

Middlesex University Research Repository:

an open access repository of
Middlesex University research

<http://eprints.mdx.ac.uk>

Rayan, Marc, 2012. The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy. Available from Middlesex University's Research Repository.

Copyright:

Middlesex University Research Repository makes the University's research available electronically.

Copyright and moral rights to this thesis/research project are retained by the author and/or other copyright owners. The work is supplied on the understanding that any use for commercial gain is strictly forbidden. A copy may be downloaded for personal, non-commercial, research or study without prior permission and without charge. Any use of the thesis/research project for private study or research must be properly acknowledged with reference to the work's full bibliographic details.

This thesis/research project may not be reproduced in any format or medium, or extensive quotations taken from it, or its content changed in any way, without first obtaining permission in writing from the copyright holder(s).

If you believe that any material held in the repository infringes copyright law, please contact the Repository Team at Middlesex University via the following email address:

eprints@mdx.ac.uk

The item will be removed from the repository while any claim is being investigated.

The utility of latency and spectral analysis methods in
evoked potential recordings from patients with
hepatic encephalopathy.

Marc Rayan December 2012

A report submitted to Middlesex University
in partial fulfilment of the requirements for the award of PhD

Contents

List of figures.....	6
Glossary.....	8
Statement of Originality.....	9
Abstract.....	10
Chapter 1: Background to the Study	11
Forward.....	12
1.0 Preface to the Background.....	13
1.1 An Introduction to Evoked Potentials.....	16
1.2 Evoked Potentials: approaches to patients with Hepatic Encephalopathy.....	20
1.3 The Liver.....	41
1.4 Evoked Potentials in patients with hepatic encephalopathy.....	67
1.5 Signal Processing, Neurophysiology and Evoked Potentials.....	90
1.6 The Research Question and Aims of this Study.....	102
Summary.....	103
Chapter 2: Methods	104
Forward.....	105
2.1 Ethics.....	106
2.2 Control population.....	106
2.3 Patient population.....	106
2.4 Study Procedure.....	107
2.5 Neuropsychiatric Assessment.....	107
2.6 Evoked Potentials.....	108
2.7. Protocols for evoked potential recording.....	109

2.7.1 VEP.....	109
2.7.2 BAEP	111
2.7.3 SEP.....	113
2.7.4 Cognitive Auditory P300	114
2.8 Frequency analysis.....	116
2.9 Statistical analysis	116
Summary.....	118
Chapter 3: Results	119
Forward.....	120
3.1 Subjects.....	121
3.2 Evoked Potential Latencies.	123
3.2.1 Visual Evoked Potential (VEP).....	123
3.2.2 Somatosensory Evoked Potentials (SSEP)	126
3.2.3 Brainstem Auditory Evoked Potentials (BAEP).	128
3.2.4 Auditory Cognitive Evoked Potential (P300).	130
3.3 EP Frequency and Power analysis (I) : Fourier Transforms ..	132
3.3.1 Fourier Transform – VEP	132
3.3.2 Fourier Transform – SSEP.....	135
3.3.3 Fourier Transform - BAEP	137
3.3.4 Fourier Transform - P300.....	139
3.4 EP Frequency and Power analysis (II): Power Spectral Density Estimates (PSD).....	141
3.4.1 PSD – VEP	141
3.4.2 PSD – SSEP	143
3.4.3 PSD – BAEP	145

3.4.4 PSD - P300	147
3.5 Sensitivity and Specificity Analysis	149
3.5.2 Sensitivity and Specificity – Frequency domain	150
3.5.3 Test combinations.....	151
Summary.....	154
Chapter 4: Discussion	155
Chapter 5: Further Work & Conclusions	171
Chapter 6: References	177
Chapter 7: Appendices	202
Appendix I	203
Figs 7.1 (above) and 7.2 (below): The 10-20 electrode placement system and EP recording device.....	203
Appendix II	204
Table 7.1: Summary table of previous VEP studies in patients with hepatic encephalopathy.....	204
Table 7.2: Summary table of previous BAEP studies in patients with hepatic encephalopathy.....	206
Table 7.3: Summary table of previous SSEP studies in patients with hepatic encephalopathy.....	208
Table 7.4: Summary table of previous cognitive (P300) studies in patients with hepatic encephalopathy.....	210
Appendix III	212
Summary table of signal analysis applications in neurophysiology	212
Appendix IV	223
Confirmation of ethics approval	223

Appendix V: Window functions.....225
Appendix VI Latencies in combined hemispheres.....226
Acknowledgments227

List of figures

Fig 1.1: Illustrative examples of Visual, Somatosensory and Brainstem Auditory evoked potentials	16
Fig. 1.2 Illustrative examples of normal P300 and Bereitschaftspotential cognitive evoked potentials	18
Fig 1.3: Views of a normal and a cirrhotic liver.....	42
Fig 1.4: Passage of gut-derived neurotoxic material into the systemic circulation.....	48
Fig 1.5: Cerebral ammonia detoxification.....	50
Fig 1.6: Disruption of central neurotransmission in hepatic encephalopathy.....	51
Fig 2.7.1: Recording electrode placement for VEP	109
Fig 2.7.2: a typical pVEP setup.	110
Fig 2.7.3: Recording electrode placement for BAEP	111
Fig 2.7.4: Upper limb recording placement.....	113
Fig 2.7.5: Recording electrode placement for P300	114
Fig 3.1: Visual Evoked Potentials.....	124
Fig 3.2: Somatosensory Evoked Potentials.....	126
Fig 3.3 : Brainstem Auditory Evoked Potentials	128
Fig 3.4: Cognitive Auditory (P300) Evoked Potential.....	130
Fig 3.5: Discrete Fourier Transforms of Visual Evoked Potentials	133
Fig 3.6: Discrete Fourier Transforms of Somatosensory Evoked Potentials	135
Fig 3.8: Discrete Fourier Transforms of Cognitive P300 Auditory Evoked Potentials	139

Fig 3.9: Power Spectral Density (PSD) Estimates of Visual Evoked Potentials	141
Fig 3.10: Power Spectral Density (PSD) Estimates of Cortical Somatosensory Evoked Potentials.....	143
Fig 3.11: Power Spectral Density (PSD) Estimates of Cortical Brainstem Auditory Evoked Potentials	145
Fig 3.12: Power Spectral Density (PSD) Estimates of Cognitive P300 Auditory Evoked Potentials	147
Fig 7.1: The 10-20 electrode placement system	203
Fig 7.2: An EP recording device; the amplifier headbox is indicated.	203
Fig 7.5: a rectangular window function	225
Fig 7.6: a comparison of window functions in the frequency domain.	225

List of abbreviations

10-20:	International 10-20 system
BAEP:	brainstem auditory evoked potential
BP:	Bereitschaftspotential
BSCN:	British Society of Clinical Neurophysiology
CNV:	contingent negative variation
EASL:	European Association for the Study of the Liver
EEG:	electroencephalography
EMG:	electromyography
EP:	evoked potential
ERP:	event related potential
FT:	Fourier Transform
GCS:	Glasgow Coma Scale
HE:	hepatic encephalopathy
ISHEN:	International Society for Hepatic Encephalopathy and Nitrogen Metabolism
LFP:	local field potential
MEG:	magnetoencephalography
MEP:	motor evoked potential
MMSE:	mini-mental state examination
NCT:	number connection test
P300:	auditory cognitive evoked potential
PHES:	psychometric hepatic encephalopathy score
PSD:	power spectral density
SD:	standard deviation
SSEP:	somatosensory evoked potential
VEP:	visual evoked potential

Statement of Originality

This study was undertaken by the author, Marc Rayan¹ and the following contributors:

The project was devised by Dr Marsha Morgan² and Professor Richard Bayford¹

Patients were recruited by Dr Marsha Morgan.

Control subjects were recruited by myself and Dr Marsha Morgan

Professor Richard Bayford provided assistance with the Matlab™ code

The following experimental studies were undertaken by myself –

- Evoked Potential recordings and analysis
- Frequency domain conversion and analysis
- Statistical analysis throughout the study

Psychometric testing was performed by Dr Marsha Morgan and Dr Sara Montagnese³, with EEG investigations performed in the Neurophysiology Department, Royal Free Hospital, Hampstead NHS Trust.

Professor Richard Bayford and Dr Huw Jones¹ advised on the statistical approaches to the data.

I undertook the writing of the thesis, with assistance from Professor Richard Bayford, Dr Marsha Morgan and Dr Richard Billings⁴.

¹ Department of Natural Sciences, Middlesex University, London

² Centre of Hepatology, Royal Free and University College Medical School, London.

³ Department of Clinical and Experimental Medicine, University of Padua, Padua, Italy

⁴ Office of the Chief Scientific Officer, Department of Health, Whitehall, London

Abstract

Evoked potentials (EPs) are small phasic potentials that are elicited in conjunction with sensory, motor and cognitive events. EP variables have been assessed in patients with cirrhosis but in general, methods were inadequately standardized and study populations incompletely characterized, leading to some studies questioning the validity of EP's in diagnosing and monitoring hepatic encephalopathy, while other studies indicated that there is only a low positive yield with these investigations. Few studies have attempted tri-modal sensory and cognitive recordings.

Recorded waveforms may demonstrate altered morphology while possessing broadly normal latencies. Since EP analysis is usually performed solely in the time domain, latency measurements do not therefore highlight morphological changes to the waveform and so abnormalities may go unreported.

The aim of this study was twofold (i) to measure sensory and cognitive EPs in patients with cirrhosis in relation to their neuropsychiatric status and (ii) to address frequency content in relation to neuropsychiatric status by examining EPs with two spectral techniques, the Fourier Transform (FT) and the Power Spectral Density Estimate (PSD).

Seventy patients with biopsy-proven cirrhosis were classified using clinical, psychometric and EEG criteria as unimpaired or as having minimal or overt hepatic encephalopathy (HE). Forty-eight healthy individuals served as controls. Visual (VEPs), brainstem auditory (BAEPs) somatosensory (SSEPs) and cognitive auditory (P300) EPs were recorded under standardized conditions.

Significant latency differences were observed in sensory EPs between patients and controls with patient subgroups differences being less significant. The cognitive auditory P300 however, distinguished the patient subpopulations from one another. Frequency shifts are observed in all EP modalities with significant differences also occurring between patient groups. The sensitivity and specificity of the frequency-domain is comparable to that of the time-domain.

Paired EP investigations analysed by latency indicate BAEP and P300 best discriminate any degree of encephalopathy; in the frequency domain it is the VEP combined with SEP and in the time-frequency domain it is the SEP. These findings suggest that EPs, when performed as a bank of multimodal tests and with spectral analysis, could provide a sensitive and specific method for the diagnosis and monitoring of hepatic encephalopathy.

The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy.

Chapter 1: Background to the Study

Forward

In this chapter Evoked Potentials (EPs) are defined as a neurological investigation, highlighting typical findings in a range of clinical applications and highlighting issues of reliability. This is followed by a methodological examination, by each recording modality, of the technical issues that need to be addressed in order to achieve consistent and reliable recordings. The role of stimulation, recording and post-processing parameters will be explored along with consideration of the effects of altering these factors. Confounding factors are identified and appropriate methodologies are then indicated.

The next section (The Liver) introduces the pathological changes in cirrhotic liver disease, and from an extensive literature review mechanisms are suggested for the clinical changes seen in hepatic encephalopathy. The diagnosis and grading of encephalopathy will be addressed noting the current problems with diagnostic test sensitivity.

The following section (EPs in patients with hepatic encephalopathy) is based on a survey the literature of previous EP investigations in this patient group. Critical scrutiny to patient inclusion criteria and encephalopathic classification is made. The neurophysiological methods will also be examined in light of positive yields for each of these investigations.

The final section of this chapter (Signal Processing, Neurophysiology and Evoked Potentials) is a broad survey of previous signal analysis studies undertaken on signals of neurophysiological origin. Signal processing methods are identified for both animal and human studies. The aims of the previous studies will also be considered and the extent of spectral analysis studies in patients with encephalopathy.

1.0 Preface to the Background

Hepatic encephalopathy is one of the major complications of cirrhosis. Patients will have clinically apparent neuropsychiatric abnormalities, encompassing a wide spectrum of mental and motor disorders; other patients, although neuropsychiatrically clinically unimpaired, will show significant abnormalities in both psychometric and neurophysiological performance. The presence of hepatic encephalopathy therefore, whether minimal or overt, has a considerable impact on the execution of complex tasks - such as driving, memory, decreased attention and health-related quality of life.

There is no “gold standard” diagnostic test for hepatic encephalopathy; as a result it is poorly diagnosed and in consequence is often sub-optimally managed. Complete management may be unattainable due to the varying presentations and impairments experienced by patients as well as multiple possible pathophysiological aspects of the disease. This brings us to the second problem: the exact pathophysiology of the syndrome is unknown. In consequence, treatment is based empirically on the current concepts of its pathogenesis. It follows that there is no one specific treatment but a number of possible treatments that can be used in tandem. Possibly a battery of the tests which each assess different aspects of cerebral function is necessary to provide a reliable diagnosis – a multidimensional approach. Currently however, many centres rely on clinical assessment to diagnose hepatic encephalopathy. Further, additional measures are used inconsistently, based on which is most easily accessible rather than one which has been validated or standardized.

Evoked potentials (EPs) have been used in earlier studies of cirrhotic patients to provide information for the detection and monitoring of encephalopathy. Both sensory and cognitive EP investigations have been reported, although few utilized multimodal investigations, and none with *four* modalities. The electrophysiological criteria for indicating the presence of encephalopathy has been determined by visual inspection of the waveform; an increase in the appropriate peak latency of the recorded waveform indicating an abnormality. Latency findings can be subject to inter-operator variability; additionally, many of the previous studies were not conducted in dedicated environments or with the most current protocols; the methodologies therefore have been frequently sub-optimal. As a result the findings of these studies have been inconsistent, and with many inconclusive reports on their validity, EPs – particularly the sensory modes – are not currently considered to be a valuable adjunct to encephalopathic screening or monitoring.

Spectral analysis methods are relatively resistant to inter-operator variation and can be automated if required. The characteristics of an EP signal allow it to be incorporated into computational software with ease; sensory and cognitive potentials have been analysed spectrally from a range of pathophysiologies but there are no reports of this form of analysis in patients with hepatic encephalopathy. Spectral methods have a further advantage in addition to the inter-operator robustness; if a patient produces an altered waveform, but the conventional latency peaks remain broadly within the normal range, the patient will be reported as a false-negative. The spectral content of such a

waveform not apparent in a visual time-domain inspection, will be altered numerically and thus with a greater possibility of an abnormal finding.

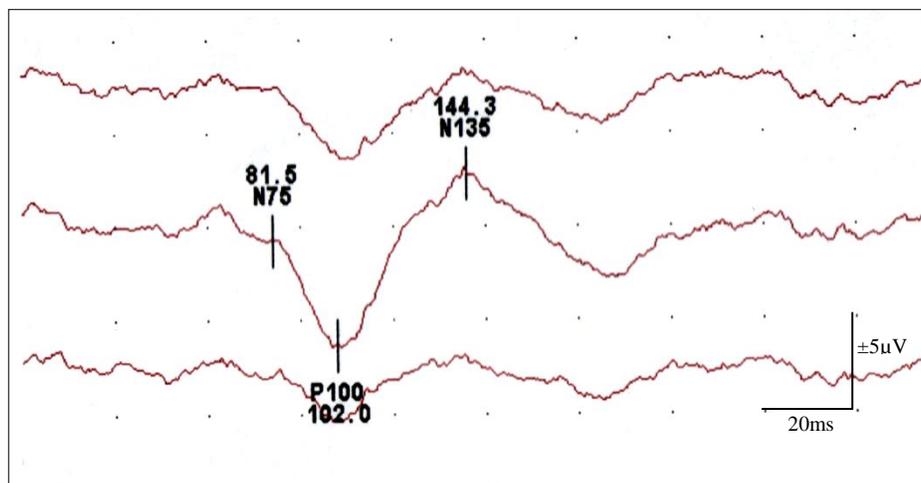
In order to evaluate the role EPs may have in the diagnosis and monitoring of hepatic encephalopathy this thesis will undertake two investigations: firstly, to record multi-modal sensory and cognitive potentials while using the most current clinical guidelines in electrophysiological recording; secondly, two spectral methods will be performed on the EP recordings. These three aspects of this investigation are unique in patients with hepatic encephalopathy.

This thesis brings together elements of liver disease, neurophysiology and signal processing. The [two] liver and signal processing sections serve to set the scene and justify the requirement of this study. The first of the neurophysiology sections illustrates the complexities of EP recording while the latter is a critique of the previous attempts to assess encephalopathic patients with EP investigations presents the current state of research. The chapter as a whole therefore, provides a broad background for the patient type, the previous work undertaken and the rationale for the research in this study.

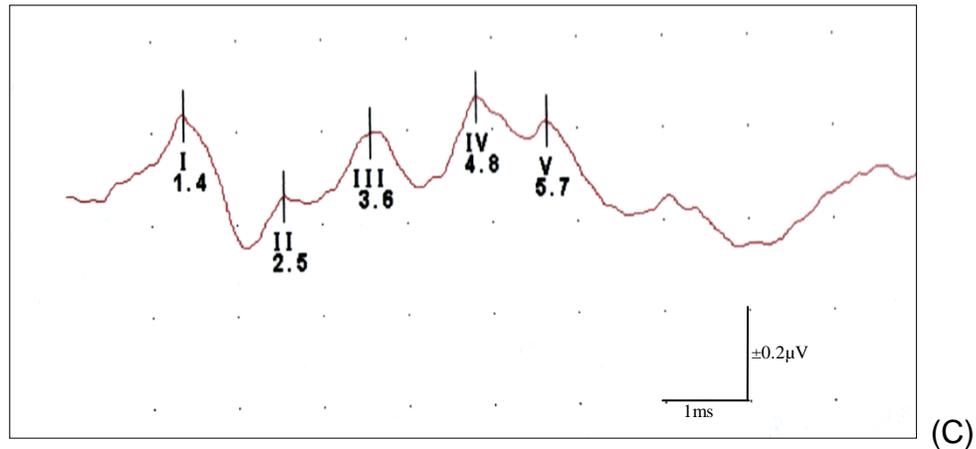
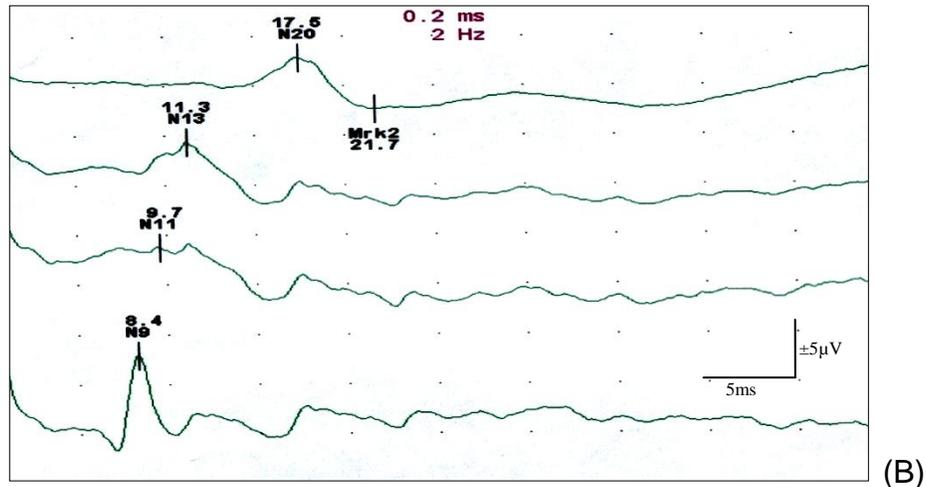
1.1 An Introduction to Evoked Potentials

An evoked potential (EP) is a potential difference, or an electrical expression, of the brain's reaction to and processing of, changes in the external environment; these changes are commonly referred to as stimuli, and the physiological changes subsequent to stimulation share electrical features of both action potential volleys and the on-going electroencephalogram (EEG). The electrical waveforms observed are usually named after the stimulus that elicited them; for example in the sensory system, a pattern reversal or strobe light flash will produce a visual evoked potential (VEP). Electrical stimulation of peripheral nerves will produce a somatosensory (SSEP) and auditory stimulation will elicit a brainstem auditory response (BAEP).

Fig 1.1 (below and overleaf). Illustrative examples of Visual, (A) Somatosensory (B) and Brainstem Auditory (C) evoked potentials, demonstrating the distinctive morphology of each sensory modality. The marked peaks bear the standardised nomenclature and are used for diagnosis. The latencies (msec) are typical for each of the peaks shown; (see appendix V for recording the montages for each of the waveforms).



(A)



These sensory EPs are also known as exogenous responses. They are non-cognitive and stimulus modality specific potentials that only indicate primary sensory afferent and cortical registration of a change.

Cognitive evoked potentials arise as a result of cognitive neuronal networks processing sensory information. These EPs are endogenous potentials; however they are not mode specific but are time locked to the central processing of afferent signals. Cognitive EPs are longer latency potentials - the best known being the P300 or event related potential (ERP). Other endogenous ERPs

include the contingent negative variation (CNV) and the pre-motor 'bereitschaftspotential' (BP).

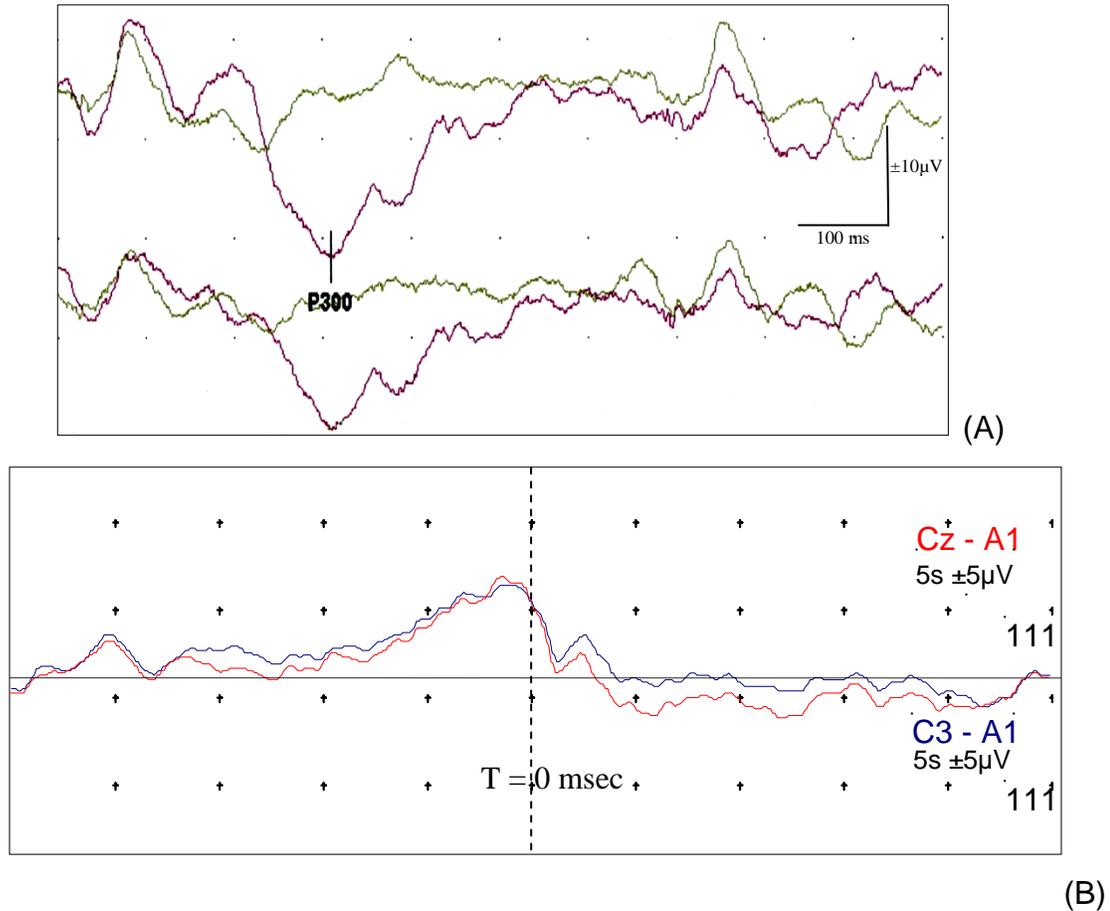


Fig. 1.2 Illustrative examples of normal P300 (A) and Bereitschaftspotential (B) cognitive evoked potentials, demonstrating modality specific morphology.

Any central neuronal activity that is triggered from the periphery and related to a time-locked cognitive process or a motor activity can be seen as an EP and therefore may have clinical applications in appraising or monitoring cerebral functions. However, EP recording in human subjects does have limitations. The data are recorded via surface electrodes and a wide range of artefacts are encountered.

The EP is a small voltage with a signal to noise ratio of less than 1; it must therefore, be extracted from the background EEG by signal averaging (Cooper *et al* 1980; Dawson 1951), a technique originally devised by George Dawson. In short, a waveform is produced by the summation of epochs – a stimulus presentation and its associated sampling of time related electrical concomitants. Waveforms obtained by this method assume that there is a perfect correlation between the onset of the stimulus and the response. The stimuli must be perfectly reproducible and if many epochs or ‘sweeps’ are required, this may become a monotonous experience for the subject.

A third issue is that of considerable inter-subject variability in the normal population. This entails large sample sizes for normative data in order to discriminate between control and abnormal responses. Sensitivity and specificity of EP findings may be further complicated by modulation of neuronal activity, for example alertness or vigilance, which could affect the consistency of repeated recordings.

Despite these limitations the EP has been found to be a reliable diagnostic investigation that yields objective and reproducible data in routine clinical practice. This has been the case since the early 1970s where the VEP highlighted clinically silent lesions in the optic tracts in patients with multiple sclerosis, and illustrates the advantage that EPs may have over clinical examinations.

1.2 Evoked Potentials: approaches to patients with Hepatic Encephalopathy.

Visual EPs

VEPs can be robust diagnostic investigations since they elicit reproducible data in clinical settings; the data is an objective and sensitive measure of the integrity sensory tracts. In addition, clinical data has been obtained in a large number of normal subjects and in patients with a range of neurological diseases. As central nervous system conduction is also monitored, changes in patient state, over time, can be monitored objectively – and thus provide a quantitative adjunct to neurological examination.

Equipment, recording conventions in clinical neurophysiology, measurements and operational considerations must be approached systematically and consistently; the operational aspect is particularly significant if investigations are to occur across several sensory modalities or on more than one recording device. Preparation of the electrode / skin interface must be thorough; contact impedances must be checked numerically, and should be consistent within the test (to balance the amplifiers) and between subjects. A value of 2-3k Ω is sufficient to promote common mode noise rejection. The positioning of the electrodes must also be consistent to allow for accurate inter-patient comparisons as well as using previously obtained databases. The 10-20 International System (Neidermeyer & da Silva F. 1982), Appendix I) is the most widely used.

Infection control requires that universal precautions be followed according to the clinical departmental guidelines. This is particularly important as we are deliberately abrading the skin in patients who may potentially be sources of hepatitis. The electrodes plug into a head-box (Appendix I) that may also house the pre-amplifiers. This in turn is connected to the analogue-to-digital converter (ADC) by a cable to a remote viewing room. Channel selection and other instrumentation can then be adjusted without distracting the patient. This also minimises the electromagnetic interference incident on the pre-amplifiers. Impedance checking may also be done remotely – with the same advantages – if the recording montages are pre-programmed.

The amplifier gain should be in excess of 100dB; this gain should be linear within a choice of selected bandwidths. A range of steps between 1-300Hz for the high pass and 100-3kHz for the low pass. The bandwidth is usually maintained by single order filters. Notch filters (50Hz) are not recommended, as they remove frequencies of interest and may introduce artefacts time-related to the stimulus, into the trace.

Signal averaging systems have a number of important parameters which must be programmed correctly, or at least known and noted in order to assist in the interpretation of the data; this may be most important in artefact recognition or as a marker of malfunction. The numbers of channels, voltage resolution, time resolution and data points per channel are the most important items. The averager processes the signal, with artefact rejection, whilst sampling / holding an epoch. As this takes a finite time the stimulus rate and total run time must be

carefully considered. Since the VEP latency is affected by state of alertness, a prolonged run-time, or number of sweeps would have the advantage of improving the signal quality, but may induce drowsiness in the patient.

A calibration signal, usually a square wave, should be injected such that the amplifiers and averager system process this input. It is more advantageous if the test signal has an independent or external origin.

The stimulators, flash or pattern reversal, must also be accurately and consistently controlled. Visual angle (pVEP) once chosen and calculated, e.g. 20°, can only be maintained if the patient-monitor distance is constant. Check sizes are also chosen from a programmable range (35' - 70'); these of course must be constant for a patient, but within a patient cohort some variations may be required to accommodate variable visual acuity. The luminance strongly affects the latency of the VEP and thus must be monitored, certainly between patients – but if possible throughout an individual recording.

Amplifiers used in clinical neurophysiology are all differential amplifiers. Therefore the + (G1) input, for example from electrode 1, becomes more positive with respect to the – (G2) input, from electrode 2. The only possible alternative is the opposite connection. The G1 and G2 inputs may both be receiving a negative potential, but if there is a difference, i.e. G2 is less negative then effectively G1 is positive. A zero output will only occur when the inputs are identical. The amplifier output will cause the display to either deflect up or down; one of the most common sources of error when reading EPs is either not knowing the amplifier configuration, or that it has been changed from a previous programme setup.

This is particularly common on multimodal devices and is responsible for a trace being abnormal yet at the same time familiar. In the absence of a universal EP convention, an efficient approach would be to mark every trace with the amplifier configuration. A calibration signal would achieve this with the added advantage of providing a system check. Similarly there is no convention on assigning the active and reference electrodes to either of the amplifier inputs. Again, consistency between both patients and between modalities in individuals will ensure that clinical data is meaningful.

Waveform nomenclature is also variable, as is demonstrated in the VEP literature set, and is usually based on either numbering the phase shifts chronologically (N1, P1, N2) or by labelling components after the value of the mean in a normal population, e.g. P100. There is a current trend however to label components after observational findings and not theoretical latencies. This however, usually causes confusion when interpreting waveforms the best approach is to be consistent and to use the system that the whole research group find the least ambiguous – possibly polarity-latency.

Latency, in msec, refers to the ‘absolute’ latency – the time between the stimulus and a predefined point on the waveform. This reference point is usually a peak, but some measurements occasionally use the peak onset – or take off. If the signal averager is software controlled and gives a digital read out, the cursors must be programmed and checked to ensure they are all reading in the same way. This will also apply to the other commonly used latency measurement – the ‘interpeak latency’. Unfortunately even manual placing of cursors may be

subjective in some cases, for example difficult waveforms such as bifids, or those with artefact. An acceptable approach would to have a strict protocol specifically for waveforms that were problematic – this would again ensure consistent data evaluation.

Amplitude measurements (mV) are measured in the same way and may present the same difficulties. Cursors can be placed either baseline to peak of the waveform – known as “absolute” amplitude or peak-peak. The signal averagers do not calculate the baseline voltage; therefore a peak cursor measurement will be subjective. Peak-peak cursors will provide an accurate voltage, but the physiological activity causing the peak of the first wave may have nothing to do with the activity that produces the second opposite peak. For example in the SSEP, the N20 is followed by the P23 but are formed in anatomically distinct structures. Since amplitude measurements are less useful as diagnostic markers – due to a greater variation within normal controls – they are most useful when comparing hemispheres in single subjects; in this case the patient is their own control. Amplitude differences can therefore, when expressed as percentage differences in a subject, be used in monitoring or longitudinal studies. In this instance either amplitude method is acceptable, providing of course that the same measurements are taken within the group and individual.

Determining the normal limits of a patient requires that each investigation (VEP, SSEP & BAEP) on each recording device have a normal population database, recorded with the same parameters that are used clinically. Most EP measurements have a normal distribution; therefore, standard deviations can

describe the upper limit of normal. As normal range must include 98% of the control population the only acceptable SD's are 2.5 and 3 (98.8% & 99.7% respectively).

In the advent of an absent or abnormal EP recording, a systematic approach to the methodology – in each modality – should be employed, based on an initial assumption that the observations are the result of technical error rather than conduction defect. This approach would be in addition to a repeated test.

Adequate stimulation would be the first question; checks therefore would follow, including controls (frequency, duration & intensity), electrical continuity and signal averager synchronisation. Amplifier input / output checks would confirm the bandwidth, gain, recording electrode selection and recording electrode continuity / impedance. A final check would be performed to confirm that the correct stimulator is correctly triggering the averager; this will include a memory check to ensure that the data is erased in between data-collection runs. If these post-recording checks are completed, and trial repetition produces clearly seen abnormal waveforms, then the possibility of technical factors can be excluded.

Brainstem Auditory EPs

The brainstem auditory evoked potential (BAEP) is the preferred neurological investigation for the cochlear (VIII) nerve and brainstem auditory pathways. As with the other EP's (visual & somatosensory) it is an objective and non-invasive test that yields reproducible potentials in clinical practice. BAEPs

have been studied in large groups of normal controls and also in patients with a wide range of neurological disorders.

The most common stimulus used for this investigation is a 'click', which is produced by delivering a square wave [electric] pulse to an earphone. The duration of the pulse is approximately 100-200 μ s, with rates that can be varied from 0.5-100Hz. The polarity of the pulse determines the initial direction of the earphone diaphragm and hence if the sound wave is a compression or rarefaction click. White noise must be available for masking the contralateral ear, since a monaural stimulus click may cross to the opposite side and subsequently alter the waveform. To avoid disturbing the patient, the headphones should be controlled such that stimulus / white noise sides can be switched remotely, with each side having separate intensity controls.

The stimulator (headphone) is calibrated with external sound level meters and the output recorded in decibels. Headphone calibration is not usually attended to on a regular basis. This can be excused in part since the relationship between the sound output in dB and the actual basilar membrane stimulation is dependent on subject variables such as shape of external canal, state of tympanic membrane and the mechanical condition of the ossicles and oval window. The subject hearing threshold *is* required as it will indicate the stimulus intensity required to produce a clear BAEP waveform. A typical stimulus intensity is 70dB HL which will be 70dB above the threshold-of-hearing level for the stimulus. If the stimulator can be adjusted in small increments (e.g. 1dB) the hearing level can be assessed accurately. The methodology used for the

determination of this threshold level should comply with the British Society of Audiology recommended procedures (revised 2008).

Surface electrodes are suitable for BAEP recordings. Electrode application will be with the conventional EEG technique, and the positions according to the 10-20 International System. Skin preparation is achieved using the EEG technique. With this modality the averaged signal is of low voltage, therefore particular attention should be paid to the electrode contact impedance, maintaining them below 5k Ω .

Amplifier gains of 100-120dB should be available, with a pre-amplifier bandwidth of 50-150Hz (highpass) and 3kHz low pass. The sweep window should have a duration of 10-12ms with the automatic repetition set to not less than 1000.

The potentials observed at the scalp subsequent to a stimulus are generated in the cochlear nerve, pons & midbrain. The action potential in the cochlear nerve appears at the ear as a negativity. The other waves (II-V) all appear at the scalp as positivities; if a non-cephalic reference is used for an ear recording wave one will have the opposite phase to waves II-V. Since waves III-V are clearer if they are recorded from the vertex, with wave II being of good amplitude from either position, the vertex or ear positions will provide maximum amplitudes. Therefore an ear (mastoid) – vertex bipolar montage can be used. Although not essential a second contralateral channel may be recorded to ensure that there is no wave I present in the non-stimulated side. Therefore the BAEP recording montage is

Ch 1: Ai - Cz

Ch2: Ac-Cz

'i' & 'c' are ipsi- and contra- to the stimulus.

To reduce muscle artefact the patient must be supine with neck support. The recording should also be as remote as the cabling will allow; this may aid the patient to fall asleep during the investigation. Any further aid to comfort is essential in this sensory modality as muscle potentials artefacts are often encountered.

Stimulus intensities of below 65dB (HL) usually result in poorly defined peaks, therefore it is of no advantage to start at a lower stimulus; 75dB (HL) is often a more effective intensity. Stimulation is monaural as abnormalities, where reported, are often unilateral.

The BAEP waveform is of low amplitude, often necessitating over 1000 sweeps to ensure a clearly defined signal. The stimulus rate will then dictate the duration of the investigation, but rates of over 10Hz elicit progressively degraded waveforms. Further, faster rates rarely reveal abnormalities that are not apparent at lower stimulation rates.

The polarity of the stimulus, i.e. to produce a compressed or rarefied sound wave) clearly alters the BAEP in normal controls. Wave I amplitude is greater with rarefied clicks due to positive pressure in the cochlea and on the window. Cochlear microphonics however, may add to and alter wave I. An alternating polarity will cancel this while having the additional benefit of reducing the amplitude of stimulus artefact seen on the trace.

The stimulus is capable of travelling by bone conduction to the contralateral ear to stimulate it at an intensity of 40 or 50dB below the ipsilateral ear. Contralateral white noise masking at 40dB below stimulus will prevent cross stimulation; this in turn can be monitored on channel two of our montage.

Finally, as suggested in visual and somatosensory investigations, repetition and superimposition should be considered mandatory. The recommended minimum intertrial variability is 0.2ms for interpeak latency (IPL) measurements (Rowe 1981). Waveforms often reveal after only 500 repetitions whether or not they will be superimposed. Repeatability, therefore, is not a major undertaking even with the sweep numbers required for this test.

Wave V is the most prominent and often first appearing peak. With a passband of 100-3kHz it occurs at 5.5ms starting above the baseline and descending farther below the base line. This below baseline trough is unique to wave V. Wave 1 appears after 1.4ms post stimulus as the first negative peak, and will be absent in the corresponding position of channel 2. Waves II – IV are inserted equidistantly between I-V; wave III is often attenuated in channel 2, can present two peaks, and in addition may be absent in channel 1 which, with no other changes is not an indication of abnormality. Waves II and IV are not commonly used for clinical interpretations; thus, the IPLs most commonly recorded are I-III, III-V & I-V.

Peak latencies can be affected by non-pathological factors. Effects stimulus intensity, polarity, bandpass and rate have been extensively studied. Stimulus frequency content is determined by the square-wave duration. An

electrical squarewave of less than 200 μ s will produce a decaying sinewave with a content of 500Hz –4000Hz. A change in frequency content will affect the amplitude and latency of all waves. A broader range for example will stimulate extended regions of the cochlea. As a wave may take 2-6ms to stimulate the entire cochlea, more than one of each BAEP component will be generated – the final waveform will consist of blended components and so appears unclear. Unfortunately, the usual first course of action when observing an unclear waveform would be to increase the stimulus intensity.

Subject factors that affect BAEP latencies include age, gender, body temperature and drugs. With increasing age after childhood there is an increase in wave latencies; there are reports however, that suggest the differences are too small, i.e. of the order of 0.1msec (Rowe 1978), to change a BAEP result from normal to abnormal when mixed gender group are used for controls. There is agreement that females have shorter peak and IPL's than males. The amplitudes are also bigger for all waves. Various studies have attributed many reasons for this but most are unconfirmed. One suggestion is that males have a higher core temperature than females. There is a correlation between peak latencies and body temperature, such that progressive lowering of the body temperature produces a wave V latency prolongation of 0.17ms/ $^{\circ}$ C below 32.5 $^{\circ}$ C (Picton *et al* 1981). In addition, BAEP latencies correlate with circadian alterations of temperature during sleep – 0.2ms/ $^{\circ}$ C (Marshall & Donchin1981).

Interpeak and peak latencies of BAEP's are not significantly affected by medications that otherwise affect the CNS. This includes patients that have had

toxic doses that have rendered the EEG isoelectric (Drummond J. *et al* 1985; Stockard & Sharbrough 1980). Anaesthesia decreases amplitude but the latencies are not significantly affected. The effects of alcohol, both acute and long terms are unclear.

Despite the factors that affect latency, the main BAEP peaks are consistent in healthy controls and have been utilised successfully in clinical practice. BAEPs have been found to be unchanged after over 8 hours of continuous testing, and unchanged within individuals tested repeatedly over several months. This, with the resistance to anaesthesia, indicates that the auditory EP modality is a particularly robust avenue of investigation.

Somatosensory EPs

SEPs are clinically utilised by such a range of practitioners that, unfortunately, there exists a multitude of protocols. This is further complicated by variable waveform nomenclature. Most SEP recording protocols have evolved from the American EEG Society (J Clin Neurol 1986 3 pp43-92) and most practices retain some of the original guidelines. Equipment, recording convention and operational issues will be based on this protocol while adjusting for methodological problems encountered at the Royal Free Hospital.

The preferred stimulus for an SEP is an electrical pulse since it can be precisely controlled and produces potentials of the greatest amplitude. The stimulator should be capable of delivering a square wave at range of frequencies (1 – 100Hz) and at a range of durations (10 μ s – 2ms). Stimulus durations

normally used are 100 μ s & 200 μ s, the higher duration used in patients with suspected peripheral neuropathies. Constant current and constant voltage stimulators are both available (voltage being more common) with no significant difference reported in the waveforms produced. Constant current devices have the advantage of allowing the stimulus to be described in mA, the amplitude being directly proportional to the number of fibres stimulated. The current from a constant voltage output is not reliably calculated since impedance is both unknown and variable.

