loud deviants in REM sleep is apparent when the probability of deviant occurrence is very rare (.05).
4. The pitch deviant will not elicit a P300 in any stage of sleep.
5. The occipital dominant positive wave around 400-450 ms in non-REM stage 2 is probably not a candidate P300. It may be a positive deflection between the larger N350 and N550 components of the K-Complex.

6. P300 latency was somewhat prolonged in tonic REM compared to phasic REM. This indicates that stimulus classification processes are prolonged in tonic REM.
7. N1, a measure of general consciousness, was larger in amplitude during phasic REM. The increase in N1 may be due to the overlapping effects of Processing Negativity, indicating that the sleeper is more attentive to external stimuli during phasic REM.
8. A late negative slow wave (SW) was found in REM sleep following loud deviants. It is affected by stimulus intensity and has an occipital maximum distribution. It may be related to PGO spikes.

Discussion

The data presented in this thesis indicate that the attention-dependent P300 typically recorded in wakefulness, can also be recorded during stage 1 when the subject detects the stimulus. It can also be recorded in REM sleep. The REM P300 is apparent only following very loud and very rare stimuli, perhaps because these loud stimuli are more biologically relevant. These data provide evidence of at least some aspect of consciousness during an apparently unconscious state, sleep. The sleeper is not always disengaged from the external world, as would seem to be implied by many behavioural definitions of sleep (see Introduction in Chapter 1). During REM sleep, the subject appears to have some awareness of their external environment in a manner similar to that during wakefulness, as evidenced by the consistent parietal P300. However, the frontal contributions to consciousness may be absent.

Some researchers have proposed that REM sleep is similar to wakefulness (Horne, 1988). The presence of a P300 in REM sleep, indicating awareness of external stimuli, would appear to provide support for this notion. Nevertheless, Hobson, Pace-Schott, Stickgold, and Kahn (1998) point out that there are many neurophysiological differences between REM and wakefulness. For

example, positron emission tomography (PET) studies indicate unique blood flow patterns in REM and wakefulness. In general, there is increased activity in the limbic regions, and decreased activity in the pre-frontal cortex in REM compared to the waking state. Furthermore, different neuronal systems will be inhibited or facilitated in the two states. For instance, the rhythmic switching between non-REM and REM sleep may be explained by activity of so-called "REM-on" and "REM-off" cells of the nervous system (Siegel, 1994). REM-on cells are active during the REM state, but inactive during wakefulness and non-REM. Contrarily, REM-off cells are active in wakefulness and non-REM, yet inactive in REM sleep. In the present group of studies, the P300 recorded during stage 1 and REM differed in its topographic characteristics from that typically elicited in the waking state. In these studies, the P300 at frontal sites was not always apparent. It was absent in stage 1 when subjects failed to respond (experiment 1), and also absent in two investigations of P300 in REM (experiments 3 and 4). Thus, it may be that frontal contributions to consciousness are much reduced during REM. The frontal lobes may contribute to the "experience" of consciousness. The presence of the parietal P300 may indicate that the very rare, loud stimulus is detected. However, such stimuli usually elicit a startle reflex in waking subjects. It is possible that the subject may not experience these loud stimuli as "startling" during REM. This may explain why aspects of the startle reflex, such as blinking, and changes in muscle tonus follow presentation of the loud stimulus during REM. The absence of affect and experience might explain another phenomenon. Very loud and longer duration stimuli will awaken the sleeper. The short duration stimuli used in the present studies did not elicit awakenings. It is possible that awakening from sleep requires prior frontal activation and arousal. The REM P300 also differed from that typically recorded in wakefulness in other ways. Subjects do not appear to

be consciously aware of less biologically relevant stimuli, such as pitch deviants. In addition, the P300 is also not elicited by personally relevant stimuli such as the subject's own name.

This ERP evidence of heightened awareness of external stimuli during episodes of REM sleep supports a sentinel theory of REM sleep function. The sentinel theory proposed that REM sleep served to periodically prepare the brain for arousal in the event that the sleeper might need to respond to threatening stimuli (e.g., predators) in the environment (Snyder, 1966). This theory did not withstand the test of time, largely because data did not support it. Contrary to the predictions of such a theory, species which were considered to be more prey than predator did not have more REM sleep. Nevertheless, the data in this thesis indicate that REM is a period of enhanced stimulus processing capabilities. This appears to be the case for both internal (i.e., dreams) and external (i.e., stimuli) processing. Perhaps the purpose of such processing capabilities during REM sleep is not merely to protect against threatening prey, but to provide sensory stimulation to the brain. Alternatively, this enhanced processing may simply represent a by-product of other functions of REM sleep.

Future Direction of Research

The studies in this thesis demonstrated that intense stimuli will elicit the P300 in REM sleep. An explanation for this intensity-elicited P300 may be that the loud stimulus was more biologically relevant. Recently, Pratt et al. (1999) failed to show a P300 response during sleep to the subject's own name. The role of stimulus meaning on the REM P300 thus needs to be further explored. Manipulation of the stimulus parameters employed in the Pratt et al. (1999) study may prove to show differences in such psychologically relevant stimuli. For example, the intensity of the word

stimuli might need to be sufficiently loud and their probability of occurrence sufficiently rare. In the Pratt et al. (1999) study, the subject's own name was delivered on 30% and 70% of trials in different conditions. Its intensity was 40 dB nHL.

In experiment 1, P300 at frontal sites was much reduced when subjects detected the target in stage 1. This frontal P300 was also absent in experiments 3 and 4 when parietal P300s were recorded in REM sleep. We proposed that the absence of a frontal P300 may be due to "functional deafferentation" of the forebrain during sleep. The contribution of the frontal lobe to consciousness may be investigated using a stimulus novelty paradigm during the sleep onset period and throughout the various stages of sleep. Fabiani and Friedman (1995) showed that novel auditory stimuli (such as environmental sounds) will elicit a frontal P3 component. Spencer, Dien, and Donchin (1999) used a dense 128 channel electrode array to demonstrate that the frontal P3 elicited by novel stimuli is distinct from the P3a which peaks earlier at frontal sites. This frontal novel P3 is absent from frontal lobe patients, but does occur at parietal sites (Knight, 1984).

To explore the nature of the REM P300, various stimulus parameters could be further investigated. For example, stimulus abruptness may contribute to the perceived intrusiveness of a stimulus. Fast rise-fall times may elicit the P300, even with pitch deviants. In addition, more infrequent levels of probability of deviant presentation need to be investigated. It is possible that only exceedingly rare pitch deviants will elicit a P300. Probability levels such as, .05, .025, and .0125 may be employed to ascertain if P300 varies as stimulus probability decreases. Another parameter which may alter the elicitation of the P300 in REM sleep is the inter-stimulus interval. In experiment 3 of this thesis, a P300 was elicited to a loud 100 dB stimulus, which was

delivered at equal probability with three other lower intensity stimuli. We speculated that this loud stimulus may have elicited the P300 because of its temporal probability rather than its probability relative to the other stimuli. In the study by Ogilvie et al. (1991), a single low intensity tone was delivered approximately every 30 seconds. This tone also elicited a P300. The role of temporal versus sequential probability may be investigated by comparing conditions in which a single stimulus is delivered at various inter-stimulus intervals.

Another way to investigate the functional similarity between REM and wakefulness is through investigation of the 40-Hz rhythm. The 40-Hz rhythm is a transient, spontaneous oscillation that is believed to be generated from activity of thalamocortical neurons (Steriade, Amzica, & Contreras, 1996). There is good evidence that the 40-Hz rhythm may be related to awareness of relevant external stimuli. It may be recorded during wakefulness and REM sleep, but is markedly reduced in SWS. Llinás and Ribary (1993) proposed that the 40-Hz rhythm was related to attention and alertness. Thus, the presence of the 40-Hz rhythm in REM sleep provides further evidence that consciousness is possible in REM. Auditory stimuli will also evoke the 40-Hz rhythm, but only in wakefulness. Llinás and Ribary (1993) therefore suggested that the enhanced cognitive processing, evidenced by spontaneous 40-Hz activity in REM sleep, occurs because the subject's attention is more directed toward internal mental events. It may be that the stimuli were not sufficiently loud to evoke the 40-Hz rhythm in REM sleep. Low intensity tones varying in tonal pitch were employed. Loud or relevant stimuli may be necessary to elicit the 40-Hz response in REM sleep.

A number of unique ERPs may be recorded during sleep. The large N350 has received some study in non-REM sleep. However, a large frontal negative wave, peaking between 300 and

400 ms, was also apparent to the loud stimuli in REM. It may be that this negativity is similar to the N350 recorded in non-REM. If so, the N350 may represent a general sleep-related phenomena, rather than a non-REM sleep phenomena. A very late 700-900 ms negativity was also apparent in REM sleep. It was speculated that this SW may be related to PGO activity since it has an occipital dominance and is larger for more intense stimuli. In addition, in Experiment 2, the SW was larger during phasic REM. This finding was not replicated in subsequent studies. Most ERP studies of sleep have focused on components ranging from 10 to 300 ms in non-REM sleep. Further studies which focus on these later negativities in REM sleep may lead to a better understanding of the nature of this SW.

This thesis relied on ERP evidence of consciousness. Additional studies could employ subjective report of mental activity and dreaming following stimulus presentation. Would these be different when a P300 is elicited in REM compared to when it is not? When P300 is elicited in REM sleep, it may be that the auditory stimuli are incorporated into dream content. This would require awakening the sleeper several times during the sleep period. This was not done in the present studies, since the presence of a P300 needed first to be established. The disruption of REM periods would have reduced the amount of data available for averaging. In Experiment 4, subjects were however questioned in the morning regarding memory for auditory stimuli during the night and possible incorporations of stimuli into dream content. There were no reports of incorporations and subjects reported hearing the stimuli only if they awoke during the night.

This dissertation did not attempt to examine the individual differences between and within subjects. This would require large sample sizes. Individual differences and variability in response to external stimuli in sleep needs to be further investigated. Recently, Voss and Harsh

(1998) examined how a subject's personality characteristics might be related to information processing at sleep onset. Individual differences in coping styles were categorized as Monitors (i.e., information seeking), and Blunters (i.e., information avoiding). Monitors were more responsive to stimuli (i.e., behavioural response) in wakefulness and stage 1. In addition, the Monitors had a larger P300 response, and a smaller N350 response. Voss and Harsh (1998) suggest that the sleep-related N350 response may reflect blocking of the stimuli. Other personality characteristics might also influence the attention-dependent, endogenous P300 component of the ERP. For example, extraversion has been shown to affect certain ERPs, most notably N1 (see Stelmack & Geen, 1992, for review). Introverts are thought to be more sensitive to external stimuli than extraverts. For instance, their pain threshold is reached earlier. Moreover, within sleep, components of the auditory brainstem evoked potential appear to peak earlier for introverts than extraverts (Stelmack, Campbell, & Bell, 1993). It is possible that they may have a P300 in REM at lower thresholds than extraverts. Introverts are not equivalent to Monitors. While they are sensitive to external stimuli, they may seek to avoid it. On the other hand, the less sensitive extraverts may seek stimulation (somewhat like the Monitors).

There may also be individual differences in alpha brain wave activity, the 8-10 Hz frequency band that is normally observed during relaxed wakefulness. These alpha waves will however also occur during sleep, particularly in disorders of non-restorative sleep (Moldofsky & Scaribrick, 1976). Scheuler, Stinshoff, and Kubicki (1983) claim that approximately 14 % of healthy young adults will exhibit alpha rhythms during non-REM sleep. They suggest that alpha may represent an indicator of depth of sleep or increased vigilance during sleep. Those individuals with high alpha in non-REM, may also be more vigilant in REM sleep, thus being

more likely to produce the P300 response. These inter-subject differences may of course also be evident within a single subject over time. The intra-subject variability in the P300 response also needs to be further investigated. For example, there may be night-to night variability due to habituation, or fluctuations in many diurnal factors (e.g., caffeine intake, mood, stress, daytime sleepiness).