Recording electrodes are of the conventional EEG type; needle electrodes may produce a cleaner and larger trace but patient comfort is a higher priority, particularly where the investigations may be prolonged. The conventional method for attaching EEG electrodes is used, i.e. using the 10-20 International System for placement system and attempting low and consistent impedances. Ground electrodes may also be of the EEG surface type; however, a flexible strip of metal, covered with saline soaked Velcro, and placed proximal to the stimulus provides the most effective patient ground. In addition, this arrangement greatly reduces stimulus artefact appearing on the trace.

Some recording systems require less operator intervention than others; a parameter, for example may be unchangeable from the manufacturers settings, alterations to accommodate new guidelines therefore, being somewhat limited. Otherwise, SEPs are normally recorded at gains of 100 – 120dB, with a pre-amplifier band of 1-30Hz highpass to 3kHz lowpass. An averaging window of 100ms should have a sampling interval of 0.2ms. If the sweep setting has an

automatic stop it should be set to 1000, and a trial should not be stopped *before* 500 stimulus repetitions unless it is exceptionally clear. It is obvious that a repeat trial is performed for superimposition.

Channel derivations are based on the principle waveforms which are recorded most effectively when the electrode site is close to the voltage generator (Chiappa *et al* 1978). A four channel montage is standard; this will record i) brachial plexus potential, the electrode being placed on Erbs' point; ii) cervicomedullary potentials, CVII for the dorsal root entry zone, and CII for the dorsal column spinal nuclei; iii) somatosensory cortical potential, electrodes placed 2cm posterior to the ipsilateral C₄ / C₃ positions of the 10-20 System. All active electrodes are referred to F_z. An upper limb SEP montage would therefore be:

Ch 1 : C₃ / C₄ – F_z
Ch 2 : CII – F_z
Ch 3 : CVII – F_z
Ch 4 : Erb - F_z

Stimulus sites for upper limb SEP are commonly median, ulnar and radial nerves. The actual site is treated to reduce contact impedance as for recording electrodes; the ground lead site should also be treated in this way. The cathode of the stimulator should be proximal, otherwise the action potential may be reduced (anodal block). Stimulus intensity should be maximal in order to stimulate all fibres; patient comfort however, will be an overriding factor. The major cause of an apparently delayed cortical potential is that only the group II (slower) afferent fibres have been activated. Unilateral stimulation is normal, as

pathologies may be literalised. Bilateral stimulation - where lateralisation is not an issue – may be feasible if obtaining a large potential is a priority. This may be the case in post-operative monitoring. The low voltage nature of the potentials generated in EP's dictates that a large number of stimuli are required. It is tempting, therefore, to increase the stimulation rate in order to reduce the investigation time. A rate of 5Hz is often reported (Pratt *et al* 1980), however many traces may have their clarity improved with a reduced rate, 2Hz being the current departmental protocol.

The typical stimulus duration is 0.2ms and will be supplied from an isolated power supply. It is helpful if this parameter is adjustable as a longer pulse width may be required to elicit a response from patients with peripheral neuropathies. Finally, it cannot be over stressed that trial repetition is mandatory; it is not unusual to record four investigations in order to ensure a measure of repeatability.

Waveform identification and therefore peak latencies can be demonstrated by example but cannot be described easily in writing. In short, the Erbs potential appears as a diphasic (positive-negative) waveform and is recorded as N9. The upper cervical chord CVII and lower brainstem CII have a merged waveform such that CVIII is a lobule in a larger peak CII, recorded as N11 & N13 respectively. The cortical potential is a well formed isolated peak recorded as N20. Absolute latencies are dependent on variables that are not all reproducible from one department to another. This may also be true when moving between different recording devices. Normal standards, therefore, should be collected for each

department and for each device; there are however, many studies available that have large numbers of normal data and that yield consistent results.

There are various non-pathological factors which may affect the results. Technical factors include stimulus intensity, rate and bandpass. In addition, patient factors may possibly include age, gender, limb temperature and medication.

Age changes in adults have not been researched as extensively as for the visual and auditory modalities. Unfortunately there are contradicting reports. Some studies that have found no significant changes in N19 & P23 latencies across a range of 19 – 70yrs, while other reports suggest there is a moderate (~0.3ms) increase in N19 latency between age 50 – 60yrs but no change thereafter. There is a similar unclear relationship between conduction times and gender.

Peripheral nerve conduction velocity is significantly affected by limb temperature; it is normal practice in electromyography to both measure and control the temperature. It is accepted that a rising of limb temperature by 1°C can shorten the N19 latency by 0.7 – 1.0ms, most protocols refer to (Bolton C. *et al* 1981; Matthews & Small 1979). Consistent limb temperature is a factor that will be relatively straightforward to achieve in a departmental study.

Short latency SEP's are particularly unaffected by drugs. Notably when barbiturates, carbamazepine or diazepam are present at a dose that affects the background EEG, the SEP is preserved. Phenytoin conversely is associated with prolonged SEP latencies.

The SEP is an objective, reproducible, non-invasive method of investigating the sensory system. Interpretation depends partly on familiarity with a large normal control database. In addition to technical expertise, a pertinent medical history from each patient will assist the waveform interpretations where they may be affected by clinical factors.

Auditory Cognitive EPs: P300

The central nervous system can respond to external stimuli in two distinct ways. In one, the exogenous potentials are modality specific and reflect physiological processing of a sensory afferent message and its cortical destination. They are therefore non-cognitive. Endogenous potentials share, in common with the former, a time-locked relationship with the stimulus. However, they are not modality specific and they reflect the activity of the pathways involved in cognitive processing of sensory information. Exogenous EPs depend on the characteristics of the stimulus while endogenous EPs depend on the nature of the task once the sensory information has arrived at the cerebral cortex.

Cognitive potentials occur with a longer latency in comparison to sensory potentials and so both cannot be easily visualised in the same recording protocol. The current strategy is to compare EPs generated by a stimulus requiring attention – the target, embedded randomly in a series of stimuli not requiring attention – non-target. The difficulty here is clearly to ensure that non-target stimulation does not elicit a cognitive response.

Cognitive potentials are generally recorded several milliseconds after sensory EPs and so are often termed long-latency endogenous EPs. The best known is the P300; others frequently studied include the contingent negative variation (CNV) and the movement related *bereitschaftspotential* (BP). The P300 is a symmetrical positive wave seen maximally over the midline in the central and parietal regions. It occurs with a latency of between 250 – 600ms, depending on the stimulus type. It is elicited by the so-called 'oddball' paradigm; this requires the presentation of unexpected infrequent stimuli randomly interspersed among frequent stimuli. The difference would be in the frequency (tone) if the stimulus was auditory, as is the case in this study.

Visual, somatosensory and auditory modalities can all elicit a P300 response (Desmedt & Robertson 1977; Snyder *et al* 1980; Barrett *et al* 1987) and their morphologies are similar, although latencies may differ. The amplitude of the P300 is affected by the probability of the target or rare stimulus. There are three factors that affect the target presentation – the overall probability, the local sequence and the interstimulus interval (ISI). The P300 amplitude increases as the target frequency decreases (Polich J. 1987). Maximum amplitudes occur with the target rate at 10-15%. Most neurophysiology departments use 20% in their clinical protocols. The local sequence refers to the preceding stimuli, such that if two target stimuli are presented sequentially the amplitude will be lower than if the rare tone was preceded by four or five common tones. In addition, if the target stimulus occurs at a regular interval it will not be unexpected and so the amplitude decreases.

The amplitude of the P300 increases as the ISI increases, or as the target probability decreases. This appears to be more significant than overall probability (Fitzgerald & Picton 1981); where ISI's are below 300ms latencies are also prolonged. A 'pseudo-random' sequence, with the appropriate ISI, will therefore contain a random presentation of rare stimuli but will avoid local the local sequence effects. The stimulus rate is usually once per second, as should be the sweep duration.

Recording issues include technical and patient factors. The commonly used bandwidths are 0.1 to 1Hz and 30 to 100Hz for high- and lowpass respectively. Three midline channels should be used – Fz, Cz and Pz, referenced to linked mastoids, A1 and A2. An eye movement channel is required to identify blink artefact; this can be used for sweep rejection by amplitude, and is particularly important for the target stimulus and channel. The P300 can be variable within a subject, therefore in common with other EPs; a repeated and superimposed trial will ensure reproducibility. However, habituation may be a problem as it can occur in long latency cognitive studies which involve a novel or rare stimulation paradigm (Polich 1986). It is currently unclear to what extent task dependent EPs are affected.

The age of the patient, their level of attention and the difficulty of the task can affect the latency of the P300. The subject must be awake and alert in order to produce a P300 waveform (Barrett *et al* 1987). Decreasing vigilance is associated with decreasing amplitude while drowsiness eliminates the evoked response. Behavioural assessment of attention is therefore advantageous and a

recordable response to the target stimulus will provide an appropriate estimate; pushing a button, for example, on the rare tone will allow the operator to assess attention while the reaction time can indicate changes in alert levels.

The difficulty in discrimination in the oddball paradigm affects the latency such that as the task becomes more challenging the P300 latency increases (Polich 1987). In an auditory paradigm for example, the latency will increase if there is insufficient difference between the two presented tones of the stimulus.

The age of the patient has a positive correlation with P300 latency (Barrett *et al* 1987; Picton *et al* 1984; Polich *et al* 1985). Latency increases by approximately 1ms per year after 20 years of age. A regression line for age and latency has not been found by all researchers however; in addition, there appears to be a reduction of latency in paediatric studies where the minimum latency varies between 15 – 25 years of age.

Cognitive EPs: current status

Research into cognitive function has attempted to correlate EP latency with clinical changes. Some alterations have been reported but specificity and sensitivity are currently unclear. Diagnosis and monitoring of dementia is one of the areas where the earliest investigations were made; studies with dementia of mixed aetiologies all reported prolonged latencies but some of these were not statistically significant. However, patients selected for Alzheimer's type (St. Clair *et al* 1985) observed a 70% abnormality with no false positives. Studies attempting to differentiate subtypes of dementia (Goodin & Aminoff 1986) are hampered by patients producing incorrect responses which may alter the latency

more than the underlying pathology. In addition, there is a significant latency overlap between the control group and patient's, particularly where the dementia is mild.

There have been many cognitive studies in patients with schizophrenia; many report alterations in amplitude but most do not report any significant change in latency (Brecher *et al* 1987). This patient group tends to have less correct responses, longer reaction times and frequently reduced motivation – all of which affect any task driven EP. In these studies there is also a great overlap between the control and patient groups (Morstyn *et al* 1983).

Other patient groups investigated include Parkinson's and Huntingdon's dementia where significant changes have been reported for auditory P300 latency (Goodin & Aminoff 1986; Rosenberg *et al* 1985). Metabolic encephalopathies have also been investigated; patient studies of chronic renal failure, Down's syndrome and alcoholism have all reported prolonged P300 latencies, including sub-groups which clinically have no cognitive impairment.

The application of cognitive EPs is problematic since there is no 'gold-standard' protocol that has been approved for clinical neurophysiology investigation. Further, the evidence of overlap between patient with control data and variability within the control group, suggests that the P300 may be limited in its usefulness as a clinical tool. In addition to instrumentation factors, patients with altered cognitive function must be closely monitored for behavioural features, as inattention and accurate task performance greatly affect the P300 outcome.

1.3 The Liver

The liver is the largest internal organ in the body and is located in the right hypochondrium. It performs several key functions in the body including metabolism of proteins, carbohydrates and lipids, formation of bile, hormone and drug inactivation and detoxification. Injury to the liver can occur acutely, usually due to infection with hepatotropic viruses or drugs, or chronically, usually as a result of chronic alcohol misuse or chronic infection with hepatitis B or C. While the majority of individuals with acute hepatitis recover, with no long-term sequelae, the development of cirrhosis is associated with a number of complications reflecting loss of hepatocellular function and/or the development of portal hypertension. These complications include; jaundice, fluid retention, bleeding from oesophageal varices and hepatic encephalopathy.

Injury to the liver

Injury to the liver can occur acutely, usually due to infection with hepatotropic viruses or hepatotoxic drugs, or chronically, mainly as a result of excess alcohol ingestion or chronic infection with hepatitis B or C. Chronic injury results, in a proportion of individuals, in the development of cirrhosis which is characterised by the presence of severe fibrosis or scarring with evidence of regeneration in the form of nodules of 'normal' liver tissue; as a result, the normal architecture of the liver is disrupted (Figure 1.3).

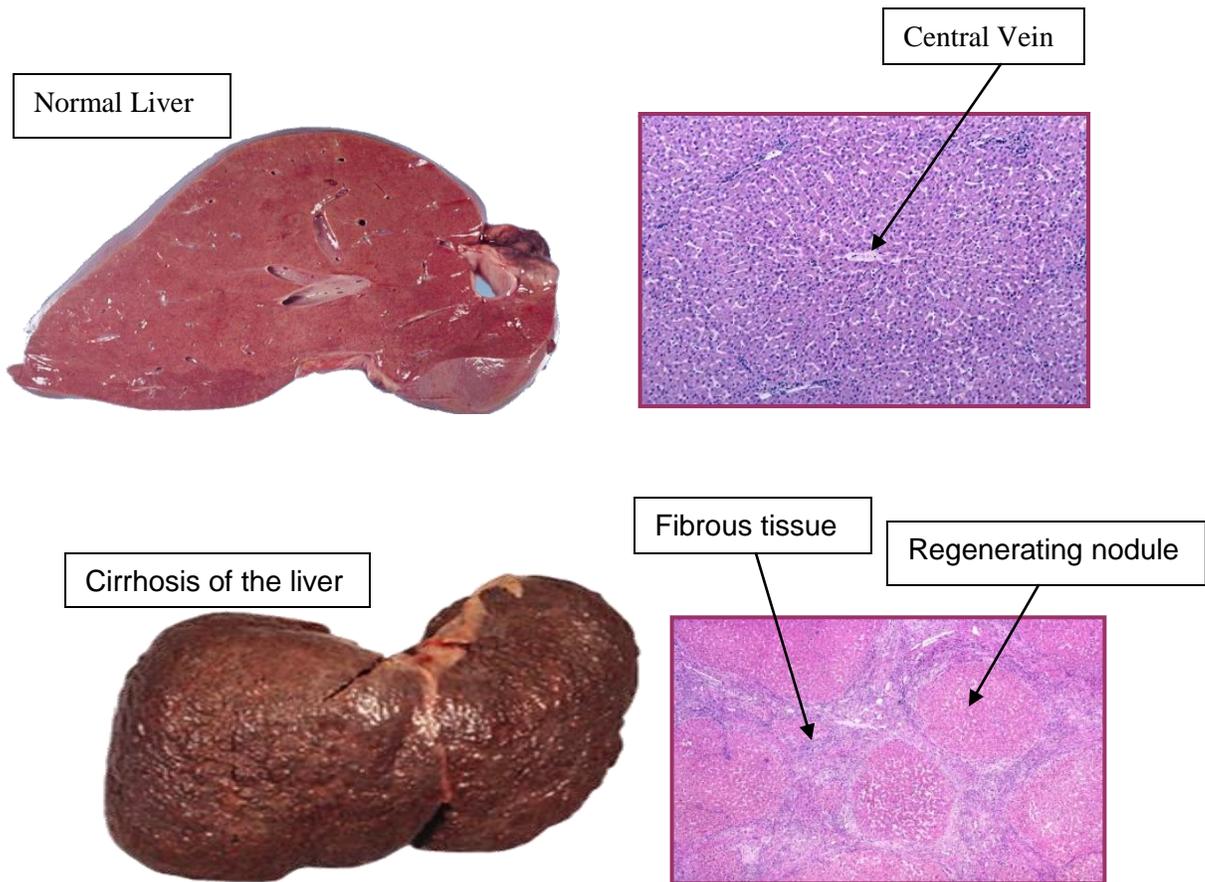


Figure 1.3: Macroscopic and microscopic views of a normal and a cirrhotic liver. The outline and surface of the normal; liver are smooth and the liver tissue arranged in orderly lobules around a central vein. The outline and substance of the cirrhotic liver are nodular and irregular with disruption of the normal architecture by fibrous bands and nodules of regenerating tissue.

Complications of Cirrhosis

The development of cirrhosis results in a reduction in the number of liver cells available to carry out its major functions. Thus, when the functioning cell mass of the liver drops below a critical level its activity is impaired. This is referred to as *hepatocellular failure*. In addition the presence of fibrosis and nodules in the liver impede the passage of blood through the liver parenchyma and this leads to an increase in the pressure within its main vascular supply

system. This is referred to as *portal hypertension*; it also has significant consequences (Table 1.1).

Table 1.1: Complications of the development of cirrhosis

Hepatocellular failure	
Function	Consequences
Breakdown of plasma bilirubin	Jaundice
Protein synthesis Albumin Clotting factors	Fluid retention - oedema Easy bruising/ bleeding
Sodium and water homeostasis	Fluid retention - oedema
Detoxification	Impaired drug handling Impaired handling of circulating neurotoxins
Portal hypertension	
Consequences	
Splenomegaly leading to: A reduction in red blood cells - anaemia A reduction in white blood cells (leucopaenia) - impaired immunity A reduction in platelets (thrombocytopenia) – poor clotting and bleeding	
Portal-systemic shunting leading to: Development of oesophageal/gastric varices – gastrointestinal bleeding Bypassing of the liver by neurotoxic material – hepatic encephalopathy Direction of the retained fluid into the abdominal cavity – ascites	

In many individuals the presence of cirrhosis is not accompanied by the development of hepatocellular failure and/or portal hypertension. They may be asymptomatic or else suffer from no more than minor lethargy and tiredness.

These individuals are referred to as having *compensated* cirrhosis. In others, however the cirrhosis becomes 'decompensated' following the development of portal hypertension and/or hepatocellular failure; they may present with jaundice, bleeding from varices; spontaneous bleeding; ascites and peripheral oedema and/ hepatic encephalopathy.

Classification of Cirrhosis

Cirrhosis is categorised in relation to its aetiology and to its functional capacity. The aetiology of is determined on the basis of clinical, laboratory, radiological and histological variables. The functional severity of their liver injury is assessed using the Pugh modification of the Child's grading system (Pugh *et al* 1973). This system is based on assessments and numerical classifications of five variable of which two are clinical and three are laboratory based. Each measure is scored from 1 to 3, with 3 indicating the most severe or greatest degree of abnormality. The sum of the five scores is used to assign a Child's grade of A, B or C with A indicating the least degree of compromise and hence the best prognosis (Pugh *et al* 1973).

Table 1.2: Child-Pugh Scoring System for functional hepatic reserve

Variable	Points scores for increasing severity		
	1	2	3
Serum bilirubin ($\mu\text{Mol/l}$) (5-17)	<34	34-50	>50
Plasma albumin (g/L) (35-55)	>35	28-34	<34
Prothrombin time (s) Seconds prolonged >16	1-4	4-6	>6
Ascites	None	Mild	Moderate or refractory
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Pugh's Score: 5-6 Child's Grade A

 7-9 Child's Grade B

 10-15 Child's Grade C

Compensated: Scores 5-7; Decompensated: Scores 8-15

Hepatic Encephalopathy

Hepatic encephalopathy (HE) is the term used to describe the neuropsychiatric abnormalities which arise in patients with acute and chronic liver disease although this thesis will be focused on the syndrome which accompanies cirrhosis. Although hepatic encephalopathy is one of the major complications of cirrhosis and has a considerable impact both on quality of life (Ferenci P 2002 *et al*; Groeneweg *et al* 1998) and survival (Merkel *et al* 1989; Butterworth 2000) it is often under-diagnosed and, therefore, sub-optimally managed.

There is general agreement that hepatic encephalopathy is a metabolic/neurophysiological rather than a structural disorder of the brain and is, therefore, potentially reversible. Approximately 20-30% of patients with cirrhosis manifest clinically overt neurological and/or psychiatric changes and are thus classified as having *overt hepatic encephalopathy*; these individuals invariably show abnormalities of psychometric performance and/or abnormalities of their electroencephalogram (EEG). A further 30-40% of patients with cirrhosis display no clinically detectable symptoms or signs, but do show impairment of psychometric and/or EEG performance; these individuals are classified as having *minimal hepatic encephalopathy*. Finally, 20-30% of patients with cirrhosis show no neuropsychiatric abnormalities and no defining psychometric or electrophysiological abnormalities: these individuals are classified as *neuropsychiatrically unimpaired*.

Pathophysiology

The pathogenesis of hepatic encephalopathy is not known. It is highly unlikely that any one factor alone is responsible for the generation of the syndrome and thus at present a “multiple-hit” hypothesis (Wright *et al* 2007) is favoured. This implicates gut derived toxins, especially ammonia, systemic inflammation and changes in the auto-regulation of cerebral haemodynamics. Other gut-derived toxins and disruption of multiple neurotransmitters and neurotransmitter systems are also implicated.

There is agreement on the importance of gut-derived neurotoxins, especially ammonia in the pathogenesis of hepatic encephalopathy. Ammonia is

derived in the gut from ingestion of nitrogenous substances which are broken down by bacterial ureases and amino acid oxidases. The majority, however, is derived from the uptake of glutamine in the intestine, which, through the actions of glutaminase, forms glutamate and ammonia (Desjardins *et al* 1999; Olde Damink *et al* 2003). In the normal liver, ammonia is detoxified by conversion to urea in periportal hepatocytes via the urea-cycle and to glutamine in perivenous hepatocytes via glutamine synthetase.

In patients with cirrhosis the fibrotic changes within the hepatic parenchyma impede the passage of blood resulting in the establishment of portosystemic shunts which allow nitrogenous breakdown products from the intestine to pass from the portal venous system directly into the systemic circulation without the interposition of the hepatic detoxifying filter. In addition, the detoxification of the products that are extracted by the liver is suboptimal in the presence of hepatocellular failure. Thus potentially neurotoxic substances can impinge directly on the brain (Figure 1.4).

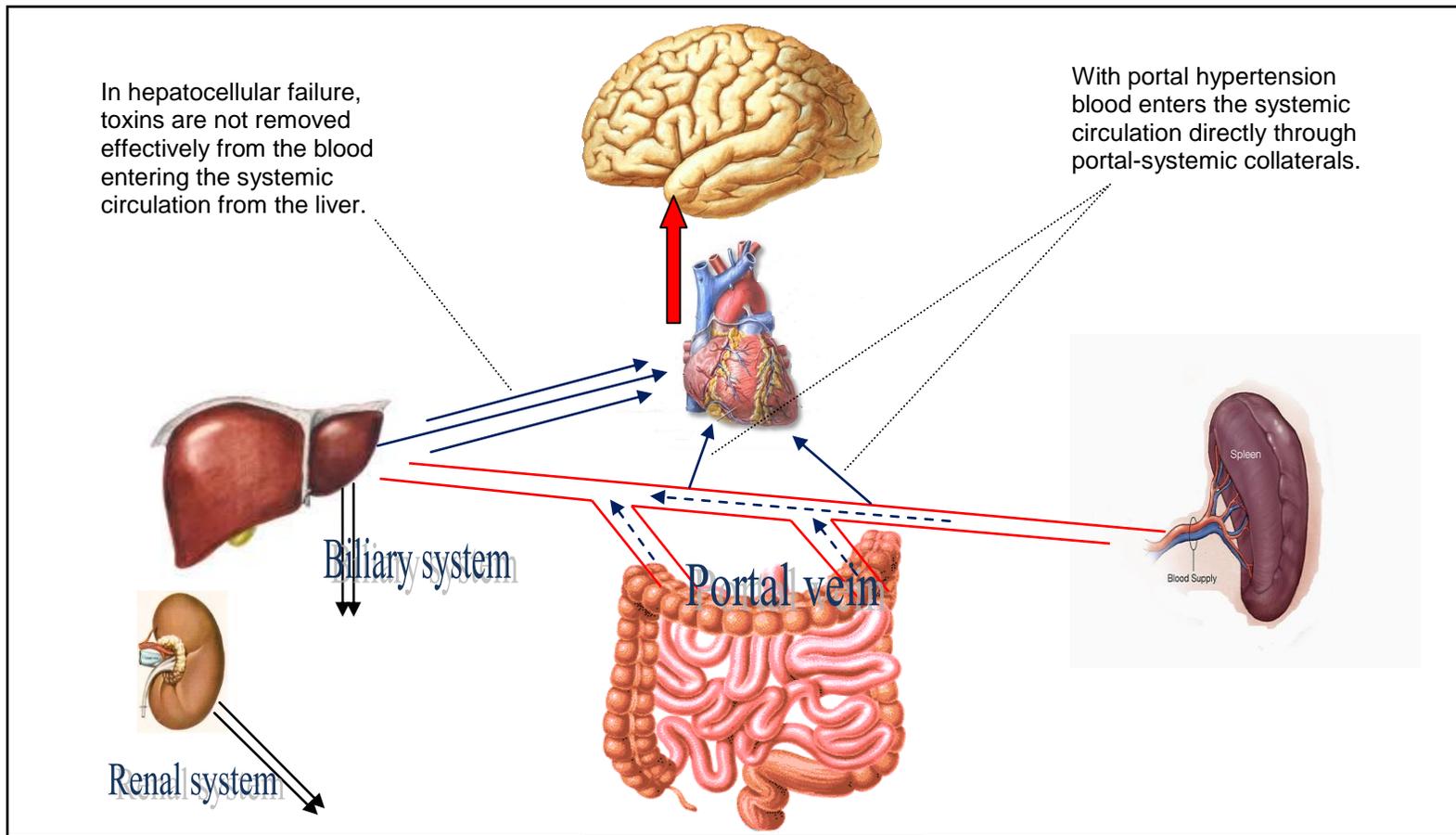


Figure. 1.4. Passage of gut-derived neurotoxic material into the systemic circulation in cirrhosis as a result of ineffective hepatic detoxification and portal systemic shunting of blood

Brain ammonia uptake has been shown to be significantly increased in both acute and chronic liver failure, due to increased permeability of the blood-brain barrier and alterations in cerebral blood flow (Butterworth 2002). Evidence from PET studies confirms that ammonia diffuses into the brain easily (Abou-Assi & Vlahcevic 2001). The brain does not possess urea cycle enzymes and thus ammonia is detoxified exclusively in astrocytes to glutamine via the enzyme glutamine synthetase. Glutamine efflux from astrocytes occurs by passive diffusion into the extracellular fluid and CSF. This may be affected by changes in pH. Therefore, in states of acute hyperammonaemia, glutamine accumulates in astrocytes, causing them to swell (Haussinger *et al* 2000) (Figure 1.5). This has been identified as the “glutamine hypothesis” (Brusilow 1986) of cerebral oedema in fulminant hepatic failure (Blei & Stolze Larsen 1999). This same brain swelling is not seen to any extent in patients with cirrhosis with hepatic encephalopathy although, *in vivo* proton-magnetic resonance-(¹H MR)-spectroscopic studies have suggested that astrocytes may show some change in their cell volume (Haussinger *et al* 1994; Laubenberger *et al* 1997).

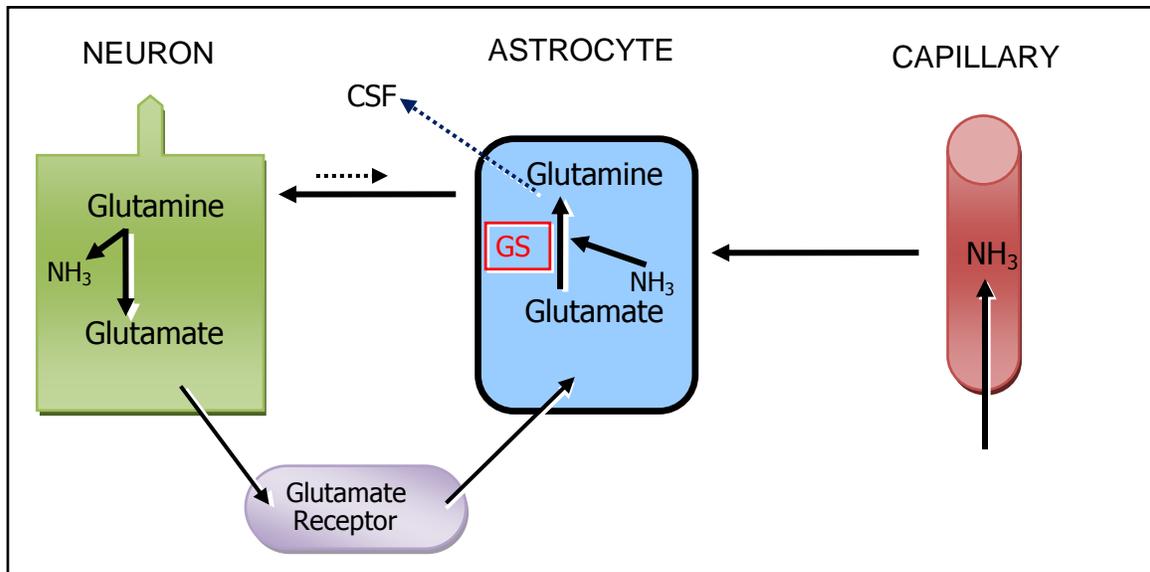


Figure 1.5. Cerebral ammonia detoxification. GS=Glutamine synthetase. Ammonia is converted to glutamine in the astrocytes by glutamine synthetase and accumulates here.

The astrocyte clearly plays a pivotal role in cerebral ammonia detoxification and in the presence of persistent hyperammonaemia develops changes of Alzheimer Type II degeneration with nuclear enlargement, peripheral margination of chromatin and prominent nucleoli. These changes can be induced experimentally in cultured astrocytes by exposure to ammonia, indicating that HE is a disorder of glial cells, with neuronal dysfunction the consequence (Haussinger *et al* 2000). Alzheimer Type II astrocytes also have altered expression of key astrocytic proteins including glial fibrillary acidic protein, glutamate transporters and “peripheral-type” (mitochondrial) benzodiazepine receptors (Butterworth 2002).

The changes in astrocytic function which occur in response to hyperammonaemia causes alterations in neurotransmission (Haussinger *et al* 2000) (Figure 1.5) Ammonia also directly inhibits excitatory postsynaptic potentials, thereby depressing overall central nervous system function. The

ammonium ion also inhibits the tricarboxylic acid cycle enzyme ketoglutarate dehydrogenase, thus inhibiting cerebral glucose metabolism (Lai & Cooper 1986). This could impair brain energy metabolism, however it appears to be a late effect in chronic liver failure. Ammonia also has a stimulatory effect on L-arginine uptake resulting in increased production of nitric oxide and inhibition of the storage of glutamate in astrocytes (Butterworth 2000).

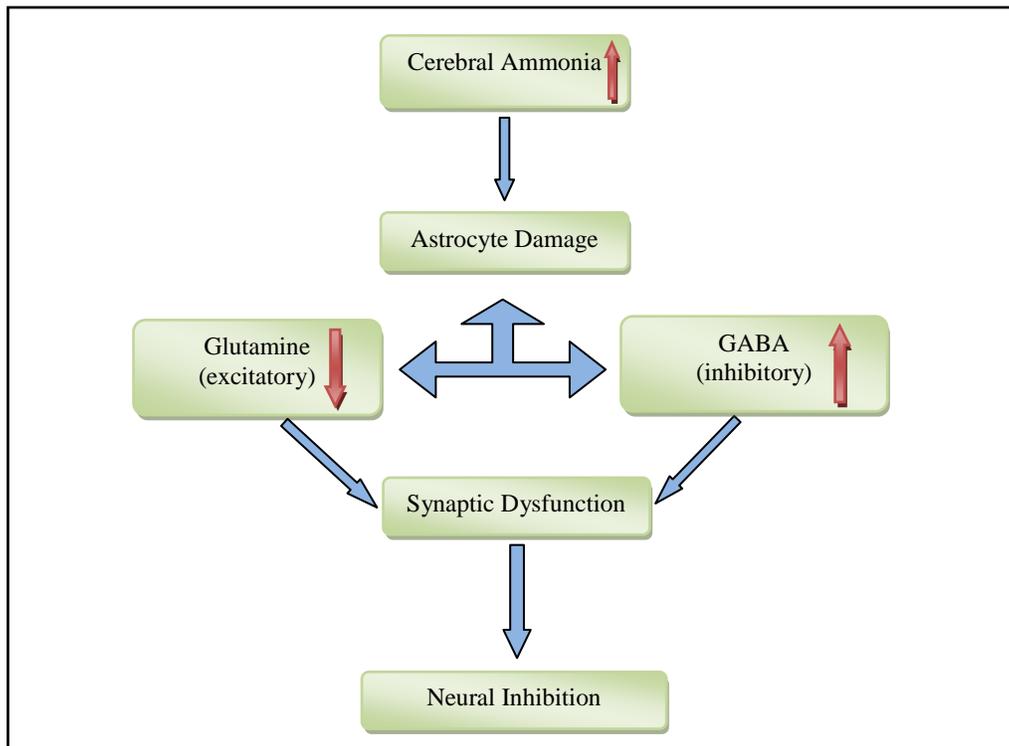


Figure 1.6. Disruption of central neurotransmission in hepatic encephalopathy. With astrocyte damage, glutamine synthetase activity and glutamate uptake decrease leading to glutamatergic synaptic dysfunction.

The evidence for the role of ammonia and glutamine in the pathogenesis of hepatic encephalopathy is compelling even though the correlation between arterial ammonia concentrations and the degree of hepatic encephalopathy is sometimes poor; hepatic encephalopathy can be present in the absence of

elevated ammonia levels and low levels of ammonia have neuroexcitatory effects (Abou-Assi & Vlahcevic 2001). However the correlation between concentrations of glutamine in the CSF and the presence and degree of hepatic encephalopathy is excellent (Romero-Gomez *et al* 2004). The severity of neuropsychiatric symptoms also correlates with brain concentration of glutamine (Butterworth 2002).

Neurotransmitters and receptors

Gene expression of a number of neurotransmitter-related proteins is increased in the presence of hepatic failure (Merkel *et al* 1989). Many of the early neuropsychiatric symptoms of hepatic encephalopathy such as altered sleep patterns have been attributed to modification of serotonin, a monoamine neurotransmitter (Abou-Assi & Vlahcevic 2001). Increased expression of the monoamine oxidase gene and thus increased activity of the enzyme and increased density of catalytic sites on the enzyme protein have been reported in autopsied brain tissue of cirrhotic patients with hepatic encephalopathy (Mousseau *et al* 1997).

Increased expression of the “peripheral-type” benzodiazepine receptor located on the outer mitochondrial membrane of the astrocyte, has been identified in brain extracts from portacaval-shunted rats and attributed to increased cerebral concentrations of ammonia and/or manganese level (Desjardins *et al* 1997; Butterworth 2000). This receptor could be involved in the maintenance of the energy metabolism of astrocytes and cholesterol uptake by

the mitochondria resulting in a generation of 'neurosteroids' (Butterworth 2000). Increase in receptor level may result in alterations of monoamine and amino acid neurotransmitter function as well as modified cerebral perfusion in chronic liver failure.

Hepatic encephalopathy also demonstrates effects on opiate and catecholamine pathways (Wright *et al* 2007). The interaction between these neurotransmitters and other key factors already identified in the pathogenesis of hepatic encephalopathy is significant and so the true influence of neurotransmitters in hepatic encephalopathy is difficult to identify (Wright *et al* 2007). For example, the uptake of tryptophan, from which serotonin is derived, is facilitated by elevated ammonia levels (Young *et al* 1975).

Diagnosis

The diagnosis of hepatic encephalopathy in patients with cirrhosis is based on historical and clinical evidence and the use of surrogate markers to delineate neuropsychiatric status.

Evidence for changes in daily living, including the sleep-wake cycle, cognitive impairments and energy levels should be sought from the patient themselves and from family and friends. A detailed neuropsychiatric examination must also be performed including a mental-state examination, cranial nerves examination and a motor and sensory examination. Patients may manifest subtle or florid clinical symptoms relating to a number of cerebral systems, e.g:

- Cortical
 - Cognitive deterioration
 - Personality change
 - Psychiatric abnormalities
 - Disturbed consciousness
- Extra-pyramidal and cerebellar
 - Dysarthria
 - Ataxia
 - Tremor
 - Cogwheel rigidity
- Spinal
 - Spasticity
 - Paraplegia

The changes in mental state are classified using West Haven criteria which were described by Conn and co-workers in 1977 (Conn *et al* 1977) and adapted from an earlier classification suggested by Parsons–Smith. (Table 1.1) (Parsons-Smith *et al* 1957)

Table 1.1. The West Haven criteria of altered mental state in hepatic encephalopathy (Blei *et al* 2001; Amodio *et al* 2004)

Grade	Description
0	<ul style="list-style-type: none"> • Lack of detectable changes in personality or behaviour. • *Asterixis absent.
I	<ul style="list-style-type: none"> • Trivial lack of awareness. • Shortened attention span. • Dyscalculia. (Impaired addition or subtraction.) • Hypersomnia, insomnia, or inversion of sleep pattern. • Euphoria or depression or anxiety. • Asterixis can be detected.

II	<ul style="list-style-type: none"> • Lethargy or apathy. • Disorientation. • Disorientation of time. • Inappropriate behaviour. • Obvious personality change. • Slurred speech. • Obvious asterixis.
III	<ul style="list-style-type: none"> • Gross disorientation. • Bizarre behaviour. • Somnolence to semi-stupor. • Confused. • Responsive to stimuli. • Asterixis generally absent.
IV	<ul style="list-style-type: none"> • Coma.

*Asterixis – flapping tremor of the wrist.

These criteria are not universally accepted, primarily because they do not allow clear differentiation between patients with Grade 0 and I. Blei and Cordoba (2001) and Ferenci *et al* (2002) have, in consequence, suggested minor modifications to the West Haven criteria but more recently Amodio *et al* have suggested a more radical modification in the hope of providing a more practical universally applicable tool (Table 1.2).

Table 1.2. Suggested modification of the West Haven criteria for the grading of mental state in patients with cirrhosis (Amodio *et al* 2004).

Grade	Description	Proposed operative definition
0	<ul style="list-style-type: none"> No abnormality detected 	
I	<ul style="list-style-type: none"> Trivial lack of awareness Euphoria or anxiety Shortened attention span Impairment of addition or subtraction 	<ul style="list-style-type: none"> Not able to complete TMT-A^a in 120 sec (individuals with ≥ 5 years of education), or Naming ≤ 7 animals in 120 sec Orientated in time and space
II	<ul style="list-style-type: none"> Lethargy or apathy Disorientation for time Obvious personality change Inappropriate behaviour 	<ul style="list-style-type: none"> Disorientation in time: (≥ 3 items incorrect): <ul style="list-style-type: none"> Day of the week Day of the month The month The year, and Orientated in place
III	<ul style="list-style-type: none"> Somnolence to semi-stupor Responsive to stimuli Confused Gross disorientation Bizarre behaviour 	<ul style="list-style-type: none"> Disorientated in place: (≥ 2 items incorrect): <ul style="list-style-type: none"> State/country Region/country City Place Floor/ward, and Disorientated in time, and Reduction of Glasgow score (8-14)ⁱ
IV	<ul style="list-style-type: none"> Coma, unable to test mental state 	<ul style="list-style-type: none"> Unresponsive to pain stimuli (Glasgow score < 8)

^a TMT-A: Trail-Making Test A (Reitan 1955)

When consciousness is impaired, the Glasgow coma scale (GCS) is used to assess the severity of the condition. It is calculated by assessing verbal, motor and ocular responses and summing the total values to provide an overall score.(Table 1.3)

Table 1.3. The Glasgow coma scale (Longmore *et al* 2005)

Best motor response		Best verbal response		Eye opening	
6	Carrying out request ('obeying command')				
5	Localizing response to pain	5	Orientated		
4	Withdraws to pain	4	Confused conversation	4	Spontaneous eye opening
3	Flexor response to pain	3	Inappropriate speech	3	Eye opening in response to speech
2	Extensor response to pain	2	Incomprehensible speech	2	Eye opening in response to pain
1	No response to pain	1	None	1	No eye opening

Use of the above criteria does not preclude the use of other tools such as the Mini Mental Score Test or Abbreviated Mental Test.

Neuropsychometric Assessment

Psychometric tests can be used to demonstrate the disturbances of cognition including perception, memory, learning, executive functions, expression (language, constructive abilities, voluntary motor control) and mental activity (attention, concentration and psychomotor speed) characteristic of this syndrome (Amodio *et al* 2004; Ortiz *et al* 2005). Weissenborn *et al* (2001b) recommend use of a test battery comprising number connection tests (NCTs) A and B, the line tracing test, the serial dotting test and the digit symbol test. This test battery which is called the psychometric hepatic encephalopathy score (PHES), although recommended by the international working party on hepatic encephalopathy is still not internationally accepted. Scores in the PHES battery however are affected by many variables including age, gender and number of years in education (Montagnese *et al* 2004). Therefore, simple thresholds of raw data are inappropriate. Tests must be appropriately validated and standardised within the populations in which they are to be applied.

Recently a system for scoring the PHES battery was devised for use in the UK which has two unique features. First it provides a new system for scoring the line tracing test (LTT). This test has two components: time (LTTt) and errors (LTTe), which have been variously considered as either dependent (Amodio *et al* 1996) or independent (Weissenborn *et al* 2001) variables. A method for scoring the LTT which is based on a model which relates times and errors has been incorporated into the new scoring schema. Second, the scoring system includes

adjustment for important confounding variables such as age, years and location of education and ethnic origin (Marks *et al* 2008).

Neurophysiological Assessment

Neurophysiological tests include electroencephalography (EEG) which records the electrical activity of the brain cortex and evoked potentials (EP) which examine discrete firing patterns of populations of cells in response to a stimulus (exogenous EP), or to a passive or active cognitive task (endogenous EP). These tests provide a more objective measure to quantitatively grade hepatic encephalopathy (Kircheis *et al* 2007). Neurophysiological tests are very useful for investigational purposes, especially in those in whom neuropsychological tests are difficult to interpret due to age or co-morbidities.

The EEG is useful for detecting, assessing and monitoring hepatic encephalopathy. The majority of EEGs recorded in healthy individuals are dominated by a basic rhythm concentrated in the posterior area of the scalp in the alpha range (8-13Hz). If the EEG is of low voltage or is dominated by fast activity this rhythm can be difficult to distinguish (Niedermeyer 1999). The main criterion for diagnosing hepatic encephalopathy by electroencephalography is slowing of the posterior basic rhythm from the alpha range (8-13Hz) towards the theta (4-7Hz) and delta (1-4Hz) ranges (Montagnese *et al* 2007).

The EEG can be analysed visually, though this is subject to inter- and intra-operator variability. Spectral EEG analysis has been shown to be a more reliable measure (Amodio *et al* 1999) as it provides an automated estimate of the

dominant EEG frequency and the component contributions. The diagnosis of hepatic encephalopathy is generally based on changes in the EEG mean dominant frequency. However, topographical changes have also been noted including 'anteriorization' of the posterior basic rhythm. This can be viewed, if prominent, on a visual analysis. It can also, however, be quantified by analytical techniques such as spatio-temporal decomposition, (SEDACA) (short epoch, dominant activity, cluster analysis) which separates the main components of the EEG and defines their distribution across the scalp. SEDACA separates the activities recorded in the EEG into sets of paired temporal and spatial components. The temporal components (waveforms) provide the time courses of the separated EEG activities, while the corresponding spatial components (head-maps) illustrate the distributions of the waveforms across the scalp. The temporal components are scaled according to their relative contribution to the original EEG. Electrode, muscle and eye artefacts are separated leaving artefact-free components available for analysis (Montagnese *et al* 2007).

Diagnostic sensitivity of the EEG varies from 43% to 100% for overt hepatic encephalopathy (Montagnese *et al* 2004; Montagnese *et al* 2007). EEG abnormalities have also been detected in 8 to 40% of clinically unimpaired patients, however this also depended on the techniques used (Montagnese *et al* 2004). In their review, Montagnese, and co-workers describe how, despite discrepancies over the sensitivity of EEG in diagnosing hepatic encephalopathy, EEG alterations in cirrhotic patients "generally reflect the patients' neuropsychiatric status, the effects of treatment, dietary manipulation, and

surgical/nonsurgical shunt creation” and “correlate well with health-related quality of life “(Montagnese *et al* 2004).

Evoked Potentials

Evoked potentials (EPs) are small phasic potentials which occur with sensory, motor and cognitive events. As described earlier there are:

- Exogenous (sensory) – elicited by direct stimulation of visual, auditory or somatosensory systems.
- Endogenous (cognitive) – cognitive activity.
- Movement-related – the preparation for movement and its execution.

All varieties of exogenous EPs have been used in studies of minimal hepatic encephalopathy. Delays in conduction were identified; however, these changes could also have been the result of alcohol consumption or diabetes. Endogenous EPs reflect cognitive changes and so would, in theory, be a better indicator of minimal hepatic encephalopathy. The P300 has been studied in cirrhotic patients with hepatic encephalopathy. The P300 is a large positive deflection in voltage at a latency of approximately 300ms in the EEG which is elicited by different tasks; the most common task used is the oddball paradigm. The amplitude of the P300 reflects the amount of attentive resources devoted to a task and the latency of the P300 reflects the speed of stimulus classification before a response is selected. The use of P300 latency elicited by the oddball paradigm has been identified as a sensitive tool to detect minimal hepatic encephalopathy in many

studies (Davies *et al* 1990b; Weissenborn *et al* 1990). However, the data are conflicting (Amodio *et al* 2005).

At present, therefore, there is no gold standard test for the diagnosis of hepatic encephalopathy and the classification of patients is based on arbitrary principles. The following schema is the one most commonly adopted.

Overt Hepatic Encephalopathy

The diagnosis is based on the elucidation of abnormalities of neuropsychiatric status and requires a detailed neurological examination and assessment of mental state using the modified West Haven criteria. These patients invariably show abnormalities in their psychometric assessment and/or the neurophysiologic assessment.

Minimal Hepatic Encephalopathy

Patients with minimal hepatic encephalopathy, by definition, show no clinical evidence of neuropsychiatric abnormalities—otherwise the diagnostic criteria applied to minimal hepatic encephalopathy are not secure. The working party for the 11th World Congress of Gastroenterology produced a consensus that at least two of the following neuropsychological tests should be used: NCT-A; NCT-B; block-design test; and digit-symbol tests, with, where possible a quantitative neurophysiologic tool such as EEG with mean dominant frequency or P300 auditory evoked potentials. They also noted that the PHES battery might prove useful (Ferenci *et al* 2002). Thus the diagnosis should be based on the absence of clinical features together with impaired performance of at least two of the four psychometric tests recommended, with adjusted normative scoring,

and/or an abnormal neurophysiological test result (Ferenci *et al* 2002; Kharbanda *et al* 2003).

Patients with minimal hepatic encephalopathy have been previously termed subclinical or latent hepatic encephalopathy, though its importance should not be minimised. These individuals, despite their apparent normalcy, show significant impairment of several activities of daily living including complex task execution such as driving (Schomerus *et al* 1981; Watanabe *et al* 1995), and there is clear evidence that their earning capacity is detrimentally affected (Ortiz *et al* 2005) as is their quality of life (Groeneweg *et al* 1998). In addition PET scanning has shown a significant decrease in glucose utilization in several cortical regions which correlate with the patient's cognitive function even in minimal hepatic encephalopathy (Lockwood *et al* 2000). It has also been shown that minimal hepatic encephalopathy predicts the occurrence of overt hepatic encephalopathy (Romero-Gomez *et al* 2001) and that it can be successfully treated (Watanabe *et al* 1997).

Neuropsychiatrically Unimpaired

Patients who are classified as neuropsychiatrically unimpaired show no clinical, psychometric or neurophysiological abnormalities.

Current Problems

Hepatic encephalopathy is an often neglected complication of chronic liver disease. It is poorly diagnosed and in consequence is often sub-optimally managed. There are two main difficulties:

1. There is no “gold standard” diagnostic test. Indeed this may be unattainable due to the varying presentations and impairments experienced by patients as well as multiple possible pathophysiological aspects of the disease. It may be that a battery of the tests described which each assess different aspects of cerebral function is necessary to provide a reliable diagnosis – a multidimensional approach. In their review of diagnostic methods for hepatic encephalopathy Montagnese *et al* (2004) described how due to the wide range of diagnostic modalities and the lack of a consensus as to the value of each, many centres either rely solely on clinical assessment to diagnose hepatic encephalopathy or inconsistently use an additional measure which is most easily accessible rather than one which has been validated or standardized. Indeed it is difficult to validate the findings of most studies as no consensus has been reached on the diagnosis and classification of patients.

2. The exact pathophysiology of the syndrome is unknown. In consequence, treatment is based somewhat empirically on the current concepts of its pathogenesis. It follows that there is no one specific treatment but a number of possible treatments that can be used in tandem.

The major treatment effort centres on reducing the generation, absorption and elimination of potentially neurotoxic material from the body. In addition there are some centrally acting agents that can be employed in specific instances:

- Decrease of ammonia load:
 - Enemata
 - Non absorbable antibiotics
 - Non absorbable disaccharides
 - L ornithine L aspartate
 - Sodium benzoate
 - Probiotics and zinc supplements

- Action on CNS:
 - Bromocriptine
 - BCAA
 - Flumazenil

The disaccharides lactulose and lactitol are the current mainstays of treatment and are usually well tolerated, cheap and effective. Some patients can experience some cramping, diarrhoea or flatulence, however once the dose is titrated, side effects are minimal. A recent meta-analysis evaluating the benefit of non-absorbable disaccharides versus placebo or no treatment in the treatment of hepatic encephalopathy showed no significant effect on hepatic encephalopathy grade or mortality (Als-Nielsen *et al* 2004). However, many trials were excluded from this analysis and lactulose continues to be effective as a therapy (Morgan *et al* 1989; Watanabe *et al* 1997). Lactulose has been shown to improve cognitive function and health-related quality of life (Prasad *et al* 2007).

In the absence of a diagnostic gold standard and without a good evidence base for current treatment regimens it is not surprising that unless symptoms are florid, hepatic encephalopathy is frequently not recognised or considered. Ortiz et al (2005) noting the lack of a well-standardised screening test identified two groups of patients who should undergo screening for minimal hepatic encephalopathy:

- Patients at risk of accidents, such as active drivers
- Patients with cognitive complaints such as; psychomotor performance; decreased attention; or poor memory, or decline in work performance.

However, the simplest approach would be to screen all patients with cirrhosis for evidence of hepatic encephalopathy and to treat accordingly or even more pragmatically to simply treat all patients with cirrhosis with lactulose or lactitol. Therefore, the search for better diagnostic tools and better insights into the pathogenesis continues.

1.4 Evoked Potentials in patients with hepatic encephalopathy

An extensive review of the literature revealed several single and multimodal EP studies; these are summarised (appendix II).

VEP.

Twenty-one VEP patient studies were carried out from 1983 – 2001. Nine of these studies were dedicated visual evoked investigations, the remainder consisting of mixed EP's (uSSEP & BAEP).

The combined patient total is 584; one study was dedicated to a paediatric patient group (Nora *et al* 2000), with age (mean \pm SD) 8.6 ± 2.5 , range 5 - 15 yrs. The mean age of the adult patients, where stated, was 45.2 ± 10.6 yr. Four papers stated the age range rather than the mean age, while eight papers did not state the range; where it was stated, the age range of the total patients in VEP studied was 13 – 78 yr.

The aetiologies varied markedly across the VEP patient group, and can be broadly divided into three sets.

Exclusively cirrhotic: 8 studies

Cirrhosis with other aetiologies: 9 studies

Alcoholic cirrhosis

Hepatitis (viral and autoimmune)

Primary Billiary Cirrhosis

Non-cirrhotic fibrosis

Extra-hepatic billiary atresia

Paracetamol overdose

Malnutrition

ALD

Cryptogenic

Non-cirrhotic liver disease: 5 studies

Cholestatic liver disease

Hepatocellular liver disease

Hepatitis B

The selection criterion for patients was not stated in five of the studies.

Four papers selected on an 'inclusion' basis, while the remainder used an 'exclusion' criterion.

INCLUSION	EXCLUSION
<ul style="list-style-type: none">• No clinical signs of hepatic encephalopathy (HE₀)• No history of alcoholism (Mehndiratta <i>et al</i> 1990)• No medication within previous 24hrs that has a known neurological effect• Normal visual acuity• Normal ERG	<ul style="list-style-type: none">• Abnormal visual acuity• Neurological disorders• Sedation within 24hrs• Psychotropic medication• Metabolic disorders including diabetes and uraemia• Overt HE (Demirturk 2001)• History of alcoholism (Nora <i>et al</i> 2000)• Malnutrition• Treatment with Lactulose & Neomycin (Zenerolli <i>et al</i> 1984)• GI bleeding

The assessment of hepatic encephalopathy was varied, but (Parsons-Smith 1957) was the system most adopted for classification.

Parsons-Smith (1957)	9
Child-Pugh	2
Adams & Foley (1953)	1
Zenerolli (1984)	1
Zeive (1987)	1
Conn / Child-Pugh	1
MMSE & Reitan	1
Not stated	3

The diagnosis of the underlying pathology was less varied, although it was not stated in five of the investigations. Serum biochemistry was used in ten studies (five of which had accompanying liver biopsy); biopsy as the exclusive method was stated in eight studies. The visual stimulus for seven studies was flash, while a pattern reversal stimulus (pVEP) was used in the remaining patient groups

Flash VEP: five of the seven studies recorded one channel only; the recording electrode positions corresponded to either the 10-20 International System (EEG) or the Modified Maudsley placements. The two groups that recorded bilaterally (Caellas *et al* 1985; Davies *et al* 1990a) did not use standard clinical neurophysiological recording electrode positions. The number of sweeps (flashes) per run was also variable, with one study not stating. Three studies were under 120 sweeps - the minimum standard – while only two were above (200 sweeps). One patient group (Sollazo *et al* 1983) used a variable number (40 – 480) with no further elaboration. Four of the groups did not state the filter pass band; all three of the remainder have either the low pass or high pass at an inappropriate setting, and with (in the one case stated) an excessively steep roll off - 60dB - compared to a typical setting of 12dB.

PVEP: one study did not describe the electrode recording positions, while three recorded from more than one parieto-occipital channel. The remaining studies all reported one occipital electrode for recording P100; all the pVEP investigations recorded from standard 10-20 electrode placement positions.

In the pVEP patient groups, one study did not state the sweep number. Four of the studies were below the clinical standard acceptable (minimum of 128) where the stated numbers were 70, 40-100, 100, 64-128. Again, where a variable sweep number is stated, there is no further elaboration. The amplifier passband was reported in four cases; three were acceptable bandwidths, but one of these described a roll off of 24dB. The remaining group stated a low pass of 1kHz which is high for visual studies and results in the trace being subject to EMG artefact.

Post processing procedures were not stated 15 of the study groups. One stated "repeated" but with no specific information; two used 4 runs and three used 2 runs. Once the reliability of the separate runs were checked by superimposition, it is unclear in these six patient groups whether they were summated to make a grand average in order to produce the reported latency values. When comparing the patient to the control group for statistical analysis purposes, only eight studies stated their working definition of abnormal latency. One used a numerical value of P100 >122ms, or interocular difference of >7ms (Demirturk 2001). The other studies used the control group and defined abnormal as latency above control mean + SD: two studies used 1SD and 3SD, while three studies used 2SD.

Of the seven studies that utilised the flash stimulus only one found no significant differences between the patient and control groups for both P100 and N145 potentials. This was the paediatric study (Nora *et al* 2000) with a patient group selected from liver transplant candidates. The stimulus & recording

parameters were in keeping with clinical neurophysiology protocols (one channel recording); therefore the result may reflect the unique nature of the patient group. In addition, although sedative medication within 24 hr of clinical examination was an exclusion criterion, chloral hydrate was given when necessary to ensure patient compliance. A second study (Demirturk 2001) did not find significant differences in VEP latencies in the patient groups but no specific control group was studied; the EP investigations were used here to study the effect of eradication of *Helicobacter pylori* and the subsequent changes in NH_3 levels. This study did not state the amplifier passband but did have a suitable sweep number (200).

The other studies all showed some differences between the patient and control groups, varying from all components, to either P100 only or N145 only. In two studies the N1 and N2 potentials also showed significant differences between controls and HE_0 groups.

In the patients who underwent pVEP's only two studies showed clear significant differences between test and control subjects; there were differences between HE_0 and HE_1 and differences obtained from N75 & P100 potentials respectively. However, of those that did not, one demonstrated evidence of a correlation of VEP to clinical evolution (Bombadari *et al* 1985). This study also reported a lower sweep number (70) than recommended and an unstated filter passband. (Guedon *et al* 1993) also reported no statistically significant differences between patient groups but control latencies were shorter. In this study the control group consisted of a mix of healthy and extra-hepatic

diagnoses. A pre- and post TIPS study (Kuba *et al* 1996) that reported no significant differences between controls and patients, had a study group of 'hepatic cirrhosis' with no specific aetiologies stated. Conversely, (Sawhney *et al* 1995) reported the VEP P100 as being not a significant marker for chronic liver disease; the aetiologies in this case were varied and included a large proportion of patients with alcoholic cirrhosis. A study of exclusively non-alcoholic cirrhotics, with HE₀₋₁, (Tarter R *et al* 1987) found that there were no correlations between P100 latencies and the extent of hepatic injury as defined by biochemical parameters – including indocyanine green clearance. However, as this was a correlation study the latencies of N75, P100 & N145 were not tested against normal (healthy) controls.

In summary, investigations with fVEP yield significant differences in 70% of the test groups. Unfortunately the flash stimulus is reported to be less sensitive to conduction defects and has a greater normal subject variation (Halliday 1973; Shahroki *et al* 1978). The role of the flash VEP may be questioned therefore in the light of pattern reversal stimulation. However, beyond the obvious issue of patient compliance, fVEP has the further advantage of stimulating wider area of the retina, and so recruiting a larger rod population into the retinal generators. Since rod and cone signals are largely processed in separate areas of the cerebral cortex, it would be advantageous to include flash stimulus in the case of an encephalopathic process that may have diffuse effects. The percentage positive yield may be further increased by a more robust EP methodology and careful selection of the patient and control groups.

It is disappointing that such a large proportion of the pVEP investigations describe no statistically significant differences. Some of this may be attributed to inappropriate instrumentation and post-processing. In addition, the selection of the patient or control types in some studies may have led to a predisposition for the pVEP P100 to become ineffective as a marker. Pattern shift stimuli are most suitable for half-field recordings - a method not reported in any of the studies. Half-field stimulation produces waveforms with better component identification and are more sensitive to early neurological effects (Halliday 1985), and can detect delays which may be masked by full-field waveforms.

The VEP investigation in patients with hepatic encephalopathy may be of use as a diagnostic marker – in contrast to the impression from some of the previous studies. Flash VEP's can possibly be made more sensitive by employing robust methodology. This could be used in addition to pattern VEP, not an alternative, again with strict methodology and the incorporation of half-field stimulation. This combined VEP can be further optimised by strict inclusion / exclusion criterion, in particular, any history of alcoholism.

BAEP

12 BAEP patient studies were carried out from 1985 – 1995. Three of these studies were dedicated auditory evoked investigations; the remainder consists of mixed EP's (Visual and / or Somatosensory). The combined patient total is 400 adults. The mean age of the patients was 41.9 ± 6.8 yr. The age range was stated in only three studies, and combines to give, for the BAEP group a range of 27 – 71yr.

The aetiologies vary markedly across the BAEP patient group, and can be divided into four sets.

Cirrhotic – unspecified, cryptogenic, idiopathic : 4 studies

Cirrhosis - Alcoholic: 1 study

Chronic active hepatitis: 6 studies

Non Cirrhotic Liver disease: – 2 studies

hepatocellular carcinoma

metabolic coma secondary to hepatic insufficiency

The selection criterion for patients was stated in all but one of the studies, (Trzepacz *et al* 1989a). Two papers selected on an 'inclusion' basis, while the remainder used an 'exclusion' criterion.

INCLUSION	EXCLUSION
<ul style="list-style-type: none">• No clinical signs of encephalopathy (HE₀)• No previous history of alcoholism• No medication within previous 24hrs• > 6weeks elapsed since previous variceal bleed• Normal tonal audiometry	<ul style="list-style-type: none">• previous h/o alcoholism• previous h/o hepatitis• psychiatric illness• neurological trauma• current medication affecting CNS• infection• diabetes, renal failure, uremia• sedation within previous 24hrs• malnutrition

The assessment of hepatic encephalopathy was varied, but Parsons-Smith (1957) was the system most adopted for classification.

Parsons-Smith (1957)	6
Zeive (1987)	1
Conn	3
MMSE & Reitan	1
Not stated	1

The diagnosis of the underlying pathology was less varied; it was not, however, stated in three of the investigations. Serum biochemistry was used in six studies (it was the exclusive diagnostic method in one study – (Mehndiratta M.M. *et al* 1990). Specific biochemical parameters were described in two patient groups; serum albumin, NH₃ and PT were common to both of these studies. The other parameters included globulin, indocyanine green clearing, alkaline phosphatase and transaminases. The other diagnostic methods were ultrasonography and histopathology.

In this EP cohort, three studies found no electrophysiological changes in the patient group (Sandford *et al* 1987; Tarter *et al* 1989; Trzepacz *et al* 1989a). One study was not strictly an investigation into encephalopathy - the study used a population of patients with cirrhotics of mixed aetiology and divided them into delirium / non-delirium groups, the later serving as the control group. The two Sandford / Tarter studies both had patient populations of non-alcoholic cirrhotics; one however (1987) did not have a separate control group, while the other, although not showing significant difference *cf* controls, did demonstrate a difference within the patient sub-groups (biliary cirrhosis chronic active hepatitis).

In addition, none of these study groups specified the use of contralateral white noise masking, and all stated their stimulation intensity in dB(SPL), with no indication of patient hearing threshold levels. It is also unclear from these three studies whether the final average used for latency calculations was superimposed from more than one run, or if two or more trials were performed for reproducibility.

In comparison to the previous VEP survey, a higher proportion of the BAEP studies reported some significant differences (*cf* controls), i.e. >70% compared with ~45%. One study reported findings only in the peak latencies (one or more of waves I-V), while one study reported changes confined to interpeak latencies (IPL's I-V or III-V). The remaining studies all demonstrated significant electrophysiological changes *cf* controls in peak and IPLs. It may be possible to increase the positive yield of this investigation by addressing some of the instrumentation issues.

Stimulation parameters are neither consistent across the positive studies nor do they correspond to current neurophysiological protocols. For example square wave type for stimulation should be alternating; of the studies surveyed one used alternating, the remaining using rarefaction (4), compression (1) or not stated. The stimulus intensity, possibly the most crucial EP attribute, was delivered relative to the hearing threshold in only four of the patient groups, the remainder using an absolute intensity which varied from 85-130dB.

White noise contralateral masking was reported in only one study (Mehndiratta *et al* 1990) but the SPL intensity is not specified. The remaining

studies did not stimulate with masking. Other stimulating parameters, i.e. rate and duration, were less variable and all fell within neurophysiological protocols.

The recording parameters, where fully stated, varied little across the studies, with amplifier passband, recording sites, number of sweeps and sample window appearing with consistent values and in keeping with current neurophysiological protocols. Stated active electrodes were A1/A2 while the reference is Cz; pre-amplifier passband is 100-3kHz. It is notable that the two of three studies that reported no significant electrophysiological changes did not fully specify the recording parameters.

Post processing was not fully reported in six of the studies. Four studies did not define 'abnormal' within their patient groups; the remainder that did specify all concurred with a reported abnormal latency as control mean+3SD. Confirming reproducibility by repeating trials was reported by only three studies (Picton *et al* 1984; Pozzessere *et al* 1988; Trzepacz *et al* 1989a). Two runs were recorded per investigation, but it is unclear whether these were simply used for superimposition or added to make a 'grand average'. Finally, only three studies clearly stated that R & L ears were stimulated separately; these studies were then able to demonstrate which changes occurred bilaterally and which, if any occurred unilaterally. The remaining reports, therefore could be presenting unilateral data or bilateral data obtained from a summation of averaged trials.

In summary, BAEPs have been successfully employed as an adjunct to psychometric testing in the cirrhotic patient group. It is feasible that the

sensitivity of the brainstem investigation could be increased with minimal alterations to the previous study methodologies. Well researched stimulus and recording parameters and a robust post processing protocol may be sufficient to increase the positive diagnostic yield. In addition, the incorporation of a strict exclusion criterion by eliminating confounding factors that affect conduction velocities (e.g. diabetes & alcoholism), may further enhance the sensitivity of this investigation.

SSEP.

13 SSEP patient studies were carried out from 1983 – 1998. One of these studies was a review article with no novel experimental data reported. One report was a single case study on a recovering liver transplant patient. Of the remaining 11, four were dedicated somatosensory evoked investigations; the remainder consisted of mixed EPs (visual and / or auditory).

The combined patient total is 552 adults. The mean age of the patients was 42.4 ± 12 yr. The age range was stated in only three studies, and combines to give, for the SEP group a range of 17 – 74yrs.

The aetiologies vary markedly across the SEP patient group, and can be divided into four sets.

Cirrhotic – unspecified, cryptogenic, idiopathic

Cirrhosis – Alcoholic

Chronic active hepatitis

Non Cirrhotic Liver disease:

hepatocellular carcinoma

metabolic coma secondary to hepatic insufficiency

The selection criterion for patients was not stated in four of the studies; two papers selected their patients on an 'inclusion' basis, while the remainder used an 'exclusion' criterion.

INCLUSION	EXCLUSION
<ul style="list-style-type: none"> • No clinical signs of encephalopathy (HE₀) • No medication within previous 24hrs 	<ul style="list-style-type: none"> • previous h/o alcoholism • psychiatric illness • neurological trauma • current medication affecting CNS • infection • diabetes, renal failure, uremia • sedation within previous 24hrs • malnutrition • GI bleeding • Overt HE

The assessment of hepatic encephalopathy was varied:

Parsons-Smith (1957)	5
Conn	4
MMSE & Reitan	1
N/A	1
Not stated	1

The diagnosis of the underlying pathology was not stated in four of the investigations. Serum biochemistry was the exclusive diagnostic method in two studies, while biopsy only was reported in three studies. The remaining reports combined biopsy and serum biochemistry, with additional methods. Specific biochemical parameters, where described, included serum albumin, NH₃ and PT (which were common to all biochemical studies). The other diagnostic methods were ultrasonography and endoscopy.

In the SEP cohort two studies categorically stated that the somatosensory evoked potential adds no further information to a cirrhotic patient study (Mehndiratta *et al* 1990; Sandford *et al* 1987), while one report suggests that the SEP only alters in moderate-severe hepatic injury (Tarter *et al* 1987).

In both negative studies, the stimulus intensities were described as producing only 'discernable' & 'minimal' thenar muscle twitches respectively. Sandford *et al* (1987), in addition, did demonstrate a L / R side asymmetry such that there was a significant difference ($p < 0.01$) for a right limb N20 peak latency; they achieved this while using a sweep of 64 per average. Mehndiratta *et al* (1990) stimulated and recorded bilaterally but simply reported N19 and P23 latencies; there is no data to suggest if one limb only was reported or if bilateral latencies were combined. Neither of these studies reported on IPLs.

Tarter (1987) suggests that SEP sensitivity is low; however this study did not set out to test cirrhotic patients against controls, but attempted to demonstrate univariate correlations between a range of assessment methods, including IGC and fasting NH_3 . There was no healthy control patient group to compare the SEP findings with.

The SEP survey, in comparison to the VEP & BAEP studies, produces the highest positive outcomes with 77% demonstrating a patient *cf* control significant difference (VEP = 45%, BAEP = 70%). Where cervicomedullary potentials were recorded in addition to the cortical components, one study found the peripheral latencies to be normal in the presence of an abnormal cortical potential. The remaining studies, reported abnormalities in both the cortical and distal potentials.

Stimulating parameters, where stated, were variable, within the positive yield, and only one reported a maximal twitch, which is specified in current clinical guidelines. Stimulus rate and polarity (i.e. prevention of anodal blocking)

were not consistent across the SEP group, and may account for the overall yield. Where the findings were moderately significant, stricter attention to stimulating parameters may have increased the differences seen between controls and patients.

Recording parameters were frequently not fully stated; where they were, there was little variation between studies and were close to accepted protocols. One exception was the sweep number which was only acceptable in six of the nine cases that stated it. Electrode positioning however, was consistent and acceptable across this study group.

Post processing was only clearly reported in two studies (Trzepacz *et al* 1989a; Yang *et al* 1986). Reproducibility, or the number of runs per trial, is unstated for most of the reports. Similarly, where right and left limbs were stimulated and recorded, only two studies reported the findings separately; therefore the findings of the remaining reports may be a combined average or a 'preferred' limb. Only three studies failed to define the 'abnormal' latency values. The remainder, however were not consistent, with mean + 1, 2, 2.5 & 3SD all being employed; one paper, despite having a large control group, used absolute latencies for their normal values (N20 <22ms, P30 25-35ms).

This is an encouraging survey, more so than the visual and brainstem auditory investigations. The SEP studies have in common with the other modalities, the need to standardise the stimulus and recording parameters; in many cases these are not in keeping with current neurophysiological protocols. A strict recording regimen may possibly improve the positive yield of this already

effective investigation. Patient selection, of course, will have to be similarly robust in order to prevent pathologies that affect the ascending pathways from becoming a confounding factor.

Cognitive P300

The overview is based on 12 patient studies carried out from 1976 – 2007. Eleven of these studies were dedicated P300 evoked investigations; the other was an assessment of the CNV investigation in this patient group. The combined patient total is 631 adults. The age of the adult patients, where stated, was 45.9 ± 12.5 yrs. The age range was indicated only in 3 papers; the age range from these studies was 19 – 75 yrs.

The aetiologies varied across the P300 patient group, and can be divided into three sets.

Exclusively cirrhotic: 4 studies

Cirrhosis with other aetiologies: 7 studies

- Hepatitis (viral B, C, B&C)
- Primary Billiary Cirrhosis
- Gauchers Disease
- Drug overdose
- Cancer
- Cryptogenic
- Haemochromatosis

Non-cirrhotic liver disease: 1 studies

- Hepatocellular liver disease

The selection criterion for patients was not stated in one of the studies. The other papers selected on an 'exclusion' basis. There was no specific 'inclusion' criterion stated, but this could be inferred in some studies from the stated methodology.

INCLUSION	EXCLUSION
<ul style="list-style-type: none"> • Language native to country of research centre • No alcohol within 2 weeks / 6 months / 2 years • Normal sensory function 	<ul style="list-style-type: none"> • Alcoholic toxicity • Sedatives or centrally acting medication • Neurological conditions • Other major diseases • Overt HE (SHE / minimal HE studies)

The assessment of hepatic encephalopathy was varied, but Conn *et al* (1977) was the most adopted for classification.

Parsons-Smith (1957)	2
Holm (1980)	1
Conn	4
Not stated / unspecified 'clinical' features	5

The diagnosis of the underlying pathology also varied, although it was not stated, or unspecified, in three of the investigations. Histology was used as the only diagnostic method in two studies, and in combination with imaging with a further one. Serum biochemistry was used in four studies; two were exclusive to the diagnosis, while one study each combined biochemistry with imaging and MMSE / NCT. This latter study was a pre- and post- transplant assessment.

Specific biochemical parameters were not described in 2 patient groups; both of the other groups specified albumin, prothrombin time and bilirubin. The remaining study also stated venous ammonia & urea levels.

The P300 stimulus for 4 studies was visual, (three exclusively and one investigation both visual and auditory), while the remaining patient groups were stimulated by auditory paradigms only.

Visual P300

Three of the four studies recorded one channel only (O_z), while the other study (Giger-Mateeva *et al* 2000) recorded all 4 midline positions. The recording electrode positions of all investigations corresponded to the 10-20 International System. The number of sweeps (flashes) per run was also variable, with one study (Giger-Mateeva *et al* 2000) using an 'approximate' with no further elaboration. Similarly, the ratio of common-to-rare varied so that in combination with the sweep numbers the patients would receive rare patterns numbering as little as 35 (Giger-Mateeva *et al* 2000) or up to 50 (Kulger *et al* 1992; Reeves *et al* 2007). In addition the rare visual angle varied in presentation, with 12.5', 15' & 200' check sizes and one not stated. Two studies utilised a larger check size for the common stimulus, and two studies utilised smaller checks for the common stimulus. Stimulus intensity (luminance) and contrast was stated in two of the four studies and was appropriate for evoking visual responses.

All four of these studies stated the filter passband; three had either the low pass or high pass at an inappropriate setting, one having a particularly narrow bandwidth.

Auditory P300

Four studies did not describe any electrode recording positions. Of the remaining five studies, three recorded from one midline channel, while only one (Amodio *et al* 2005) recorded from the other midline electrode positions. All the auditory P300 investigations recorded from standard 10-20 electrode placement positions.

In the auditory P300 subgroup two studies did not state the sweep number while another stated a 20 minute limitation. The presentation of the rare tone varied in terms of the number of stimuli, with 32, 40, 150 & 160 stated. Six studies used 1000Hz & 2000Hz pairs of stimuli, one study 500Hz & 100Hz pairs and one study not stating. The rare tone was of higher frequency than the common tone for five studies and the opposite for the remaining two.

Stimulus intensity was stated in five of the eight auditory studies. Where given the value was appropriate for this sensory modality; however only one study indicated dB SPL, but no study stated how the intensity related to patient hearing threshold.

The amplifier passband was reported in all but one case; however, only one study (Gallai *et al* 1995) stated a bandwidth that would be acceptable for recording in a clinical neurophysiology department.

Post processing procedures were stated in all but one of the study groups. Of these, seven identified the waveform morphology and so defined the peak for latency measurement. Abnormality was defined statistically in seven studies, by amplitude in two studies and not stated in two studies. Statistical abnormality

defined with a control group latency used mean + 2SD in six studies and +2.5SD in one.

The reliability of the separate runs was not checked, in any study, by repetition and superimposition of sequential recordings. Therefore it is unclear whether the latency measurements are obtained from a 'grand average'.

Of the four studies that utilised visual stimulation only one found no significant differences between the patient and control groups for the P300 latency. The stimulus parameters were in keeping with clinical neurophysiology protocols for VEP recording, however the amplifier bandwidth for recording was excessively narrow and only 35 rare presentations were averaged. In addition the exclusion criteria were particularly concise, stating only neurological and hepatic diseases. The aetiologies and assessment of HE was not stated. The other visual studies all showed some differences between the patient and control groups; all studies indicate that cirrhotics can be distinguished from controls while one study discriminated between cirrhotic and non-cirrhotic liver disease, but could not separate unimpaired and minimal HE.

In the patients who underwent auditory P300's two studies stated that P300 findings are unspecific and provide little additional information in this patient group. One study (Hollabach *et al* 1997) described no significant differences between encephalopathic subgroups (HE₀, HE₁ & HE₂), although there was a clear difference in latencies between all patients and controls. There was however, no correlation between P300 latency and Child's score. A similar finding occurred in the other study (Amodio *et al* 2005) where the P300 is only

found to be altered where the EEG and psychometry are also abnormal. The former investigation stated appropriate recording parameters but did not state sweep number, instead indicating a variable rare tone presentation ratio. The latter study did not use the latency values for analysis but age-adjusted the measurements and obtained reference values from a database of control latencies. In addition to having no control data for the actual study, the stated highpass filter was relatively low, giving this patient group the narrowest recording passband of all the studies.

It appears that cognitive evoked potentials can highlight neurological alterations in patients with hepatic encephalopathy. Although there have been relatively few studies in this modality, the majority of investigations have indicated significant differences between patients and controls despite non-standardised stimulation, recording and post-processing protocols. The use of a more robust EP methodology may also highlight differences between the patient groups, particularly if strict inclusion / exclusion criteria and HE classification methods are adhered to.

To conclude, the P300 cognitive evoked potentials have been recorded in patients with hepatic encephalopathy; findings in previous studies are variable, but, when used as part of an array of investigations, they may be useful in monitoring neuropsychiatric status.

1.5 Signal Processing, Neurophysiology and Evoked Potentials.

Introduction: waveform analysis.

The reading and interpretation of electrophysiological data has traditionally been undertaken by direct visual observation of the recording. This involves a level of inherent subjectivity in judgement, which can, by definition, result in significant intra- and inter-observer variability in interpretation. There is, therefore a need to provide an adjunct to direct interpretation a mathematically derived instrument which would remove the subjectivity of interpretation and which might provide further interpretive detail and meaning to the signal.

In physiological recordings a waveform can be defined as a single valued function of time or, $f(t)$. Much of medical literature omits labelling the 't' axis because the shape of the waveform is considered to be more important than any further attempt at a quantitative analysis. A waveform may be periodic, e.g. $f(t) = f(t + T)$, for a respiratory signal where T represents the time period between contributions, or $f(t) = \sin 2t$, for a 2 cycle/second sine wave.

In neurophysiology and other disciplines involving biological signals waveforms are non-periodic. These more arbitrary waveforms such as an ongoing 'background' EEG need to be broken down into a sequence of ordered segments, each contributing to f at a delayed time t , such that $f(t)$ at $t = nT$ (the period between each contribution). A set of functions will then be related through the process of integration.

Continuous repeating waveforms can be represented by a summation of simple sinusoidal waves: the *Fourier Series*. This effectively breaks waveforms such as the EEG down into component sine and cosine waves. Raw EEG data is non-periodic and so the Fourier Transform converts a continuous signal into another function from waveform $x(t)$ to $X(\omega)$ or $X(f)$:

$$X_{(\omega)} = \int_{-\infty}^{\infty} x(t)e^{-j\omega t} . dt$$

..... (eqn 1) where ω is in rad)*

Or,

$$X_{(f)} = \int_{-\infty}^{\infty} x(t)e^{-j2\pi ft} . dt$$

..... (eqn 2) where f is in Hz)*

*(after Kaiser 1994)

This is simpler to process and interpret and as it is independent of the signal source it may be applied as a general analytical tool. If a waveform contains periodic and non-periodic components the Fourier Transform will reveal the quantitative contribution of each to the overall signal – in terms of its frequency spectrum – and can then be examined in the frequency-domain. The Fourier transform may be implemented by digital computation, the Fast Fourier Transform (FFT), which is an efficient version of the discrete or DFT.

The spectral density of a wave can be used to determine the power of a signal as a function of the frequency distribution, and is often associated with descriptions of finite stochastic waves. Power spectral density (PSD) is an

indication of the energy in the waveform per unit frequency (dB/Hz). The power is usually the square of the FFT magnitude while the frequencies are either normalised or scaled with various constants (e.g. $1 / 2\pi$). The standard non-parametric PSD method is a periodogram, which provides an *estimate* of the energy present. It is an estimate because window functions are employed to correct spectral bias – the consequence of a truncated epoch in a finite time-series; smoothing is also required because the variance of a given frequency in an unprocessed FFT spectrum does not decrease as the number of computed samples increases.

While the spectral density function PSD is derived from the FFT an intermediate stage, the so called autocorrelation function, which employs the Fourier cosine transform only, must be calculated. As a result the magnitude and frequency information is retained but the phase information is lost. The method of autocorrelation and the limitations of FFT will be revisited in the final discussion.

A survey of spectral analysis of EPs is summarised in table 7.5 (Appendix III).

A brief survey of signal analysis: animal studies

Signal analysis for neurophysiological investigations has been utilized for the past three decades. Ongoing and elicited neuronal activity has been studied for all sensory modalities while some motor system investigations have also been undertaken.

Some studies have investigated aspects of instrumentation and were performed on healthy animals. For example, characteristics of stimulus parameters have been investigated for auditory (Kawasaki 1993; Finneran & Houser 2007), somatosensory (Beysen 2004), and visual EPs (Asher 2007). The latter study which was undertaken in macaque monkeys also demonstrated that noise bands can be easily identified. The spectral analysis methods were all based directly on a FFT or a FFT derived spectral estimate for example PSD. The other approaches to analysis include investigations into the correction of spectral leakage (Felix *et al* 2005; Felix 2009), which involves further calculations to derive coherence coefficients. Methods of invasive electrocortical recording have also been studied (Franowicz & Barth 1995).

Signal analysis has been often employed to assess the effects on the nervous system of anaesthetics, analgesics and sedatives (Freye & Hartung 1983; Kaplan 1977; Russell *et al* 1995), have been reported using FFT based methods, including compressed spectral array (CSA). Although these studies all reported power band changes in the background raw EEG, changes in the somatosensory evoked potentials were only reported in the time-domain, i.e. amplitude and latency measurements. Notable exceptions were early studies of the dose-effects

of the inhaled anaesthetic halothane, (Guarino 2004; Imas *et al* 2004) where PSD was used to analyse the VEPs. Investigations of the relationship between CNS anatomy and physiology using signal analysis have also utilised EPs. The effect of cell specific neurotoxins has helped to highlight functional areas of the hippocampus (Heale *et al* 1995); here the activity of discrete cell populations in sensory processing is monitored by frequency-domain analysis. More recently a relationship between the sensory and higher cortical areas been demonstrated (Liang 2002). In visual processing in the macaque monkey PSD and coherence analysis has shown that a synchrony develops in the anticipation of a sensory input. The electrophysiological activity isolated in this case was of an oscillatory nature in the beta-range.

Central nervous system trauma, particularly hypoxic ischaemia, has been studied using spectral analysis of both SEPs and MEPs (Simpson *et al* 1993a; Simpson *et al* 1993b; Brauna *et al* 1996).

Aspects of induced CNS dysfunction have been highlighted in the frequency domain, again with multi-modal sensory EPs. PSD analysis of VEPs has been undertaken in pharmacologically induced epileptic seizures (Fernandez-Guardiola 1988). The visual system is also the sensory mode of choice for investigations into experimental diabetes (Yargicoglu 1998). A particularly relevant metabolic model – induced hepatic encephalopathy - has been studied using both visual and auditory EPs (De Groot *et al* 1985). Unfortunately, although PSD was carried out on the raw EEG data the VEP and BAEP were studied for latency and morphology changes only.

A brief survey of signal analysis: human studies

Signal processing has also been used, albeit sporadically in human studies. Much of the work undertaken in healthy subjects has centred on the investigation of various aspects of instrumentation in physiological recordings.