The studies in this thesis demonstrate that the P300 may be recorded during REM sleep following loud, rare deviant stimuli. There is no evidence for either a pitch-elicited or an intensity-elicited P300 in non-REM stage 2 sleep. These data provide evidence that conscious processing is possible in REM sleep. The research studies proposed above may provide a better understanding of the stimulus parameters and individual differences which contribute to the expression of consciousness during sleep.

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Appendix A:**Scalp Topography of the Auditory Evoked K-Complex in Stage 2 and Slow Wave Sleep**

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Summary

During non-REM sleep, a very large amplitude waveform, the K-Complex may be elicited upon presentation of an external stimulus. The present study compared the scalp distribution of a prominent negative wave peaking at about 550 ms and a later positive wave peaking between 900 and 1300 ms in stage 2 and Slow Wave Sleep (SWS). Nine subjects spent a single night in the laboratory. They were presented with an 80 dB SPL 2000 Hz auditory tone pip every 15 seconds. The EEG was recorded from 29 electrode sites and referenced to the nose. A K-Complex was elicited on 34% of trials in stage 2 and on 46% of trials in SWS. A negative wave peaking at 330 ms was larger on trials in which the K-Complex was elicited than on trials in which it was not. The large amplitude N550 was readily observable on trials in which the K-Complex was elicited but could not be observed on trials in which it was not. The N550 was bilaterally symmetrical and was maximum over fronto-central areas of the scalp in both stage 2 and SWS. It inverted in polarity at the mastoid and inferior parietal regions. The scalp distribution of N550 significantly differed between stage 2 and SWS. It showed a sharper decline in amplitude over parietal and posterior-inferior areas of the scalp in stage 2 compared to SWS. A later P900 was maximum over centro-frontal areas of the scalp and was also bilaterally symmetrical. It showed a significantly sharper decline in amplitude over widespread inferior areas during SWS. Because the scalp maps of the N550 and P900 are different in stage 2 and SWS, their intra-cranial sources must also be different.

Introduction

The K-Complex was first described almost sixty years ago as an extremely large waveform that occurs in non-REM (NREM) sleep in response to an external stimulus (Loomis et al. 1939; Davis et al. 1939). They also observed that the K-Complex could occur spontaneously in the absence of any apparent external stimulus. Most authors now agree that the evoked K-Complex consists of an early negative wave peaking from 300-350 ms ("N350") followed by a second much larger amplitude negative wave peaking from 500-600 ms ("N550") after stimulus onset. The K-Complex terminates with a late positive wave peaking from 900-1200 ms ("P900") (Halasz et al. 1985; Ujaszsi & Halasz 1988; Bastien & Campbell 1992; Niiyama et al. 1995). Although the early N350 wave is considered to be part of the K-Complex (Ujaszsi & Halasz 1988), it can also be recorded when the larger N550-P900 cannot be elicited (Bastien & Campbell 1992; Harsh et al. 1994).

The K-Complex is most often elicited using auditory stimuli since constancy of sensory input to the sleeping subject can be assured. The probability of eliciting a K-Complex is highest for loud and abrupt (fast rise-time) acoustic stimuli (Halasz et al. 1985; Bastien & Campbell 1992). The probability of eliciting a K-Complex is affected by the physical characteristics of the stimulus. For example, more K-Complexes will be elicited with high than low intensity stimuli. Nevertheless, if the K-Complex is elicited, the amplitude of the N550-P900 wave remains constant (Bastien & Campbell 1992). The amplitude of N550 is however affected by the rate of stimulus presentation (Bastien & Campbell 1994; Colrain et al. submitted). It is larger when stimuli are presented at very slow rates (for example, at inter-stimulus intervals of 30 s). Bastien

and Campbell (1994) have noted that this may be due to the effects of habituation or alternatively, to a very long "psychological" refractory period for the K-Complex. The amplitude of the N350 is much more readily altered. It is larger on trials when a K-Complex is elicited compared to when it is not (Bastien & Campbell 1992; Harsh et al. 1994). Furthermore, it is affected by the intensity of the stimulus and its rate of presentation (Bastien & Campbell 1992; 1994).

The scalp distribution of the K-Complex remains poorly understood. This is because most studies have used a small number of electrode placements, thus limiting spatial resolution. Those studies that have employed midline electrode placements are in general agreement that the large N550 is maximum over fronto-central areas of the scalp (Bastien & Campbell 1992; Harsh et al. 1994; Sallinen et al. 1995). On the other hand, standard clinical texts have claimed that the general "K-Complex" is maximum over central areas of the scalp. Recently, Niiyama et al. (1995) employed a large 21 channel array of electrodes. They recorded during stage 2 of sleep and averaged only those trials in which a K-Complex was elicited by an auditory stimulus. They noted that the average of all activity in a 300 to 800 ms period following stimulus onset was bilaterally maximum over the fronto-central areas of the scalp. It decreased in amplitude over posterior and lateral regions. Unfortunately, the averaging of activity over such a wide latency range will tend to blur the contribution of the various negative and positive components. Numminen et al. (1996) recorded magnetic (MEG) and electric (EEG) activity from 122 and 4 channels respectively. The large negative N550 component of the K-Complex was easily and consistently identified in the EEG. It was quite variable and often difficult to observe in the MEG. Similarly, Iramina and Ueno (1996) have noted that in a single subject the electric N550

component of the K-Complex was distributed over fronto-central areas of the scalp while the magnetic N500 was difficult to observe.

The late positive wave, P900, has not been extensively studied. Bastien & Campbell (1994) used a midline frontal, central and parietal montage and noted that it was maximum over frontal areas of the scalp in stage 2. Their data indicated that it was slightly more posterior in stage 4 but the difference was not significant. Niiyama et al. (1995) did not measure the P900. None of the MEG studies reported P900 data (Iramina and Ueno 1996; Numminen et al. 1996).

The K-Complex can be elicited in stages 2, 3 and 4 of NREM sleep. Stages 3 and 4 are often collapsed to form Slow Wave Sleep (SWS). Little is known about the intra-cranial generators of the N550 and P900 waves in these stages of sleep. SWS is acknowledged as being "deeper" than stage 2. Because subjects are more difficult to awaken in SWS, it is possible that the sources responsible for the generation of the K-Complex are different than in stage 2. The purpose of the present study was therefore to compare the scalp topography of the N550 and P900 waves of the auditory K-Complex during stages 2 and SWS. Components that have different scalp topographies must have different intra-cranial generators (Picton et al. 1995). Spatial resolution was increased by recording from 29 scalp sites. Temporal resolution was increased by only examining activity around the peaks of N550 and P900.

Methods

Subjects

Nine self-reported good sleepers (6 female, 3 male), aged 18 to 31 (Mean = 22.8, SD = 3.7 years)

volunteered to spend a single night in the sleep laboratory. Participants were instructed to refrain from alcohol and caffeine use 24 hours prior to the experiment. All participants read and signed a consent form which provided details of the purpose and the procedures involved in the study. Each was provided with an honourarium for their participation.

Physiological recording

The EEG was recorded from 29 scalp sites using tin electrodes mounted in an elastic cap. The cap was held secure to the head during the night by means of a chin strap. The EEG electrodes were placed over pre-frontal (Fp1, Fp2), frontal (F9, F7, F3, Fz, F4, F8, F10), pre-central (FC3, FC4), central-temporal (T7, C3, Cz, C4, T8), pre-parietal (CP3, CP4), parietal (P9, P7, P3, Pz, P4, P8, P10) and occipital sites (O1, O2). In addition, two electrodes were placed over the left and right mastoids (M1, M2). The reference was the tip of the nose. Vertical eye movements and blinks were recorded from tin electrodes placed over the supra- and infra-orbital ridges of the left eye. Horizontal eye movements were recorded from electrodes placed at the outer canthus of each eye. Inter-electrode impedance was below 2 kOhm for the EEG electrodes and 5 kOhm for the EOG electrodes. An electrode placed between Cz and Fz (FCz) served as the ground.

The high filter was set at 35 Hz. The time constant was 2 s. Electrophysiological signals were digitized at a sampling rate of 128 Hz using a 12-bit A/D converter. Stimulus presentation, data acquisition and their subsequent analysis were controlled by InstEP SystemsTM hardware/software.

Procedure

Auditory stimuli were delivered binaurally via earphone inserts. The earphones assured constancy of stimulus input in spite of head movements during the night (Campbell & Bartoli 1986). Stimuli were delivered in blocks of 60 trials. The stimulus was an abrupt (2 ms rise-and-fall time) 70 dB SPL 2000 Hz tone pip having a total duration of 52 ms. The inter-stimulus interval (ISI) was 15 s. These stimulus characteristics have been shown to be optimal for eliciting a K-Complex (Bastien & Campbell 1992). Stimulus presentation began approximately 30 min after sleep onset. Stimuli were presented in sleep stages 2, 3 and 4. When the EEG showed signs of arousal or motor artifact, stimulus presentation was halted. A total of 360 stimuli were presented to all subjects in both stages 2 and SWS.

Data Scoring and Analysis

The continuously recorded EEG was classified into stages 2, 3 and 4 of sleep by two experienced scorers, based on 15 s epochs. Stages 3 and 4 were grouped together to form SWS. In cases of stage ambiguity, the data were rejected from further analyses.

The continuous EEG was reconstructed off-line into discrete trials or "sweeps". A sweep began 100 ms prior to stimulus presentation and continued for 1900 ms following it. The single trial sweeps were then classified into those in which a K-Complex could and could not be identified (KC+ trials and KC- trials respectively). The K-Complex was defined as a large amplitude (greater than 75 μ V) negative-positive wave, maximum over frontal or central areas of the scalp. The negative wave had to peak between 450 and 750 ms and the positive wave between 800 and 1300 ms. This algorithm was employed to control for the possibility of

including random background noise as a K-Complex. This was especially problematic in SWS when isolated large amplitude delta waves could have been mistakenly identified as K-Complexes. Single trials were then sorted according to stage of sleep (stage 2 or SWS), the presence or absence of K-Complexes ("KC+" or "KC-" trials) and electrode site. They were then averaged to reduce random, background activity.¹

A series of peak deflections was measured at each electrode site. N350 was measured at Cz as the maximum negative peak occurring between 250 and 450 ms. N550 was measured at Fz as the maximum negative peak occurring between 450 and 700 ms. P900 was also measured at Fz as the maximum positive peak occurring between 700 and 1300 ms. Isocontour voltage distribution maps were computed for the peak of N550 and P900 within stage 2 and SWS. All data points within a ± 10 ms window of the peak of N550 and P900 were averaged. A spherical spline algorithm (Perrin et al. 1989) was employed to compute the maps, using the original nose reference. For purposes of comparison to the Niiyama et al. (1995) and Colrain et al. (submitted) results, the data were also re-referenced to linked mastoids.

Statistical Analyses

Comparison of scalp distribution data is fraught with statistical problems (Picton et al., 1995). A simple approach to compare the stage 2 and SWS scalp variation would be to run a 2-way ANOVA (Electrode x Stage). Changes in scalp distribution would be evaluated as an interactive effect. Unfortunately, one of the assumptions of the ANOVA is that of "additivity". Experimental effects add a constant to a baseline condition. Voltage differences across the scalp produced by intra-cranial source strength are often, however, multiplicative. McCarthy and Wood (1985) have

proposed a method to circumvent this problem by normalizing scalp data in different conditions. The peak amplitudes of N550 and P900 were scaled to eliminate differences between stage 2 and SWS. The data were then normalized in each stage of sleep by computing the maximum and minimum values of each peak over the scalp. The minimum value was subtracted from the amplitude at each electrode site and then divided by the difference between the maximum and minimum. The ANOVAs were thus run on these normalized data. Comparisons are made on relative rather than on absolute amplitude data.