The effects of stimulus parameters for SEPs for example, rate and intensity (Rush JL 1976) was one of the earliest major studies to employ the FFT to highlight redistribution and enhancement of harmonic energy. The stimulus for motor evoked potentials (MEPs) also requires careful parameter selection (Kiers 1993), and the effects of intensity, coil size state of subject alertness and muscle tone can be visualised by the FFT method. In the visual system the effects of varying stimulus frequency, contrast, luminance and of monochromatic sensitivity have all been investigated and demonstrated with FFT derived PSD; the positions of the peaks in the α - and δ -power EEG bands are stimulus frequency dependant (Srinivasan 2006). Similarly, the dominant spectral bands are altered for different contrasts and also for mono- versus binocular stimulation (Spileers 1992), whilst the dominant FFT harmonic also varies as a function of wavelength (colour) and intensity of the light stimulus (Frederiksen 1993).

Filter application also lends itself to study by PSD methods, particularly in evaluation or comparison of digital and analogue methods. Digital filters can be selected to impose zero shift, confirmed by FFT, or compared with analogue filters for specific EP bandwidths (Kavanagh *et al* 1988; Skuse & Burke 1990; Shimoyama 2000). The PSD is also well suited for final evaluation of autocorrelation/coherence analytical methods that attempt to improve signal to

noise ratios (Dobie & Wilson 1989; Winterer 1999). This has been studied extensively as EP signals have such a low amplitude with respect to the background EEG.

Post-stimulus oscillations are of interest in somatosensory cortical function since middle latency (fast) and longer latency (slow) activity can confirm involvement of other structures possibly involved in cognitive processes. FFT based power analysis of cortical activity has revealed dominant power bands phase locking with stimulus (Cheron 2007). However, the analysis used the EP measurements in the time-domain only.

Functional neurodevelopment has been the subject of study for many years. Evaluation of sensory maturation in humans can be investigated with FFT analysis for raw EEG and EP data. Median and maximum power bands can show maturational alterations in the visual system (Manas *et al* 1997), with hemispheric asymmetries. Unfortunately, the VEP recordings were assessed for latency only. In the auditory system dominant spectral bands gradually evolve from a distinct neonatal appearance to an adult profile by migrating to higher frequencies (Lippe & Martinez-Montes 2009).

Other evoked potentials that are not used in routine neurophysiology have also been investigated with signal processing methods. Emotional responses albeit by visual stimulation and occipital recording) produce FFT spectral bands that relate to the amplitude of skin conductance, i.e. emotional effect (Keil 2008). Olfaction potentials have also been investigated in a comparison of FFT and

Fractal Dimension (FD) analysis (Murali & Vladimir 2007); it is of interest here that FFT was insensitive to stimulation but the FD is altered for a range of stimuli.

Signal processing methods have been employed to evaluate the effects of psychoactive drugs, such as chlorpromazine and olanzapine-and antivertiginous neuroactive medications such as cinnarizine, dimenhydrinate and betahistine on neurophysiological variables in healthy individuals. Significant drug dose / plasma level dependant changes were observed in the EEG power spectra with both psychoactive and neuroactive drugs, (Hubl 2001; Laurian & Baumann 1981; Schneider 2003). Changes were also observed in various sensory and cognitive EPs but the measurements were limited to assessments of wave latency and amplitude.

Somatosensory EPs are frequently used for surgical and anaesthetic monitoring and coma assessment. Spectral SEP changes have been noted in studies of analgesia and depth of anaesthesia for SEPs (Bromm 1987), and AEPs (Capitano 1997), although later studies only used the α -power on EEG for assessment although AEP amplitudes were monitored (Bischoff 1998; Plourde 1997). Similarly evaluations of coma monitoring methods (with FFT derived CSAs) study auditory and somatosensory EPs for latency only, in both diagnosis of brain death (Shiogai 1989; Shiogai 1993), and for predictive outcome (Liesiene 2006; Tsubokawa 1990).

Signal analysis methods have been applied to study neurophysiological recording in patients with diverse disease states. Patients with multiple sclerosis (MS) are frequently tested with multimodal sensory EPs; early FFT studies of

VEPs indicated that spectral peaks are altered in the pathological state (Bobak 1983; Trick *et al* 1984), although latency measurements may increase the diagnostic yield. Similarly, early studies of the main peaks in BAEP recordings revealed reduced power across the three main bands (Kamath *et al* 1987).

Diagnosis of epilepsy and evaluation of patients suffering from migraine attacks are frequent sources of referral for neurophysiology and neurology investigations; although imaging and the EEG are the main investigations performed, the role of EPs has been evaluated. Focal seizures (Meador *et al* 1988a; Meador *et al* 1988b; Nuwer 1988) show altered PSD profiles on the EEG, normalising post-surgery, while encephalopathic myoclonus has a spectral profile similar to cortical myoclonus (Canafoglia 2003). In the latter case, however, (Nuwer 1988) the SEP has been used only for latency measurements. FFT data from VEPs recorded during migraine attacks in adults (de Tommaso 1998) highlight dynamic changes in the main frequency components during the acute phase, while in juveniles (Marrelli 2001), there are significant differences between patients and controls.

Studies within the field of psychiatry have also encompassed evoked potentials and signal processing methods. Visual and auditory EPs have been analysed with PSD in patients with schizophrenia and show reduced power and frequency shifts (Jutai *et al* 1984; Krishnan 2009); a study of a subpopulation of psychiatric patients with substance abuse (Braverman & Blum 1996), has revealed significant power changes in the EEG spectra, but the EPs, including the cognitive P300, were only assessed for latencies. Cognitive decline has also

been documented in Alzheimer's and older people with non-specific decline. A VEP study compared FFT windowing techniques (Moody *et al* 1989) in this patient group, suggesting misclassifications were possible. Further studies again quantified the EEG and not the EP (i.e. P300 latency measurements only) (Zappoli 1991a; Zappoli 1991b); this also occurred for evaluations of treatment of this patient group (Gallois 2002; Zimmermann 1982).

Studies on a diverse range of metabolic pathologies have embraced neurophysiological waveforms as possible indicators of systemic function and in turn have performed PSD analysis on the recorded data. The effects on the central nervous system of hypoglycaemia, (VEP), prolonged kidney dialysis (VEP & SEP), treatments for phenylketonuria (VEP), lead toxicity (all sensory & P300) and effects from the Chernobyl incident (SEP), have all been investigated (Araki & Sato 2000; Harrad *et al* 1985; Lewis *et al* 1978; Loganovsky 2000; Pietz 1995). However, all of these studies performed quantitative analysis on the raw EEG data, but utilised the EP only for latency.

There is a frequent occurrence of neurophysiological studies where the EEG is spectrally analysed - with positive results - but concomitant EP recordings are reported in the time-domain. In addition to the above, paediatric investigations of autism and other learning difficulties (Ogawa 1989; Pinkerton 1989) fall into this category. Studies in on the effects of lesions caused by stroke have also used EPs in this way with sensory (Iwayama 1979; Kusske *et al* 1980) and motor investigations (Gerloff 2006) used for latency confirmation, although the latter

study did employ EEG coherence analysis to investigate MEP stimulus following responses.

The metabolic pathology of greatest interest in this study hepatic encephalopathy, and attempts to utilise neurophysiological data for detection and grading also has a long history. The earlier studies noted EEG α - and θ - power reductions in PSD in chronic liver disease in the presence of neuropsychiatric change (Trzepacz *et al* 1989b), while treatment of the condition resulted in clinical improvement and (Higuchi 1994) an increase in α -power increased and decrease in δ -power. In these cases SEP, BAEP (former) and P300 (latter) were all recorded but used for latency confirmations. Visual EPs have also been adopted, as an adjunct to the EEG, for use in diagnosis of hepatic and encephalopathy and while spectral analysis of the EEG reveals a shift towards the lower frequencies (Kuba *et al* 1996), the EPs demonstrated sensitivity to the clinical state, but again in the time-domain. Spectral EEG analysis has also been accompanied by cognitive P300 investigation (Amodio *et al* 2005). The EP did not appear to correlate well with cognitive alteration ($R = -0.03$; $p=0.76$); spectral EEG was the most sensitive but only latencies were measured for the P300.

There has been an interest in using signal processing methods to quantify neurophysiological waveforms since the mid 1970's. Both animal and human experimental models have demonstrated that spectral peaks are observable and can be altered under a range of conditions. Instrumentation variables have been addressed, with some useful studies on EP stimulus parameter effects.

Investigations into animal and clinical physiology have been most varied, with a wide range of non-neurological pathologies studied with EEG and EP quantification. Signal processing of EPs within both neurology and hepatology appears to have been directed into EEG investigation, with the EPs used in the morphological context and a reduced emphasis on its signal content.

1.6 The Research Question and Aims of this Study

There is currently no 'gold standard' for the diagnosis or monitoring of hepatic encephalopathy; the research question in this thesis is - can the evoked potential investigation, in combination with frequency analysis, provide a method for identifying this syndrome.

The aims of this study are:

1.

Biopsy-proven cirrhotic patients, with independently classified neuropsychiatric status, will be subjected to VEP, BAEP, SEP and P300 investigations; latency data will be analysed to determine if there are significant alterations to latency occur between patients and healthy control subjects; comparisons will also be made between unimpaired, minimal and overtly encephalopathic patient groups.

2.

Voltage data from EPs will be examined by the spectral methods FT and PSD to determine if there are significant power and/or frequency differences between patient and control subjects and between patient groups.

3.

Where significant differences exist, to determine which analysis method (time-domain, frequency-domain or time-frequency combination), has the greater ability to identify neuropsychiatric status.

Summary.

EPs are quantitative, reproducible and sensitive extension of the neurological examination; they are an objective and primarily numerical measure of cortical function. Each EP modality requires careful attention to stimulation and recording parameters which must be standardised across the patient groups. Patient clinical history is required as is maintenance of compliance during the investigation.

Hepatic encephalopathy is a neuropsychiatric complication of liver cirrhosis. The underlying pathophysiology is not fully understood and it may involve several processes. There is no diagnostic gold standard for hepatic encephalopathy and therefore may be under-diagnosed leading to quality of life issues.

Previous EP recordings in patients with hepatic encephalopathy reveal low to modest positive yields; (VEP 45%, BAEP 71%, SSEP 77% and P300 66%). Some studies have inappropriate acquisition methodologies or unsuitable post processing methods, while others have inconsistent selection criteria with heterogeneous test groups. Neuropsychiatric characterisation was also inconsistent and often incomplete.

An extensive survey of the literature reveals that spectral analysis methods have been performed on signals of neurophysiological origin; the previous studies range from animal sources to various human pathologies. EEG and EP frequency and power analysis has also been studied in normal subjects to investigate instrumentation issues. However, there are comparatively few studies specifically in hepatic encephalopathy; in these cases the EEGs were spectrally analysed while the EPs used for latency and morphological comparisons only. Frequency analysis of sensory and cognitive EP recordings therefore, has not been undertaken.

The inconclusive EP history in this patient group combined with the absence of spectral processing form the basis of the research question: can spectrally analysed EPs identify neuropsychiatric changes in patients with hepatic encephalopathy.

The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy.

Chapter 2: Methods

Forward

The previous chapter highlighted some of the issues that have hindered the diagnosis of hepatic encephalopathy. These include patient selection criteria and classification of encephalopathy which have been variable across the previous studies. The EP methodology has been similarly inconsistent; many studies have utilized unsuitable instrumentation parameters while few have investigated multiple modalities.

The patient population groups in this study were subject to standardised inclusion and exclusion criteria. The assessments of mental state and psychometric performance were made under standardized conditions by consistent observers. Similarly the EP recordings were performed by a single observer using the clinically appropriate protocols and a consistent environment throughout. All patients and controls were systematically investigated, across all modalities, within an hour of neuropsychiatric assessment.

The previous chapter also surveyed the application of frequency analysis in signals of neurophysiological origin. The majority of the previous studies investigated the EEG with much less emphasis on EP signals. A range of signal processing methods have been employed, although there has been very little application specifically in encephalopathic subjects for either animal models or clinical studies. The Fourier Transform and the power spectral density estimate are the two most reported analysis methods; these were both applied systematically to the multimodal EP signals obtained from the patients and controls.

2.1 Ethics.

The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) and Good Clinical Practice (European guidelines). The protocol was approved by the Royal Free Hampstead NHS Trust Ethics Committee, Ref 6112 (appendix IV). All participating subjects provided written, informed consent.

2.2 Control population.

A control population of 48 healthy volunteers (25 men, 28 women; mean age 39.8 [range 22-68] years) were recruited from colleagues working at the Royal Free Hospital, London, all with no previous neurological history. None had a history of liver disease, drank alcohol in excess of 20g daily, took prescription or over-the-counter medications or was visually impaired.

2.3 Patient population.

The test population consisted of 70 patients (47 men, 78 women; mean age [range] 55.1 [37-80] years) with biopsy-proven cirrhosis who were recruited between 2004 and 2008. The aetiology of the cirrhosis was determined using clinical, laboratory, radiological and histological variables. The severity was assessed using the Pugh modification of the Child's grading system (Pugh et al 1973). All patients were clinically stable at the time of the study. Patients were excluded if they were over 80 years of age; if they had suffered an episode of major hepatic decompensation within seven days of the assessment date; had significant cardiac, respiratory or renal failure; insulin dependent diabetes

mellitus; cerebrovascular disease; epilepsy; a history of significant head injury or other conditions likely to affect cerebral function. Patients were also excluded if they had misused alcohol or drugs in the previous three months; if their manual dexterity was impaired; if they could not speak English or obey spoken commands; or were taking psychoactive medications.

2.4 Study Procedure

Each recruited subject was assessed in a single session lasting approximately two hours. All EP assessments were completed in a dedicated recording room by a trained neurophysiologist. Electrode position notation corresponds to the 10-20 International System measuring system (Neidermeyer & da Silva 1982). The EEGs and psychometric tests were performed in a dedicated clinical neurophysiology department. The procedures were carried out in the same order using a standard set of instructions from scripted texts.

2.5 Neuropsychiatric Assessment

Patients were clinically assessed by two hepatologists, working independently, and their mental state classified, using the West Haven Criteria (Conn *et al* 1977) as either clinically unimpaired or as showing features of overt hepatic encephalopathy. Psychometric performance was assessed using the PHES battery (Weissenborn *et al* 2001) which comprises five paper and pencil tests: digit symbol (DS), number connection A (NCT-A) and B (NCT-B), serial dotting (SD), and line tracing (LTT), which has both time and error components (LTTt

and LTTe). The PHES data in the original patient population were adjusted and scored using UK normative reference data (Marks *et al* 2008). Composite scores of less than two standard deviations below mean reference values were considered abnormal (Saxena *et al* 2001). Neuropsychiatric status on the day of the study was classified as: (i) unimpaired – no clinical evidence of hepatic encephalopathy and no psychometric abnormality; (ii) minimal hepatic encephalopathy - no clinical abnormalities but impaired psychometric performance; (iii) overt hepatic encephalopathy - clinically evident neuropsychiatric disturbances.

2.6 Evoked Potentials

Evoked potentials for visual, brainstem auditory, somatosensory (median nerve) and auditory cognitive modalities were recorded in one continuous session using an Oxford Medelec Synergy™ 8-channel dedicated recording device. Stimulation, recording and instrumentation settings were applied using BSCN approved protocols. Waveform peaks were identified as follows: VEPs - N75, P100 and N145; SEPs - N9, N13 and N20; BAEPs waves I – V; Cognitive Auditory - P300 (see Figs 1.1 & 1.2). All runs were repeated and inspected for superimposition prior to saving and analyzing. Measurement cursors for latency were placed on waveform peaks using Synergy Reader™ version 10 software.

2.7. Protocols for evoked potential recording.

2.7.1 VEP

Recording sites

- Active electrodes are placed at O₁, O_z, and O₂
- Reference electrode at Fz
- Ground is placed at Cz
- Bipolar/common reference montage

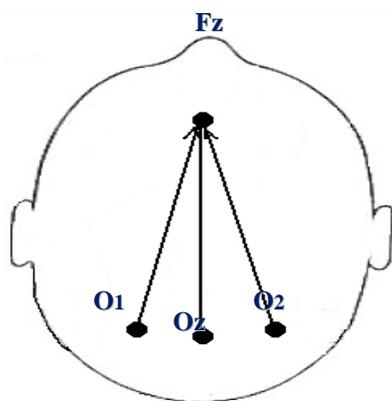


Fig 2.7.1: Recording electrode placement for VEP

Recording parameters

- Minimum of 1-100Hz bandwidth
- No notch filter
- 250msec timebase
- 120 reversals per trial
- At least two reproducible traces

Pattern Stimulation

- Check size: two sizes - 1° and 15' checks
- Contrast: >75%
- Reversal rate: 1.9/sec
- Luminance: white >280cd/m²
- Visual Field: >15deg

Other Techniques.

- Position patient with line of sight slightly above fixation point
- Ensure head is supported and try to relax patient.
- Ensure proper refraction and that the pupil is not occluded (e.g.. ptosis)
- Watch for fixation, and monitor on-going EEG - encourage patient to remain alert
- Monitor blink rate



Fig 2.7.2 (above): a typical pVEP setup.

2.7.2 BAEP

Recording sites

- Bipolar montage: Channel 1 Cz-A1; Channel 2 Cz-A2
- Active electrodes: A1/A2
- Reference electrode: Cz
- Ground: Fp_z

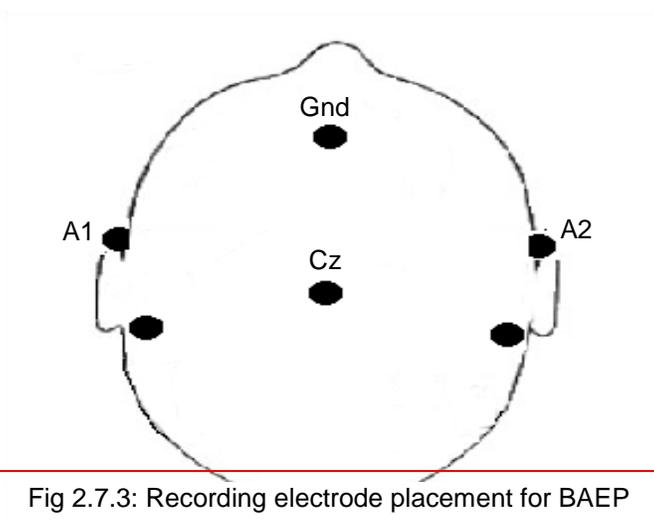


Fig 2.7.3: Recording electrode placement for BAEP

Recording parameters

- Time-base:
 - Threshold: 15 - 20ms
 - Neurological: 12 - 15ms
- Filters:
 - Threshold - 20Hz - 1.5 KHz
 - Neurological - 100Hz - 5 KHz
- Sweep: 1500 - 3000
- Reps: 2 - 3
- Masking noise: 40dB below stimulus intensity

Stimulus parameters

- Stimulus Type: broad band click
- Stimulus Rate: 8-11/sec
- Stimulus Intensity: 80-100dB
- Stimulus Click Polarity: 'Alternating' (condensation/rarefaction)

Masking noise

- The stimulus is conducted by the skull and may reach the opposite ear and although being attenuated by 50-60dB, can still excite it.
- Masking noise is constant white noise and is used to prevent this cross-stimulation during testing.
- Intensity of 40dB below the stimulus intensity.

2.7.3 SEP

Upper limb recording – median nerve

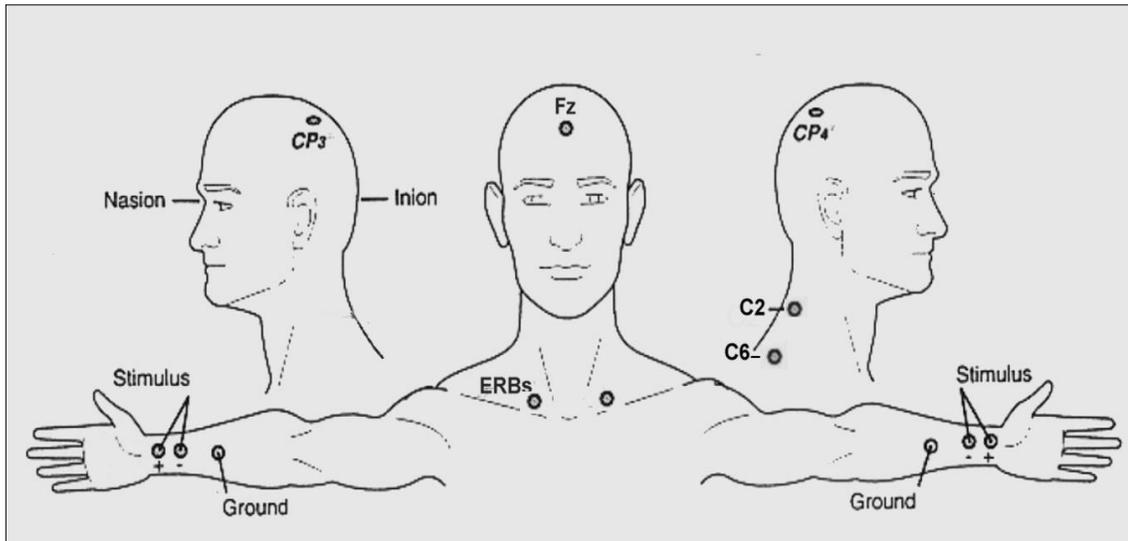


Fig 2.7.4: Upper limb recording placement.

Recording parameters

- Time base: (upper limb): 50ms
- Filter bandpass: 1-5Hz - 2.5-3KHz
- Epochs: 200 - 250

Stimulus parameters

- Electrical pulse-constant current
- Duration: 0.05 - 0.5ms
- Rate: 2 – 5Hz
- Intensity: approx. 3x sensory threshold

2.7.4 Cognitive Auditory P300

Recording sites

- Bipolar montage:
 - Channel 1: F_z -A1/A2
 - Channel 2: C_z - A1/A2
 - Channel 3: P_z - A1/A2
 - Channel 4: O_z - A1/A2
- Active electrodes: F_z , C_z , P_z & O_z
- Reference electrode: A1/A2 - combined
- Ground: Fp_z

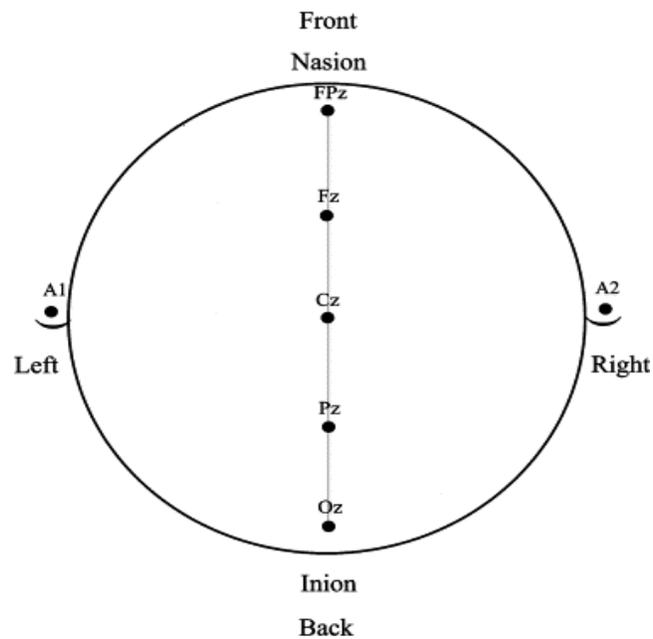


Fig 2.7.5: Recording electrode placement for P300

Recording parameters

- Time-base:500ms
- Filters: 0.10Hz - 100Hz
- Sweep: 100
- Reps: 2 (odd / even averaged separately)

Stimulus parameters

- Stimulus Type: pure tone 'pip', pseudorandom
 - Common probability: 0.8
 - Rare probability: 0.2
- Stimulus frequency :
 - Common 1000Hz
 - Rare 1500Hz
- Stimulus Rate: 1/sec
- Stimulus Intensity: 50dB above hearing threshold

2.8 Frequency analysis

Each patient recording comprised of two superimposed EP runs, per modality, for each hemisphere; left and right side waveforms were then added to make a grand EP average for the patient. The signal voltage data from each EP modality was exported and processed in Matlab™ (version 7.2). Each waveform was subjected to Fourier Transform (FT) and power spectral density estimates (PSD) computation. The frequencies of the main and secondary harmonics (peaks) were identified from the FTs; the maximum power, mean power and frequency at maximum power were recorded from the PSDs.

2.9 Statistical analysis.

Statistical analysis was performed with Minitab™ (version 16). Distributions of EP data sets were tested by the Anderson-Darling test. Differences between non-normally distributed group variables were examined using the Kruskal-Wallis test; subsequent comparisons within the group made with the Mann-Whitney test.

The sensitivity and specificity of each EP investigation was assessed using ROC curve analysis; this was performed for the main latency peaks identified for each EP modality. Similarly for the frequency domain, ROC analysis determined sensitivity and specificity for each parameter: PSD (peak power, mean power and frequency at peak power) and FT (main and secondary harmonics).

The ROC data was used to determine a threshold for each parameter to distinguish between neuropsychiatrically impaired and unimpaired patients. For

practical purposes, the control and unimpaired patients were combined into one group and the minimal and overt HE were combined into a second.

Multivariate analysis was performed by binary logistic regression, using the thresholds obtained from the ROC analysis, to determine the diagnostic validity of the latency and frequency tests in various combinations.

Summary

A population of patients and controls were recruited, selected and assessed for their degree of neuropsychiatric impairment; EPs were then performed in multi-sensory and cognitive modes. Voltage data was subjected to frequency and power analysis by FT and PSD methods, the observer / operator being blind to the neuropsychiatric state of the patient. The latencies of the main components, the frequencies of the main peaks and the power at these peaks were statistically analysed. Comparisons between controls and patients and between patient groups were made, as were sensitivity / specificity of time and frequency domain investigations, both separately and in combinations.

The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy.

Chapter 3: Results

Forward.

Controls and patients with hepatic encephalopathy were recruited, adhering strictly to standardised inclusion and exclusion criteria. Multimodal evoked potentials were obtained for the four test populations: (controls, unimpaired patients and patients with minimal and overt hepatic encephalopathy).

EP latency data for each modality were examined by non-parametric comparative statistical analysis to determine whether any differences occurred between patients and controls or between patient sub-groups. The frequency domain data (FT and PSD) from each EP modality was similarly examined by comparative statistical methods.

Both time-domain and frequency-domain data were applied in ROC calculations to determine the sensitivity and specificity for latency, frequency and power thresholds in each EP modality. Using thresholds with appropriate sensitivity and optimal AUC's (area under curve) latency, frequency and power values were selected for multivariate binary logic regression analysis, in order to distinguish between the neuropsychiatrically impaired and non-impaired.

3.1 Subjects.

The liver disease was alcohol-related in 50 (71%), cryptogenic in 3 (4%); alcohol/hepatitis B or C infection in 5 (7%); primary biliary cirrhosis in 3 (4%) and miscellaneous in 9 (13%). Functionally, 36 (51.5%) of the patients were classified as Child's Grade A, 20 (28.5%) as Child's Grade B, and 14 (20%) as Child's Grade C (Table 3.0.1).

Evoked Potentials were recorded from 48 healthy control subjects (25 men, 23 women; mean [range] age 39.8 [22 – 68] years), and from 70 patients with biopsy-proven cirrhosis (47 men, 23 women; mean [range] age 55.1 [37 – 80] years). On the day of study, 27 (38.6%) of the 70 patients were classified as neuropsychiatrically unimpaired, 13 (18.6%) as having minimal and 30 (42.6%) as having overt hepatic encephalopathy. PHES and EEG variables were significantly different between controls and patient subgroups (Table 3.0).

Table 3.0: Demographic and assessment variables in the study population, by degree of neuropsychiatric impairment.

	Controls (n=48)	All patients (n=70)	Unimpaired (n=27)	Minimal HE (n=13)	Overt HE (n=30)
Age	39.8 (22-68)	55.1 (37-80)	54.5* (43-76)	57.2* (43-76)	54.7* (37-78)
Gender (% male)	52	67	55	69	76
Child-Pugh (5-15)		7.3±2.9 (5-14)	5.7±1.9 (5-12)	5.7±1.0 ^{♦♦} (5-8)	9.9±2.6 ^{##} (6-14)
PHES (z score)		-1.6±1.7 (-8.1; 1.1)	-0.5±0.8 (-1.8; 0.8)	-2.0±1.4 [#] (-4.1; 1.1)	-3.0±2.0 ^{##} (-8.1; -1.5)
PHES abnormal n (%)		16 (29.6)	0 (0)	7 (53.8)	9 (60)
EEG MDF P3-P4 (Hz)		9.4±2.2 (4.9-16.9)	10.5±1.3 (8.1-12.4)	9.6±1.7 [♦] (6.4-12.3)	8.0±2.7 ^{##} (4.9-16.9)
Theta P3-P4 (%)		26.4±17.4 (5.7-72)	14.4±6.6 (5.7-29.8)	31.8±19.3 [#] (10.9-72)	37.9±15.5 ^{##} (8.0-65.5)
Abnormal EEG report: n (%)		25 (42)	0 (0)	7 (54)	18 (86)

Data are expressed as mean ± standard deviation (range) or absolute number (%).

PHES data were available in 54 patients (unimpaired = 26, minimal = 13, overt 15);

P₃P₄ spectral EEG data were available in 60 patients (unimpaired = 26, minimal = 13, overt = 21).

Significance of the differences between the control population and the patient subgroups:

*p <0.001

Significance of the differences between the unimpaired patients and the patients with minimal/overt HE: # p <0.005; ## p <0.001

Significance of the differences between the patients with minimal and overt HE:

♦ p <0.05; ♦♦ p <0.001

Table 3.0.1: Liver disease aetiology in the study population, by degree of neuropsychiatric impairment

Aetiology	Unimpaired (n=27)	Minimal HE (n=13)	Overt HE (n=30)
Alcohol-related n (%)	22 (81.5)	11 (84.6)	17 (56.6)
Alcohol + HCV n (%)	1 (3.7)	2 (15.4)	2 (6.7)
PBC n (%)	-	-	3 (10)
Cryptogenic n (%)	1 (3.7)	-	2 (6.7)
Haemochromatosis n (%)	1 (3.7)	-	-
NASH n (%)	1 (3.7)	-	1 (3.3)
n/a n (%)	1 (3.7)	-	5 (16.7)

Data is expressed as absolute number (%); aetiology data was available for 64 patients. HCV – hepatitis C virus; PBC – primary biliary cirrhosis; NASH – non-alcoholic steatohepatitis

3.2 Evoked Potential Latencies.

3.2.1 Visual Evoked Potential (VEP).

Three patients (2 overt and 1 unimpaired) were unable to tolerate stimulation for both eyes; these data sets are derived from one hemisphere only. One overt patient could not tolerate the test. One overt patient had an unreadable N145 peak; their N145 data set therefore comprised of one hemisphere.

VEP latencies were prolonged in all three patient subgroups compared to the healthy controls (Table 3.1); the N75 and P100 peak latencies were significantly prolonged in all three patients' subgroups (Figure 3.1) while the differences in latencies between patients and controls were less marked for the N145 peak. A significant difference for the N145 also occurred between the unimpaired patients and those with minimal HE.

Table 3.1: VEP latencies in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment

Evoked Potential VEP (ms)	Controls (n = 48)	Unimpaired (n=27)	Minimal (n= 13)	Overt (n=29 / 28*)
N75	73.7±5.5	80±8.2***	79.1±8.8**	81.5±7.2****
P100	101.7±4.5	106.5±5.5***	110.1±7****	108.1±7.7****
N145*	137.0±10.2	137.8±10.8 ^o	145.6±12*	142.2±9.9

Data are expressed as (mean ± SD) for VEP latencies

Significance of the difference from controls: *p < 0.05; **p < 0.01; *** p < 0.005; ****p < 0.001; *****p < 0.0001.

Significance of the difference from patients with minimal HE: ^op < 0.05;

(Overleaf Fig 3.1: Visual Evoked Potentials)

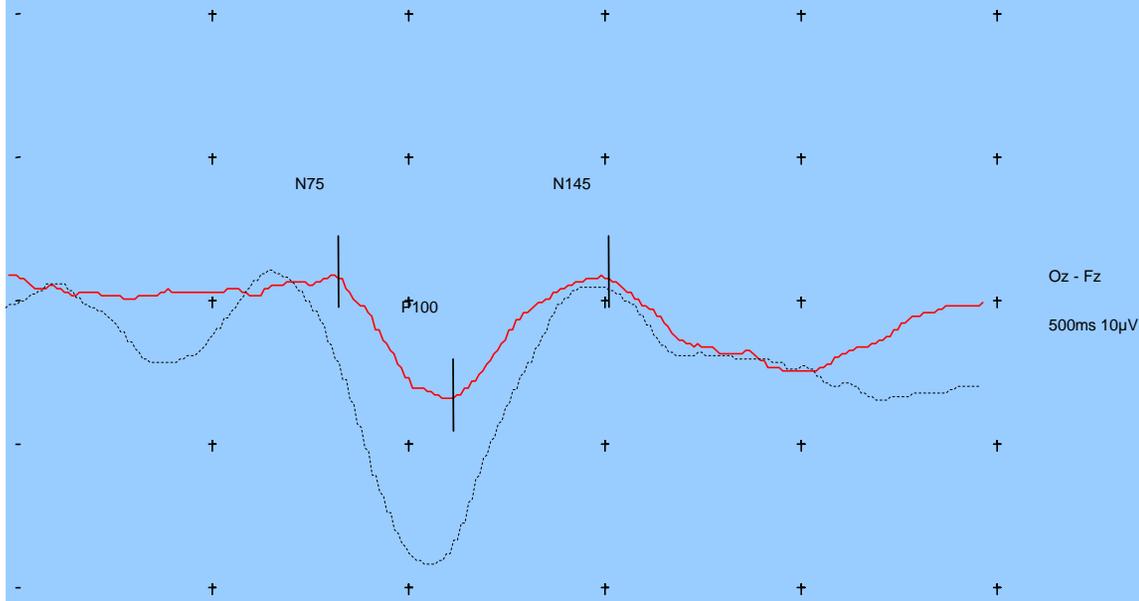


Figure 3.1: Visual Evoked Potentials

● LEFT: Recordings obtained from pattern stimulation with main waveforms marked:

----- control,
 ——— patient with overt HE

● BELOW: Box plots of results from controls, and patient groups: unimpaired, minimal HE, overt HE

Data are median, mean and 95%CI; significant differences are inset.

* p = <0.05
 ** p = 0.01
 *** p = 0.005
 **** p = 0.001
 ***** p = <0.0001

Visual Evoked Potential

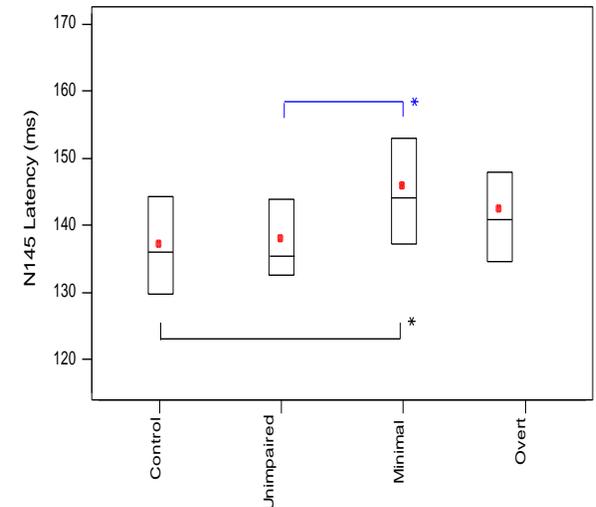
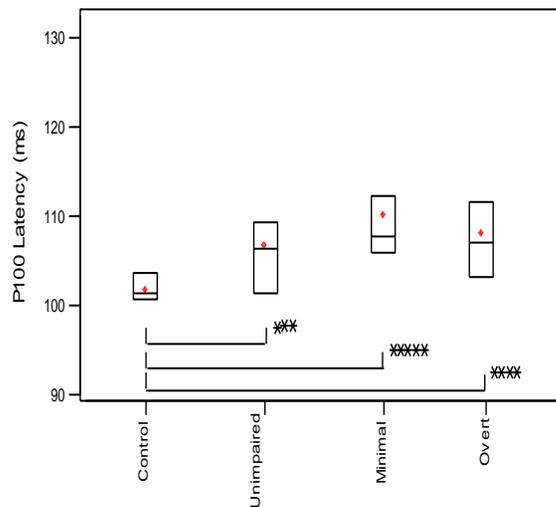
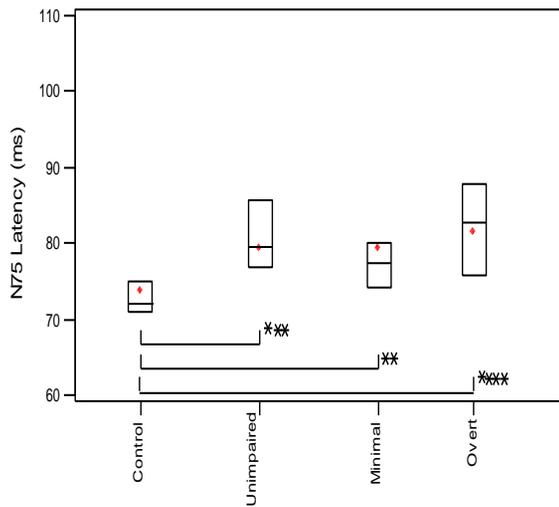
Visual Evoked Potential

Visual Evoked Potential

(means are indicated by solid circles)

(means are indicated by solid circles)

(means are indicated by solid circles)



3.2.2 Somatosensory Evoked Potentials (SSEP)

Two overt patients were unable to tolerate stimulation on both limbs; these data sets are derived from one hemisphere only. One overt patient could not tolerate the test. Two patients (one minimal HE and one unimpaired) had an unreadable N20 peak for one hemisphere; their data sets were unilateral for N20.

Significant differences are found between the control and all patient groups for the N9 and N13 peaks, the overt HE patients having the greatest difference and the minimal HE the least (Table 3.2). The cortical N20 component demonstrates significant latency differences between the controls and the unimpaired and overt patients but not for the minimal HE group. There are no differences observed between the patient groups for any of the peaks.

Table 3.2: SEP latencies in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n = 48)	Unimpaired (n=27)	Minimal (n= 13)	Overt (n=29)
SEP (ms)				
N9	9.9±0.7	11.0±1.2***	10.6±1.4*	11.3±0.8*****
N13	13.2±0.9	14.7±1.4***	14.8±1.8**	14.9±0.9*****
N20	19.1±1.0	20.9±1.5*****	20.4±2.0	20.9±1.5*****

Data are expressed as (mean ± SD) for SEP latencies

Significance of the difference from controls: *p < 0.05; **p <0.01; *** p <0.005; ****p <0.001; *****p <0.0001.

(Overleaf Fig 3.2: Somatosensory Evoked Potentials)

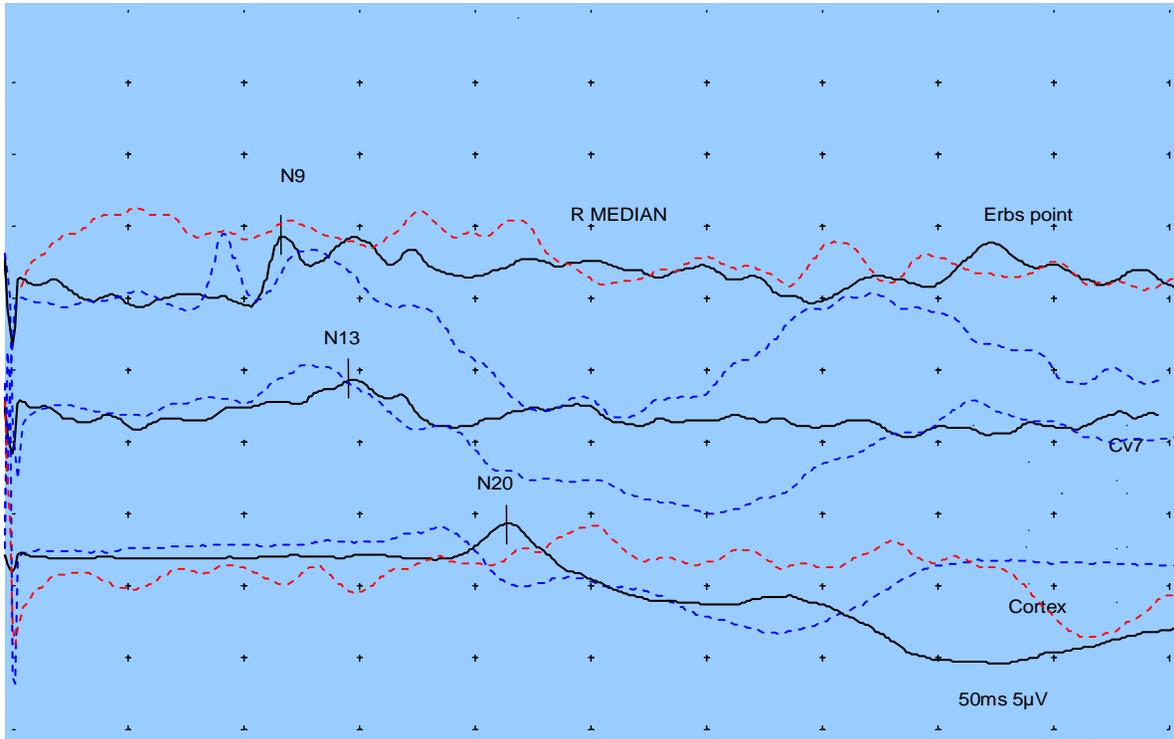


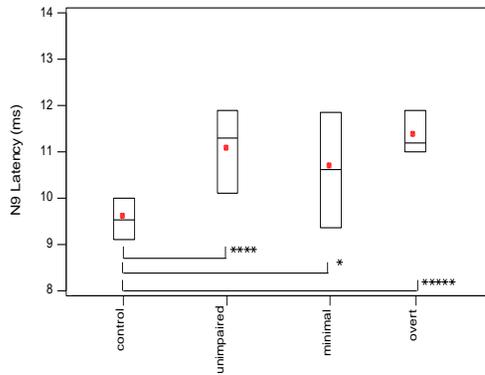
Figure 3.2: Somatosensory Evoked Potentials

- LEFT: Waveforms obtained by electrical stimulation of the median nerve at the wrist, with peripheral, cervico-medullary and cortical markers. SSEP data from:
 - control
 - patient with minimal HE
 - - - patient with overt HE
- BELOW: Box plots of results from controls and patient groups: unimpaired, minimal HE, and overt HE. Data are median, mean and 95% CI; significant differences are inset.

* p = <0.05
 ** p = 0.01
 *** p = 0.005
 **** p = 0.001
 ***** p = <0.0001

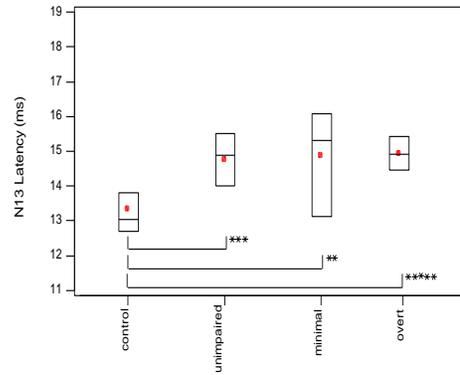
Somatosensory Evoked Potential N9

(means are indicated by solid circles)



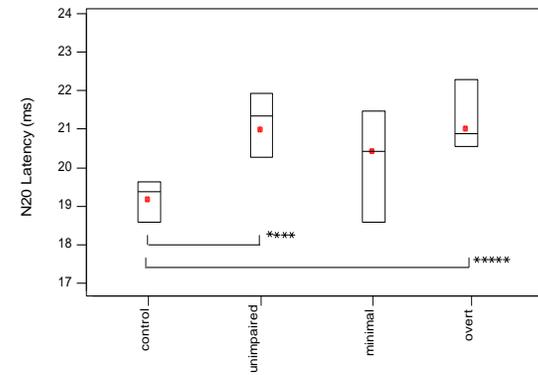
Somatosensory Evoked Potential N13

(means are indicated by solid circles)



Somatosensory Evoked Potential N20

(means are indicated by solid circles)



3.2.3 Brainstem Auditory Evoked Potentials (BAEP).

One unimpaired and three overt patients were unable to tolerate stimulation of both ears; these data sets are derived from one hemisphere only. Two patients (one overt HE and one unimpaired) had an unreadable 'wave V' peak for one hemisphere; the wave V data for these patients comprise of one hemisphere only.

Significant differences occur between the unimpaired and overt HE patients vs. controls for BAEP wave III (Table 3.3, Fig 3.3). Wave V demonstrated similar changes where all three test groups were significantly different from the control group. Additionally, between patient groups the overt HE's significantly differed in latency from the unimpaired patients.

Table 3.3: BAEP latencies in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

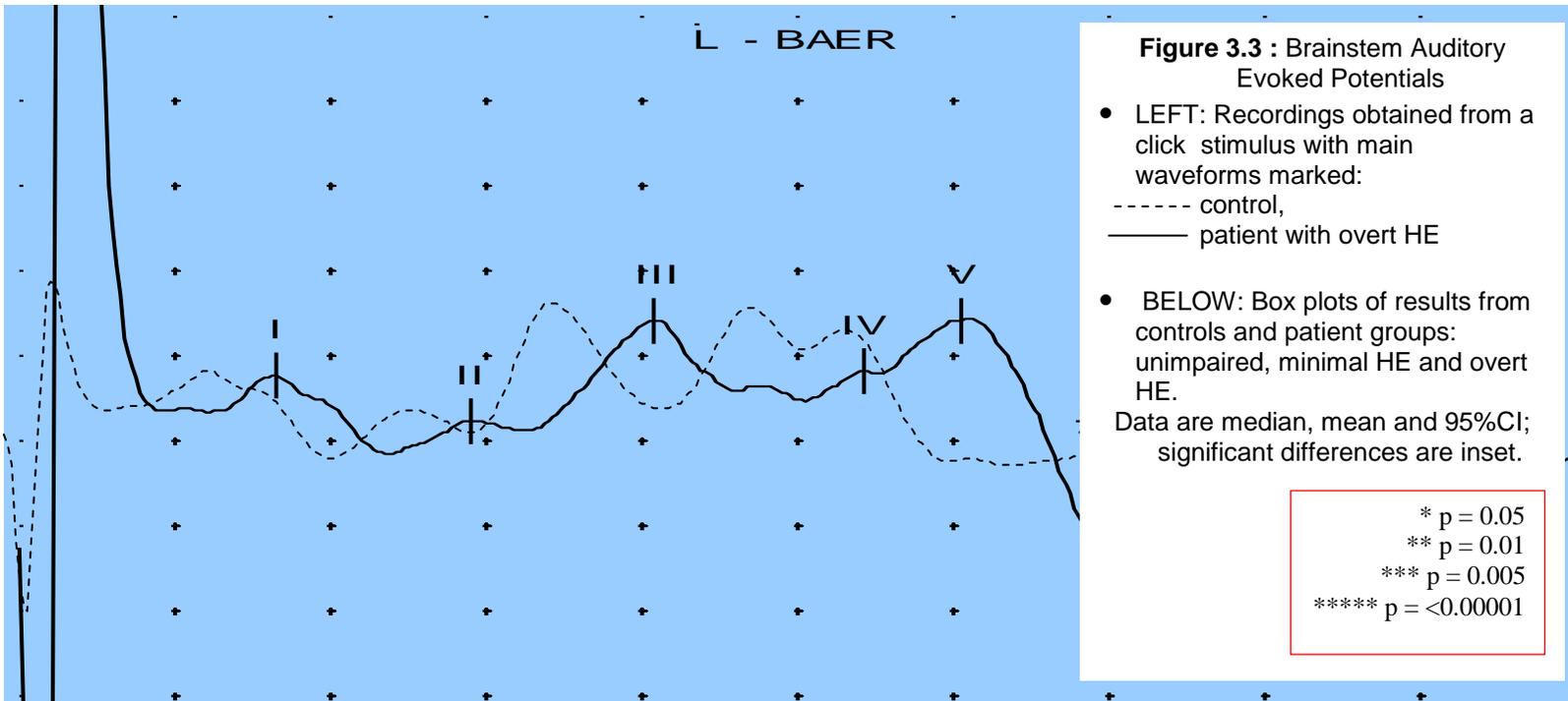
Evoked Potential	Controls (n = 48)	Unimpaired (n=27)	Minimal (n= 13)	Overt (n=30)
BAEP (ms)				
Wave III	3.7±0.2	3.8±0.2**	3.7±0.2	3.9±0.2***
Wave V	5.6±0.2	5.8±0.2*+	5.8±0.3**	5.9±0.3*****

Data are expressed as (mean ± SD) for BAEP latencies

Significance of the difference from controls: *p < 0.05; **p <0.01; *** p <0.005; *****p <0.0001.

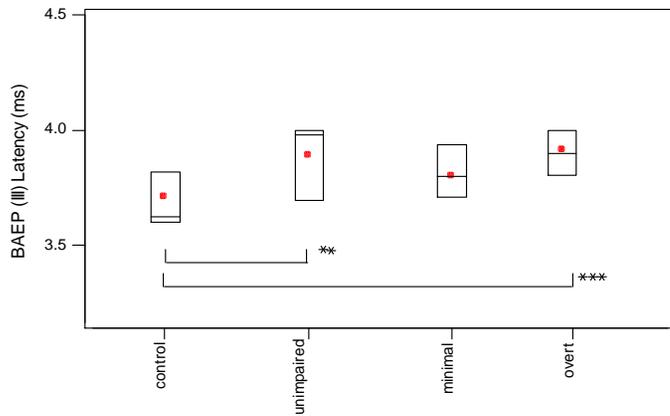
Significance of the difference from overt HE: +p < 0.05.

(Overleaf Fig 3.3 : Brainstem Auditory Evoked Potentials



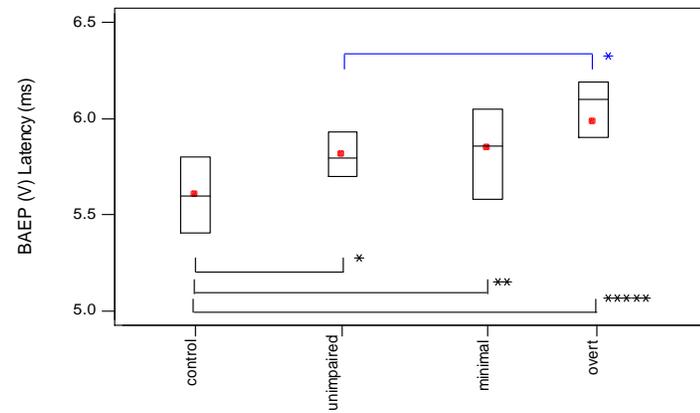
Brainstem Auditory Evoked Potential wave III

(means are indicated by solid circles)



Brainstem Auditory Evoked Potential wave V

(means are indicated by solid circles)



3.2.4 Auditory Cognitive Evoked Potential (P300).

Four overt patients were unable to comply with the test. One overt and one minimal HE patient were co-operative but had unreadable waveforms. One unimpaired patient was co-operative but was unable to understand the instructions of the test; their waveform was excluded.

Cognitive P300 latencies were prolonged for all patient groups compared to the control population, with the overt HE group more significantly than the unimpaired or the minimal HE patients (Table 3.4). The mean P300 peak latency of the overt HE patient group was also significantly prolonged compared to the two other patient populations, the minimal HE more than the unimpaired patient group (Fig 3.4)

Table 3.4: P300 latencies in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n = 48)	Unimpaired (n=26)	Minimal (n= 12)	Overt (n=25)
Auditory Cognitive (ms)				
P300	306.5±19.4	354.3±55.4 ^{***+}	351.8±44.9 ^{****+}	401.5±42.4 ^{*****}

Data are expressed as (mean ± SD) for the P300 latencies – derived from electrode Pz

Significance of the difference from controls: **p <0.01, *** p <0.005, *****p <0.0001.

Significance of the difference from patients with overt HE: +p < 0.05, +++p <0.005.

(Overleaf Fig 3.4: Cognitive Auditory (P300) Evoked Potential

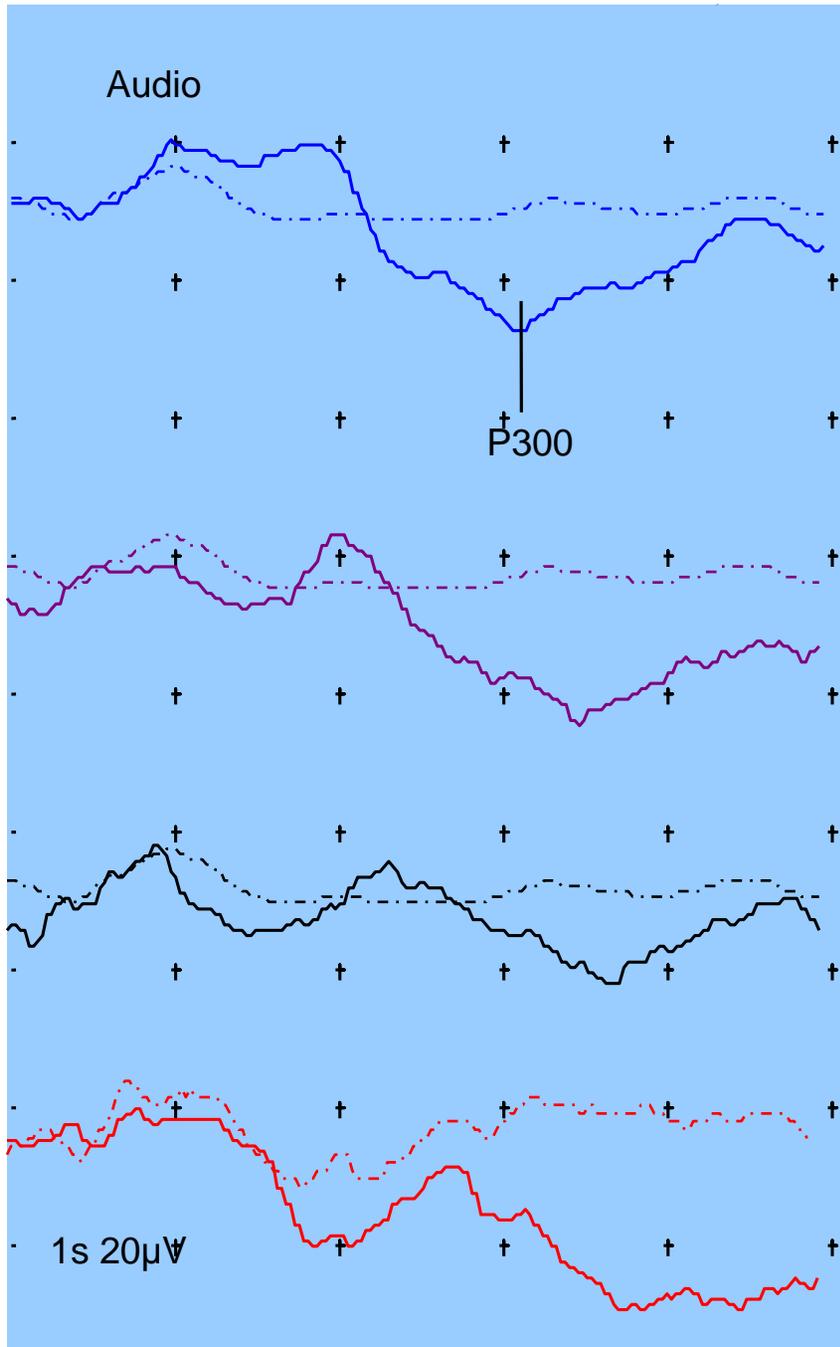


Figure 3.4: Cognitive Auditory (P300) Evoked Potential

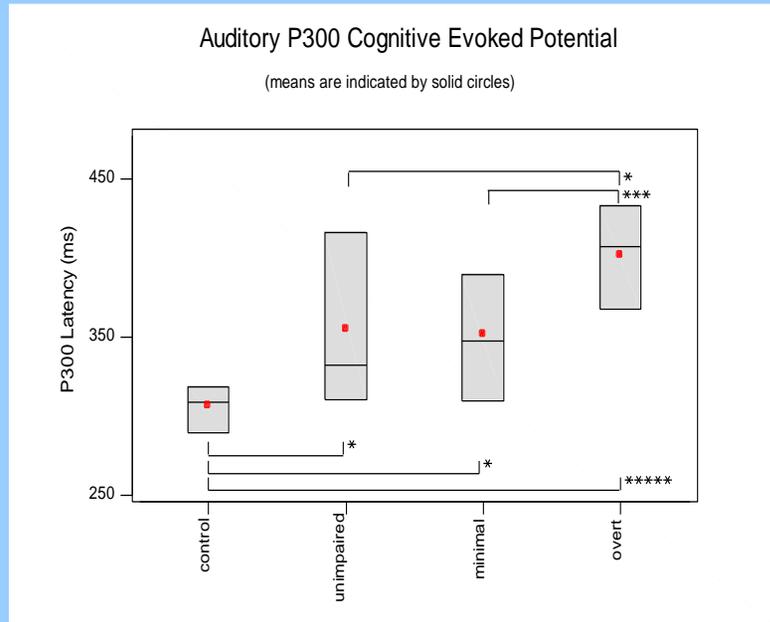
- LEFT: Waveforms obtained from an auditory 'odd-ball' stimulus; the frequent tone is the dashed line, the rare tone average is solid. The P300 marker is present in the control subject:

— control subject
— unimpaired
— minimal HE
— overt HE

- BELOW: Box plots of results from controls and patient groups: unimpaired, minimal HE and overt HE.

Data are median, mean and 95% CI; significant differences are inset.

* $p = <0.01$
 *** $p = <0.005$
 ***** $p = <0.0001$



3.3 EP Frequency and Power analysis (I): Fourier Transforms

Voltage data was exported for frequency and power computation for a population of controls and patients. The control group consisted of 17 EP data sets (13 men, 4 women; mean age [range] 43.8 [22 – 68] years). 41 sets of patient EP data were analysed; (22 men, 21 women; mean age [range] 54.2 [41 – 78] years). On the day of the EP recordings the patients were classified into their neuropsychiatric status as either unimpaired (n = 18; 44%) or having minimal (n = 11; 27%) or overt (n = 12; 29%) hepatic encephalopathy.

3.3.1 Fourier Transform – VEP

All patient waveforms comprised of a bilateral grand averaged VEP. 76% (13/17) of the controls demonstrated two or more main frequency peaks (Fig 3.5), compared with 56% (10/18) unimpaired HE, 45% (5/11) minimal HE and 8% (1/12) overt HE.

For the main and secondary peaks there were significant differences between the patients and controls (Table 3.5). The main peak (FFT-1) in the healthy controls had a higher frequency than the unimpaired and the overt HE patients. Similarly, for the secondary peak (FFT-2) the control group frequency was higher than for both the unimpaired and the minimal. Statistical analysis for the overt HE group was not possible as the FFT-2 peak was absent in all but one patient. There are also differences between patient groups. For the secondary peak frequency FFT-2, the unimpaired group has a higher frequency than the minimal HE's.

Table 3.5: VEP frequency in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

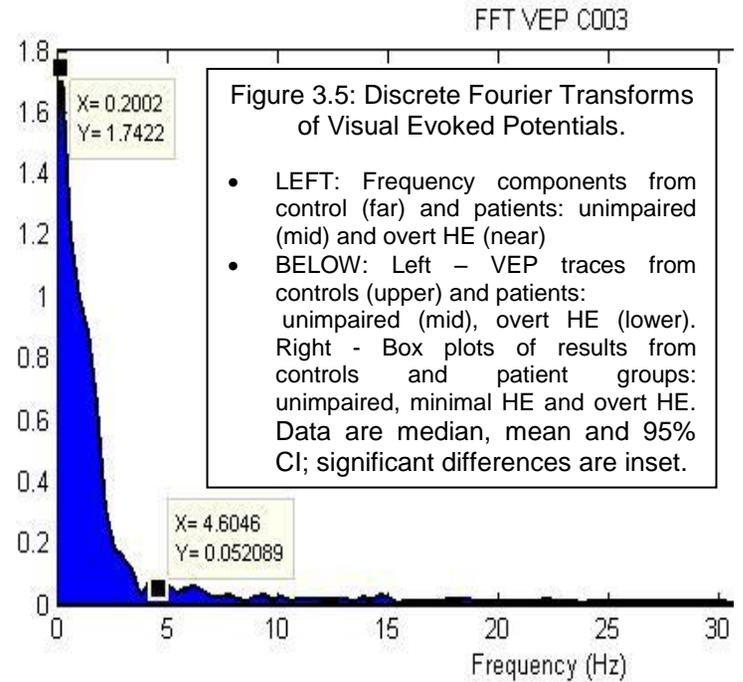
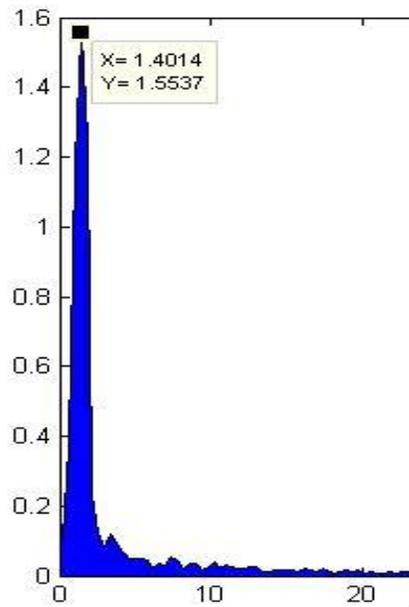
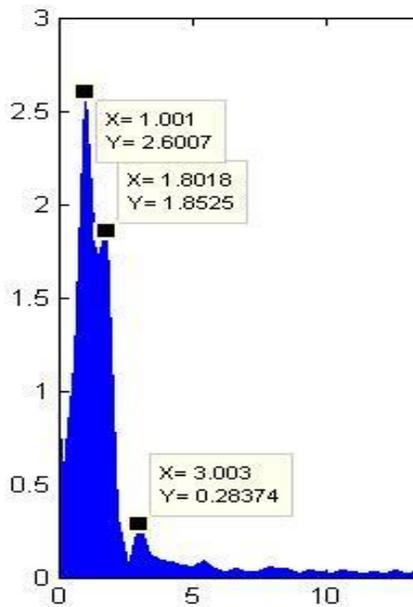
Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
VEP (Hz)				
FFT-1	1.02±0.2	0.59±0.4****	0.84±0.5	0.63±0.4*
FFT-2	1.98±0.5	1.53±0.2** ^{oo}	1.16±0.4****	1.40±0

Data are expressed as (mean ± SD) for VEP frequency components.

Differences from controls: *p < 0.05, **p < 0.01, *** p < 0.005, ****p < 0.001, *****p < 0.0001.

Differences from: minimal HE: ^op < 0.05, ^{oo}p < 0.01

(Overleaf Fig 3.5: Discrete Fourier Transforms of Visual Evoked Potentials).



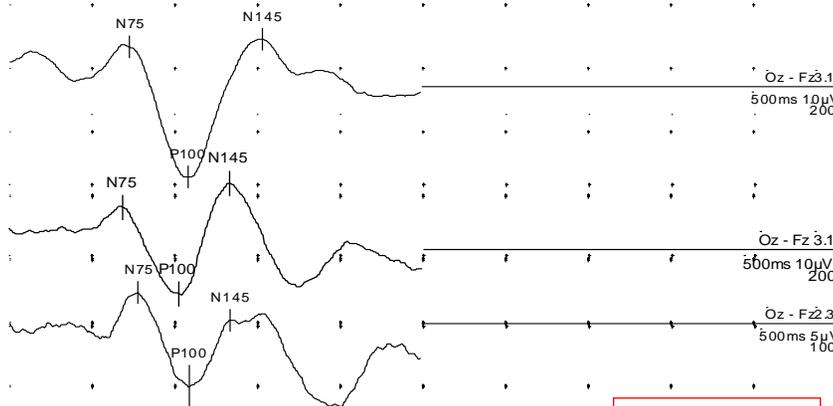
L - VEP

Visual Evoked Potential FFT-1 component

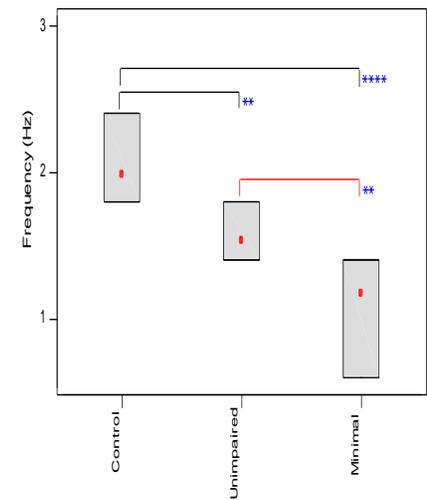
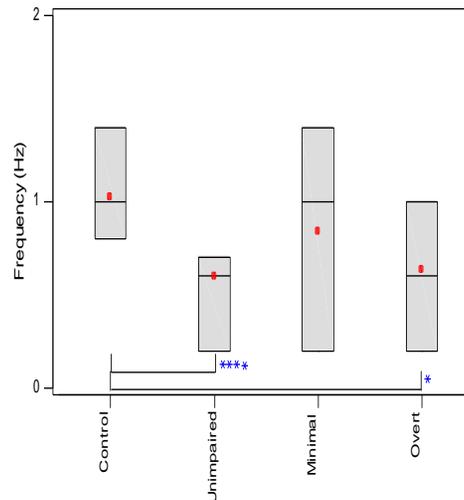
(means are indicated by solid circles)

Visual Evoked Potential FFT-2 component

(means are indicated by solid circles)



* $p < 0.05$
 ** $p < 0.01$
 **** $p < 0.001$



3.3.2 Fourier Transform – SSEP

The data from one overt patient was derived from a unilateral signal.

Two components, the main (FFT-1) and secondary (FFT-2) are observed in the frequency domain (Figure 3.6). Two peaks occur in all healthy controls and in all patients across the HE groups; the independence of FFT-1 sub-populations was rejected, but accepted for FFT-2. The unimpaired HE group had a significantly higher frequency than the healthy controls, minimal HE and overt HE patients (Table 3.6)

Table 3.6: SSEP frequency in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
SSEP (Hz)				
FFT-1	6.44±2.8	7.59±4.2	5.80±2.6	6.64±2.9
FFT-2	25.88±6.7	35.53±10.99**** ^{ooo+}	28.80±8.33	27.56±11.0

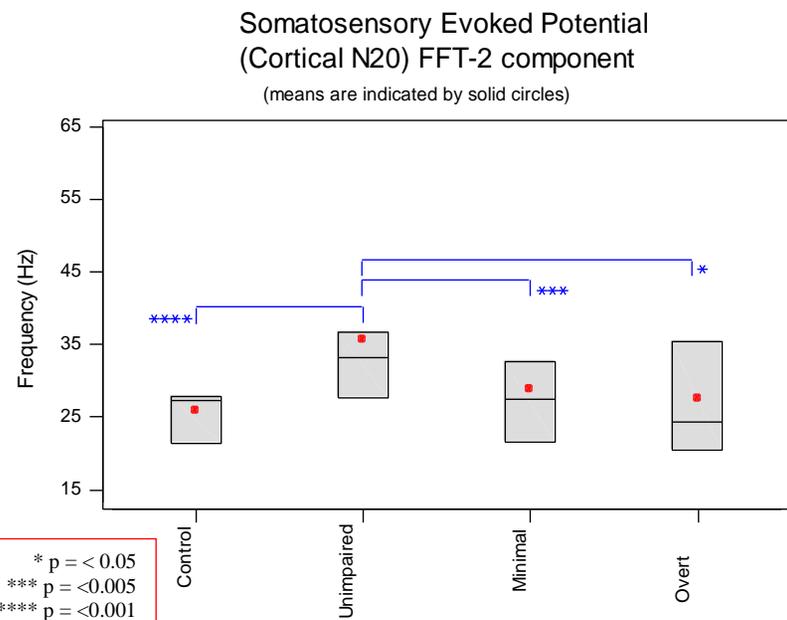
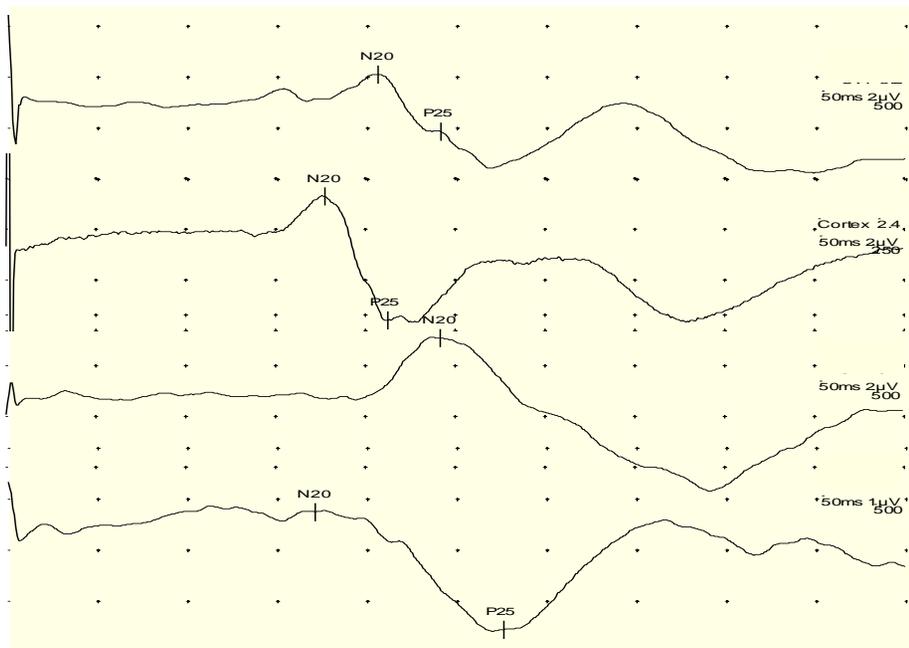
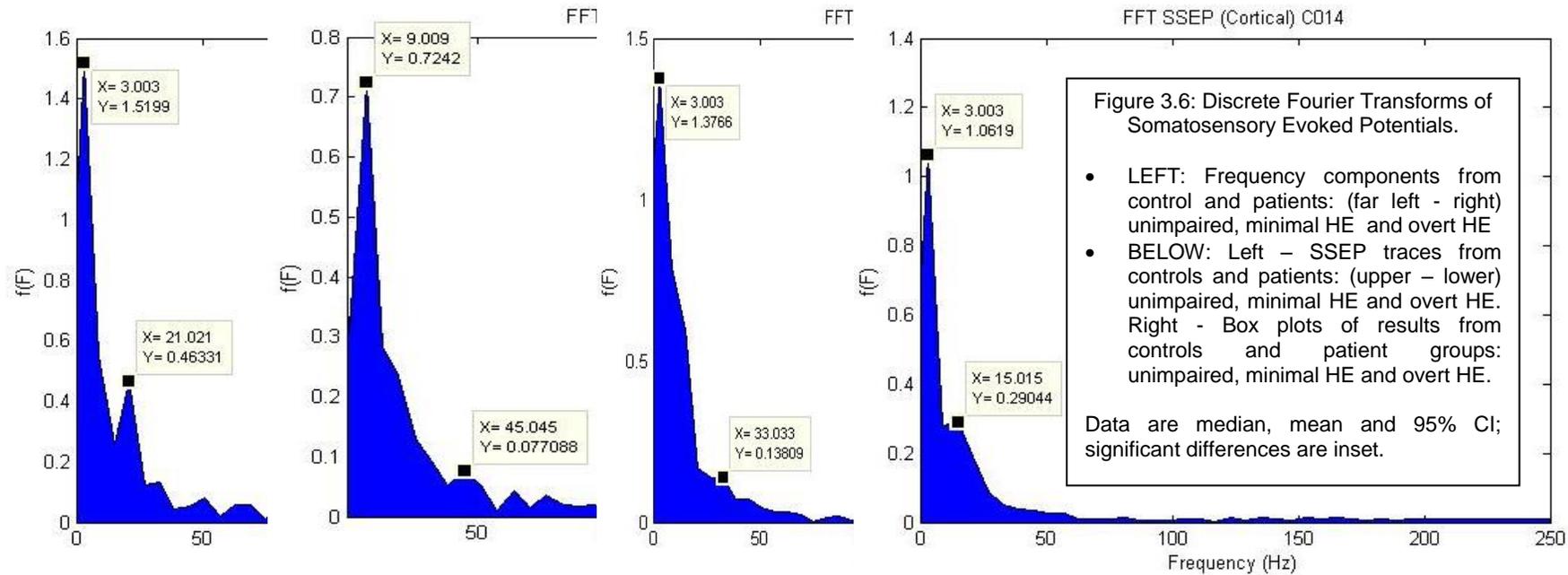
Data are expressed as (mean ± SD) for SSEP frequency components.

Differences from controls: ****p <0.001.

Differences from overt HE: +p < 0.05.

Differences from: minimal HE: ^{ooo}p < 0.005.

(Overleaf Fig 3.6: Discrete Fourier Transforms of Somatosensory Evoked Potentials).



* $p < 0.05$
 *** $p < 0.005$
 **** $p < 0.001$

3.3.3 Fourier Transform – BAEP

The data from one overt patient was derived from a unilateral signal.

Two components, the main (FFT-1) and secondary (FFT-2) are observed in the frequency domain. The two peaks occur in all healthy controls and across all patient groups (Fig 3.7).

The unimpaired patients demonstrated a significantly higher frequency compared to the controls and other patient groups for the FFT-1 peak. No other inter group differences were observed.

For the secondary FFT-2 peak the minimal and overt HE patients had a significantly lower frequency compared to the healthy controls and the unimpaired groups (Table 3.7)

Table 3.7: BAEP frequency in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

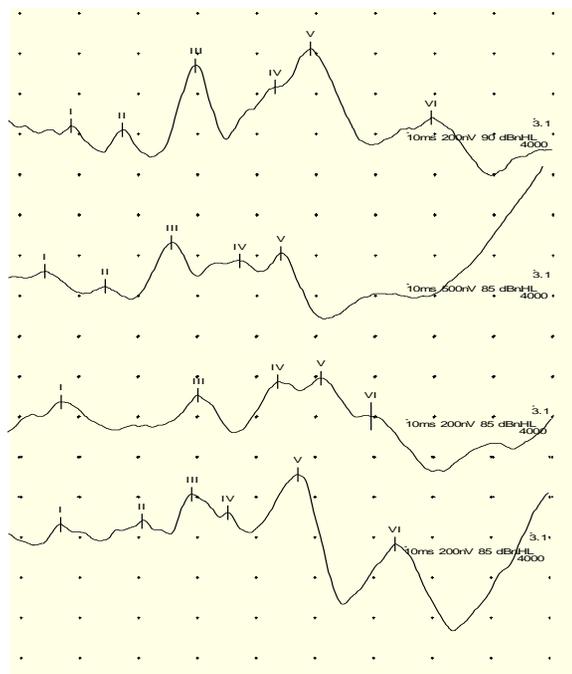
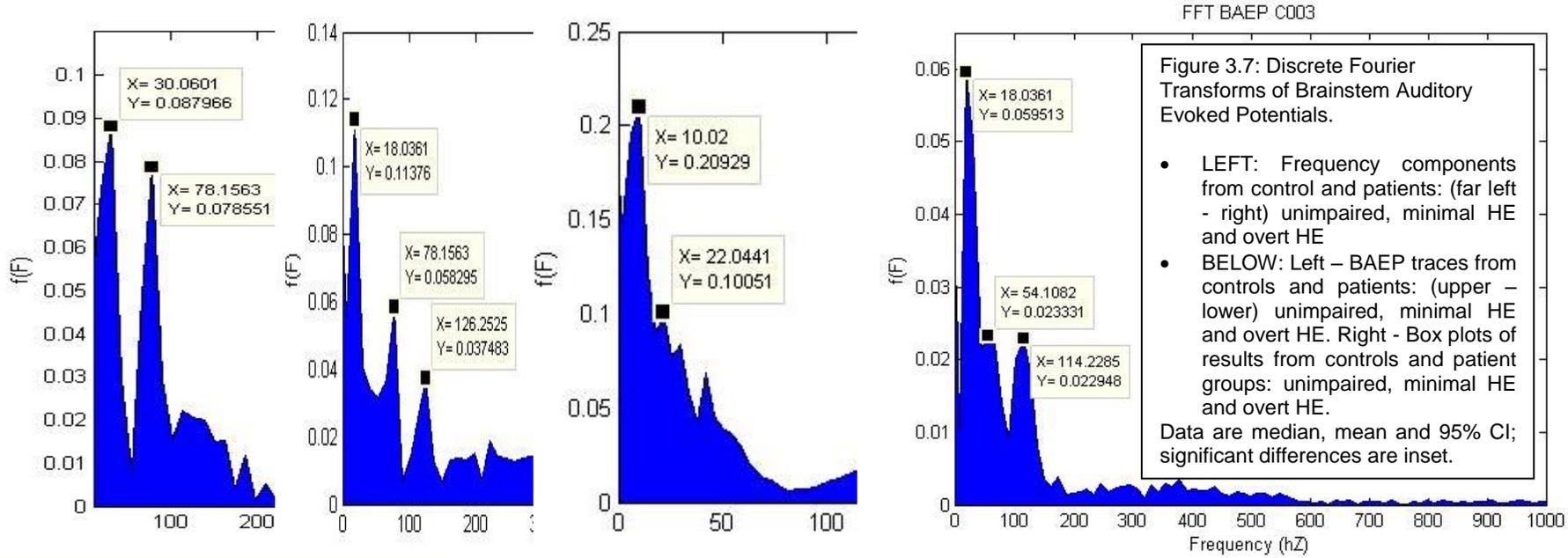
Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
BAEP (Hz)				
FFT-1	31.57±22.1	57.17±38.35* ^{ooo} +++	32.25±27.6	24.01±6.0
FFT-2	94.49±32.2	89.07±31.1 ^{oo} +++	62.50±32.1***	58.89±22.1***

Data are expressed as (mean ± SD) for BAEP frequency components.

Differences from controls: *p <0.05, ***p <0.005

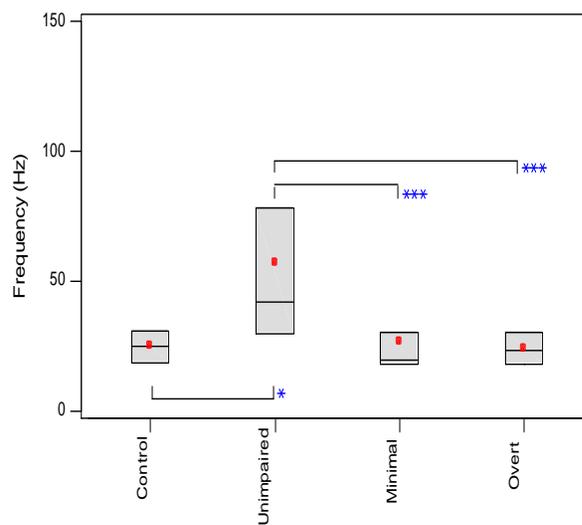
Differences from overt HE: +++p < 0.005.

Differences from: minimal HE: ^{oo}p < 0.01, ^{ooo}p < 0.005



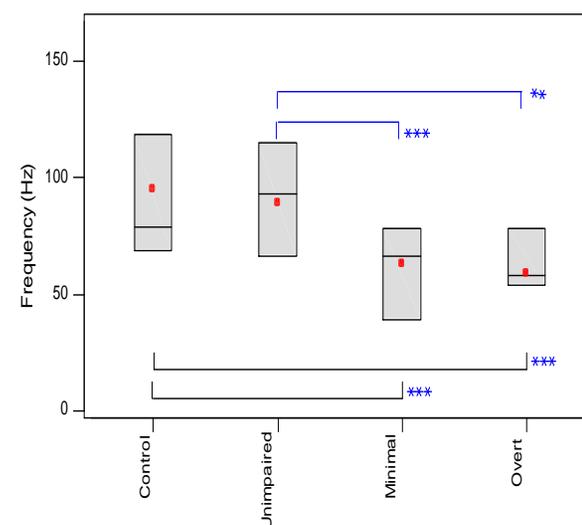
Brainstem Auditory Evoked Potential FFT-1 component

(means are indicated by solid circles)



Brainstem Auditory Evoked Potential FFT-2 component

(means are indicated by solid circles)



3.3.4 Fourier Transform – P300

All patient waveforms consist of a signal midline signal (derived from electrode Pz). Consistency was confirmed by averaging odd and even sweeps separately and adding superimposed signals to make an average for the individual. Two main frequency peaks, the main (FFT-1) and secondary (FFT-2) are observed in the auditory P300; these occurred in all healthy controls and in all individuals for the patient sub-groups (Figure 3.8).

The unimpaired patients had a significantly higher main (FFT-1) peak frequency compared to the minimal and overt HE patients (Table 3.8). No significant differences were observed for the secondary peak.