Hassainia et al. (1994) have developed a method to compare scalp distribution maps in different conditions (or in this study, between different stages of sleep). This procedure has recently been extended to include the differences between McCarthy-Wood normalization method. The amplitude for N550 and P900 were initially measured at each of the 29 electrode sites within each stage 2 and SWS. These data were then corrected using the McCarthy-Wood procedure. A map of the scalp distribution of the data was computed by using the four-nearest neighbour interpolation method. This resulted in a 64 x 64 pixel matrix of data points. A different map was computed for each subject in both stages 2 and SWS. A mean and variance map was then computed for each stage of sleep. A paired t-test was computed for each pixel to compare Stage 2 and SWS data. Given the large number of t-tests, there is considerable chance of random significance. However, if significance were truly due to chance, differences should emerge at random pixel locations. On the other hand, if significant differences emerge over consistent, consecutive regions, this is highly unlikely to be due to chance. Therefore, in this study differences between scalp distribution maps are considered to be significant only if they appear in consistent and circumscribed areas.

Results

Across all subjects, a K-Complex was elicited on 34% of the trials in stage 2 and on 46% of the trials in SWS. This difference was however not significant, $t(8) = 1.36$, $p > .05$. Figure 1 presents superimposed averages of individual subject K-Complexes that were elicited during stage 2. The thicker line represents the average of each of the individual subject's averaged K-Complexes (i.e., the grand average). As can be observed, although there is some variability among the subjects, the grand average is a good reflection of the general trend. Grand averages will therefore be employed to illustrate all other results.

----- Insert Figure 1 here -----

Figure 2 illustrates KC+ and KC- grand averages across the midline scalp sites. K-Complexes in both stage 2 and SWS are collapsed together in this figure. KC+ trials consisted of an early N350 peak followed by the very large amplitude N550. A late P900 appeared more as a slow wave than as a distinctive peak. Only a very small amplitude N550 and P900 was visible on KC- trials. N350 was initially measured at Cz, where it was maximum. It peaked earlier on KC- trials (290 ms) than on KC+ trials (330 ms), but the difference was not significant. Its amplitude did not differ between stages 2 and SWS ($F < 1$). It was significantly larger during KC+ than KC- trials, $F(1,8) = 9.62$, $p < .01$). There was no interaction between the stage of sleep and the presence of the K-Complex ($F < 1$).

————— Insert Figure 2 here —————

Figure 3 illustrates the grand averages of KC+ trials at all 29 electrode sites during stage 2 and SWS. The large amplitude N550 peaked later (570 ms) in stage 2 than in SWS (525 ms), but this difference was not significant ($F < 1$). N550 was maximum over fronto-central areas of the scalp in both stages 2 and SWS. Its amplitude was symmetrical. There were no significant differences in amplitude between homologous electrode sites over the left and right hemispheres. N550 inverted in polarity at the mastoids and at inferior parietal sites during both stage 2 and SWS. The scalp distribution maps of the two stages for the peak of N550 are presented in the upper portion of Figure 4. As may be observed, N550 is maximum over fronto-central areas of the scalp in both stages 2 and SWS. N550 declines in amplitude in posterior areas. However, this voltage attenuation over posterior regions was sharper for stage 2 sleep than for SWS. Thus in Figure 4, the distance between neighbouring contours is shorter in stage 2 than in SWS.

————— Insert Figures 3 & 4 here —————

The polarity inversion may have been due to the choice of a nose reference. The data were thus re-referenced to linked mastoids. Figure 5 presents the stage 2 averaged K-Complex using the algebraically derived mastoid reference. Although N550 remained maximum over fronto-central areas of the scalp, no polarity inversion was observed at the lateral and inferior parietal regions.

----- Insert Figure 5 here -----

N550 did not have identical scalp distributions in the two stages of sleep. The negativity extended in a more posterior direction in SWS than in stage 2. The data were normalized using the McCarthy-Wood correction procedure. The nose reference was used for this purpose. Statistical mapping (Figure 6) failed to reveal differences in the scalp topography over fronto-central regions. N550 was however significantly larger over widespread parietal and inferior-parietal regions during SWS, $t(8)$ ranging from 4.00 to 3.79, $p < .05$.

----- Insert Figure 6 here -----

P900 peaked significantly later in stage 2 (1130 ms) than in SWS (962 ms), $t(7) = 6.07$, $p < .01$. The lower portion of Figure 4 presents the isocontour spline maps at the peak of the positive wave within stage 2 and SWS. Its amplitude was symmetrical. There were no significant differences in amplitude between homologous electrode sites over the left and right hemispheres in either stage 2 or SWS. There was no evidence of polarity inversion in either stage 2 or SWS, when either a nose or linked mastoids reference was employed. P900 was maximum over frontal areas of the scalp in Stage 2 and gradually decreased in amplitude over posterior and lateral regions. In SWS, P900 was more centro-frontally distributed. It declined more rapidly in amplitude over posterior and inferior regions. Thus in Figure 4, the distance between neighbouring contours is shorter in SWS than in stage 2. The McCarthy-Wood normalization procedure was again applied to the scalp distribution data. The relative (normalized) amplitude of

P900 at midline and superior regions did not significantly differ between stage 2 and SWS.

Normalized P900 amplitudes were significantly larger during stage 2 over widespread inferior frontal, temporal and parietal as well as occipital areas of the scalp, t ranging from 7.86 to 2.33, $p < .05$.

Discussion

The overall probability of eliciting a K-Complex (about 0.40) is consistent with other studies that have used stimuli presented at this rate and intensity (Bastien & Campbell 1994). K-Complexes did occur more often in SWS than in stage 2, although the difference was not significant. The failure to find significance in the proportion of K-Complexes during stage 2 and SWS is also consistent with other studies (Bastien & Campbell 1992; 1994; Ujjaszi & Halasz 1988). It is possible that the higher proportion of K-Complexes in SWS is a confound of the classification procedure. Isolated delta waves occurring at random between 450 and 750 ms could have been misclassified as K-Complexes. Recent studies have however indicated that the frequency spectrum of delta waves (1-4 Hz) is higher than those of a slow oscillation (0.6-1 Hz) identified as the K-Complex (Achermann & Borbély 1997; Amzica & Steriade 1997). Furthermore, previous studies in our lab (Bastien & Campbell 1992; 1994) have employed identical classification procedures. These studies should also have reported a higher proportion of K-Complexes in SWS if isolated delta waves had been similarly misclassified. This was not the case. In both studies, K-Complexes were reported to occur more often in stage 2 than SWS, although again the differences were not significant.

N350 was larger on trials in which the N550 was elicited compared to when it was not. Bastien and Campbell (1992, 1994); Harsh et al. (1994); Niiyama et al. 1995, 1996; Sallinen et al. (1996) and Colrain et al. (submitted) have also reported a similar N350-N550 relationship. Bastien and Campbell (1992) and Harsh et al. (1994) have hypothesized that the N350 might act as a trigger for the larger N550 wave. The N350 must reach a threshold amplitude before the N550 could be elicited.

During stage 2 sleep, the peak of the large negative wave (N550) occurred earlier in the present study (at about 570 ms in stage 2) than that reported by Colrain et al. (about 610 ms). This may be due to their lower intensity or use of an external loudspeaker. The peak latency is similar to that observed in other studies when earphone inserts or headphones have been employed (Bastien & Campbell 1992; Salisbury & Squires 1993; Niiyama et al. 1996). N550 peak latency was shorter in SWS than in stage 2. Although this difference was not significant, it is consistent with other studies (Salisbury & Squires 1993). The peak of P900 did occur significantly earlier in SWS than in stage 2. Thus, the K-Complex process appears to occur more rapidly in SWS than in stage 2. There is little support for this claim in the literature. Such latency differences are not easily explained. It is possible that there is greater synchrony in SWS than stage 4. The prolonged latency in stage 2 would thus be due to latency "jitter" (the peaks of N550 and P900 occurring at variable times from trial-to-trial). Latency jitter would however produce a broad, lowered amplitude waveform. If anything, N550 was larger in stage 2 than in SWS.

It is possible that the variance in the peak latencies in stage 2 and SWS reflects the activity of the timing of different intra-cranial generators. A novel finding in this study was that the scalp distributions of N550 and P900 were not identical in stages 2 and SWS. N550 was

more widely dispersed in SWS. Although it was attenuated from anterior to posterior sites, this decline in amplitude was sharper in stage 2 than in SWS. Waveforms whose scalp distribution vary must have different intra-cranial generators (Picton et al. 1995). The intra-cranial generators of the N550 wave must therefore be different during stage 2 than in SWS. While it is possible that at least some of the dipole sources are identical, others must be different in order to account for the different scalp topographies.

Maps of scalp potentials must be interpreted with considerable caution. Scalp distribution maps are not maps of the underlying intra cranial sources (Picton 1995). The maxima and minima do not necessarily occur over the areas of the cortex that are most active. Thus, while N550 was maximum over fronto-central areas of the scalp in both stage 2 and SWS, it is quite possible that the location of the generator could be at some distant site. The actual generator sites of the K-Complex are not known. Case reports of human patients indicate that the thalamus may at least be implicated in the generation of the K-Complex (Tinuper et al. 1989; Weisz et al. 1995). Steriade and his colleagues claim that this may not be the case. They have identified a slow, 0.3-0.8 Hz cortical oscillation that they associate with the K-Complex in both the cat and the human. (Amzica & Steriade 1997; Steriade et al. 1993). This slow oscillation persists following removal or disconnection of the thalamus. Although the slow oscillation appears to be cortically generated, it has not been extensively studied. Moreover, the extent to which this low frequency wave is equivalent to the K-Complex remains to be determined.

Neither the human patient nor the animal studies have described different candidate generator sites in stage 2 and SWS. Equivalent intra cranial source dipoles can be predicted from scalp-recorded potential maps. This is called the "inverse problem". Finding the possible multiple

source solutions for the scalp-recorded N550 will not be easy. There is no unique solution to the inverse problem. A major limitation for all dipole methods is that a large number of possible discrete models (or solutions) can be found that will equally well explain the scalp distribution variance. Constraints must therefore be put on the number of possible dipole solutions. A commonly-used constraint is that the solution must be physiologically plausible. This requires that reasonable candidates for the generator site(s) of the K-Complex be known. Such knowledge is usually obtained from either implanted electrodes in animals, lesion data from both animals and humans or from modern scanning techniques such as the PET or fMRI in "normal" human brains. PET and fMRI studies of the K-Complex in non-patient populations have not been carried out. As already mentioned, there is a paucity of both animal and patient data. The very large amplitude of the N550 implies that it is probably generated by a near-field cortical source. MEG studies indicate that this is not a tangentially oriented dipole. Numminen et al. (1996) observed a very large N500 in their EEG sleep recordings but could not easily observe it in the MEG. This may be because the intra-cranial source of the electric K-Complex is radially oriented toward the scalp. The MEG is not sensitive to radial sources. These dipole sources must however be different in stage 2 and SWS.

Niiyama et al. (1995) employed a relatively large electrode array to examine the scalp distribution of all activity in the 300-800 ms latency range. The spatial resolution has been increased in the Colrain et al. (submitted) and the present study by recording from 29 scalp sites. Moreover, the temporal resolution has been increased by measuring activity in a small time interval (± 10 ms in this study) around the peak or at the peak (Colrain et al.) of the large N550 waveform. Both Niiyama et al. and Colrain et al. employed a mastoid/ear reference compared to

the nose reference used in the present study. The present study employed electrode placements (F9/F10; M1/M2; P9/P10) that were inferior to the Sylvian plane (located just above the T7 and T8 electrodes). This created unequal distances between electrodes, hence creating "holes" in the data. Niiyama et al. (1995) and Colrain et al. employed equidistant but less inferior placements. In spite of the variance in the placement of the reference and the active scalp electrodes, the large N550 peak is consistently maximum over fronto-central areas of the scalp in stage 2 and SWS. It was bilaterally symmetrical in both stages of sleep. There was thus no evidence of asymmetry of the K-Complex in any of the studies. Colrain et al. employed both auditory and respiratory stimuli to elicit the K-Complex. Niiyama et al. (1996) have also examined the midline scalp topography of apparently spontaneous-occurring K-Complexes. In all cases, during stage 2 of sleep, a fronto-central topography was noted. The intra-cranial generators of the large N550 wave therefore seem to be independent of the modality of stimulus presentation. As Colrain et al. point out, in spite of the fact that all three laboratories have reported a fronto-central distribution of the large amplitude N550 wave, there is far less consensus in the general sleep literature. Indeed, in both general textbooks and in clinical guidelines, a central-vertex placement continues to be recommended for the "optimal" recording of the K-Complex. In clinical sleep recordings, the "K-Complex" is observed in the ongoing EEG. The only component that is readily identifiable in the raw EEG is the large N550 waveform. All three studies that have used multi-electrode placements indicate that the N550 wave is not maximum over the vertex.

It is possible that in the present study the criteria used for the classification of single trial K-Complexes may have confounded the results. In order to be classified as a K-Complex, the large negative-positive wave (N550-P900) had to be maximum over frontal or central sites. This

would, of course, exclude K-Complexes that were not maximum over either the frontal or central scalp sites. The finding of a fronto-central scalp topography might thus be due to the fact that only frontally or centrally distributed K-Complexes were included in the average. If this were the case, it would have been expected that valid K-Complexes that did not have either a frontal or central maximum would have been included in trials in which a K-Complex could not be identified (i.e., KC- trials). This was not the case. There was no evidence of the large N550 at *any* scalp site in the KC- averages. Also, Colrain et al. (submitted) identified their single trial K-Complexes at a central electrode placement. They nevertheless also observed a frontal maximum N550.

Different studies have employed different reference sites. The choice of a reference site is not incidental. An inversion of N550 was observed at sites inferior to the Sylvian fissure when a nose reference was employed. This suggests that the equivalent source dipole may be along the Sylvian plane. It is possible however that the nose is active at the time of the large amplitude N550. Its amplitude remained very large at the most anterior sites (Fp1 and Fp2). It is not unreasonable to expect that at least a small proportion of this frontal voltage dispersion may have extended as far inferior as the tip of the nose. When the data were algebraically re-referenced to the mastoid, no inversions were apparent. It is of course also reasonable to assume that the mastoid is active at the time of N550. Unfortunately, no reference placed on the head is truly inactive since fields generated in the brain spread through volume conduction to all areas of the scalp. Other distant, non-cerebral sites have been proposed, such as a balanced sterno-vertebral placement (Stephenson and Gibbs, 1951), but even these sites will pick up activity from the base of the brain. Furthermore, the use of a reference that links two different sites (for example the

sternum and cervical vertebra) has the disadvantage that the virtual location of the actual reference is unknown.

There has been very little systematic investigation of the P900 waveform. Indeed, most K-Complex studies fail to report data related to it. P900 usually appears as a slow wave rather than as a distinctive peak. Its "maximum" peak amplitude is therefore not easily identified. P900 was distributed over centro-frontal areas of the scalp. Like N550 it was bilaterally symmetrical. There was no evidence of a polarity inversion. Its amplitude declined more rapidly over inferior regions of the scalp during SWS compared to stage 2. Very few studies have examined the intracranial generators of the P900 wave. Again, however, they must be different in stages 2 and SWS because of the differing scalp topographies in these two stages of sleep.

Footnote

1. Latency, amplitude and scalp distribution criteria were used in this and most other studies that have attempted to identify single trial K-Complexes. Such criteria are necessary in order to prevent high amplitude background "noise" from being mistakenly considered to be a K-Complex. There are problems with this definition. A 75 μ V amplitude would have eliminated lower amplitude waveforms falling within the latency range from being considered as K-Complexes. If this were the case, the average of the trials in which no K-Complexes were deemed to have been elicited should have contained at least a small amplitude N550-P900. There was no evidence of negative activity in the 450-750 ms range in the KC- average.

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Figure Legends

Figure 1. Averages of all K-Complex trials for each of the subjects are superimposed. As may be observed, while there is some inter-subject variability, the N350, N550 and P900 waveforms are discernible in each subject. The overall mean of all subjects (the "grand" average) is thus a good representation of the central trend. In this figure (and in all others), negativity at the scalp relative to the reference is shown as a downward deflection. The large negative wave (N550) is maximum at Fz and decreases in amplitude over posterior sites. Note that it inverts in polarity at the mastoids.

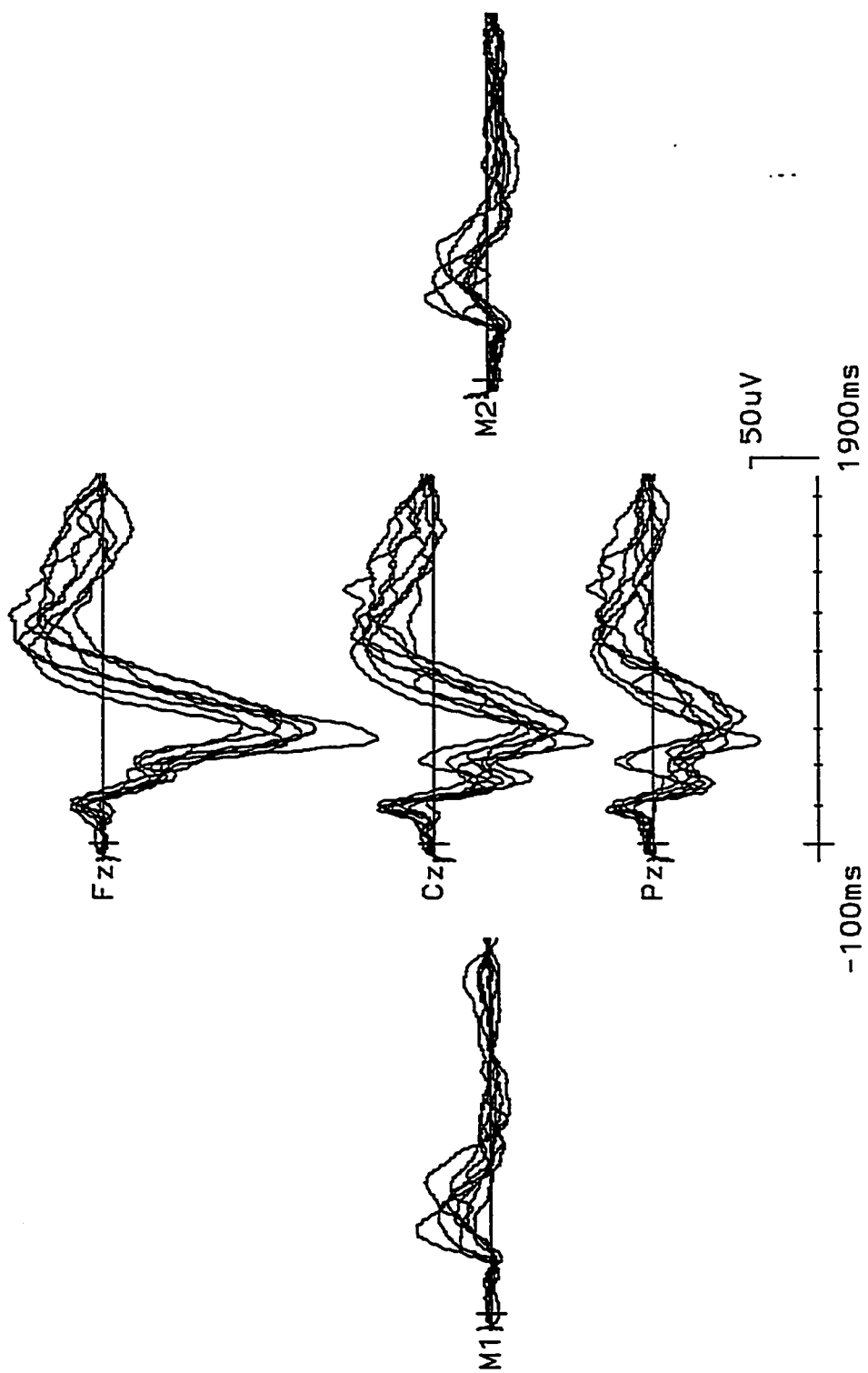
Figure 2. Grand averages of trials in which a K-Complex was elicited (KC+) and in which it was not elicited (KC-). Note that the very large amplitude N550 is difficult to detect on KC- trials. An earlier N330 was visible on both KC+ and KC- trials, but is attenuated in the latter.

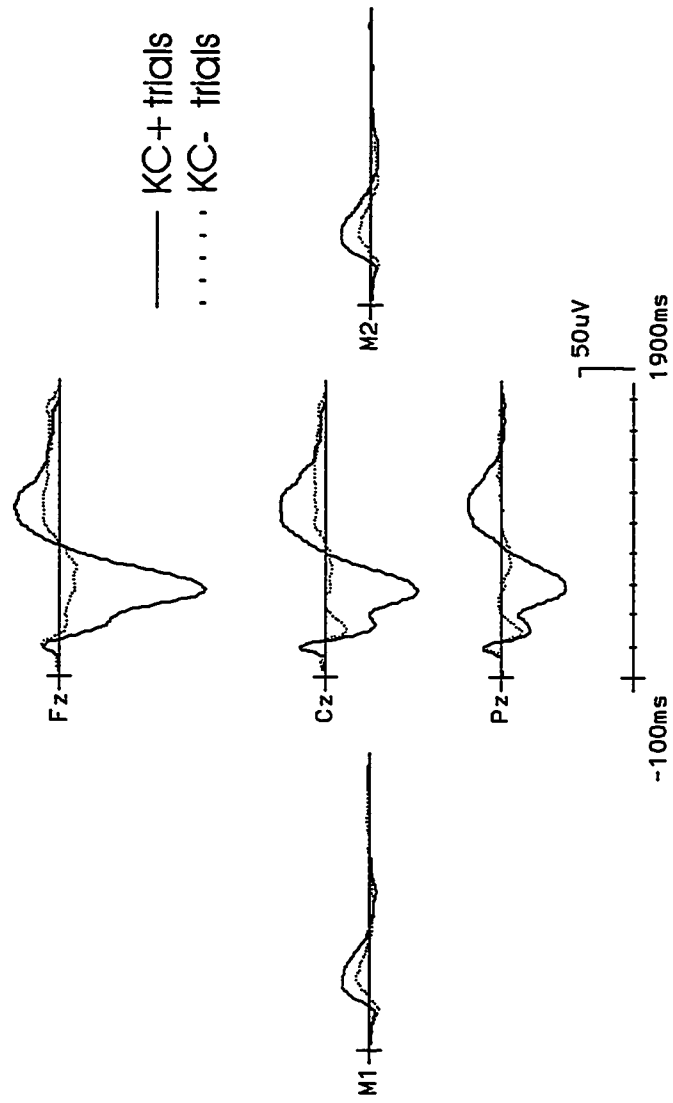
Figure 3. Scalp distribution of the K-Complex in stages 2 and SWS. The data represent averages of all trials in which K-Complexes were elicited. All scalp sites were referenced to the nose. The large amplitude N550 and the smaller amplitude P900 peaked earlier in SWS than in stage 2. N550 is maximum over fronto-central areas while P900 is maximum over centro-frontal areas of the scalp. Both are bilaterally symmetrical.