Table 3.8: P300 frequency in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

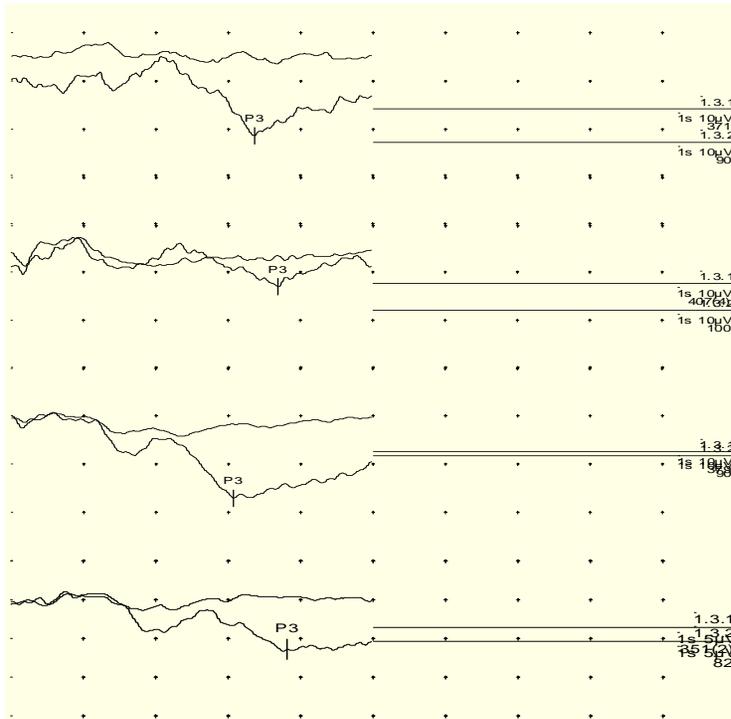
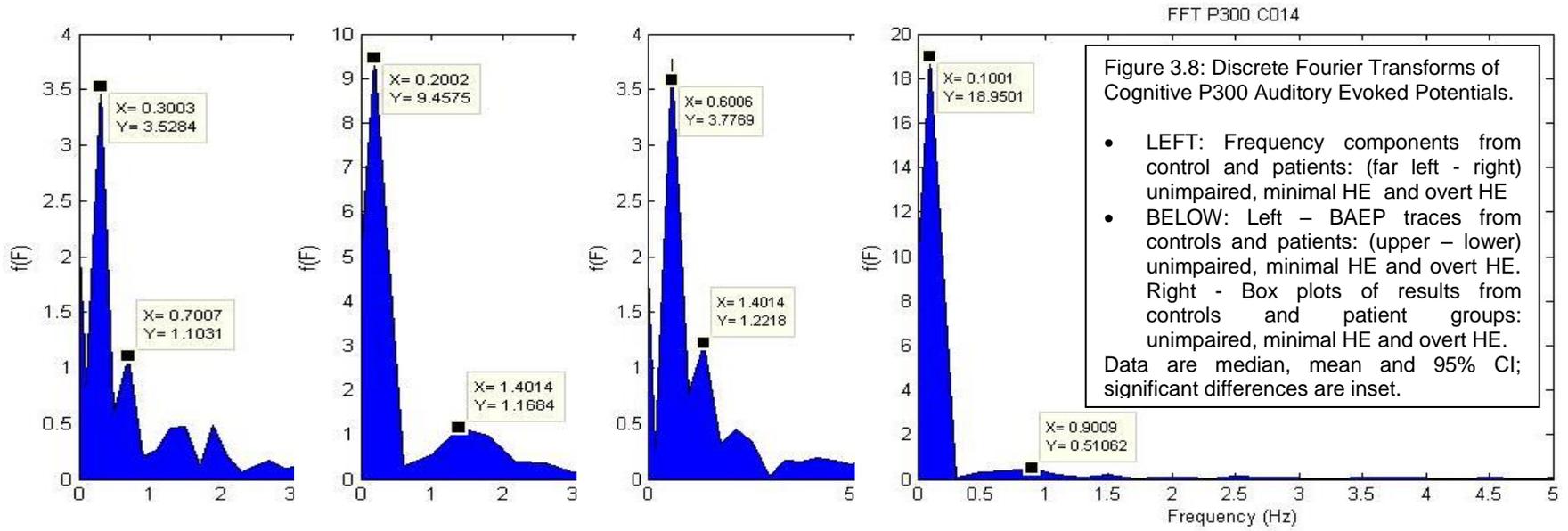
Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n=11)	Overt (n=12)
P300 Auditory Cognitive (Hz)				
FFT-1	0.29±0.1	0.35±0.1 ^{oo+++}	0.28±0.1	0.32±0.2
FFT-2	2.02±0.5	1.93±0.5	1.87±0.6	2.05±1.1

Data are expressed as (mean ± SD) for P300 frequency components.

Differences from overt HE: +++p < 0.005.

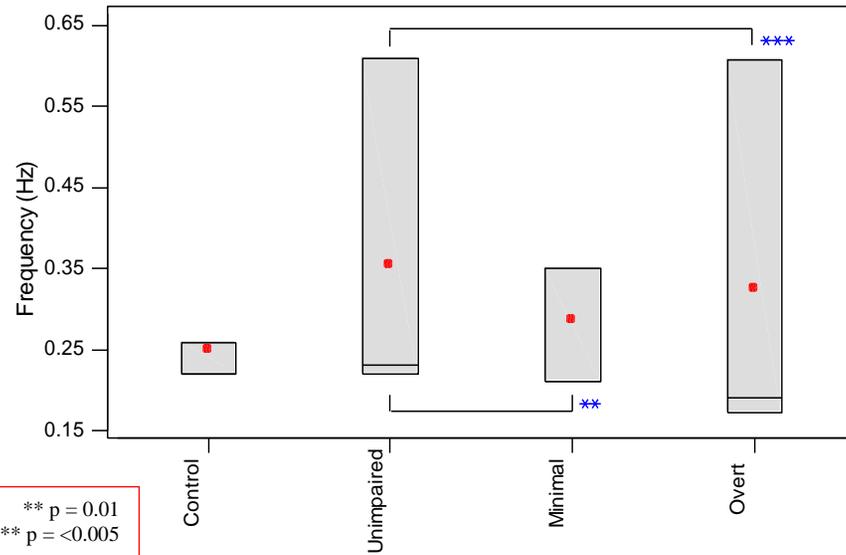
Differences from: minimal HE: °°p < 0.01.

(Overleaf Fig 3.8: Discrete Fourier Transforms of Cognitive P300 Auditory Evoked Potentials).



Cognitive P300 evoked potential FFT-1 component

(means are indicated by solid circles)



3.4 EP Frequency and Power analysis (II): Power Spectral Density Estimates (PSD)

3.4.1 PSD – VEP

There is a significant reduction in peak frequency between the controls and both the minimal HE and overt HE patients (Figure 3.9). There are also differences within the patient groups; a significant reduction in peak frequency is observed between the unimpaired patients and both the minimal HE and overt HE groups (Table 3.9). No significant differences occurred between any group for the VEP maximum power or mean power.

Table 3.9: PSD estimates of VEPs in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n=17)	Unimpaired (n=18)	Minimal (n=12)	Overt (n=12)
VEP				
P _(max) dB/Hz	-4.31±4.3	-6.37±5.7	-4.52±3.5	-4.66±5.2
P _(mean) dB/Hz	-58.57±5.1	-51.29±31.6	-58.39±4.9	-58.42±3.9
F _(peak) Hz	12.11±3.9	11.12±4.2 ^{ooo} ++++	7.95±4.6 ^{****}	6.1±3.5 ^{****}

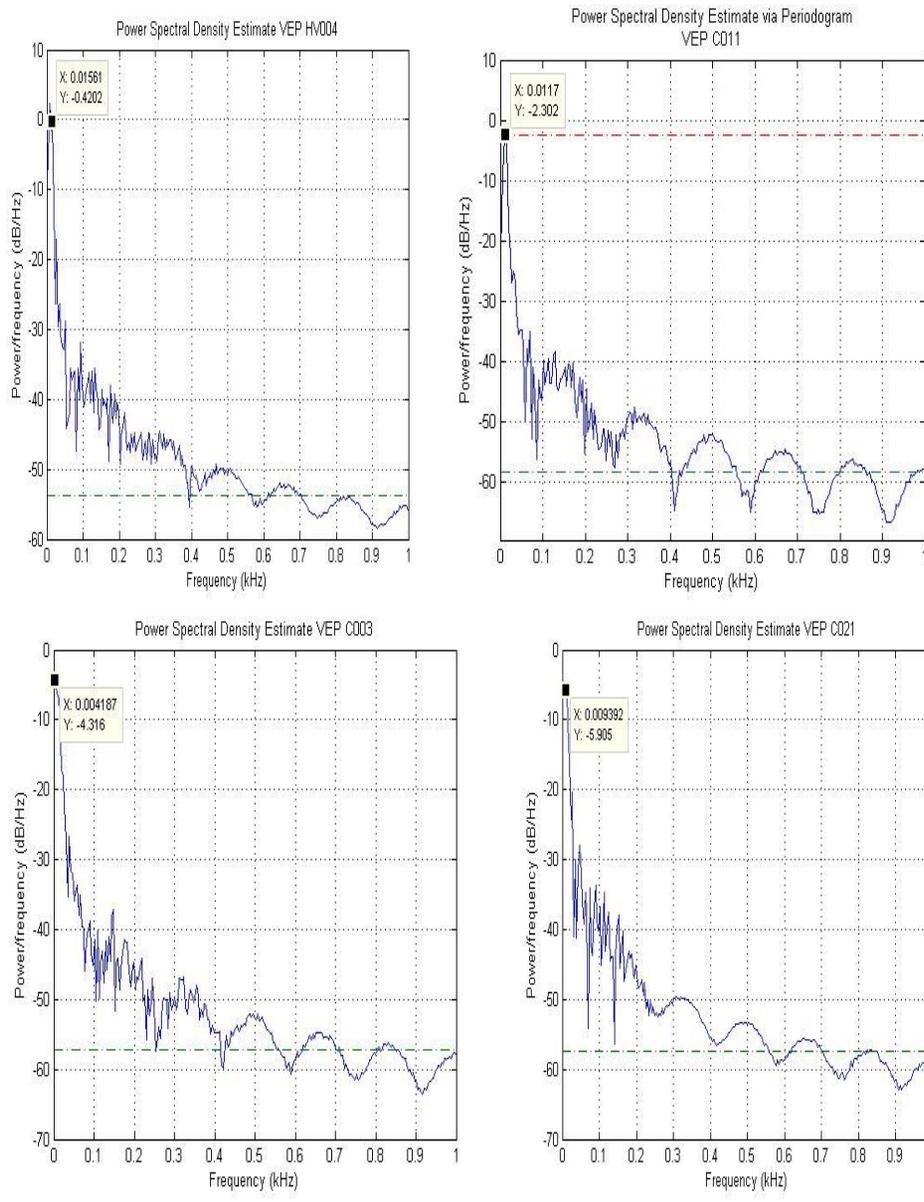
Data are expressed as (mean ± SD) for VEP frequency at peak power.

Differences from controls: ****p <0.001.

Differences from overt HE: ++++p <0.001.

Differences from: minimal HE: ^{ooo}p <0.005.

(Overleaf Fig 3.9: Power Spectral Density (PSD) Estimates of Visual Evoked Potentials).



VEP - PSD: Frequency at peak power

(means are indicated by solid circles)

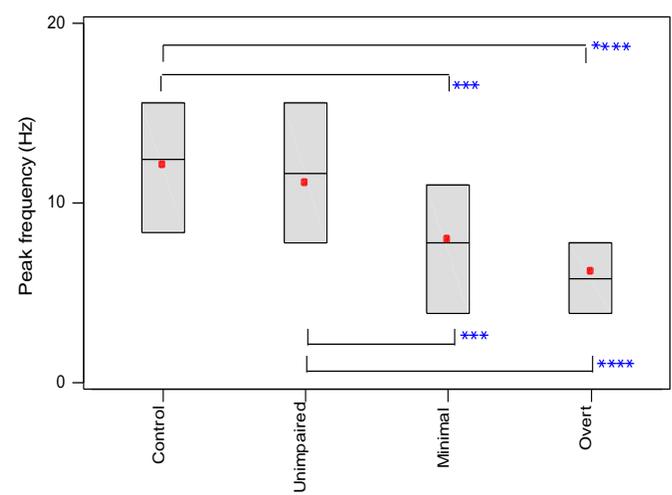


Figure 3.9: Power Spectral Density (PSD) Estimates of Visual Evoked Potentials.

- LEFT:** PSD components from control (upper-far) and patients: unimpaired (upper-near), minimal HE and overt HE (lower-far & near)
- ABOVE:** Box plots of results from controls and patient groups: unimpaired, minimal HE and overt HE.

Data are median, mean and 95% CI; significant differences are inset.

*** $p = <0.005$
**** $p = <0.001$

3.4.2 PSD – SSEP

There is an increase in peak cortical SSEP power for all patient groups compared to the controls (Figure 3.10), with the greatest significances seen with the unimpaired and minimal HE patients. Differences between patient sub-populations are not significant at the 0.05 level.

There is a significant reduction of the frequency at peak power across all patient groups compared to the control subjects; there is also significant decrease in frequency at peak power between the unimpaired and minimal HE patients (Table 3.10).

Table 3.10: PSD estimates of SSEPs in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

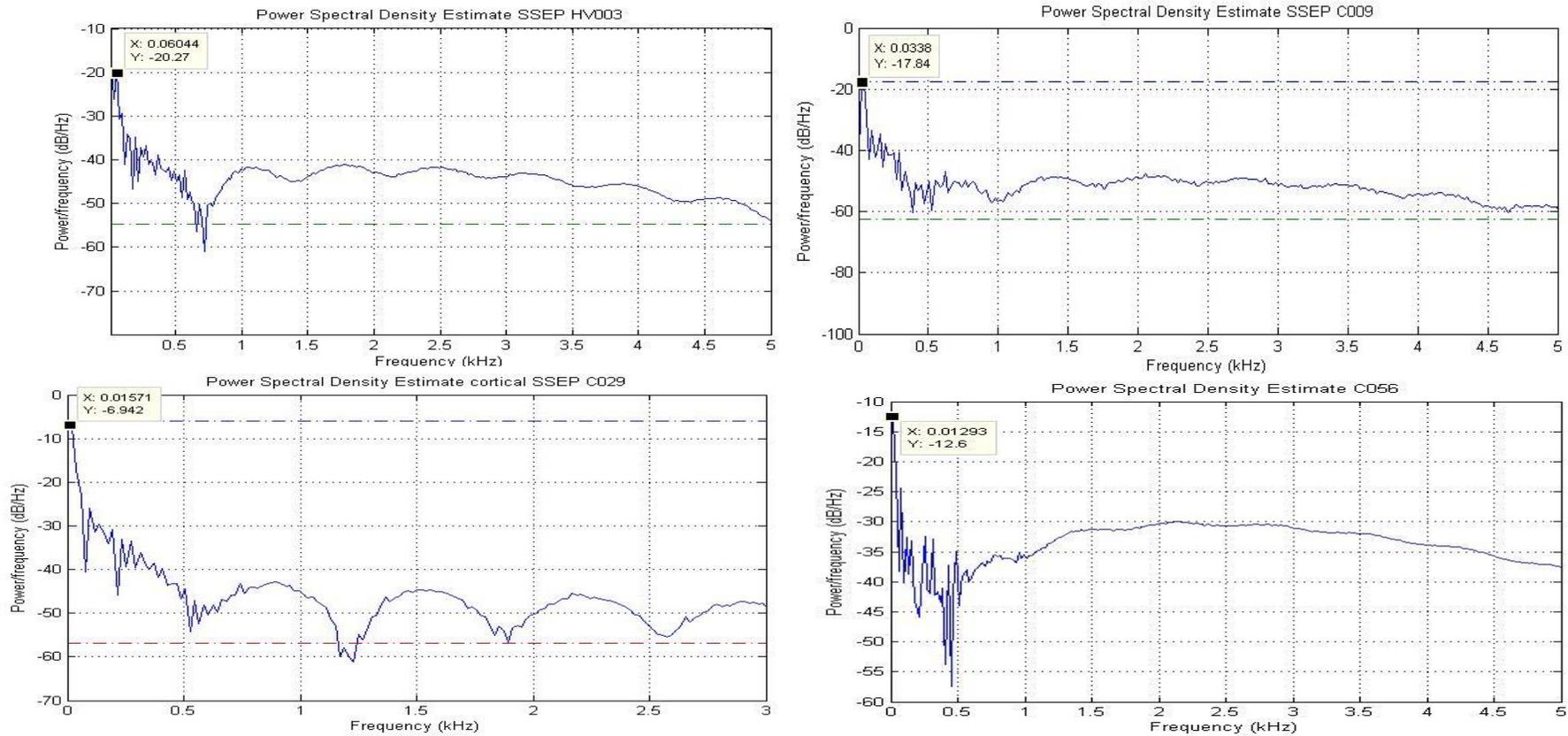
Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
SSEP				
P _(max) dB/Hz	-21.24±4.0	-15.91±6.6****	-14.07±5.2*****	-14.49±6.9*
P _(mean) dB/Hz	-52.20±5.7	-51.94±21.9	-44.58±35.7	-43.8±35
F _(peak) Hz	69.70±14.0	41.76±28.6*** ^{oo}	25.72±13.48****	30.66±27.37****

Data are expressed as (mean ± SD) for SSEP peak power and frequency at peak power.

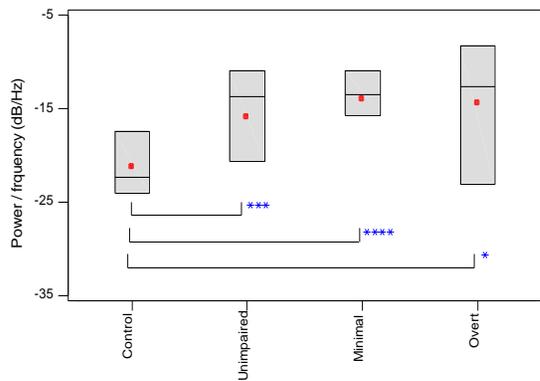
Differences from controls: *p < 0.05, *** p <0.005, ****p <0.001, *****p <0.0001.

Differences from: minimal HE: ^{oo}p <0.01.

(Overleaf Fig 3.10: Power Spectral Density (PSD) Estimates of Cortical Somatosensory Evoked Potentials).



Maximum power of cortical SSEPs
(means are indicated by solid circles)



Cortical SSEP - frequency at peak power
(means are indicated by solid circles)

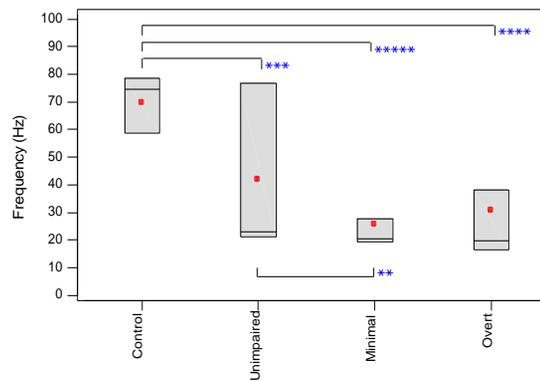


Figure 3.10: Power Spectral Density (PSD) Estimates of Cortical Somatosensory Evoked Potentials.

- **ABOVE:** PSD components from control (upper-left) and patients: unimpaired (upper-right), minimal HE and overt HE (lower-left & right).
- **LEFT:** Box plots of results from controls and patient groups: unimpaired, minimal HE and overt HE.

Data are median, mean and 95% CI; significant differences are inset.

*p = 0.05
 **p = <0.01
 *** p = <0.005
 **** p = <0.001
 *****p = <0.00001

3.4.3 PSD – BAEP

There is a reduction in the frequency at peak power of the patients compared to the healthy controls, which is highly significant for the minimal and overt HE groups (Figure 3.11). The frequency reduction of the minimal and overt HE patients is also significant compared to the unimpaired group (Table 3.11).

Table 3.11: PSD estimates of BAEPs in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
BAEP				
P _(max) dB/Hz	-37.97±2.8	-38.23±3.8	-37.37±4.7	-34.96±24.9
P _(mean) dB/Hz	-47.5±63.7	-73.82±7.2	-65.27±41.0	-36.0±74.6
F _(peak) Hz	174.9±83.9	152.4±81.1 ^{○○○○++}	87.39±36.0 ^{*****}	83.96±20.5 ^{*****}

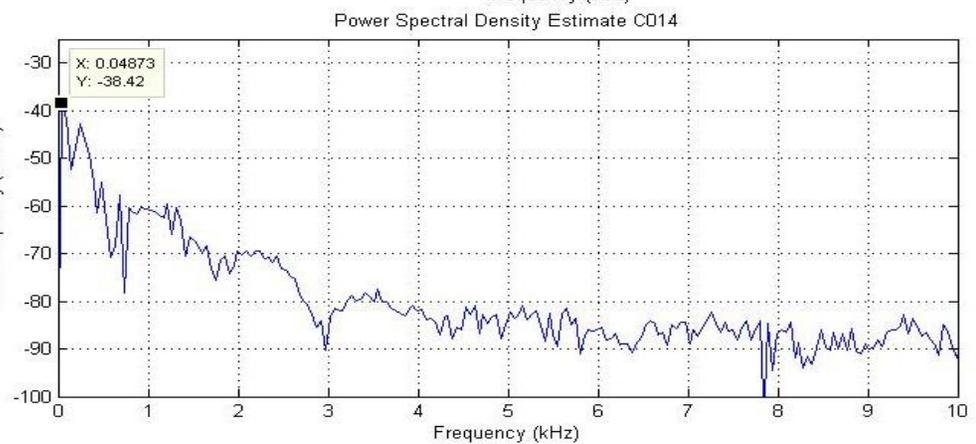
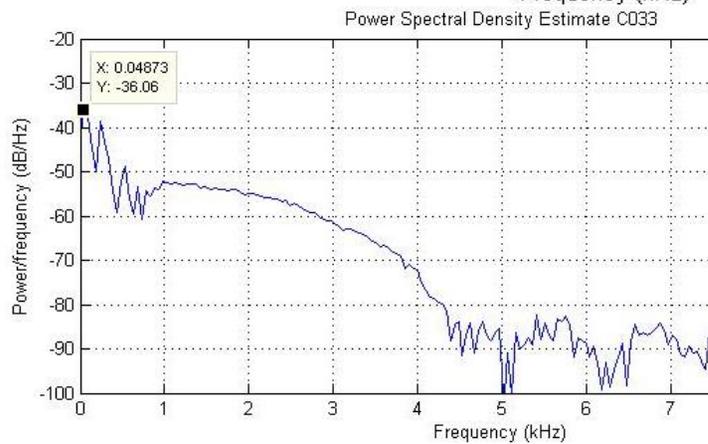
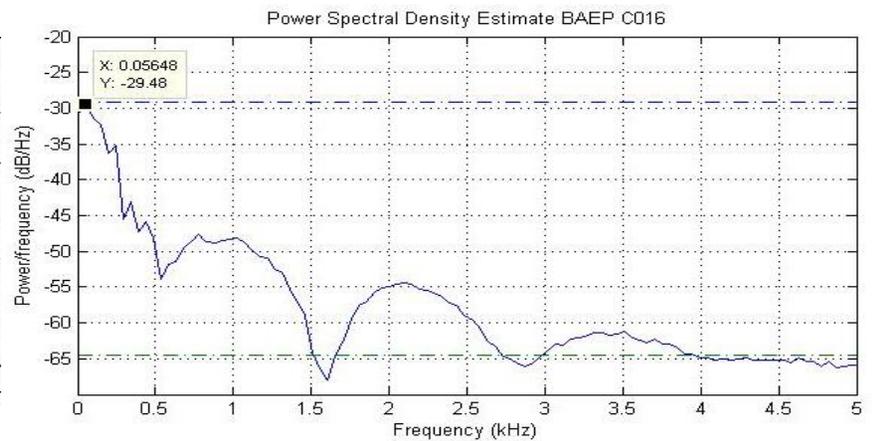
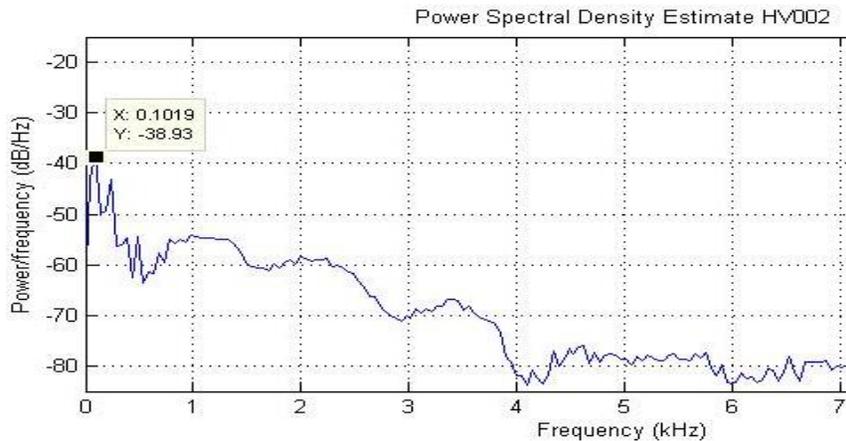
Data are expressed as (mean ± SD) for BAEP frequency at peak power.

Differences from controls: ****p <0.001, *****p <0.0001.

Differences from: minimal HE: ○○○○p <0.001

Differences from overt HE: ++p <0.01.

(Overleaf Fig 3.11: Power Spectral Density (PSD) Estimates of Cortical Brainstem Auditory Evoked Potentials).



BAEP - Frequency at Peak Power

(means are indicated by solid circles)

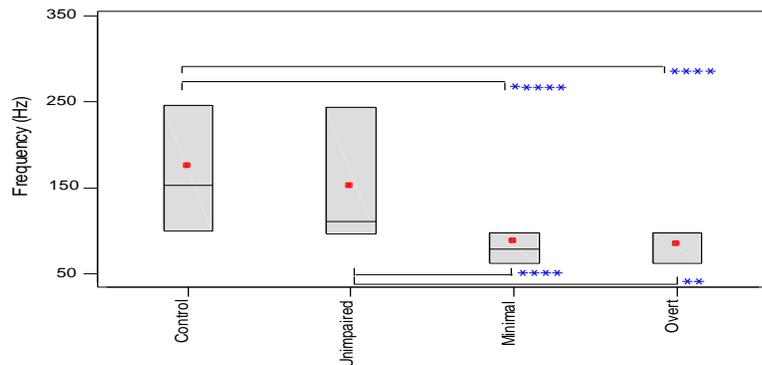


Figure 3.11: Power Spectral Density (PSD) Estimates of Cortical Brainstem Auditory Evoked Potentials.

- ABOVE:** PSD components from control (upper-left) and patients: unimpaired (upper-right), minimal HE and overt HE (lower-left & right).
- LEFT:** Box plots of results from controls and patient groups: unimpaired, minimal HE and overt HE.

Data are median, mean and 95% CI;
 significant differences are inset.

**p = <0.01
 **** p = <0.001
 ***** p = <0.0001

3.4.4 PSD - P300

A significant reduction in peak frequency occurs in all patient groups compared to the healthy controls (Figure 3.12); differences in frequency reduction are also significant between patient sub-groups (Table 3.12). Unimpaired patients have a significantly higher peak frequency than the minimal and overt HEs, while the peak frequency of the minimal HE patients is also significantly higher than the overt HEs.

Table 3.12: PSD estimates of Cognitive P300 potentials in healthy control subjects and patients with cirrhosis, by degree of neuropsychiatric impairment.

Evoked Potential	Controls (n = 17)	Unimpaired (n=18)	Minimal (n= 11)	Overt (n=12)
P300 Auditory Cognitive				
P _(max) dB/Hz	-2.13±4.1	-1.07±8.6	-0.15±6.6	-0.88±5.8
P _(mean) dB/Hz	-39.85±5.7	-41.29±3.2	-41.81±3.8	-42.59±3.9
F _(peak) Hz	7.71±2.6	5.87±2.3 ^{****} ++++	3.83±1.5 ^{*****} +	2.64±0.9 ^{*****}

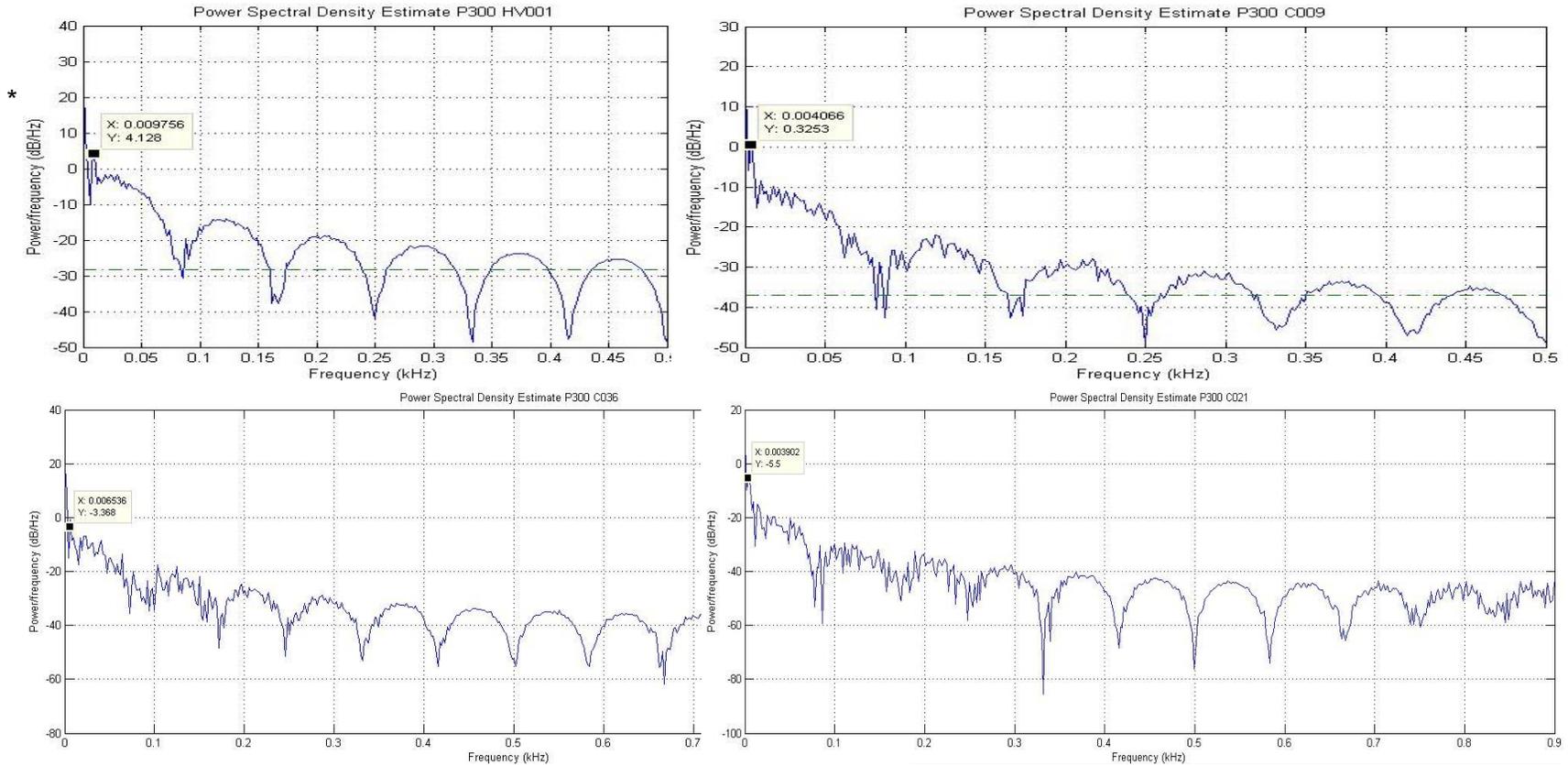
Data are expressed as (mean ± SD) for P300 frequency at peak power.

Differences from controls: *p < 0.05, *****p <0.0001.

Differences from overt HE: +p < 0.05, ++++p <0.001

Differences from: minimal HE: ****p <0.001,

(Overleaf Fig 3.12: Power Spectral Density (PSD) Estimates of Cognitive P300 Auditory Evoked Potentials).



P300 Cognitive EP - Frequency at peak power.
(means are indicated by solid circles)

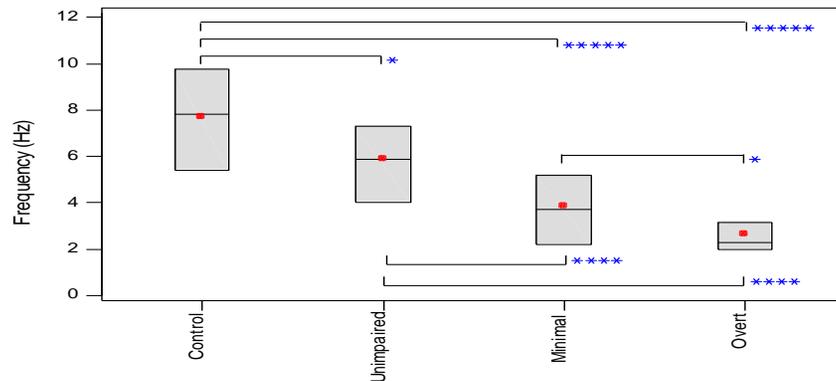


Figure 3.12: Power Spectral Density (PSD) Estimates of Cognitive P300 Auditory Evoked Potentials.

- ABOVE:** PSD components from control (upper-left) and patients: unimpaired (upper-right), minimal HE and overt HE (lower-left & right).
- LEFT:** Box plots of results from controls and patient groups: unimpaired, minimal HE and overt HE.

Data are median, mean and 95% CI; significant differences are inset.

*p = <0.05
 *** p = <0.001
 *****p = <0.0001

3.5 Sensitivity and Specificity Analysis

ROC curve analysis identified thresholds which could discriminate any degree of hepatic encephalopathy. The 'negative' group consisted of the data from controls and unimpaired patients combined; the 'positive' group consisted of the minimal and overt HE patient data combined. Where there are multiple latency peaks for an EP the optimal waveform marker is highlighted (tables 3.13 & 3.14).

3.5.1 Sensitivity and Specificity – EP latencies

Table 3.13: Latency thresholds of evoked potentials distinguishing healthy controls and unimpaired patients from minimal and overt HE patients.

Evoked Potential / threshold (ms)		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Area under curve
VEP						
>74.15	N75	80.0	54.0	55.7	78.8	0.67
>103.0	P100	73.3	51.5	52.3	72.8	0.68
>145.05	N145	43.2	82.3	63.8	66.7	0.62
SSEP						
>9.95	N9	81.8	53.8	60.2	77.7	0.73
>13.55	N13	86.3	50.0	59.5	81.1	0.75
>20.05	N20	79.5	59.6	62.7	77.4	0.72
BAEP						
>1.54	wave I	75.0	40.8	52.9	64.8	0.59
>2.75	wave II	55.6	56.2	53.0	58.8	0.56
>3.76	wave III	78.2	49.0	57.6	71.8	0.68
>5.0	wave IV	69.5	40.8	51.0	60.2	0.62
>5.75	wave V	76.1	51.0	57.9	70.6	0.72
Cognitive Auditory						
>336.5	P300	86.8	70.0	73.3	84.9	0.80

3.5.2 Sensitivity and Specificity – Frequency domain

Table 3.14: Frequency and power thresholds of evoked potentials distinguishing healthy controls and unimpaired patients from minimal and overt HE patients.

Evoked Potential / threshold		Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Area under curve
VEP						
FFT-1(Hz)	<1.0	80.56	33.33	46.0	70.8	0.58
FFT-2(Hz)	<1.4	81.8	58.6	58.2	82.0	0.71
P _{max} (dB/Hz)	<-4.09	61.11	44.68	43.8	62.0	0.52
P _{mean} (dB/Hz)	<-56.16	77.78	34.78	45.7	68.9	0.51
F _{Pmax} (Hz)	<11.03	75.0	54.35	53.7	75.5	0.66
SSEP						
FFT-1(Hz)	<8.05	50.0	41.3	37.5	53.9	0.52
FFT-2(Hz)	<28.74	67.65	44.44	46.2	66.1	0.58
P _{max} (dB/Hz)	>-18.62	82.35	43.48	50.7	77.7	0.66
P _{mean} (dB/Hz)	>-56.89	73.53	45.65	48.8	71.0	0.56
F _{Pmax} (Hz)	<33.78	85.29	58.7	59.3	85.0	0.70
BAEP						
FFT-1(Hz)	<30.06	77.1	52.1	53.2	76.4	0.66
FFT-2(Hz)	<78.15	43.75	43.75	45.2	64.4	0.63
P _{max} (dB/Hz)	<-37.15	65.71	41.7	44.3	63.3	0.60
P _{mean} (dB/Hz)	<-75.99	52.9	52.1	43.58	61.1	0.52
F _{Pmax} (Hz)	<98.74	85.7	56.25	58.0	84.8	0.72
Cognitive Auditory P300						
FFT-1(Hz)	<0.24	65.7	29.79	39.8	55.2	0.55
FFT-2(Hz)	>1.61	66.7	41.67	44.6	63.69	0.51
P _{max} (dB/Hz)	>-2.92	63.89	47.92	46.4	65.3	0.55
P _{mean} (dB/Hz)	<-42.03	63.89	62.5	54.6	71.0	0.60
F _{Pmax} (Hz)	<5.73	80.56	56.25	56.5	80.4	0.75

3.5.3 Test combinations

Tables 3.15-17

EP components with the best sensitivity and specificity were combined in pairs to evaluate the diagnosis of any degree of encephalopathy (highlighted: tables 3.13 & 3.14) Pairs of markers were analysed by binary logic regression using the thresholds derived from ROC analysis. EP component pairs were combined in the time-domain, frequency-domain and time-frequency domains.

The optimal EP markers for distinguishing any degree of encephalopathy in the time-domain are the P300 and 'wave V' of the BAEP. In the frequency domain the optimal pair of markers is the second FT peak of the VEP (FFT-2) and the frequency at maximum power of the cortical (N20) somatosensory potential. When the time and frequency domains are combined for logic regression analysis, the optimal pair of markers is both somatosensory – the N20 latency and frequency at peak power.

3.15 Significance comparisons of different test combinations – time domain		
Test combination	Evoked potential <u>latency</u> marker	P value
Combination 1	vN75	0.02
	sN20	0.001
Combination 2	vN75	0.001
	b(wave V)	0.001
Combination 3	vN75	0.02
	P ₃₀₀	0.001
Combination 4	sN20	0.001
	b(wave V)	0.002
Combination 5	sN20	0.003
	P ₃₀₀	0.001
Combination 6	b(wave V)	0.002
	P ₃₀₀	0.001

v – visual; s – somatosensory; b – brainstem; P₃₀₀ – auditory cognitive

3.16 Significance comparisons of different test combinations – frequency domain		
Test combination	Evoked potential <u>spectral</u> marker	P value
Combination 1	vFFT-2	0.04
	sF _(Pmax)	0.002
Combination 2	vFFT-2	0.05
	bF _(Pmax)	0.003
Combination 3	vFFT-2	0.04
	P ₃₀₀ F _(Pmax)	0.01
Combination 4	sF _(Pmax)	0.01
	bF _(Pmax)	0.01
Combination 5	sF _(Pmax)	0.004
	P ₃₀₀ F _(Pmax)	0.02
Combination 6	bF _(Pmax)	0.01
	P ₃₀₀ F _(Pmax)	0.07
v – visual; s – somatosensory; b – brainstem; P ₃₀₀ – auditory cognitive FFT-2 – secondary Fourier peak; F _(Pmax) – frequency at maximum power		

3.17 Significance comparisons of different test combinations – frequency and time domains		
Test combination	Evoked potential marker	P value
Combination 1	P ₃₀₀	0.001
	sF _(Pmax)	0.02
Combination 2	P ₃₀₀	0.001
	bF _(Pmax)	0.02
Combination 3	b(wave V)	0.001
	sF _(Pmax)	0.003
Combination 4	b(wave V)	0.001
	bF _(Pmax)	0.006
Combination 5	sN20	0.001
	sF _(Pmax)	0.002
Combination 6	sN20	0.001
	bF _(Pmax)	0.003
s – somatosensory; b – brainstem; P ₃₀₀ – auditory cognitive F _(Pmax) – frequency at maximum power		

The identified optimal marker combinations were assessed for their sensitivity and specificity in each domain (Table 3.18). Latency measurements in the time domain have sensitivity and specificity of 66% and 85% respectively; the frequency domain demonstrates a modest improvement in sensitivity (70%) with a small comparative loss of specificity (83%). The combination of time and frequency domains demonstrates an improved sensitivity (68%) over latency markers alone with a minor loss of specificity (83%).

Table 3.18
Sensitivity and specificity of paired variables in distinguishing any degree of encephalopathy.

3.18 Comparison of sensitivity and specificity of EP markers tested in pairs.			
Test combination	Evoked potential markers	Sensitivity (%)	Specificity (%)
Time domain	b(wave V) + P ₃₀₀	66	85
Frequency domain	vFFT-2 + sF _(Pmax)	70	83
Time-frequency domains	sN20 + sF _(Pmax)	68	83
s – somatosensory; b – brainstem; P ₃₀₀ – auditory cognitive F _(Pmax) – frequency at maximum power			

Summary

Multimodal EPs were obtained from healthy controls and patients pre-diagnosed with cirrhosis, who presented as either unimpaired, or with minimal or overt encephalopathy. In all sensory modalities (VEP, SSEP and BAEP) patients demonstrated statistically significant prolonged latency differences compared to the controls, in at least one of the waveform markers. This was also observed in the cognitive (P300) potential. The P300 also demonstrated significant differences between the three patient groups; differences between patient groups are less marked in VEPs and BAEPs.

FT analysis of sensory EPs demonstrated a trend of frequency reduction in the main or secondary component as neuropsychiatric impairment increases. Between patient group differences were clearly highlighted in all sensory modalities, but less so for differences between patients and controls. Similar differences occur between groups for the cognitive FTs but these are less marked. The PSD analysis highlighted significant differences between controls and patients for frequency at peak power (all modalities) and peak power (SSEP). Between patient group differences are also well illustrated across all EP modalities.

Multivariate analysis demonstrates that paired waveform markers from either the frequency domain or combined time-frequency domain have a comparable sensitivity and specificity to the time-domain only waveform markers.

The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy.

Chapter 4: Discussion

Control Latency Data

The latency data recorded for the sensory evoked potentials of healthy controls was in keeping with previous normative studies with large test groups. For visual measurements the mean latency for the control groups P100 component was either the same or within 1ms of past research (Chiappa *et al* 1985; Hughes *et al* 1987); in addition, the spread of the data was narrower in this study giving smaller SDs than in the previous literature. Both the mean and SD's of the main somatosensory components, N9, N13 and N20 are near identical with previously published normal studies while the auditory EP components - waves I, II and III – are within 0.2ms of the previous literature and all have similar SD's. The control data obtained were also in keeping with the normal values for the Neurophysiology Department's normal data set for the EP recording device used for this research. The cognitive auditory P300 latency is not currently utilised as a diagnostic tool in clinical neurophysiology, hence normative data is limited. There are conflicting findings due to a wide range of normal values. However, the mean latency and spread of the data for this group fell within the ranges of larger previous studies (Polich *et al* 1985).

Age and Gender.

Gender percentage was balanced within the control group but this could not be achieved for the patient group, although there was no significant gender bias within the HE sub-populations. Similarly, the control and patient group could not be age matched; the age difference between the patient groups was again not significant.

Differences in the visual P100 latency between genders do not become significant until later in life, at approximately >65yrs (Allison *et al* 1984). Other latency differences between genders have been reported, but occur for stimulus parameters (check-size) which were not used in this study. Gender therefore, is not a confounding factor when considering the latency of the control group. Since the mean age of the study group is below 65yrs this, and only 15% of the patients are over 65yrs, the age and gender bias will not be a confounding factor in comparative analyses. Controllable factors such as the stimulus parameters are more likely to have a measurable effect on the latencies. No significant differences between age and gender are reported for the other sensory modalities. The cognitive auditory P300 latency is subject to attention, vigilance and stimulus parameter factors but no significant variability between genders has been reported. It is unclear whether age affects latency in the normal population, with earlier studies finding both for and against a trend of increasing latency with age. Again, the findings are hampered by data sets, for age, having very wide distributions. The entire age range of the control group had a P300 latency of within 1SD of a reported regression line between age and latency (Polich *et al* 1985). Only 10% of the patients exceeded the control group's upper age range and these also fell within 1SD of the variability of age with latency.

Handedness and Hemispheres.

The issue of handedness is of interest in evoked potential studies as sensory EP recordings are made for each hemisphere; cognitive measurements are not an issue as they are derived from mid-line electrodes. The previous

studies utilising evoked potentials in patients with hepatic encephalopathy stated patient and control numbers but it was unclear whether either or both hemispheres were used for recording. Therefore a study of ...“N = 20”...may have had 40 recordings in total with left and right hemispheres averaged for each patient before being added to the overall mean \pm SD analysis. Alternately, one side may have remained unreported, using an unstated criteria and the analysis undertaken such that the number of samples is equal to the number of patients. The third option would be that the left and right hemisphere data were added into an average of the population, the sample number being double the patient number. In this study, measurements for each hemisphere were recorded and the average for each individual used throughout the analysis. This has the advantage of guaranteeing statistical sample independence between patients and so maintains a level of robustness for the statistical tests. These latency data, if added hemispherically would effectively double the sample size for all patients and controls. A calculation exercise made with this sample arrangement increases levels of significance typically from $p = <0.005$ or <0.001 to $p = <0.0001$, (appendix VII). More importantly further significant differences are uncovered *between* HE populations, particularly between both unimpaired and minimal HE compared to overt HE for somatosensory and auditory EPs, and minimal HE compared to unimpaired for visual EPs. These samples are not independent as an underlying pathophysiological process that may be affecting one individual would influence two sets of recorded data. This is also true of the current method, taking an average for an individual. However, the data strongly

suggests that clinical recordings of larger populations would enhance significant differences between population groups and uncover further distinctions between HE sub-groups.

Data reduction during EP acquisition.

In order to perform frequency analysis on the evoked potentials the voltage values were exported and converted into the appropriate formats. Fourier transforms of this data type are normally dependant on the sample frequency and the epoch duration. Initially, upon observing a poor frequency resolution it was discovered that a significant amount of data reduction is built into the EP recording software, presumably to reduce the file size. Given the sample rate and sweep duration it appears that only one in five data points are saved. Subsequently, the FTs were obtained by using a 'sampling interval' calculated from the *actual* number of data points for the sweep, adjusting for each EP modality. It is unclear if any previous research encountered this problem. Although the FT analysis eventually used a Matlab™ code to compensate for the data loss and produce appropriate spectra there is still an issue with frequency resolution. Since power spectral density estimates (PSD) are also based on the FFT, these also are affected. Cursor movement for frequency measurement appears to follow discrete steps with a modest resolution. It follows therefore, that if additional data points are available for the time-frequency domain conversion the frequency resolution would increase so reducing the minimum step between cursor points. This may change the frequency measurements, particularly for the PSD data, and so alter the final statistical findings.

Alternative signal processing techniques.

The ongoing EEG activity can be considered background noise from which the EP waveforms were extracted by signal averaging. It is assumed when obtaining measurements of the final waveform, that the signal had a constant time correlation with the stimulus onset and that the morphology of the EP was consistent throughout. Should this not be the case, the EP signal becomes incorporated into the noise and the amplitude becomes reduced. Similarly, the noise content of the sweep must have zero correlation with the stimulus and the amplitudes of the noise should be constant across all frequencies. The EEG as a signal does not comply with these noise prerequisites, particularly where pathological conditions may generate a rhythmic background; further, the EP may not comply with signal requirements particularly where there is a global metabolic process affecting the whole of the CNS, such as hepatic encephalopathy. The morphology of the final EP waveform is the average of all the morphologies generated by each stimulus, therefore the cursor placement measures the average latency for a given component.

These uncertainties could be overcome by presenting waveform data such that each point had an overall average with its \pm standard deviation. The device used in this study and for most routine clinical applications discards an earlier epoch once the next one has been added to the recalculated average. A large amount of data is lost which could be useful in highlighting the small variations in response that occur for individual stimuli. This also precludes other signal

analysis methods that would require the waveforms for every stimulus-response epochs in an EP investigation, such as auto-regression analysis.

The FT and PSD frequency domain representations used in the study both require the sampled epoch to be a stationary signal, that is, the interval must start and stop at zero amplitude. A window is imposed on the sampled epoch in order to force the start and end of the signal to zero and achieve stationarity. The default window in Matlab™ is 'rectangular', the simplest (Appendix VI) which effectively attenuates only the epoch end points. This appears to be advantageous as the signal content is mostly preserved. However, the consequence of a sudden onset and offset is to cause a rippling artefact – spectral leakage; this is clearly demonstrated in the EP waveforms, particularly the VEP and P300 investigations (figs 3.9 & 3.12). This cyclic fall to zero amplitude potentially removes data that will cause erroneous cursor placement. The alternate windows in common use, for example Hamming, Kaiser and Blackman-Harris, solve the problem of spectral leakage; however, all have steep roll-offs in the lower frequency part of the bandwidth where there is the most physiological activity, and so are also unsuitable (Appendix VI).

While spectral leakage repeats at integer values of the sample rate, this is always some distance away from the area of interest in the spectra since the epoch has been band-limited during the EP recording process; the lowpass filter being no higher than the Nyquist frequency for each recording modality. No cursor measurements were made for frequencies above the low-pass settings for

any of the EP modalities and under these two circumstances the rectangular windowing was employed throughout the study.

Neurophysiological processes.

Evoked potential components can be characterised by latency, amplitude and topographical features as well as external attributes that elicit the response, such as the stimulus variables and the recording montage. The concept of an EP generator is somewhat unclear as it can indicate an anatomical structure that is the origin of the signal, or a theoretical model of a voltage source. It is assumed that the EP signal arises from a synchronous activity of a neuronal population; this is not easily modelled as each neuron possesses multiple excitatory and inhibitory inputs and exists in a complex neural network which has a given orientation to the convoluted cortex of the brain and thence surface recording electrodes. A single EP component therefore, may arise from a combination of responses from separate cell populations which may have been activated by simultaneous parallel pathways, and may not have been in response to the originating axonal volley.

The PSD analysis of the VEP investigations in this study reveals a peak power at frequencies (~15Hz) that correspond to durations of ~65ms; (in the control group and increasing with encephalopathic severity). This duration corresponds to the main wave centred on the P100 component; however, simply looking at the width of the wave may not reveal all of the underlying activity. The FT's indicate that there are two main harmonics contributing to the waveform at ~1Hz and 2Hz. The main wave is composed of these two harmonics; however, if

they originate from different neural networks or processes, they may be differentially affected by the underlying pathophysiology. This is suggested in this study as the two harmonics reveal different behaviours across the patient groups, with the second harmonic absent from patients with overt encephalopathy. Both FFT and PSD spectral methods therefore, can make useful contributions to monitoring or visualising the on-going physiological processes.

The somatosensory and brainstem auditory EPs, by contrast, have PSD frequencies at peak power which do not appear to be in keeping with the FFT components or the duration of the main waveforms. For example, in the SEPs the PSD peak is lower at ~70Hz and in the BAEPs it is higher at ~ 175Hz; (in both modalities the frequencies decrease with increasing severity). Electrographic (invasive EEG) recordings made in patients in preparation for brain surgery have revealed well replicated gamma (>30-90Hz) and high-gamma (≥ 200 Hz) frequencies; these bandwidths have demonstrated, in other research areas, power fluctuations which are linked to information processing (Crone *et al* 2006). It has been assumed that gamma band activity does not transition through the skull with sufficient power to be detected by surface electrodes. However, auditory and somatosensory stimuli have been combined in cognitive studies using surface electrode recordings (Onton & Makeig 2009); high-density EEG data contains gamma-band activity that overlaps spatially with lower frequency bands. The SEP and BAEP investigations in this study have bandwidths that allow recording of gamma activity; further, the signal averaging process overcomes the attenuation from the skull and scalp transition. It follows

that in the frequency domain activities, such as the gamma-band, can be identified and monitored that would not be apparent in latency-only measurements. As gamma activity in combined EPs can be modulated by stress level, mood changes and attention (Kisley & Cornwell 2006), PSD findings in BAEP and SEP investigations may be particularly sensitive to physiological changes that occur in encephalopathy.

The FFT components of the auditory cognitive P300 EP shared a similar feature to the VEP finding in that the Fourier harmonic of 2.6 Hz (duration ~380ms) corresponds to the duration of the main positive wave. However, the PSD peak frequency of 8Hz (decreasing with increasing encephalopathic state) corresponds to a wave of duration of 130ms. This corresponds to the P3a component of the P300 which is normally 75-100ms earlier than the main peak, with a latency of 250-280ms. The main peak is termed the P3b when the earlier component is elicited. The dissociation of P3a from P3b normally occurs when the rare tone includes a 'non-target' or 'deviant' tone; however it can be elicited from the simpler P300 'odd-ball' paradigm if the subject is told not to listen or if there is difficulty in discriminating the rare tone (Comerchero & Polich 1999). The cognitive potential, and more importantly this 8Hz component of the P300 waveform, was the only EP to demonstrate significant differences between healthy controls and all patient groups *and* significant differences between all patient groups. It follows therefore that neuronal networks that are associated with cognition and attention combined are particularly affected by the

pathophysiological changes of encephalopathy; these networks can be clearly identified by the PSD application.

Anomaly of patients with minimal encephalopathy.

Both sensory and cognitive EPs demonstrate a trend of increasing latency compared to controls, the extent of delay increasing with worsening neuropsychiatric state. One patient group, the minimal HE, was either consistently anomalous in regard to latency, or had a comparatively wider variance, where there was significant overlap with unimpaired and overt patient values; (it remained significantly altered compared to the control groups). A possible explanation for this is a reflection of the way HE status is classified for this group. One patient was not encephalopathic on clinical examination but had both abnormal PHES and EEG findings. Of the remaining (12) patients half presented with abnormal PHES scores but normal EEG, while the other half presented with normal PHES scores but abnormal EEG. The minimal HE group may possibly contain a physiologically heterogeneous population; such a group could produce a relatively broader variance, with patient data overlapping significantly with control values, and also the anomalies observed in the between-patient group mean latencies. This feature in the neuropsychiatric classification is a result of the absence of a gold standard test for HE, and so the diagnosis of the patients remains potentially sub-optimal.

The observed trend of a reduction in peak frequency with increase in encephalopathic state is consistent across all EP modalities. This is highlighted in either the main FT component or in the frequency at peak power, and appears to

be resistant to the anomaly in minimal HE classification. This is particularly marked in the FTs for the sensory EPs and the PSDs in the cognitive potentials. The main advantage of frequency domain analysis over latency measurement is seen where alterations in waveform morphology may not be reported as abnormal if the peak measurements still occur within normal limits. Altered morphology in this study was particularly marked in the cortical SSEP and BAEP (figs 3.6 and 3.7 respectively). Given that varying clinical presentations, for example, within the minimal HE group may occur because of varying pathophysiological aspects, the waveform morphology may also be altered variably, producing the wide latency variance. However, the EP generators may not be affected by this aspect of the underlying physiology so that the frequency components are altered to a lesser extent within the group but change significantly between groups. This could be tested by sub-dividing the minimal HE group into, for example, 'minimal: a' – PHES abnormal and 'minimal: b' – EEG abnormal.

ROC Thresholds.

The choice of threshold latencies following ROC analysis was made to provide a balance between sensitivity and specificity. Where there were multiple parameters for each test, i.e. the individual latency peaks, the chosen marker possessed the greatest AUC (area under curve). It could be argued that the thresholds may not be appropriate clinical neurophysiology application given that 'abnormal' latencies are usually pre-defined as control mean + 2SD. Using the latency values obtained in this study such a definition would significantly drive

down the sensitivity. For example, the SSEP (N20) and BAEP (wave V) potentials decrease in sensitivity from 79.5% and 76% respectively to 46%. The P300 is less affected decreasing 86% to 79%. On this basis the logical regression analysis may appear not to be fully robust when utilising the higher, balanced sensitivity and specificity thresholds, although this does not change the AUC for each parameter. Patients presenting for neurophysiological testing will have been previously screened for cirrhosis, the results being used as part of a battery of tests to assist in the assessment of neuropsychiatric status; therefore, the strict use of mean+2SD is not necessarily indicated for this patient group. It may be possible therefore to establish a set of thresholds that are unique to cirrhotic patients with encephalopathy.

Frequency domain studies also produce multiple markers for each EP modality and so a range of sensitivity and specificity values can be considered for further analysis. Each EP has at least one marker with an appropriate ROC-AUC which immediately indicates that the frequency domain compares favourably in the ability to discriminate neuropsychiatric status. The issue of choice with frequency and power thresholds is not so apparent here as there is little data available which can be used to base a pre-determined normal–abnormal definition.

Current utility of EPs in patients with hepatic encephalopathy.

The initial research into the role of EPs in patients with hepatic encephalopathy occurred from 1983-2001 for sensory modalities and 1990-2007 for cognitive investigations, (Appendix II). Inconsistent findings have led the

ISHEN working party to the general consensus that EPs are a useful adjunct to the neurological examination but are often preserved in the presence of an altered EEG (Guerit *et al* 2009); cognitive potentials may be more sensitive to overt HE but it too may not be as reliable as the EEG. Their conclusion, using the graded recommendation scheme, (EASL (European Association for the Study of the Liver) 2009), suggests that further research is required as the current evidence is of moderate quality and the variability invokes a weak recommendation only.

Work has continued using EPs in this area although not with the intensity of the original investigations. The P300 has been further evaluated as a detection method for minimal HE with some success (Teodoro *et al* 2008) . As a result the P300 has been incorporated into the diagnostic criteria to categorise minimal HE for investigations in related areas. Minimal HE has been defined as patients with an abnormal P300 latency and one or more psychometric tests in studies of the role of arterial ammonia (Bragagnolo *et al* 2009), venous ammonia (Sharma & Sharma 2010b) and evaluation of the response to lactulose treatment (Sharma *et al* 2009). Categorising patients with the psychometry and the P300 EP has also been adopted for studies evaluating recent developments, such as the utility of critical flicker frequency in assessing patients recovering from minimal HE (Sharma *et al* 2010a). Cognitive function has been further investigated in the minimal HE group, but again related to time-domain; it has been reported that the shorter latency mismatch negativity (MMN) has a reduced area in cirrhotic

patients with minimal HE than cirrhotic patients without minimal HE (Felipo *et al* 2012). MMN area also increases with increasing PHES score.

Latency studies with sensory EPs therefore, appear to have currently lost some favour in clinical application. Motor EPs however, have been utilised in animal models, where investigation of the underlying mechanism of encephalopathy continues. Alterations in MEP amplitude and latency occur with induced liver failure (Oria *et al* 2010); further, experimental drug treatment of HE can be assessed for by monitoring the preservation of MEPs (Oria *et al* 2012).

Both the original and more recent EP investigations for this patient group were confined to latency measurements. Few studies have explored beyond the time-domain, and where this has occurred the EP latency data has not been used for comparison. For example, source localisation has been obtained from sensory EP recordings in patients with HE through brain activity reconstruction (Blauenfeldt *et al* 2010). VEPs were not sensitive to neuropsychiatric state, but the SSEP source demonstrated an increasing degree of lateralisation with increased level of HE. Frequency domain analysis has been applied to EMG with MEG signals in coherence studies of the motor system (Timmermann *et al* 2008), which demonstrated a significant lowering of frequency with increased grade of HE.

EP utility in this study.

Few researchers have utilised multimodal EP studies, while most highlighted inappropriate EP methodology or patient neuropsychiatric classification. In this study of multiple sensory and cognitive EPs the data allowed for regression

analysis with a bank of tests in time, frequency and time-frequency domains. Multivariate binary logic regression from the time-domain has indicated a *combination* of selected modalities and markers successfully discriminates normal from encephalopathic status; markers tested in pairs also have an improved specificity than a lone EP mode. A similar approach with frequency analysis demonstrates that a comparable discrimination of neuropsychiatric state can also be made. A combination of the time and frequency parameters produces a discrimination that is a modest improvement over time-domain analysis alone.

One of the contentions of this study was that frequency content is not addressed by latency measurements, yet these markers continue to be exclusively used in clinical waveform reporting. The findings from this small study group has provided evidence that when time and frequency are used together patients with altered waveforms, but with normal or near-normal peak measurements will have a higher chance of being screened positively where they are neuropsychiatrically impaired.

The utility of latency and spectral analysis methods in evoked potential recordings from patients with hepatic encephalopathy.

Chapter 5: Conclusions & Further Work

Conclusions.

The scope of this investigation was twofold: to determine the suitability of evoked potential recording in patients with hepatic encephalopathy, and secondly, to determine if frequency domain signal processing could be additionally applied as an adjunct to investigating neuropsychiatric status.

This study has clearly demonstrated that the EP latencies of cirrhotic patients are prolonged in comparison to healthy controls. Delayed latency in one EP modality may not be reflected as abnormalities in the other sensory systems; this study has confirmed that all modalities should be investigated. The patient cohort studied here demonstrated that the combinations of brainstem auditory and cognitive modalities are most suitable for distinguishing encephalopathy.

The alteration of frequency with worsening neuropsychiatric state has been observed consistently across all EP modalities; in addition, contrast is better differentiated between the patient subgroups in the frequency domain. In this patient group, frequency-domain processing was found to distinguish neuropsychiatric status with a sensitivity and specificity similar to that of time-domain processing. In this form of processing however, it is the visual and somatosensory modalities that are the most useful in distinguishing neuropsychiatric state.

When frequency and time domains are combined to detect encephalopathy it is the somatosensory EP that is most useful. In this cohort the SSEP latency and frequency variables provide a sensitivity that is a modest improvement over latency measurement alone. This study therefore, suggests

that a combination of frequency and time domain processing will achieve the optimal sensitivity for investigating neuropsychiatric status.

The findings of this study indicate that a bank of EPs, combined with spectral analysis, could provide a sensitive and specific method for the monitoring the neuropsychiatric status of patients with hepatic encephalopathy.

*

Summary

This study has two purposes; i) to determine the utility of evoked potentials in the diagnosis of encephalopathy, using the standard latency measurements; ii) to investigate the role of signal processing in EPs by determining if frequency analysis can add to the diagnostic potential of EPs.

- sensory and cognitive modalities demonstrate delayed latencies in these patients: figs 3.1-3.4
- combinations of EP latency markers are sensitive to encephalopathic state: table 3.15
- the frequency domain demonstrates clear alterations in patients; differences between neuropsychiatric states are more apparent than latency: figs 3.5-3.12
- combinations of frequency parameters are sensitive to encephalopathic state: table 3.16
- in the detection of encephalopathy frequency domain measurements have comparable sensitivity and specificity to latency measurements: table 3.18

Further work.

Before this study can be repeated instrumentation issues should first be addressed. Waveform acquisition must be made so that there is no data reduction prior to signal averaging or recording. In addition, it is advantageous if every recorded epoch is saved. This would allow further work to benefit from higher resolution for measurements; the preservation of raw data would also allow additional signal analysis calculations, such as autoregression, component analysis and changes in phase relationships. The role of windowing on physiological waveforms can be investigated by both revisiting the data from this study and for a future repeat study. As many of the proprietary windows were unsuitable for the bandwidths typical of EP recordings alternatives could be investigated that are custom designed.

The numbers of patients and controls investigated, with their respective age and gender ratios, was determined by the time limits of this investigation. A larger prolonged repeat of this study would immediately serve to validate the results observed. It would also allow further investigations of sensitivity and specificity thresholds and hence suggest appropriate latency and frequency values specifically for the use of EPs in screening for neuropsychiatric impairment. If a repeat EP study were to have a multi-centre approach, additional demographic factors affecting test sensitivity may be uncovered, for example cultural and educational differences. The control subjects could also be psychometrically tested to reduce the possibility of including individuals with sub-clinical features, due to undisclosed alcohol use. In addition, a larger subject

cohort would enable the aetiologies to be further restricted to include, for example, alcohol-related patients only.

There are additional EP modalities that can be investigated. Motor evoked potentials were not addressed in this study; the bereitshaftspotential (BP) is a non-invasive and painless stimulation that is appropriate for this patient group. The BP is a pre-motor cortical component and therefore may be subject to alterations in waveform morphology or latency where neuropsychiatric state is compromised. The auditory cognitive P300 potential can be further examined for its component waveforms the P3a and P3b. This would require a more complex stimulus paradigm than was available for this study, but recent changes in EP recording devices could allow for the additional programming. An analogous paradigm may also be developed for a visual cognitive potential.

In this study six patients presented for repeat recordings, although only one session from each individual was included in the analysis in order to maintain independence of samples. Three of these patients presented with different levels of neuropsychiatric impairment at each session; all three patients demonstrated an improvement in at least two EP modalities with improvement in HE state. This suggests that a prolonged repeat study could incorporate longitudinal investigations of recurring patients. Variations in latency and frequency components with altered state may therefore be further clarified by serial recordings.

Chapter 6: References

Reference List

- Abou-Assi, S Vlahcevic Z R. "Hepatic encephalopathy, Metabolic consequence of cirrhosis often is reversible." Postgraduate medicine 109(2): (2001): 52--70.
- Alessandrini, M. "Posturography frequency analysis of sound-evoked body sway in normal subjects." European Archives of Oto-Rhino-Laryngology vol./is. 263/3 (2006): 248-52.
- Allison T., Hume A. L. Wood C. C. Goff W. R. "Developmental and aging changes in somatosensory, auditory and visual evoked potentials." Electroencephalogr Clin Neurophysiol 58: (1984): 14--24.
- Als-Nielsen B, Gluud LL and Gluud C. " Non-absorbable disaccharides for hepatic encephalopathy: systematic review of randomized trials." BMJ 328: (2004): 1046.
- Amodio P, Marchetti P Del Piccolo F de Tourtchaninoff M Varghese P Zuliani C Campo G Gatta A and Guérit J M. "Spectral versus visual EEG analysis in mild hepatic encephalopathy." Clinical Neurophysiology, 110: (1999): 1334--1344.
- Amodio P, Montagnese S Gatta A Morgan M Y. " Characteristics of minimal hepatic encephalopathy." Metabolic Brain Disease 19: (2004): 253--267.
- Amodio P, Quero JC Del Piccolo F Gatta A Schalm SW. "Diagnostic tools for the detection of subclinical hepatic encephalopathy: comparison of standard and computerized psychometric tests with spectral-EEG." Metab Brain Dis 11: (1996): 315--327.
- Amodio P, Valenti P Del Piccolo F et al. "P300 latency for the diagnosis of minimal hepatic encephalopathy: Evidence that spectral EEG analysis and psychometric tests are enough." Digestive and Liver Disease 37: (2005): 861--868.
- Araki S, Sato. "Subclinical neurophysiological effects of lead: A review on peripheral, central, and autonomic nervous system effects in lead workers." American Journal of Industrial Medicine.37/2(193-204), (2000): 0271-3586.

- Arkhipova NA, Shevelev IN Grokhovskii NP. "The spatial organization of bioelectrical activity during the treatment of the chronic pain syndrome." Zhurnal Vysshei Nervnoi Deiatelnosti Imeni I.P.Pavlova, 43/4(730-7), (1993): 0044-4677.
- Asher, I. "Comparison of direction and object selectivity of local field potentials and single units in macaque posterior parietal cortex during prehension." Journal of Neurophysiology, May 2007, vol./is. 97/5 (2007): 3684-95.
- Barrett G, Neshige R Shibasaki H. "Human auditory and somatosensory event-related potentials: Effects of response condition and age." Electroencephalogr Clin Neurophysiol 66: (1987): 409--419.
- Beyssen, C. "Electrocorticogram spectral analysis and somatosensory evoked potentials as tools to assess electrical stunning efficiency in ducks." British Poultry Science, June 2004, vol./is. 45/3 (2004): 409-15.
- Bischoff, P. "Effect of remifentanil on clinical and electroencephalographic parameters of depth of anesthesia in balanced anesthesia with propofol, enflurane or isoflurane." Anaesthesiologie und Reanimation, 23/5(116-23) (1998): 0323-4983.
- Blauenfeldt RA, Olesen SS Hansen JB Graversen C Drewes AM. "Abnormal brain processing in hepatic encephalopathy: evidence of cerebral reorganization?" Eur J Gastro Hepat 22: (2010): 1323--30.
- Blei A T and Stolze Larsen F. " Pathophysiology of cerebral edema in fulminant hepatic failure." J Hepatol 1999 31: (1999): 771--776.
- Blei A T, Cordoba J et al. "Practice guidelines. Hepatic Encephalopathy." The American Journal of Gastroenterology 96: (2001): 1968--1976.
- Bobak, P. "Pattern electroretinograms and visual-evoked potentials in glaucoma and multiple sclerosis." American Journal of Ophthalmology, vol./is. 96/1(72-83), (1983): 0002-9394.
- Bolton C. et al. "The effects of temperature on human compound action potentials." J Neurol Neurosurg Psychiatry 44 (1981): 407--413.

- Bombadari G. et al. "Visual Evoked Potential Recordings in Hepatic Encephalopathy and their Variations During Branched Chain Amino-acid Treatment." Hepato-gastroenterol 32 (1985): 3--7.
- Boyes WK, Bercegeay M. "Momentary brain concentration of trichloroethylene predicts the effects on rat visual function." Toxicological Sciences, September 2005, vol./is. 87/1 (2005): 187-96.
- Boyes WK, Hunter E Gary. "Topical exposure of the eyes to the organophosphorus insecticide malathion: lack of visual effects." Journal of Applied Toxicology, 19/6(473-83) (1999): 0260-437.
- Boyes, W. K. "Acute perchloroethylene exposure alters rat visual-evoked potentials in relation to brain concentrations." Toxicological Sciences vol./is. 108/1 (2009): 159-72.
- Bragagnolo MA Jr, Teodoro V Lucchesi LM Ribeiro TC Tufik S Kondo M. "Minimal hepatic encephalopathy detection by neuropsychological and neurophysiological methods and the role of ammonia for its diagnosis." Arq Gastroenterol. 46: (2009): 43--9.
- Brauna JC, Hanley DF Thakor NV. "Detection of neurological injury using time-frequency analysis of the somatosensory evoked potential." Electroencephalography & Clinical Neurophysiology 100/4(310-8), (1996): 0013-4694.
- Braverman ER, Blum K. "Substance use disorder exacerbates brain electrophysiological abnormalities in a psychiatrically-ill population." Clinical Electroencephalography, 1996, vol./is. 27/4 Suppl(5-27), (1996): 0009-9155.
- Brecher M, Porjesz B Begleiter H. "The N2 component of the event-related potential in schizophrenic patients." Electroencephalogr Clin Neurophysiol 66: (1987): 369--375.
- Bromm, B. "Assessment of analgesia by evoked cerebral potential measurements in humans." Postgraduate Medical Journal, 1987, vol./is. 63 Suppl 3/ (1987): 9-13.

- Brusilow SW. "Hepatic encephalopathy." N Engl J Med 1986 314: (1986): 786-In: Blei A T and Stolze Larsen F. Pathophysiology of cerebral edema in fulminant hepatic failure. J Hepatol 1999; 31: 771-776.
- Butterworth R F. "Complications of cirrhosis III. Hepatic encephalopathy." J Hepatol 32 (suppl 1): (2000): 171--180.
- Butterworth R F. "Pathophysiology of hepatic encephalopathy: A new look at ammonia." Metab.Brain Dis 17: (2002): 221--227.
- Caellas et al. "Visual Evoked Potentials in Hepatic Encephalopathy." The Lancet Feb 16 (1985): 394--395.
- Canafoglia, L. "Rhythmic cortical myoclonus in a case of HIV-related encephalopathy." Movement Disorders, December 2003, vol./is. 18/12 (2003): 1533-38.
- Capitano, L. "On-line analysis of AEP and EEG for monitoring depth of anaesthesia." Methods of Information in Medicine, 36/4-5(311-4), (1997): 0026-1270.
- Cheron, G. "Pure phase-locking of beta/gamma oscillation contributes to the N30 frontal component of somatosensory evoked potentials." BMC Neuroscience,2007, vol./is.8/(75) (2007): 1471-2202.
- Chiappa K., Choi S. & Young R. "The results of a new method for the registration of human short latency somatosensory evoked responses." Neurology 28 (1978): 385.
- Chiappa K., Gill E. M. Lentz K. E. "Effect of check size on P100 latency." Electroencephalogr Clin Neurophysiol 61: (1985): 29--30.
- Comerchero MD, Polich J "P3a and P3b from typical auditory and visual stimuli." Clin Neurophysiol 110: (1999): 24-30.
- Conn H O, Leevy C M Vlahcevic Z R Rodgers J B Maddrey W C Seeff L and Levy L L. "Comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy. A double blind controlled trial." Gastroenterology 72: (1977): 573--83.

Cooper, R. Osselton J. W. and Shaw J. C. "EEG Technology." Butterworths, London 3rd Ed (1980).

Crone, N. E., Sinai, A., and Korzeniewska, A.. " High-frequency gamma oscillations and human brain mapping with electrocorticography". Prog Brain Res: (2006): 159: 275–295.

Davies MG, Rowan MJ Feely J. "Flash Visual Evoked Responses in the Early Encephalopathy of Chronic Liver Disease." Scand J Gastroenterol 25 (1990): 1205--1214.

Davies MG, Rowan MJ MacMathuna P Keeling PW Weir DG Feely J. "The auditory P300 event-related potential: an objective marker of the encephalopathy of chronic liver disease." Hepatology 12: (1990): 688--694.

Dawson, G. D. "A summation technique for detecting small signals in a large irregular background." J.Physiol. 115, (1951): pp1-2.

De Groot GH, Schalm SW De Vlieger. "Objective measurement of hepatic encephalopathy in pigs by means of spectral analysis and evoked responses." Brain Research, vol./is. 360/1-2(298-303), (1985): 0006-8993.

de Tommaso, M. "EEG spectral analysis in migraine without aura attacks." Cephalalgia, 18/6(324-8).0333-1024 (1998).

De Vera, L. "Reptilian waking EEG: slow waves, spindles and evoked potentials." Electroencephalography & Clinical Neurophysiology 90/4(298-303), (1994): 0013-4694.

Demirturk L. "The effect of *Helicobacter pylori* Eradication on Gastric Juice and Blood Ammonia Concentrations and on Visual Evoked Potentials in Cirrhotics." Helicobacter 6 (2001): 325--330.

Desjardins P, Bandeira P Raghavendra Rao VL Ledoux S Butterworth RF. "Increased expression of the peripheral-type benzodiazepine receptor-isoquinoline carboxamide binding protein mRNA brain following portacaval anastomosis." Brain Res 758: (1997): 255--58.

- Desjardins P, Rao KV Michalak A et al. "Effect of portacaval anastomosis on glutamine synthetase protein and gene expression in brain, liver and skeletal muscle." Metab Brain Dis 14: (1999): 273--280.
- Desmedt JE, Robertson D. "Differential enhancement of early and late components of the cerebral somatosensory evoked potentials during forced-paced cognitive tasks in man." J Physiol. 271(3): (1977): 761--782.
- Dobie RA, Wilson MJ. "Analysis of auditory evoked potentials by magnitude-squared coherence." Ear & Hearing, vol./is. 10/1(2-13), (1989): 0196-202.
- Drissi, H. "Wavelet transform analysis of visual evoked potentials: Some preliminary results." ITBM-RBM vol./is. 21/2(84-91), (2000): 1297-9562.
- Drummond J. et al. "The effect of high dose sodium thiopental on brainstem auditory and median nerve somatosensory evoked responses in humans." Anesthesiol 63 (1985): 249--254.
- EASL (European Association for the Study of the Liver). "Clinical Practice Guidelines: Management of Chronic hepatitis B." J Hepatol 50: (2009): 227--42.
- Ehara, Y. "Time-varying spectral analysis of auditory evoked potentials (ABR, MLR, SVR)." Japanese Journal of Medical Electronics and Biological Engineering vol./is. 32/3(188-195), (1994): 0021-3292.
- Felipo V, Ordoño JF Urios A El Mili N Giménez-Garzó C Aguado C González-Lopez O Giner-Duran R Serra MA Wassel A Rodrigo JM Salazar J Montoliu C. "Patients with minimal hepatic encephalopathy show impaired mismatch negativity correlating with reduced performance in attention tests." Hepatology 155(2): (2012): 530--9.
- Felix LB, Moraes JE Miranda de Sa AM Yehia HC Moraes MF. "Avoiding spectral leakage in objective detection of auditory steady-state evoked responses in the inferior colliculus of rat using coherence." Journal of Neuroscience Methods, June 2005, vol./is. 144/2 (2005): 249-55.

- Felix, L. B. "Post-processing of auditory steady-state responses to correct spectral leakage." Journal of Neuroscience Methods vol./is. 181/1(145-149), (2009): 0165-270.
- Ferenci P, Lockwood A Mullen K Tarter R Weissenborn K Blei A T and the members of the working party. "Hepatic Encephalopathy-Definition, Nomenclature, Diagnosis, and Quantification: Final Report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998." Hepatology 35: (2002): 716--721.
- Fernandez-Guardiola, A. "Naloxone facilitates sensory precipitation of focal and generalized seizures: evoked potentials and power spectral analysis in the cat." Experimental Neurology vol./is. 101/2(159-75), (1988): 0014-4886.
- Finneran JJ, Houser DS. "Bottlenose dolphin (*Tursiops truncatus*) steady-state evoked responses to multiple simultaneous sinusoidal amplitude modulated tones." Journal of the Acoustical Society of America, vol./is. 121/3 (2007): 1775-82.
- Fitzgerald PG, Picton J. "Temporal and sequential probability in evoked potential studies." Can J Psychol 35: (1981): 188--200.
- Florence, G. "Electroencephalography (EEG) and somatosensory evoked potentials (SEP) to prevent cerebral ischaemia in the operating room." Neurophysiologie Clinique, February 2004, vol./is. 34/1 (2004): 17-32.
- Franowicz MN, Barth DS. "Comparison of evoked potentials and high-frequency (gamma-band) oscillating potentials in rat auditory cortex." Journal of Neurophysiology, 74/1(96-112), (1995): 0022-3077.
- Frederiksen, J. L. "Spectral sensitivity of visual evoked potentials elicited by shifting monochromatic checkerboard patterns-1. Normal observers." Clinical Vision Sciences vol./is. 8/4(365-374), (1993): 0887-6169.
- Freye E, Hartung. "Bremazocine: an opiate that induces sedation and analgesia without respiratory depression." Anesthesia & Analgesia, May 1983, vol./is. 62/5(483-8), (1983): 0003-2999.

- Gajraj RJ, Doi M. "Analysis of the EEG bispectrum, auditory evoked potentials and the EEG power spectrum during repeated transitions from consciousness to unconsciousness." British Journal of Anaesthesia, 80/1(46-52), (1998): 0007-912.
- Gallai V., Alberti A. Balo S. Mazzotta G. Clerici C. Gentili G. Firenze C. Morelli A. "Cognitive event-related potential in hepatic encephalopathy." Acta Neurol Scand 91: (1995): 358--361.
- Gallois, P. "Comparison of spectral analysis and non-linear analysis of EEG in patients with cognitive decline." Neurophysiologie Clinique, November 2002, vol./is. 32/5 (2002): 297-302.
- Gerloff, C. "Multimodal imaging of brain reorganization in motor areas of the contralesional hemisphere of well recovered patients after capsular stroke." Brain, March 2006,(791-808), vol./is. 129/Pt 3 (2006): 1460-2156.
- Giger-Mateeva VI , Reimslag FCC Reits D Liberov B Jones EA Spekreijse H. "Visual event-related potentials in cirrhotic patients without overt encephalopathy." Metab Brain Dis 15.3 (2000): 179--191.
- Goodin DS, Aminoff MJ. "Electrophysiological differences between subtypes of dementia." Brain 109: (1986): 1103--1113.
- Groeneweg M, Quero JC De Bruijn I Hartmann IJC Essink-Bot ML Hop WCJ Schalm SW. "Subclinical hepatic encephalopathy impairs daily functioning." Hepatology 28: (1998): 45--49.
- Guarino, I. "A chronic implant to record electroretinogram, visual evoked potentials and oscillatory potentials in awake, freely moving rats for pharmacological studies." Neural Plasticity, 2004, vol./is.11/3-4 vol./is. 11/3-4 (2004): 241-50.
- Guedon et al. "Detection of subclinical Hepatic Encephalopathy with pattern reversal evoked potentials and reaction time to light: can malnutrition alter the results?" Eur J.Gastro Hepat 5 (1993): 549--554.

- Guerit JM, Amantini A Fischer C Kaplan PW Mecarelli O Schnitzler A Ubiali E Amodio P. "Neurophysiological investigations of hepatic encephalopathy: ISHEN practice guidelines." Liver Int. 29: (2009): 789--96.
- Halliday A.M. "Visual Evoked Responses in the Diagnosis of Multiple Sclerosis." BMJ 4 (1973): 661--664.
- Halliday A.M. "Visual evoked potentials in demyelating disease." In: Demyelinating Disease: Basic and Clinical Electrophysiology Ed: SG Waxman & JM Ritchie, Raven Press, New York (1981): 201--215.
- Halliday A.M. "The value of half-field stimulation in clinical visual evoked potential testing." In: Evoked Potentials. Neurophysiological and Clinical Aspects Ed: C Morocutti & PA Rizzo, Elsevier, Amsterdam (1985): 293--313.
- Harrad RA, Cockram CS Plumb AP Stone S Fenwick. "The effect of hypoglycaemia on visual function: a clinical and electrophysiological study." Clinical Science, , vol./is. 69/6(673-9), (1985): 0143-5221.
- Harrison RW, Ashton CH. "Do cholesterol-lowering agents affect brain activity? A comparison of simvastatin, pravastatin, and placebo in healthy volunteers." British Journal of Clinical Pharmacology, March 1994, vol./is. 37/3 (1994): 231-36.
- Hasegawa S, Abe. "Fourier analysis of pattern visual evoked potentials and changes of the harmonic component in longstanding optic neuritis." Acta Societatis Ophthalmologicae Japonicae 96/11(1449-57), (1992): 0029-203.
- Haussinger D, Kircheis G et al. "Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema?" Journal of Hepatology 32: (2000): 1035--1038.
- Haussinger D, Laubenberger J vom Dahl S Ernst T Bayer S Langer M et al. "Proton magnetic resonance spectroscopic studies on human brain myo-inositol in hypoosmolarity and hepatic encephalopathy." Gastroenterology 107: (1994): 1475--1480 In: Haussinger D, Kircheis G et al. Hepatic encephalopathy in

- chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema?. Journal of Hepatology 2000; 32: 1035-1038.
- Heale VR, Vanderwolf CH Leung LS. "Title: The neurotoxins colchicine and kainic acid block odor-induced fast waves and olfactory-evoked potentials in the dentate gyrus of the behaving rat." Brain Research, 690/2(157-66).0006-8993 (1995).
- Higuchi, K. "Effects of an infusion of branched-chain amino acids on neurophysiological and psychometric testings in cirrhotic patients with mild hepatic encephalopathy." Journal of Gastroenterology & Hepatology 9/4(366-72), (1994): 0815-9319.
- Hollabach S., Kullman F. Frund R. Lock G. "Auditory event-related cerebral potentials (P300) in hepatic encephalopathy - topographic distribution and correlation with clinical and psychometric assessment." Hepato-gastroenterol 44: (1997): 1002--1012.
- Hubl, D. "Multilead quantitative electroencephalogram profile and cognitive evoked potentials (P300) in healthy subjects after a single dose of olanzapine." Psychopharmacology, November 2001.vol./is.158/3(281-8), 0033-3158 (2001).
- Hughes JR, Stone JL Fino JJ Hart LA. "Usefulness of different stimuli in visual evoked potentials." Neurology 37.4 (1987): 656--62.
- Imas OA, Ropella KM Wood JD Hudetz AG. "Halothane augments event-related gamma oscillations in rat visual cortex." Neuroscience, 2004, vol./is. 123/1 (2004): 269-78.
- Iwayama, K. "Changes in electroencephalograms and somatosensory evoked potentials of patients with unilateral cerebrovascular lesions." Brain and Nerve, vol./is. 31/10(1049-1056), (1979): 0006-8969.
- Jutai JW, Gruzelier JH Connolly JF Manchanda. "Schizophrenia and spectral analysis of the visual evoked potential." British Journal of Psychiatry, vol./is.145/(496-501), (1984): 0007-1250.
- Kaiser G. "Windowed Fourier Transforms." In: A Friendly Guide to Wavelets Birkhäuser (1994): 44--59.