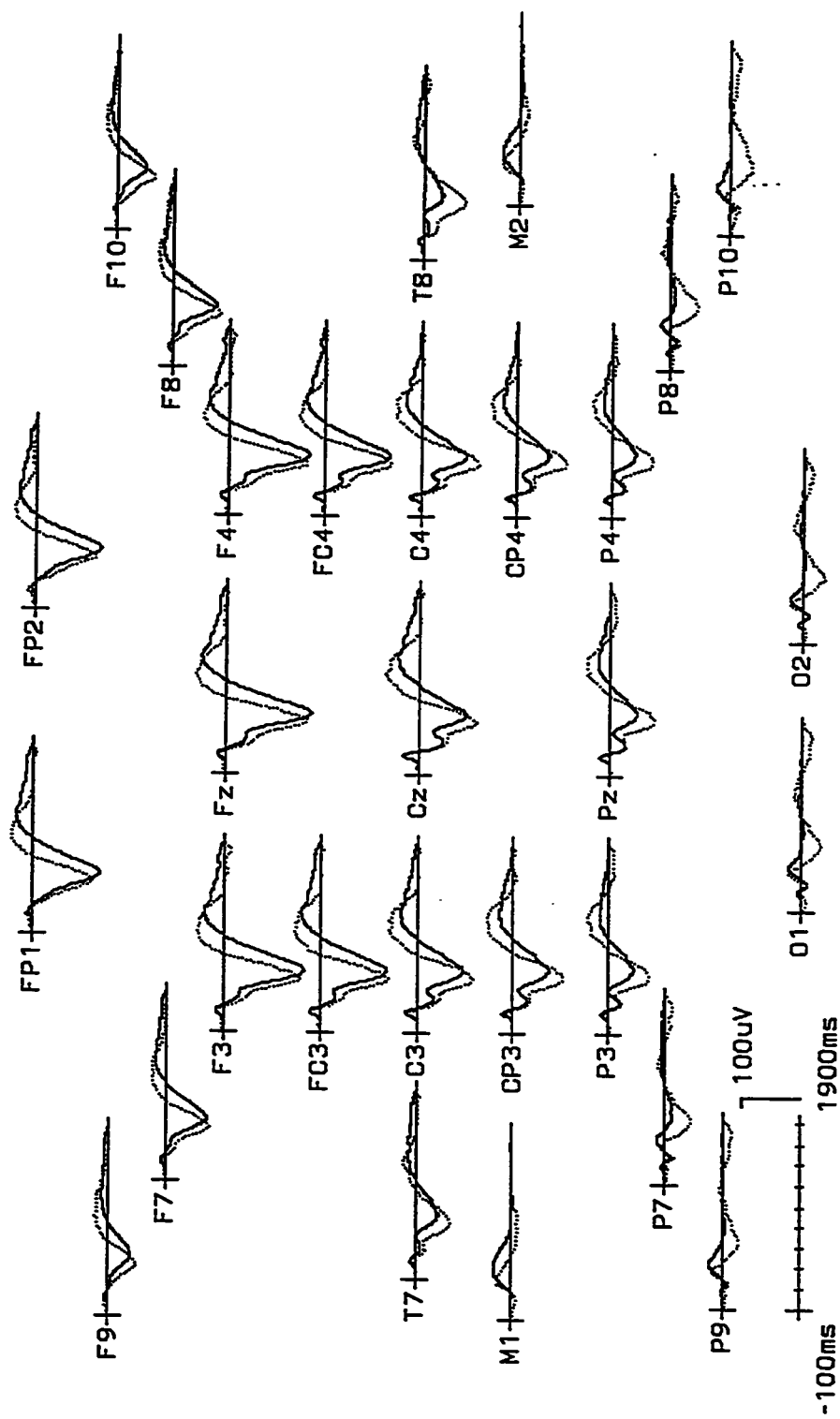
Figure 4. Scalp distribution maps of the N550 (upper portion) and P900 (lower portion) during stage 2 and SWS. The maps present the scalp distributions of the average of all data points within ± 10 ms of the peak latency of each peak in each stage. The solid lines represent contours for positive voltages and the dashed lines represent contours for negative voltages. Distance between contours is 8 μ V for the N550 and is 4 μ V for the P900.

Figure 5. Scalp distribution of the K-Complex during stage 2 when the data were re-referenced to linked mastoids. The N550 is still maximum over fronto-central areas of the scalp. However, the polarity inversion that was noted when a nose reference was employed is no longer visible with the mastoid reference.

Figure 6. Statistical mapping of the N550 wave during stage 2 and SWS. The McCarthy-Wood correction procedure was employed because of the violation of the additivity assumption of the usual ANOVA procedure. All data were normalized to the maximum peak of N550. Thus the "mean" of 0 represents the areas at which N550 was maximum (in the case of a negative peak, a "smaller" mean value is of course indicative of a larger amplitude). The minimum possible amplitude was scaled to 1.00. As may be observed, significant differences emerged over posterior-inferior sites. Although N550 declined in amplitude at posterior and inferior sites in both stage 2 and SWS, this reduction in amplitude was significantly larger during stage 2.

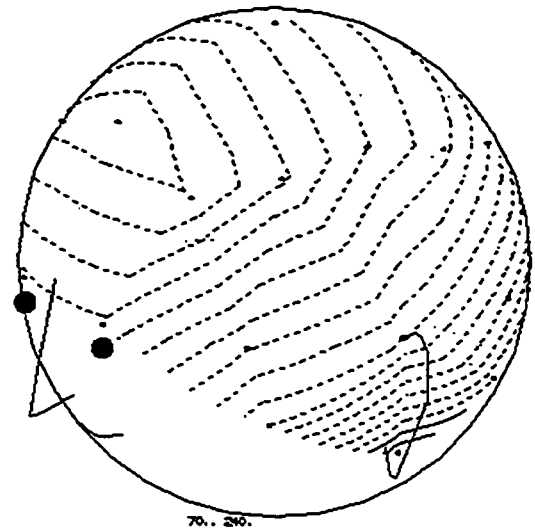
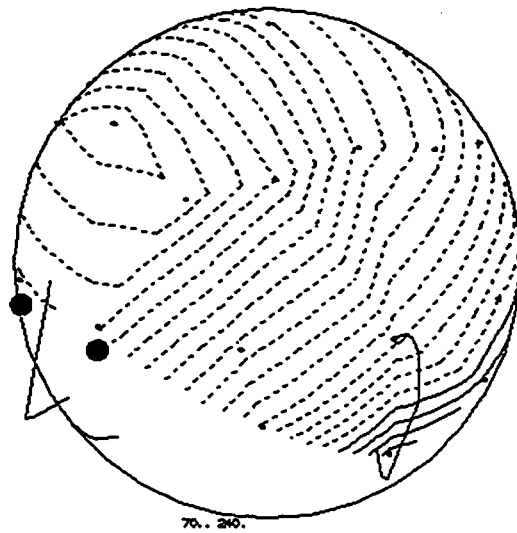




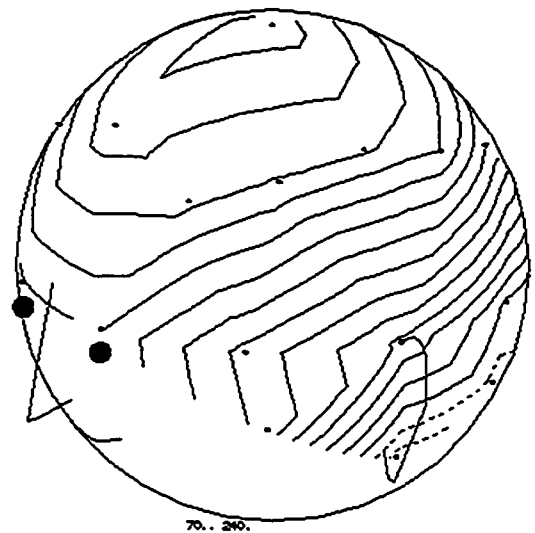
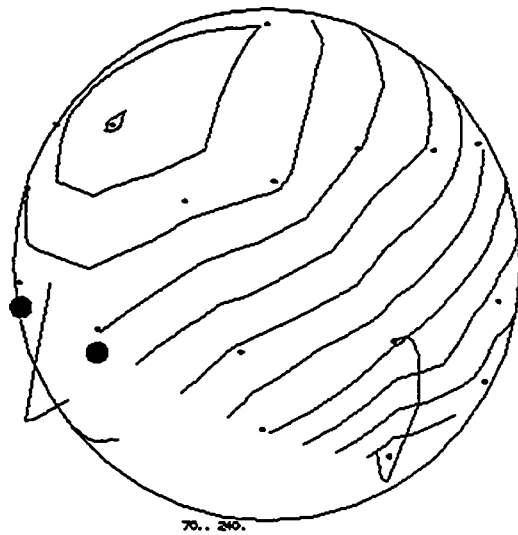


— Stage 2 Slow Wave Sleep

N550

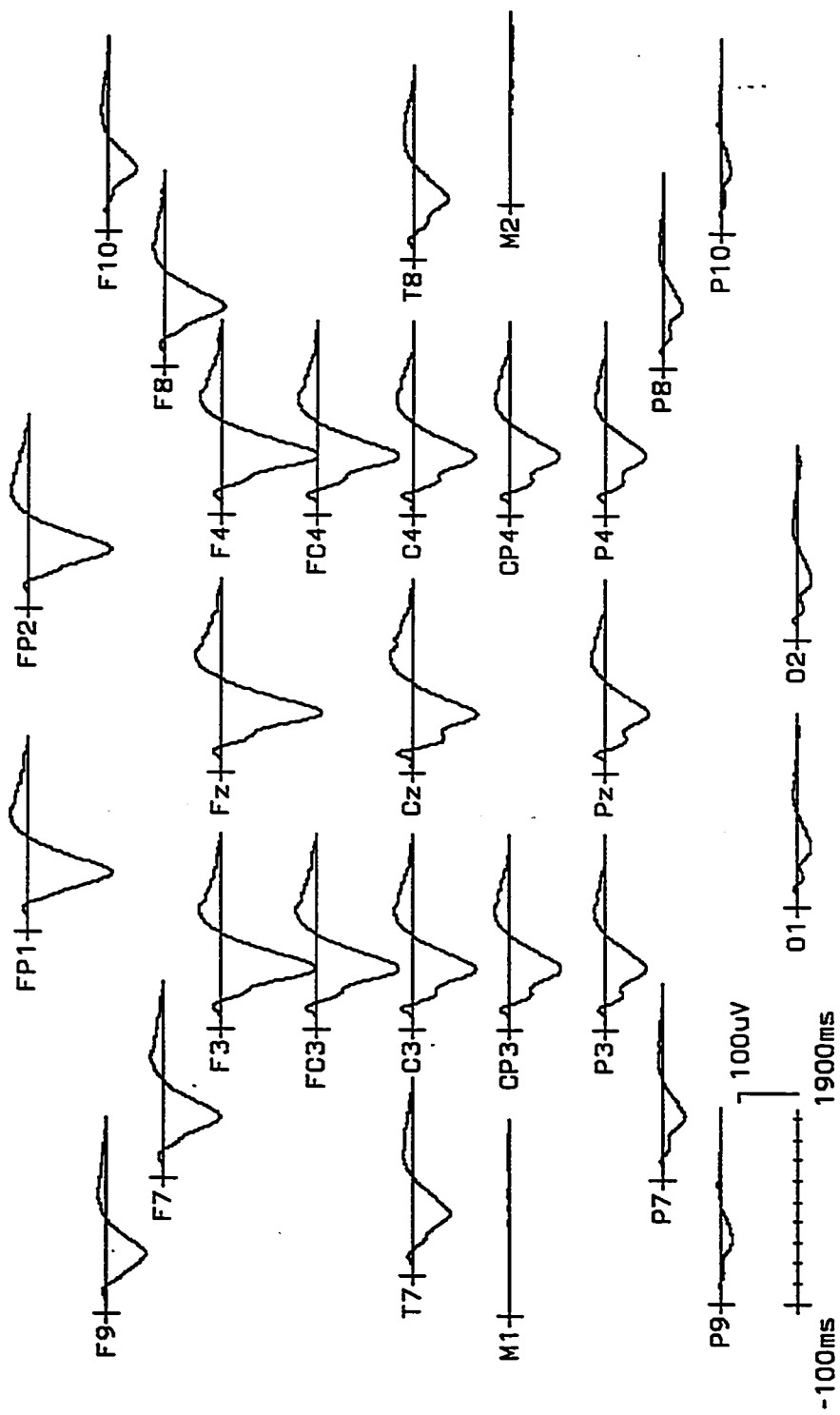


P900

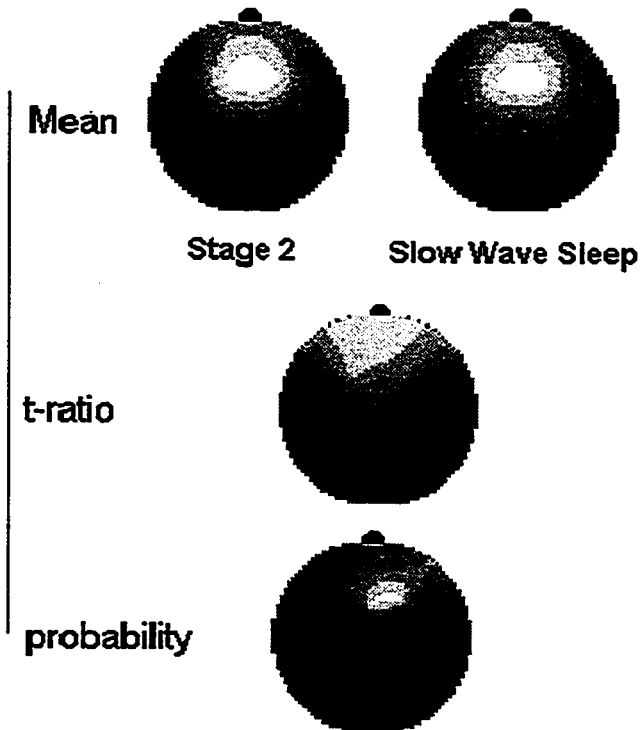


Stage 2

Slow Wave Sleep



Paired t-test (N550)
McCarthy-Wood Correction



Mean	t-ratio	prob.
0.94	3.70	.05
0.87	3.40	.13
0.80	3.10	.22
0.72	2.80	.30
0.65	2.50	.38
0.58	2.20	.46
0.51	1.90	.55
0.44	1.60	.63
0.36	1.30	.71
0.29	1.00	.79
0.22	0.70	.88
0.15	0.40	.96
0.08	0.10	.99

Digimed-
InstEP Systems

Appendix B:**The Role of the Spindle in Human Information Processing of High Intensity Stimuli during Sleep**

Cote, K.A., Epps, T. & Campbell, K.B.

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Summary

Sleep spindles are 12-14 Hz oscillations in EEG which are thought to inhibit or "gate" information processing. Event-Related Potentials (ERPs) may be employed to probe the extent of information processing during sleep. Previous research indicates that ERPs elicited by moderate intensity stimuli show increased positivity (or further removal of negativity) when stimuli are presented concurrent with spindles. However, the effectiveness of spindles to inhibit the processing of much louder stimuli remains unknown. The purpose of the present study was to investigate the extent of this gating, by using a range of stimuli including those that are loud and intrusive. Eight good sleepers were recorded during a single night. Auditory stimuli were delivered randomly at 0, 60, 80 or 100 dB SPL. Trials were sorted offline by sleep stage, stimulus intensity, and spindle characteristic (i.e., spindle absent, spindle present). During the sleep onset period, the often reported changes in ERPs were observed — N1 decreased and P2 increased in amplitude. In stage 2 sleep, P2 was affected by the presence of spindles, particularly when stimulus intensity was loud. Its amplitude was largest when spindles occurred following the onset of the stimulus. Spindles might therefore be a consequence of the inhibition of information processing especially when confronted by loud, intrusive external stimuli.

Introduction

Spindle oscillations are phasic events in the electroencephalographic (EEG) recording of non-REM sleep. They were first described by Loomis, Harvey, and Hobart (1935a, 1935b) as rhythmic 12-14 Hz periodic events lasting from 1 to 1.5 seconds. Spindle activity is thought to be generated in the thalamus as a result of a network of synaptic interactions involving inhibitory neurons (i.e., GABA) of the reticular thalamic nucleus, thalamocortical cells and cortical pyramidal neurons (Steriade and Llinás 1988; Steriade et al. 1993). Steriade and Amzica (1998) proposed that during sleep, the depolarizing component of a cortically-driven slow wave (less than 1 Hz) serves to trigger thalamic spindles. These rhythmic oscillations are due to the inhibitory post-synaptic potentials (IPSPs) of thalamic reticular neurons. Thus, a possible role of the spindle may be to gate synaptic transmission through the thalamus, thereby allowing sleep maintenance through inhibition of processing sensory information from the external environment.

Event-related potentials (ERPs) offer a method to probe the extent of information processing during human sleep (Campbell et al. 1992). ERPs are changes in electrical activity of the nervous system as elicited by an external physical stimulus or an internal psychological event (Picton et al. 1995). They are often used to investigate information processing associated with changing levels of attention and arousal. ERPs consist of a series of negative and positive deflections or "components". The late "vertex" potential of the auditory ERP consists of "N1" (negative wave peaking at approximately 80-100 ms), and "P2" (positive wave peaking at approximately 175-225 ms) deflections. These late components have been shown to be markedly

affected by sleep (Williams et al. 1962; Fruhstorfer & Bergström 1969; Noldy et al. 1988; Nielsen-Bohlman et al. 1991; Ogilvie et al. 1991; Campbell et al. 1992; Salisbury et al. 1992; Harsh et al. 1994; Bastuji et al. 1995; Winter et al. 1995; de Lugt et al. 1996; Elton et al. 1997). In general during non-REM sleep, when a sufficiently long time constant is employed and baseline-to-peak measurements are used, N1 decreases while P2 increases in amplitude (Campbell et al. 1992). Campbell et al. (1992) suggested that this increase in amplitude of the positive wave, P2, and decrease in the amplitude of the negative wave, N1, is due to the removal of a long-lasting negative wave during sleep. This slow negative wave, labelled "Processing Negativity" (Näätänen, 1982) overlaps and summates to the N1 and P2 components during wakefulness. Processing Negativity is thought to reflect the additional processing that is received by attended stimuli. The changes in the N1-P2 vertex complex at sleep onset and during non-REM sleep therefore are considered to reflect inhibition of information processing.

Recently, Elton et al. (1997) provided ERP evidence for the inhibitory effects of sleep spindles during stage 2 of non-REM sleep. ERPs to auditory stimuli were analyzed over a 1 second epoch or "sweep time". Trials were sorted into those in which spindles were present during the sweep (SP, or Spindle Present trials) or those in which spindles were absent (SA, or Spindle Absent trials). During SA trials, the usual effect of sleep was noted — N1 decreased in amplitude while P2 increased in amplitude compared to the waking period. During SP trials, P2 increased further in amplitude. This increased positivity in the ERP for trials in which spindles were present was interpreted as reflecting additional inhibition of information processing over and above that usually noted during stage 2 sleep.

The purpose of the present study was to extend the methodology employed by Elton et al.

(1997) in investigating the role of the sleep spindle on human information processing during sleep. Elton et al. (1997) presented a moderate intensity, 65 dB SPL binaural auditory stimulus. The extent to which spindles can protect the sleeper against the intrusive effects of much louder stimuli has yet to be determined. Auditory tone pips ranging from low (60 dB) to high (100 dB) intensity were therefore presented in this study. The extent of information processing may well be different for trials in which the spindle occurs at the same time as stimulus presentation, compared to when the spindle occurs after the stimulus has already been delivered. In the present study, trials in which spindles were identified were further separated into those in which the spindle occurred concurrently with or following stimulus presentation. Furthermore, Elton et al. (1997) presented stimuli at a constant, fixed rate. It is possible, although unlikely, that the sleeping participants might have anticipated the stimulus. The anticipation of the stimulus, rather than the processing of the stimulus per se, might have caused the changes observed in the ERP. To control for this possibility, stimuli were occasionally omitted (i.e., not presented). If changes in the ERP were due to anticipation of the stimulus, then ERPs should also be apparent in the no stimulus condition.

Methods

Participants

Ten healthy good sleepers volunteered to spend one night in the sleep laboratory. Data were later rejected for two participants because of insufficient sleep during the night. Statistical analyses are

therefore based on the data from eight participants (4 female) aged 19 to 30 (M age = 22.3, SD = 4.0 years). All participants were right-handed, nonsmokers, and were free from medication at the time of study. None reported a history of neurologic disorder. None reported disordered sleep as indicated by the Yoshitoke Fatigue Questionnaire (Yoshitoke 1978) and a sleep/wake history questionnaire. Hearing was verified using an audiometer to be within 15 dB ISO at 500, 1000, 1500 and 2000 Hz frequencies. Prior to the recording sessions participants were instructed to abstain from naps, and from alcohol and caffeine. Participants signed informed consent and received a \$25 honorarium for their participation in the study. This study was conducted according to the guidelines for ethical principles of the Medical Research Council of Canada.

Recording

The EEG was recorded from gold electrodes placed at midline (Fz, Cz, Pz) and temporal (T7, T8) sites, and referenced to the tip of the nose. A vertical EOG was recorded from electrodes placed at the supra- and infra-orbital ridges of the right eye. A horizontal EOG was recorded from the outer canthi of each eye. The physiologic signals were amplified using a Nihon Kohden model 4314B polygraph, with the high frequency filter set at 35 Hz, and a time constant of 1 second. EEG and EOG were digitized using a 12-bit analogue-to-digital (A/D) converter with a sampling rate of 256 Hz per channel.

Auditory Stimuli

Auditory stimuli were 1000 Hz tone pips, having a total duration of 55 ms and a rise-and-fall time of 5 ms. Stimulus intensity was set at either 0, 60, 80, or 100 dB SPL. Each intensity was

delivered at random with an equal probability ($p=.25$) of occurrence. Stimuli were presented at a fixed inter-stimulus interval (ISI) of 2000 ms. The stimuli were presented in blocks of 480 trials. Each intensity was therefore presented 120 times within each block. The order of presentation of the various intensities was randomized. All auditory stimuli were synthesized using an InstEP Systems 16-bit waveform generator card and were presented monaurally to the right ear via a modified hearing aid device. The hearing aid system assured constancy of auditory input in spite of possible head movements. A Bruel and Kjaer 2209 sound-level meter equipped with a 2 cm³ coupler was used to calibrate the auditory intensities at the beginning and end of each night.

Procedure

All participants underwent a screening procedure in which they were tested for normal hearing and completed sleep questionnaires. Upon arrival at the laboratory, electrodes were affixed, a hearing aid device was fitted and a pre-sleep questionnaire was completed. EEG was recorded and stimuli were delivered during relaxed wakefulness while the subjects read a book. Subjects were then permitted to fall asleep. Presentation of auditory stimuli began after consolidated sleep onset latency (i.e., 5 minutes continuous stage 2 sleep). A minimum of two blocks of 480 trials were presented in stages wake, 2 and slow wave sleep (SWS).

Data Analysis

An on-line spindle detector was used to identify 11 to 15 Hz activity in the EEG. The spindle analyzer consisted of sharp analogue filters having a bandpass of 11-15 Hz. Spindles were later verified by visual inspection and included only 11 to 15 Hz activity that exceeded 25 μ V and had

a duration of 0.5 seconds. The continuous physiological signals were sorted into sleep stages by an experienced rater who used standard Rechtschaffen and Kales (1968) procedures. In cases of stage ambiguity, the epochs were excluded from further analysis. Stage 2 sleep was separated into first and second halves of the night in order to determine time of night effects on information processing.

The continuous EEG and EOG were reconstructed into discrete epochs ("sweeps") offline. A sweep consisted of 256 data points beginning 100 ms prior to stimulus presentation and continued for 900 ms following it. Trials in which the EEG exceeded $\pm 150 \mu\text{V}$ were rejected from further analysis. This effectively removed those trials in which K-complexes were elicited. During the waking state, trials in which the EOG exceeded $\pm 100 \mu\text{V}$ were rejected. Single trials were stored on disk for subsequent off-line analysis. ERP waveforms were later digitally filtered in the frequency domain (employing an inverse FFT algorithm) using a low pass filter of 15 Hz.

The data were sorted on the basis of stimulus intensity, stage of sleep and the presence or absence of a spindle activity. Trials were initially sorted into those in which a spindle was present during the sweep and those in which it was absent (SA category). When spindles were identified during the sweep, they were further sorted according to those that occurred concurrently with stimulus presentation (SC category), and those that occurred following stimulus presentation (SF category). Figure 1 illustrates the detection of spindle activity (11 to 15 Hz) by the on-line spindle detector (filter).

————— Insert Figure 1 about here —————

The N1 peak is often difficult to detect during sleep because its amplitude is attenuated to near baseline level. For this reason, the amplitude of N1 was measured using a data point averaging method. N1 was defined as the average of all data points from 75 to 125 ms following stimulus onset. A distinctive P2 is however usually visible in both waking and sleeping states. The peak amplitude of P2 was therefore measured relative to the average of all data points in the pre-stimulus interval (the "baseline"). P2 was initially measured at Cz as the maximum positive peak between 175 and 250 ms. Its amplitude was subsequently measured at all other sites at this latency.

Results

The amplitudes of N1 and P2 did not differ between the first and second halves of the night ($F < 1$ in all cases). Data from both early and late stage 2 sleep were therefore collapsed. This increased the number of trials that were available for sorting and averaging according to the different spindle categories, thereby further reducing background EEG noise. Spindles were present on approximately 37 % of trials in stage 2 sleep. For each intensity, an average of 80 and 72 trials were identified per subject as SC and SF categories respectively. There were insufficient data from all participants during SWS to permit reliable sorting and averaging of the different categories of spindle activity. The effects of stimulus intensity and sleep spindle activity on ERPs are therefore reported for stage 2 sleep across the entire night.

Wakefulness and Stage 2 Sleep

The grand average ERP waveforms in waking and stage 2 states are shown in Figure 2. The latencies of N1 and P2 were somewhat delayed during stage 2 (peaking at 110 and 205 ms, respectively), compared to wakefulness (peaking at 93 and 192 ms, respectively), but this difference was not significant ($F < 1$).

----- Insert Figure 2 about here -----

Differences between the waking and stage 2 sleep data were determined using a two-way ANOVA with repeated measures on Sleep Stage (wake and stage 2) and Intensity (0, 60, 80, 100 dB SPL). Data from the Cz site, where N1 and P2 were largest, were used for the analysis. A main effect of Sleep Stage was found for the amplitude of N1, $F(1, 7) = 18.16$, $p < .05$. N1 was significantly larger (i.e., more negative) during wakefulness and became markedly attenuated to near baseline levels during stage 2 sleep. An interaction between Intensity and Sleep Stage was also found for the amplitude of N1, $F(3, 21) = 12.54$, $p < .05$. During wakefulness, N1 significantly decreased in amplitude from 100 dB to 60 dB to 0 dB SPL. Stimulus intensity had no significant effect on N1 amplitude during sleep.

The amplitude of P2 showed a small increase in stage 2 of sleep. However, neither the main effect of Sleep Stage nor the Sleep Stage by Intensity interaction reached significance, $F < 1$ in both cases. A statistically significant main effect of Intensity was found for the amplitude of P2, $F(3, 21) = 22.08$, $p < .05$. P2 amplitude was significantly larger (i.e., more positive) for the 100 dB SPL intensity compared to the 0, 60 and 80 dB SPL intensities.

Effects of Spindle Activity in Stage 2 Sleep

No ERPs were visible following the 0 dB "stimulus" in either waking or sleeping states. All subsequent statistical analyses were therefore based on when a stimulus was actually presented (i.e., following 60, 80, or 100 dB SPL stimuli). Figure 3 illustrates the grand averages when trials were sorted according to spindle absent (SA), spindle concurrent (SC) and spindle following (SF) categories.

————— Insert Figure 3 about here —————

Elton et al. (1997) have indicated that spindle activity has its largest effects at centro-parietal regions. For this reason, separate two-way ANOVAs with repeated measures on Spindle category and stimulus Intensity were run at each electrode site. No significant main effects or interactions were found at any electrode site for the amplitude of N1. The mean P2 amplitude for each spindle category and stimulus intensity are presented in Figure 4. The means and standard deviations are provided in Table 1. There were no significant main effects or interactions for the amplitude of P2 at the temporal (T7, T8) sites. At the midline frontal (Fz) site, there was a main effect for Intensity, $F(2,14) = 11.16$, $p < .01$, but the effect of Spindle category was not significant. At Cz, the Spindle by Intensity interaction was significant, $F(4,28) = 3.33$, $p < .05$. For the 100 dB intensity, P2 was larger when a spindle followed stimulus presentation (SF category) compared to when it occurred concurrently with the stimulus (SC category) or when the spindles were absent (SA category). P2 amplitude did not significantly vary between SC and SA categories. Differences were much smaller following 80 dB SPL, although the effects were consistent with

those in the louder intensity category. Although a similar trend was noted for the Pz data, the interaction did not reach significance. The main effect for Intensity at Pz was significant, $F(2, 14) = 28.70$, $p < .0001$, and the main effect for Spindle category tended toward statistical significance, $F(2, 14) = 3.52$, $p < .06$. Again, P2 was largest when spindles followed stimulus presentation. Spindle activity had no significant effect when stimulus intensity was set to 60 dB SPL.

----- Insert Figure 4 and Table 1 about here -----

Discussion

In the waking state, clear N1 (peaking at 110 ms) and P2 (peaking at 205 ms) waveforms were observed. The amplitudes of N1 and P2 increased with increasing stimulus intensity. This is consistent with many previous studies (see Näätänen and Picton 1987). No auditory evoked potential was visible following stimulus omission (i.e., when stimulus intensity was set to 0 dB) during either waking or sleeping states. There is thus no evidence that the N1-P2 waveform can be explained by anticipation of the stimulus.

Näätänen and Picton (1987) have indicated that N1 and P2 are affected by both “exogenous” and “endogenous” factors. On one hand, manipulation of the exogenous, physical characteristics of the stimulus will affect these components. On the other hand, manipulations of endogenous, psychological constructs will also affect N1 and P2. For example, directing the subject’s attention may cause N1 to increase, but P2 to decrease in amplitude, due to the

presumed overlapping effects of the Processing Negativity (Näätänen, 1982). Campbell et al. (1992) have however suggested that the apparently exogenous effects on N1 may be explained by an attentional confound. In the example of stimulus intensity, as the stimulus becomes increasingly louder, the subject may no longer be able to ignore it. The same argument cannot account for the P2 data. P2 increases in amplitude as stimulus intensity is increased, but decreases in amplitude when the subject is more attentive.

During stage 2 sleep, N1 was reduced to near baseline level, regardless of stimulus intensity. This also replicates many previous studies (Fruhstorfer & Bergström 1969; Noldy et al. 1988; Nielsen-Bohlman et al. 1991; Ogilvie et al. 1991; Campbell et al. 1992; Salisbury et al. 1992; Bastuji et al. 1995; Winter et al. 1995; de Lugt et al. 1996). Näätänen (1990) has suggested that N1 may act as a transient-detector system that triggers internal attention. N1 may subserve “conscious perception of auditory stimuli in general... without indicating what the stimulus is” (p. 212). There is thus little evidence of “general” consciousness during stage 2 of sleep, even for the very loud 100 dB tone pip. The 100 dB intensity has been shown to elicit a startle reflex in the waking state (Schupp et al. 1997). It would appear, therefore, that inhibition of gating of information processing during non-REM sleep is remarkably efficient from quite low to quite high stimulus intensities.

P2 increased in amplitude from wakefulness to stage 2 of sleep, although the difference did not attain statistical significance. This increase in amplitude has also been reported in other studies (Noldy et al. 1988; Ogilvie et al. 1991; Harsh et al. 1994). Although not all studies report this finding (see Campbell et al. 1992).

Spindle activity will modulate the N1-P2 effect. Elton et al. (1997) observed a significant

increase in P2 amplitude when spindles occurred simultaneously or shortly after presentation of moderate intensity, binaural 65 dB SPL stimuli. The present study employed monaural stimuli. Binaural stimuli are perceived as louder than monaural stimuli. The 65 dB binaural stimulus employed by Elton et al. (1997) would probably have been perceived to be as loud as the 80 dB monaural tone pip.

In the present study, spindles had no significant effect on N1-P2 amplitude following the low intensity 60 dB stimulus. The gating that normally occurs during stage 2 sleep may be sufficient to inhibit information processing without the additional "support" of spindles for low intensity stimuli. P2 was however affected by spindle activity for the louder 80 and 100 dB stimuli.

The increase in the amplitude of P2 for high intensity stimuli in the presence of spindles is consistent with further inhibition of information processing during stage 2 sleep. The increased positive shift was also noted by Elton et al. (1997) when spindles occurred concurrently or following presentation of moderate intensity auditory stimuli. The present study and that of Elton et al. Also indicate that spindle activity will have its greatest effects over centro-parietal areas of the scalp. The present study indicated that spindles will affect the processing of very loud, 100 dB stimuli to a much greater extent than moderate or low intensity stimuli. Importantly, the increase in P2 amplitude was largest when spindles occurred *following* stimulus presentation. In these trials, inhibition therefore appears to occur before the actual onset of the spindle. Thus, inhibition of information processing may not occur as a result of spindle activity. Rather, it may be that inhibition of information processing must occur initially, prior to spindle generation. Spindles may therefore be a consequence of the inhibition of information processing rather than

the cause of it. This interpretation assumes that scalp-recorded activity is an accurate reflection of the thalamic-generated spindle (Steriade and Amzica 1998). There is, of course, considerable attenuation between the spindle source(s) in the thalamus and the scalp. The onset of the spindle may therefore occur some time before that observed on the scalp.

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Table 1

Mean Amplitude (μ V) and Standard Error (in Parentheses) for P2 at Fz, Cz, Pz During Stage 2 Sleep Across Spindle Absent (SA), Spindle Concurrent (SC) and Spindle Following (SF) Trials

Spindle Category	Intensity (dB SPL)	Fz	Cz	Pz
SA	60	1.94	2.43	1.96
		(1.19)	(1.70)	(1.98)
	80	4.09	5.49	5.38
		(3.14)	(3.39)	(4.50)
	100	6.56	9.72	9.64
		(4.78)	(4.15)	(6.12)
SC	60	1.26	0.61	-0.11
		(5.61)	(3.99)	(3.70)
	80	3.20	4.98	4.55
		(2.96)	(3.41)	(4.49)
	100	5.55	8.92	9.34
		(7.29)	(8.44)	(8.89)
SF	60	2.43	3.72	3.37
		(4.30)	(4.16)	(5.16)
	80	4.17	6.69	6.71
		(3.61)	(4.56)	(4.80)
	100	10.46	17.55	16.24
		(6.08)	(8.02)	(7.58)

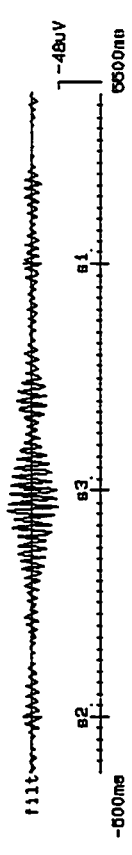
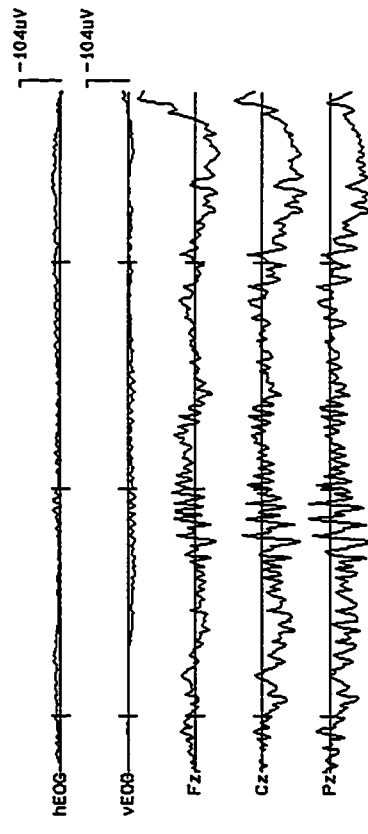
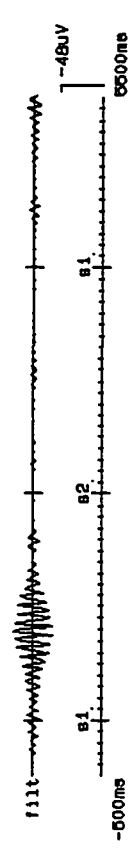
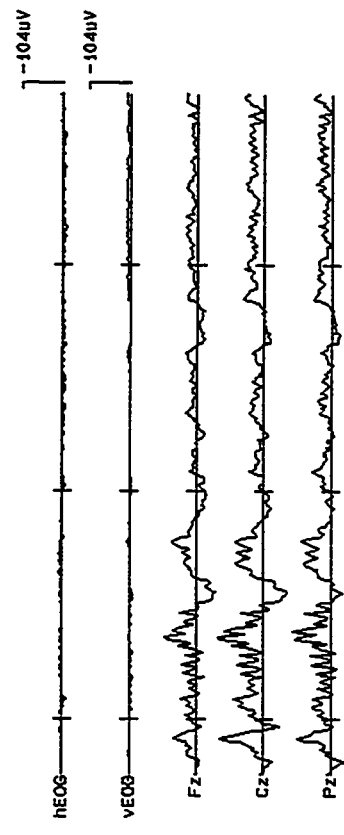
Figure Legends

Figure 1. Detection of Spindle Activity. Two brief epochs of continuous physiologic recordings are illustrated. The "filt" channel represents the on-line filtering of 11-15 Hz activity. Spindles can be observed in the background EEG at Fz, Cz, and Pz sites. The bottom line depicts the stimulus presentation sequence. Left side: This auditory stimulation sequence represents a 6 second sweep in which two 0 dB control stimuli (S1) are followed by an 80 dB tone pip (S3). A spindle occurs concurrently with the second S1 stimulus. This trial would accordingly be binned into the "spindle concurrent" (SC) category. Right Side: A train of 60 dB (s2) and 80 dB (s3) stimuli is illustrated. A spindle occurs following the first S2 stimulus. This trial would therefore be binned into the "spindle following" (SF) category. No spindles are apparent following or concurrent with the next two stimuli. These two trials would therefore be placed in the "spindle absent" (SA) category.

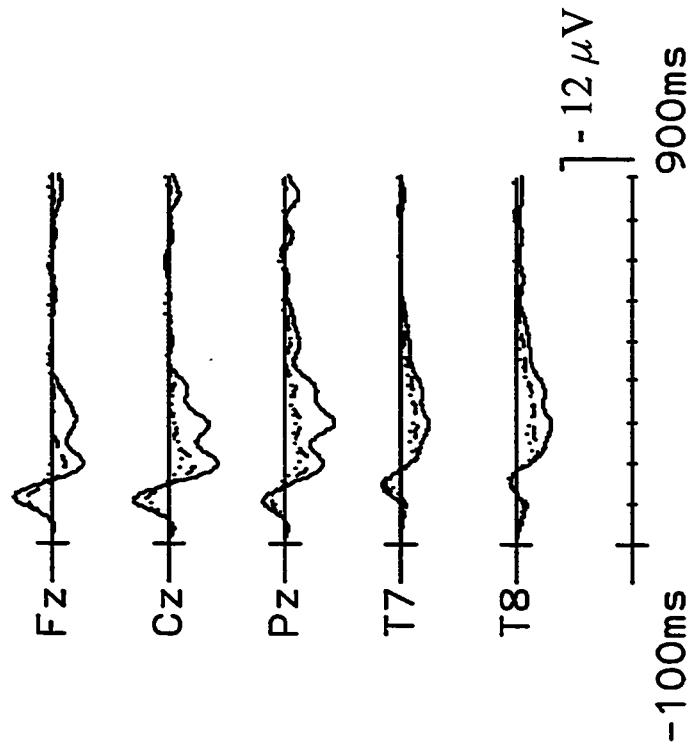
Figure 2. The Effect of Varying Stimulus Intensity on ERPs in Wakefulness and Stage 2 Sleep. Left side: The grand average ERPs to 60, 80, and 100 dB SPL stimuli in wakefulness are illustrated. No ERPs were visible following 0 dB stimuli and are therefore not illustrated. N1 and P2 are clearly visible at fronto-central sites. Right side: During stage 2 sleep, N1 is attenuated to baseline level, while P2 increases in amplitude relative to wakefulness. The P2 component of the ERP is largest following the loudest intensity. The late frontal negativity (peaking from 300 - 600 ms) may represent the presence of evoked K-complexes in the averaged ERP.

Figure 3. The Effect of Spindle Activity on ERPs in Stage 2 Sleep. The grand average waveforms for spindle absent (SA), spindle concurrent (SC) and spindle following (SF) categories are illustrated for 60 (left side), 80 (centre), and 100 dB SPL (right side) conditions. The amplitude of the P2 component is largest for the loudest intensity condition. For 80 and particularly 100 dB, P2 is larger when the spindle follows stimulus presentation (SF) compared to when it occurs concurrent with the stimulus (SC) or when it is absent (SA). In this figure, a "zoom" was made of the first 400 ms of the ERP sweep so that the later large amplitude sleep-related negativities are not visible. Note also that the amplitude scale has been doubled from the previous figure.

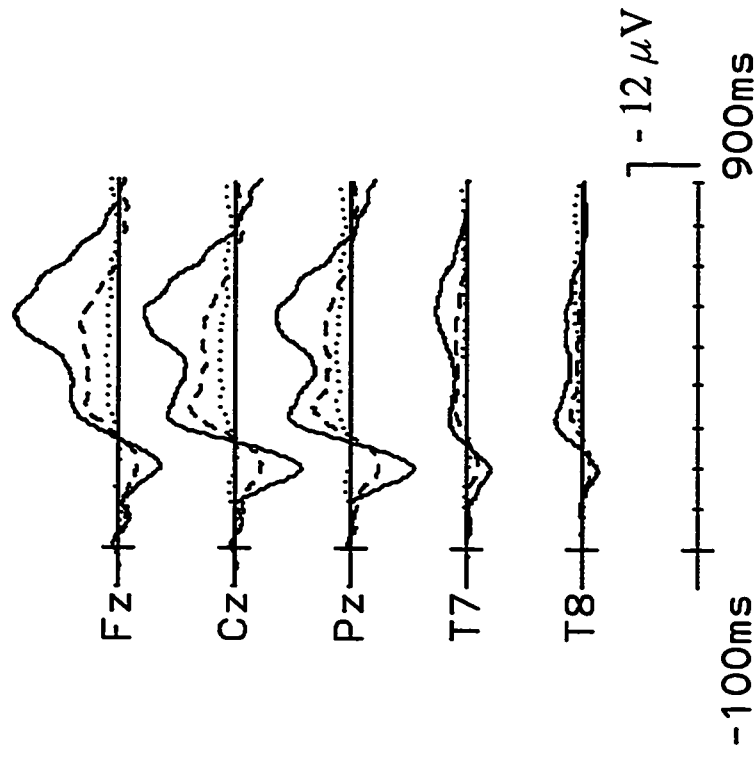
Figure 4. P2 Amplitude in the Presence and Absence of Spindles: Differences Across Stimulus Intensities and Electrode Site. The amplitude of P2 increases with each increase in stimulus intensity. P2 amplitude is largest for the SF condition. The effect of spindle activity is largest over centro-parietal areas for the louder stimuli.



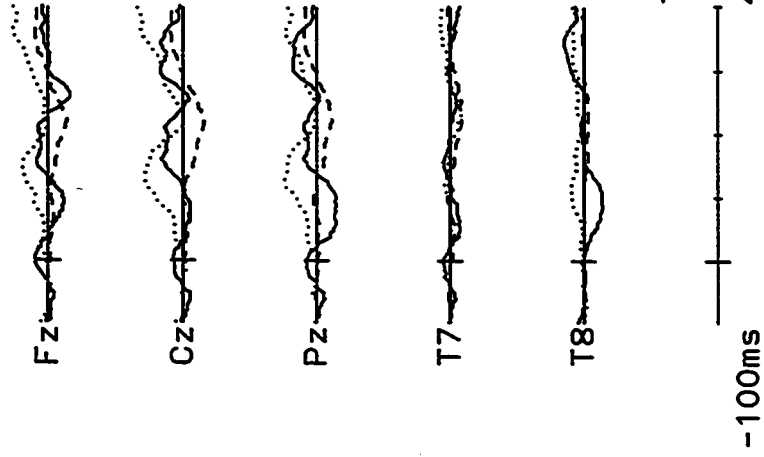
Stage Wake



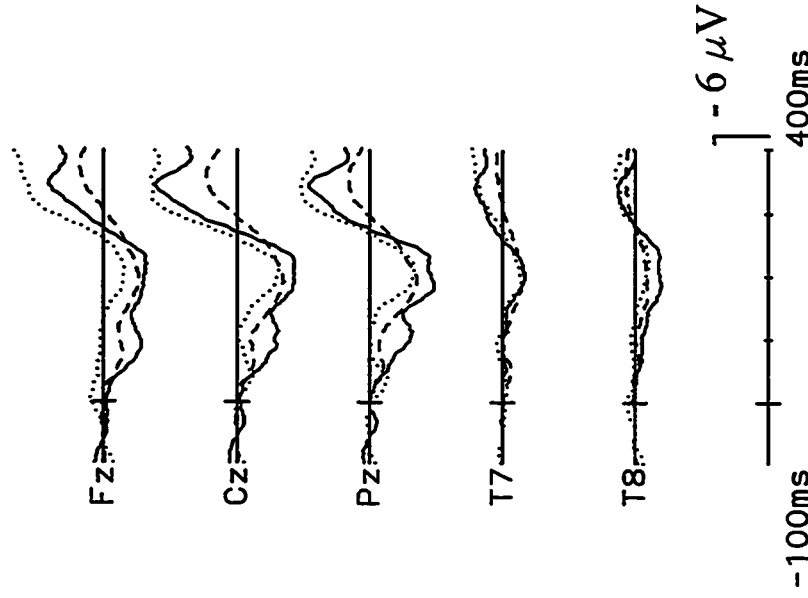
Stage 2 Sleep



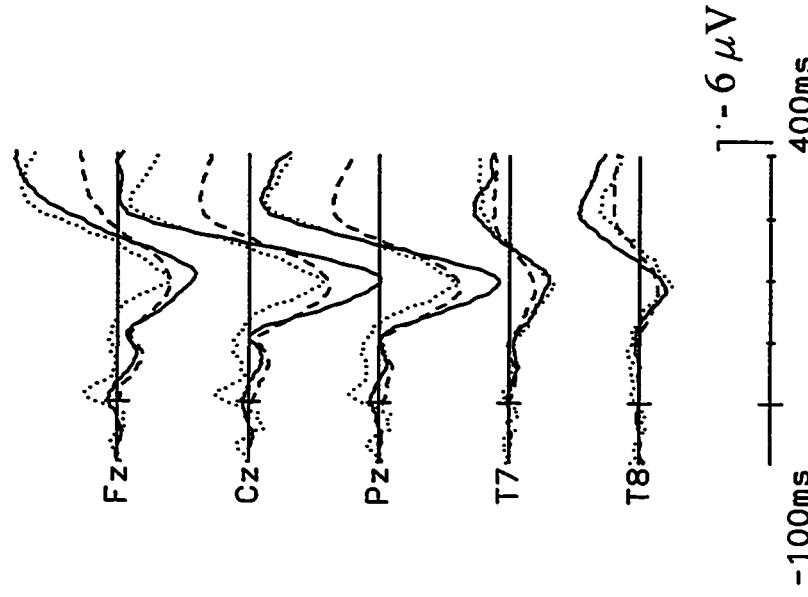
60 dB SPL



80 dB SPL



100 dB SPL



..... Spindle Concurrent (SC)
 - - - - - Spindle Absent (SA)
 ————— Spindle Following (SF)

60 dB 80 dB 100 dB

P2 Amplitude (uV)

Fz Cz Pz

Fz Cz Pz

Fz Cz Pz

..... Spindle Concurrent
(SC)
--- Spindle Absent
(SA)
— Spindle Following
(SF)