- Kamath MV, Reddy SN Ghista DN Upton AR. "Power spectral analysis of normal and pathological brainstem auditory evoked potentials." International Journal of Bio-Medical Computing, vol./is. 21/1(33-54), (1987): 0020-7101.
- Kaplan, B. J. "Phenobarbital and phenytoin effects on somatosensory evoked potentials and spontaneous EEG in normal cat brain." Epilepsia, vol./is. 18/3(397-403), (1977): 0013-9580.
- Kavanagh KT, Domico WD Franks. "Digital filtering and spectral analysis of the low intensity ABR." Ear & Hearing, vol./is. 9/1(43-7), (1988): 0196-202.
- Kawasaki, Y. "Power spectral analysis and digital filtration of brain stem auditory evoked potentials in dogs." American Journal of Veterinary Research, 54/11(1822-6), (1993): 0002-9645.
- Keil, A. "Electrocortical and electrodermal responses covary as a function of emotional arousal: A single-trial analysis." Psychophysiology vol./is. 45/4(516-523), (2008): 0048-5772.
- Kharbanda P S, Saraswat V A Dhiman R K. "Minimal hepatic encephalopathy: diagnosis by neuropsychological and neurophysiologic methods." Indian J Gastroenterol. 22 Suppl 2: (2003): S37--S41.
- Kiers, L. "Variability of motor potentials evoked by transcranial magnetic stimulation." Electroencephalography & Clinical Neurophysiology, December 1993, vol./is. 89/6(415-23) (1993): 0013-4694.
- Kircheis G, Fleig W E Görtelmeyer R Grafe S Häussinger D. "Assessment of low-grade hepatic encephalopathy: A critical analysis." J Hepatol 47: (2007): 642--650.
- Kisley, M. A., Cornwell, Z. M.. "Gamma and beta neural activity evoked during a sensory gating paradigm: Effects of auditory, somatosensory and cross-modal stimulation". Clinical Neurophysiology 117: (2006): 2549–2563.
- Klopp, J. Face-selective spectral changes in the human fusiform gyrus. Clinical Neurophysiology, 110/4(676-82), 1388-2457. 1999.

- Korinthenberg, R. "Prospective neurophysiological study in children treated for acute lymphoblastic leukaemia: serial EEG during treatment and long-term follow up with evoked potentials." European Journal of Pediatrics vol./is. 150/2(127-31), (1990): 0340-6199.
- Krishnan, G. P. "Steady state and induced auditory gamma deficits in schizophrenia." NeuroImage vol./is. 47/4(1711-1719), (2009): 1053-8119.
- Ku, Y. "Prefrontal cortex and somatosensory cortex in tactile crossmodal association: An independent component analysis of ERP recordings." PLoS ONE vol./is. 2/8, (2007): 1932-6203.
- Kuba M.et al. "Advanced Electrophysiological Diagnostics of Hepatic and Portosystemic Encephalopathy." Acta Medica 39 (1996): 21--26.
- Kuba, M. "Advanced electrophysiological diagnostics of hepatic and portosystemic encephalopathy." Acta Medica 39/1(21-5) (1996): 1211-4286.
- Kulger C.F.A., Lotterer E. Petter A. Wensing G. Taghavy A. Hahn E. G. Fleig W. E. "Visual event-related P300 potentials in early portosystemic encephalopathy." Gastroenterology 130: (1992): 302--310.
- Kulger CFA, Petter A Taghavy A Lotterer E Wensing G Hahn EG Fleig WE. "Dynamics of cognitive brain dysfunction in patients with cirrhotic liver disease: an event-related P300 potential perspective." Electroencephalogr Clin Neurophysiol 91: (1994): 33--41.
- Kusske JA, Romiti JA Porter RW Verzeano. "Somatosensory-evoked potentials and slow oscillations in patients with cerebral lesions." Neurological Research, vol./is. 2/1(47-67), (1980): 0161-6412.
- Lai JCK, Cooper AJL. "A-ketoglutarate dehydrogenase complex: kinetic properties, regional distribution and effects of inhibitors." J Neurochem 47: (1986): 1376--1386. In: Butterworth R F, Complications of cirrhosis III. Hepatic encephalopathy. J Hepatol 2000; 32 (suppl 1): 171-180.

- Laubenberger J, Haussinger D Bayer S Gufler H Hennig J and Langer M. "Proton magnetic resonance spectroscopy of the brain in symptomatic and asymptomatic patients with liver cirrhosis." Gastroenterology 112: (1997): 1610--1616 In: Haussinger D, Kircheis G et al. Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema?. Journal of Hepatology 2000; 32: 1035-1038.
- Laurian S Le PK, Baumann. "Relationship between plasma-levels of chlorpromazine and effects on EEG and evoked potentials in healthy volunteers." Pharmacopsychiatria, November 1981, vol./is. 14/6(199-204), (1981): 0720-4280.
- Lewis EG, Dustman RE Beck EC. "Visual and somatosensory evoked potentials characteristics of patients undergoing hemodialysis and kidney transplantation." Electroencephalography & Clinical Neurophysiology vol./is. 44/2(223-31), (1978): 0013-4694.
- Liang, H. "Synchronized activity in prefrontal cortex during anticipation of visuomotor processing." Neuroreport, November 2002, vol./is. 13/16 (2002): 2011-15.
- Liesiene, R. "Evaluation of the functional brain state in comatose children." Medicina (Kaunas, Lithuania), vol./is. 42/5 (2006): 355-61.
- Lippe S., Martinez-Montes. "Electrophysiological study of auditory development." Neuroscience vol./is. 164/3(1108-1118), (2009): 0306-4522.
- Lockwood AH, Weissenborn K Burchert W Bokemeyer M Donnelly KZ Manns MP. "Correlations between neuropsychological test performance and brain glucose metabolism in non-alcoholic cirrhotics." In: Yurdaydin C, Bozkaya H, eds. Advances in Hepatic Encephalopathy and Metabolism in Liver Disease. Ankara: University Press (2000): pp319--324.
- Loganovsky, K. N. "Vegetative-vascular dystonia and osteoalgetic syndrome or chronic fatigue syndrome as a characteristic after-effect of radioecological disaster: The Chernobyl accident experience." Journal of Chronic Fatigue Syndrome vol./is. 7/3(3-16), (2000): 1057-3321.

Longmore M, Wilkinson I B Rajagopalan S R. Oxford Handbook of Clinical Medicine. 6th ed Oxford University Press (2005).

Manas S, Sanchez JL Moreno. "[The combined application of visual evoked potentials and the quantified electroencephalogram improve discrimination of cerebral maturity]. [Spanish] La aplicacion conjunta del potencial evocado visual y el electroencefalograma cuantificado mejora la discriminacion de la maduracion cerebral." Revista de Neurologia, August 1997, vol./is. 25/144.(1181-6), 0210-0010 (1997).

Marrelli, A. "Spectral analysis of visual potentials evoked by pattern-reversal checkerboard in juvenile patients with headache." Headache, September 2001, vol./is. 41/8 (2001): 792-97.

Marshall N.K.& Donchin E. "Circadian variation in the latency of brainstem responses and its relation to body temperature." Science 212 (1981): 356--358.

Matthews W.& Small D. "Serial recording of visual and somatosensory evoked potentials in multiple sclerosis." J Neurol Sci 40 (1979): 11--21.

McLaughlin TJ, Koff RS. "Feedback EEG in the detection of subclinical hepatic encephalopathy: a preliminary report." International Journal of Psychophysiology, vol./is. 8/2(137-43), (1989): 0167-8760.

Meador KJ, Loring DW King DW Gallagher BB Rogers OL Smith JR Flanigin HF. "Spectral power of human limbic evoked potentials: relationship to seizure onset." Annals of Neurology, vol./is. 23/2(145-51), (1988): 0364-5134.

Meador KJ, Loring DW Huh. "Spectral analysis of sphenoidal evoked potentials predicts epileptic focus." Epilepsia, vol./is. 29/4(434-9), (1988): 0013-9580.

Mehndiratta M.M.et al. "Comparative Evaluation of Visual, Somatosensory and Auditory Evoked Potentials in the Detection of Subclinical Hepatic Encephalopathy in Patients with Nonalcoholic Cirrhosis." J.Am Gastro 85 (1990): 799--803.

- Merkel C, Bolognesi M Angeli P Noventa P Caregaro L Sacerdoti D Gatta A. "Prognostic indicators of survival in patients with cirrhosis and esophageal varices without previous bleeding." Am J Gastroenterol 84: (1989): 717--722.
- Montagnese A, Amodio P and Morgan M Y. "Methods for diagnosing hepatic encephalopathy in patients with cirrhosis: A multidimensional approach." Metab Brain Dis 19. 281- (2004): 312.
- Montagnese S, Jackson C and Morgan M Y. "Spatio-temporal decomposition of the electroencephalogram in patients with cirrhosis." J Hepatol 46: (2007): 447--458.
- Moody EB Jr, Micheli-Tzanakou E Chokroverty. "An adaptive approach to spectral analysis of pattern-reversal visual evoked potentials." IEEE Transactions on Biomedical Engineering, vol./is.36/4(439-47), (1989): 0018-9294.
- Morgan M Y, Alonso M and Stanger L C. "Lactitol and lactulose for the treatment of subclinical hepatic encephalopathy in cirrhotic patients A randomised, cross-over study." J Hepatol. 8: (1989): 208--217.
- Morstyn R, Duffy FH McCarley RW. "Altered P300 topography in schizophrenia." Arch Gen Psychiatry 40: (1983): 729--734.
- Mousseau DD, Baker GB Butterworth RF. "Increased density of catalytic sites and expression of brain monoamine oxidase A in humans with hepatic encephalopathy." J Neurochem 68: (1997): 1200--8.
- Murali S., Vladimir. "Analysis of fractal and fast Fourier transform spectra of human electroencephalograms induced by odors." International Journal of Neuroscience vol./is. 117/10(1383-1401), (2007): 0020-7454.
- Muttray, A. "Acute effects of an organic solvent mixture on the human central nervous system." European Journal of Medical Research, September 2005, vol./is. 10/9 (2005): 381-88.
- Nagaraj HC, Radhakrishnan S Srinivasan. "Acquisition and analysis of brainstem auditory evoked responses of normal and diseased subjects by spectral

- estimation." Frontiers of Medical & Biological Engineering, 10/1(67-75), (2000): 0921-3775.
- Nakamura E, Lee KP So. "Effects of endogenous and exogenous nitric oxide on electrical responses of circular smooth muscle isolated from the guinea-pig stomach antrum." Journal of Smooth Muscle Research, October 2004, vol./is. 40/4-5 (2004): 183-98.
- Neidermeyer E.& da Silva F. "Electroencephalopathy - Basic Principles, Clinical Applications and Related Fields." 2nd Ed., (1982): Urban & Schwarzenberg,- Baltimore-Munich.
- Niedermeyer E. "The normal EEG of the waking adult." Electroencephalography: basic principles, clinical applications and related fields. In: **Niedermeyer E., Lopes de Silva F., editors**. Baltimore: Williams and Wilkins. 4th Edition. (1999): 149--173.
- Nora D.B.et al. "Evoked Potentials for the Evaluation of Latent Hepatic Encephalopathy in Paediatric Liver Transplant Candidates." Journal of Paediatric Gastroenterology and Nutrition 31 (2000): 371--376.
- Nuwer, M. R. "Frequency analysis and topographic mapping of EEG and evoked potentials in epilepsy." Electroencephalography & Clinical Neurophysiology, vol./is. 69/2(118-26), (1988): 0013-4694.
- Ogawa, T. "Hemispheric asymmetries in preterm infants: Autoregressive spectral analysis of visual and auditory evoked potentials." Brain and Nerve, vol./is. 32/1(97-105), (1980): 0006-8969.
- Ogawa, T. "Neurophysiological study of autistic children." Brain & Development March 1989 vol./is. 21/2 (1989): 163-69.
- Olde Damink SW, Jalan R Deutz NE et al. " The kidney plays a major role in the hyperammonemia seen after simulated or actual GI bleeding in patients with cirrhosis." Hepatology 37: (2003): 1277--1285.

- Onton J., and Makeig S. "High-frequency broadband modulations of electroencephalographic spectra." Frontiers in Human Neuroscience 3: (2009): 1-18.
- Oppenheim, A. V. and R. W. Schaffer. "*Discrete-Time Signal Processing*." Prentice-Hall (1989): 447--448.
- Oria M, Chatauret N Chavarria L Romero-Giménez J Palenzuela L Pardo-Yules B Arranz JA Bodega G Raguer N Córdoba J. "Motor-evoked potentials in awake rats are a valid method of assessing hepatic encephalopathy and of studying its pathogenesis." Hepatology 52: (2010): 2077--85.
- Oria M, Romero-Giménez J Arranz JA Riudor E Raguer N Córdoba J. "Ornithine phenylacetate prevents disturbances of motor-evoked potentials induced by intestinal blood in rats with portacaval anastomosis." J Hepatol. 56 (2012): 109--14.
- Ortiz M, Jacas C and Córdoba J. " Minimal hepatic encephalopathy: diagnosis, clinical significance and recommendations." J Hepatol 42: (2005): S45--S46.
- Parsons-Smith B G, Summerskill W H J Dawson A M and Sherlock S. "The electroencephalograph in liver disease." Lancet 2: (1957): 867--871.
- Picton TW, Stapells DR Campbell KB. "Auditory evoked potentials from the human cochlea and brainstem." J.Otolaryngol. 9 (1981): 1--41.
- Picton TW, Stuss DW Champagne SC Nelson RF. "The effects of age on human event-related potentials." Psychophysiology 21: (1984): 312--325.
- Pierelli F. et al. "Electrophysiological study in patients with chronic hepatic insufficiency." Acta Neurol **85** (1985): 284--291.
- Pietz, J. "Effect of high-dose tyrosine supplementation on brain function in adults with phenylketonuria." Journal of Pediatrics, December 1995, vol./is. 127/6(936-43), (1995): 0022-3476.

- Pinkerton, F. "A neurophysiological study of children with reading, writing and spelling difficulties." Developmental Medicine & Child Neurology vol./is. 31/5(569-81), (1989): 0012-1622.
- Plourde, G. "Ketamine increases the amplitude of the 40-Hz auditory steady-state response in humans." British Journal of Anaesthesia, 78/5(524-9), (1997): 0007-912.
- Polich J, Howard L Starr A. "Effects of age on the P300 component of the event-related potential from auditory stimuli: Peak definition, variation and measurement." J Gerontol 40: (1985): 721--726.
- Polich J. "Normal variation of P300 from auditory stimuli." Electroencephalogr Clin Neurophysiol 62: (1986): 236--240.
- Polich J. "Task difficulty, probability and inter-stimulus interval as determinants of P300 from auditory stimuli." Electroencephalogr Clin Neurophysiol 68: (1987): 311--320.
- Pozzessere G.et al. "Brainstem auditory evoked potentials in toxic, metabolic and anoxic coma." Rivista di Neurologica 58 (1988): 183--188.
- Prasad S, Dhiman R K Duseja A Chawla Y K Sharma A Agarwal R. "Lactulose improves cognitive functions and health-related quality of life in patients with cirrhosis who have minimal hepatic encephalopathy." Hepatology 45: (2007): 549--559.
- Pratt H., Politoski D., Starr A. "Mechanically and electrically evoked somatosensory evoked potentials in humans: Effects of stimulus presentation rate." Electroencephalogr Clin Neurophysiol 49: (1980): 240-249.
- Pugh, R. N., Murray-Lyon, I. M., Dawson, J. L., Pietroni, M. C., & Williams, R. "Transection of the oesophagus for bleeding oesophageal varices", British Journal of Surgery, 60: (1973): 646-649.
- Reeves R.R., Struve A. Rash C. J. Burke R. S. "P300 cognitive evoked potentials before and after liver transplantation." Metab Brain Dis 22: (2007): 139--144.

- Reitan R M. " The relation of the trail making test to organic brain damage." J Consult Psychol. 19: (1955): 393--394.
- Restuccia, D. "Influence of cholinergic circuitries in generation of high-frequency somatosensory evoked potentials." Clinical Neurophysiology, August 2003, vol./is. 114/8 (2003): 1538-48.
- Romero-Gomez M, Boza F Garcia-Valdecasas MS Garcia E Aguilar-Reina J. "Subclinical hepatic encephalopathy predicts the development of overt hepatic encephalopathy." Am J Gastroenterol 96: (2001): 2718--2723.
- Romero-Gomez M, Ramos-Guerrero R Grande L et al. " Intestinal glutaminase activity is increased in liver cirrhosis and correlates with minimal hepatic encephalopathy." J Hepatol 41: (2004): 49--54.
- Rosenberg C, Nudelman K Starr A. "Cognitive evoked potentials (P300) in early Huntingdon's disease." Arch Neurol 42: (1985): 984--987.
- Rowe, MJ. "Normal variability of brainstem auditory evoked response in young and old adult subjects." Electroencephalogr Clin Neurophysiol 44: (1978): 459--470.
- Rowe, MJ. "Brainstem auditory evoked potentials in neurological disease: A review." Ear Hear 2: (1981): 41--51.
- Rush JL, Kusske JA Porter RW Verzeano. "Driving of slow oscillations in the human somatosensory system." Electroencephalography & Clinical Neurophysiology vol./is. 41/2(168-78), (1976): 0013-4694.
- Russell GB, Vance WT Graybeal JM. "Attenuation of midazolam-induced EEG activation in rats by both flumazenil and hyperbaric oxygen." Journal of Neurosurgical Anesthesiology, 7/4(271-9), (1995): 0898-4921.
- Sandford S.L., Tarter R. E. et al. "Sensory information processing in patients with non-alcoholic cirrhosis." J.Neurol Sci 80 (1987): 296--276.
- Sawhney I.M.S.et al. "Electrophysiological Assessment of Early Hepatic Encephalopathy in Chronic Liver Disease." Neurology India 43 (1995): 11--17.

- Schneider, D. "Influence of 3 antivertiginous medications on the vigilance of healthy volunteers." International Journal of Clinical Pharmacology & Therapeutics, April 2003, vol./is. 41/4 (2003): 171-81.
- Schomerus H, Hamster W, Blunck H, Reinhard K, Mayer K, Dölle W. "Latent portasystemic encephalopathy. I. Nature of cerebral functional defects and their effect on fitness to drive." Dig Dis Sci 26: (1981): 622--630.
- Shahroki F, Chiappa KH, Young RR. "Pattern shift visual evoked responses: Two hundred patients with optic neuritis and/or multiple sclerosis." Arch Neurol 35: (1978): 65--71.
- Sharma P, Sharma BC, Sarin SK. "Predictors of nonresponse to lactulose for minimal hepatic encephalopathy in patients with cirrhosis." Liver Int. 9: (2009): 1365--71.
- Sharma P, Sharma BC, Sarin SK. "Critical flicker frequency for diagnosis and assessment of recovery from minimal hepatic encephalopathy in patients with cirrhosis." Hepatobiliary Pancreat Dis Int. 9: (2010): 27--32.
- Sharma P, Sharma BC. "Predictors of minimal hepatic encephalopathy in patients with cirrhosis." Saudi J Gastroenterol 116(3): (2010): 181--7.
- Shimoyama, I. "Flash-related synchronization and desynchronization revealed by a multiple band frequency analysis." Japanese Journal of Physiology vol./is. 50/5(553-559), (2000): 0021-521.
- Shiogai, T. "Evaluation of electrophysiological neuromonitoring in the diagnosis of brain death: Part III. Compressed spectral arrays." Brain & Nerve, vol./is. 41/3(309-18), (1989): 0006-8969.
- Shiogai, T. "Relationship between cerebral circulatory arrest and loss of brain functions--analysis of patients in a state of impending brain death." Clinical Neurology 33/12(1328-30), (1993): 0009-918.
- Simpson RK Jr, Contant CF, Robertson CS, Goodman JC. "Spectral analysis of corticomotor evoked potentials in spinal cord injury. Part 1. Acute studies." Neurological Research, 15/6(367-72), (1993): 0161-6412.

- Simpson RK Jr, Contant CF Robertson CS Goodman JC. "Spectral analysis of corticomotor evoked potentials in spinal cord injury. Part 2. Chronic studies." Neurological Research, 15/6(373-8), (1993): 0161-6412.
- Skuse NF, Burke D. "Power spectrum and optimal filtering for visual evoked potentials to pattern reversal." Electroencephalography & Clinical Neurophysiology, vol./is. 77/3(199-204), (1990): 0013-4694.
- Snyder E., Hillyard S. A., Galambos R. "Similarities and differences among the P3 waves to detect signals in three modalities." Psychophysiology 17: (1980): 112-122
- Sollazo D. et al. "Evoked Potentials (Visual and Somatosensory) in Hepatic Encephalopathy: a Preliminary Report." Arch Psicol Neurol Psichiat 44(3) (1983): 318--321.
- Spileers, W. "Contrast modulated steady state visual evoked potentials (CMSS VEPS) measuring static and dynamic contrast sensitivity." Clinical Vision Sciences, vol./is. 7/2(93-106), (1992): 0887-6169.
- Srinivasan, R. "Steady-state visual evoked potentials: distributed local sources and wave-like dynamics are sensitive to flicker frequency." Brain Topography vol./is. 18/3 (2006): 167-87.
- St.Clair DM, Blackwood DHR Christie JE. "P3 and other long latency auditory evoked potentials in presenile dementia Alzheimer type and alcoholic Korsakoff syndrome." Br J Psychiatry 147: (1985): 702--706.
- Stejskal, L. "Experience of 500 cases of neurophysiological monitoring in carotid endarterectomy." Acta Neurochirurgica vol./is. 149/7 (2007): 681-88.
- Stockard J.& Sharbrough F. "Unique contributions of short latency somatosensory evoked potentials in patients with neurological lesions." Prog Clin Neurophysiol 7 (1980): 231--263.
- Tarter R.et al. "Relationship Between Hepatic Injury Status and Event Related Potentials." Clinical Electrophysiology 18 (1987): 15--19.

- Tebano MT, Luzi M. "Effects of cholinergic drugs on neocortical EEG and flash-visual evoked potentials in the mouse." Neuropsychobiology 40/1(47-56), (1999): 0302-282.
- Teodoro V, Bragagnolo Jr M Lucchesi L Kondo M Tufik S. "Evaluation of the event-related potential (ERP-P300) in patients with hepatic cirrhosis without encephalopathy." Arg Gastroenterol. 45: (2008): 82--6.
- Timmermann L, Butz M Gross J Ploner M Südmeyer M Kircheis G Häussinger D Schnitzler A. "Impaired cerebral oscillatory processing in hepatic encephalopathy." Clinical Neurophysiology 119: (2008): 265--72.
- Trick GL, Trobe JD Dawson WW Trick LR McFadden. "Power spectral analysis of visual evoked potentials in multiple sclerosis." Current Eye Research, vol./is.3/10(1179-86), (1984): 0271-3683.
- Trzepacz P.T., Sclabassi R. J. & Van Thiel D. H. "Delirium: A subcortical Phenomenon?" J Neuropsychiatry 1(3) (1989): 283--289.
- Tsubokawa, T. "Prediction of outcome of prolonged coma caused by brain damage." Brain Injury, vol./is. 4/4(329-37), (1990): 0269-9052.
- Watanabe A, Sakai T Sato S Imai F Ohto M Arakawa Y Toda G et al. "Clinical efficacy of lactulose in cirrhotic patients with and with-out subclinical hepatic encephalopathy." Hepatology 26: (1997): 1410--1414.
- Watanabe A, Tuchida T Yata Y Kuwabara J. "Evaluation of neuropsychological function in patients with liver cirrhosis with special reference to their driving ability." Metab Brain Dis 10: (1995): 239--248.
- Weissenborn K, Ennen J C Schomerus H Rückert N and Hecker H. "Neuropsychological characterization of hepatic encephalopathy." J Hepatol 34: (2001): 768--773.
- Weissenborn K, Scholz M Hinrichs H Wiltfang J Schmidt FW Kunkel et al. "Neurophysiological assessment of early hepatic encephalopathy." Electroencephalogr Clin Neurophysiol 75: (1990): 289--295.

- Winterer, G. "Cortical activation, signal-to-noise ratio and stochastic resonance during information processing in man." Clinical Neurophysiology, 110/7(1193-203), (1999): 1388-2457.
- Wright G, Jalan R. "Management of hepatic encephalopathy in patients with cirrhosis." Best Practice & Research Clinical Gastroenterology 21: (2007): 95--110.
- Yang S., Chu N. & Liaw Y. "Somatosensory Evoked Potentials in Hepatic Encephalopathy." Gastroenterology 89 (1986): 625--30.
- Yargicoglu, P. "The effects of cadmium and experimental diabetes on VEP spectral data and lipid peroxidation." International Journal of Neuroscience, 93/1-2(63-74), (1998): 0020-7454.
- Young SL, Lal S Feldmuller F et al. "Relationships between tryptophan in serum and CSF, and 5-hydroxyindolacetic acid in CSF of man: effects of cirrhosis of the liver and probenecid administration." J Neurol Neurosurg Psychiatry 38: (1975): 322--30 In: Abou-Assi, S, Vlahcevic Z R. Hepatic encephalopathy, Metabolic consequence of cirrhosis often is reversible. Postgraduate medicine 2001;109(2): 52-70.
- Zappoli, R. "Topographic bit-mapped event-related neurocognitive potentials and clinical status in patients with primary presenile mental decline chronically treated with nicergoline." Current Therapeutic Research - Clinical and Experimental vol./is. 49/6(1078-1097), (1991): 0011-393.
- Zappoli, R. "Effect of physiological and pathological aging processes on topographic bit-mapped cognitive evoked potentials in presenile subjects." Acta Neurologica 13/6(569-73), (1991): 0001-6276.
- Zeneroli M.L.et al. "Visual Evoked Potential: a diagnostic tool for the assessment of hepatic encephalopathy." Gut 25 (1984): 291--299.
- Zimmermann, P. "Electrophysiological profile of neurotropics (hydergine, piracetam, pyritinol) in organic brain disease in the aged." Drug Development Research, 1982, vol./is. 2/5(481-488), (1982): 0272-4391.

Chapter 7: Appendices

Appendix I

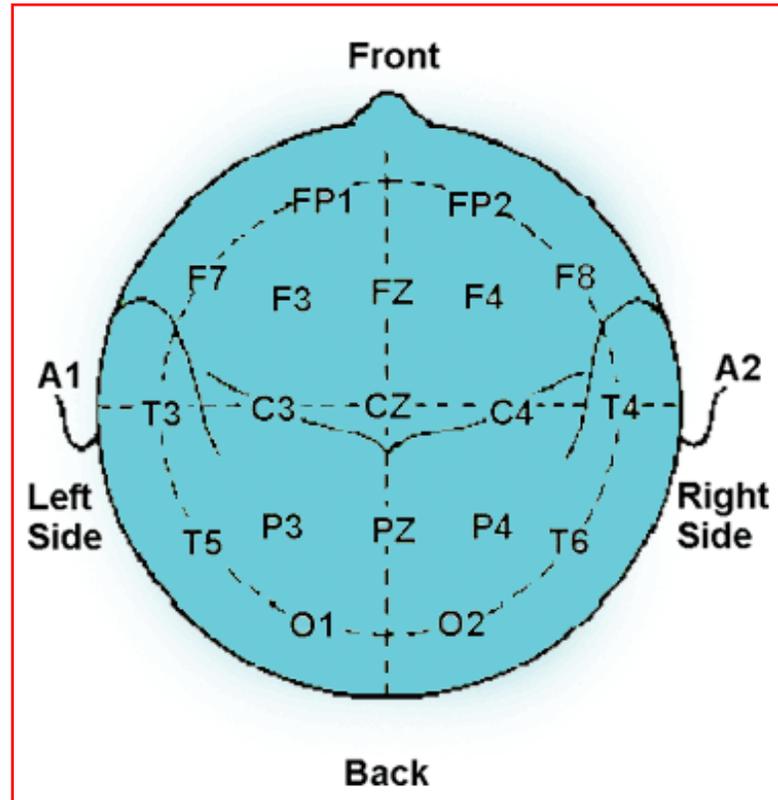
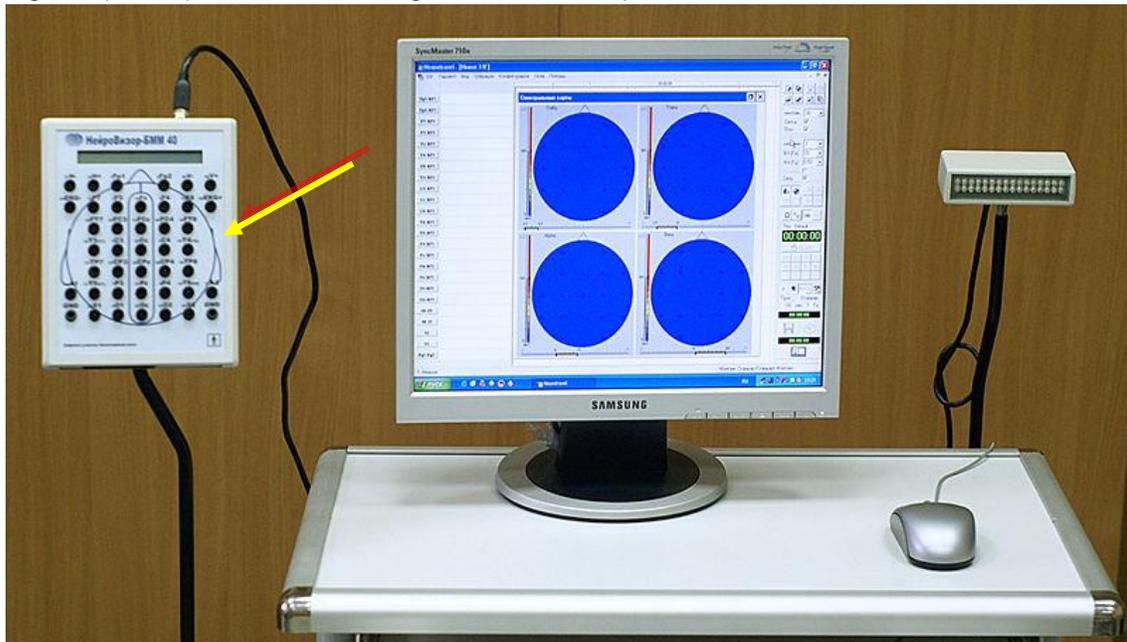


Fig 7.1 (above): The 10-20 electrode placement system

Fig 7.2 (below): An EP recording device; the amplifier headbox is indicated.



Appendix II

Table 7.1: Summary table of previous VEP studies in patients with hepatic encephalopathy.

Publication period	1983 - 2001		
Number of studies	21 20 adult		
Number of patients	584, of which 569 adult	Age: 45.2±10.6* yrs (range 13 – 78) Paediatric study 8.6±2.5* yrs (range 5-15)	* mean±SD
Diagnoses of liver pathology	Serum biochemistry* and biopsy 10 Biopsy 8 exclusive method Others not stated	common serum parameters: albumin prothrombin time ammonia levels	
Aetiologies	Cirrhotic only: 8 Cirrhosis with other aetiologies*: 9 Non-cirrhotic liver disease: 5	* Alcoholic cirrhosis Hepatitis (viral and autoimmune) Primary Billiary Cirrhosis Non-cirrhotic fibrosis Extra-hepatic billiary atresia Paracetamol overdose Malnutrition ALD Cryptogenic	

Patient selection criterion	<p><u>Inclusion:</u></p> <ul style="list-style-type: none"> No clinical signs of hepatic encephalopathy (HE₀) No history of alcoholism (Mehndiratta et al 1990) No medication within previous 24hrs that has a known neurological effect Normal visual acuity Normal ERG 	<p><u>Exclusion:</u></p> <ul style="list-style-type: none"> Abnormal visual acuity Neurological disorders Sedation within 24hrs Psychotropic medication Metabolic disorders including diabetes and uraemia Overt HE (Demirturk 2001) History of alcoholism (Nora 2000) Malnutrition Treatment with Lactulose & Neomycin (Zenerolli 1984) GI bleeding
Assessment of encephalopathy	<ul style="list-style-type: none"> Parsons-Smith (1957) 9 Child-Pugh 2 Adams & Foley (1953) 1 Zenerolli (1984) 1 Zeive (1987) 1 Conn / Child-Pugh 1 MMSE & Reitan 1 Not stated 3 	
EP Investigation	<p>Flash 7: 5/7 one channel recording; bilateral studies not 10-20 system</p> <p>Pattern 14: 3 bilateral recordings only. 20 reported 10-20 electrode system</p>	Post processing not stated in 15 studies
Results	<p>Flash: 6/7 studies demonstrate at least one VEP component significantly different vs control group</p> <p>Pattern: 2 studies clear significant differences</p>	

Table 7.2: Summary table of previous BAEP studies in patients with hepatic encephalopathy.

Publication period	1985 - 1995	
Number of studies	12 (adult data)	
Number of patients	400	Age 41.9±6.8* (range 27 – 71yrs) *Mean ± 1SD
Diagnoses of liver pathology	Cirrhosis Fibrosis (1) Hepatocellular carcinoma (1)	Serum biochemistry (exclusive: 1) Histopathology Ultrasonography Not stated (3)
Aetiologies	Unspecified cirrhosis (exclusive:2) Chronic active hepatitis Cryptogenic Idiopathic Alcoholic cirrhosis Coma following hepatic insufficiency	
Patient selection criterion	<u>Inclusion:</u> Normal tonal audiometry No clinical signs of hepatic encephalopathy (HE ₀) > 6 weeks post variceal bleed	<u>Exclusion:</u> previous h/o alcoholism previous h/o hepatitis psychiatric illness neurological trauma current medication affecting CNS infection diabeties, renal failure, uremia sedation within previous 24hrs malnutrition

Assessment of encephalopathy	Parsons-Smith (1957) 5 Conn (1977) 3 MMSE & Reitan (A & B) 1 Zieve (1987) N/A (Coma study using GCS) 1	
EP Investigation	Stimulation: monoaural with white noise: (4) - remainder not stated Threshold + SPL: 4 SPL only: 4 10 – 20 recording system 9; not stated in other studies	Three dedicated studies; remainder mixed EP's Post processing not fully stated in 6 studies
Results	<u>Changes v controls</u> Latencies waves I – V: 2 IPL's:1 Both waves I – V and IPL's: 5 No significant electrophysiological changes:3	

Table 7.3: Summary table of previous SSEP studies in patients with hepatic encephalopathy.

Publication period	1983 – 1998		
Number of studies	13 (adult data)	11 Experimental studies 1 review paper 1 Case study	
Number of patients	552	Age 42.4±12* (Range 17 – 74)	
			* mean±SD
Diagnoses	Cirrhosis only studies (2) WD (1) 'LD' (4) Mixed patient group: Active hepatitis ALD Cryptogenic	Serum biochemistry* and biopsy Biopsy (3) exclusive method Biochemistry (2) exclusive Ultrasonography not stated (4)	*common serum parameters: albumin prothrombin time ammonia levels
Aetiologies	Hepatitis B Hepatitis C Hepatitis B & C Alcoholic cirrhosis Cholestatic LD Hepatocellular disease Hepatocellular Carcinoma Not stated (2)		

<p>Patient selection criterion</p> <p>Patient selection criterion (cont.)</p>	<p><u>Inclusion:</u></p> <p>No clinical signs of hepatic encephalopathy (HE₀) No history of alcoholism (Mehndiratta et al 1990) No medication within previous 24hrs that has a known neurological effect</p> <p>Not stated (4)</p>	<p><u>Exclusion:</u></p> <p>Neurological disorders (including psychiatric) Sedation within 24hrs Psychotropic medication Metabolic disorders including diabetes and uraemia Overt HE History of alcoholism Malnutrition GI bleeding Renal failure Infection</p>
<p>Assessment of encephalopathy</p>	<p>Parsons-Smith (1957) 5 Conn 4 MMSE & Reitan 1 Not stated 1 N/a 1 (liver transplant case study)</p>	
<p>EP Investigation</p>	<p>Median nerve (12) Median & Peroneal nerves (1) Cortical recording only 3 Cortical & Central 3 Cortical, Central & Peripheral 5</p>	<p>Post processing*: incomplete data (9)</p> <p>* Reproducibility, no. trials per average, Summing R & L limb data</p>
<p>Results</p>	<p>Only 2 reports suggest that SEP adds no further information, while a single paper suggests this modality is only sensitive to moderate-severe hepatic injury. One paper – unspecified SEP abnormalities. Peroneal SEP reported as abnormal. Remaining papers find changes in cortical potentials; only 2 of the studies containing central & peripheral potentials reported significant differences.</p>	

Table 7.4: Summary table of previous cognitive (P300) studies in patients with hepatic encephalopathy.

Publication period	1976 - 2007		
Number of studies	12 (all adult)		
Number of patients	631	Age: 45.9±12.5* yrs (range 19 – 75) [#] Age not stated in 5 studies (n = 275)	* mean±SD # from 3 studies; remainder not stated
Diagnoses of liver pathology	Unspecified clinical observations: 1 Not stated: 2 Cirrhotic: 6 Chronic liver disease: 2 Transplant patients: 1	Histology Imaging Biochemical liver function	
Aetiologies	Alcoholic cirrhosis 1° billiary cirrhosis idiopathic cirrhosis Chronic hepatitis non-A, non-B hepatitis drug abuse cancer Gauchers disease	Not stated : 3 studies	
Patient selection criterion	<u>Inclusion:</u> Not stated: 1 Language native to country of research centre No alcohol within 2 weeks / 6 months / 2 years Normal sensory function	<u>Exclusion:</u> Not stated: 1 Alcoholic toxicity Sedatives or centrally acting medication Neurological conditions	

		Other major diseases Overt HE (SHE / minimal HE studies)
Assessment of encephalopathy	Parsons-Smith (1957) 2 Conn 4 Not stated 3 + (unspecified clinical features 2) Holm (1980) 1	
EP Investigation	Variable 'rare' stimulus incidence 12%, 15%, 20%; not stated in 1 study Visual P300: large & small visual angle for rare. Variable: 12.5', 15', 64', 200' Auditory P300: low & high tones used as 'rare', (0.5, 1 & 2kHz). Stimulus intensity variable, stated in 4 studies; no thresholds. Recording (visual & auditory) positions stated / complete in 6 studies only	One study – CNV Post processing not stated in one study, incomplete in 5 studies.
Results	P300: sensitive, similar to NCT-B & superior to EEG / improved evaluation – 8 studies (2 visual) P300 no discrimination / unspecific findings – 4 studies (1 CNV, 1 visual)	

Appendix III

Table 7.5: Summary table of signal analysis applications in neurophysiology

NOTES:

- ¹Local Field Potentials
- ²Steady State
- ³Hyperbaric Oxygen
- ⁴Transient Frequency Response Characteristic
- ⁵Electrocorticography
- ⁶Maximun Entropy
- ⁷Fractal Dimension analysis
- ⁸Slow Wave Index
- ⁹Dorsal Root Entry Zone
- ¹⁰ Non-Linear Prediction Model (Mathworks™)

	Investigation type			Signal Analysis	EP	EEG / LFP ¹	Outcome	Study Group
Animal Studies	Normal	Dog	Instrumentation: digital filter effect	Power spectra	BAEP	0	FFT of EP demonstrates 4 main power bands	(Kawasaki Y1993)
		Lizard	Effect of body temp / sleep on power	“	fVEP	√	Peak power increases with temperature; VEP morphology only studied.	(De Vera L 1994)
		Rat	Invasive cortical surface recording	“	AEP & (SS) ²	0	Spatiotemporal investigation of middle latency AEP components (not related to gamma oscillations)	(Franowicz MN1995)
		Macaque	Relationships between sensory & higher cortical areas	spectral power; coherence	vLFP	√	Power and coherence analysis suggest synchronised anticipation pathways	(Liang2002)
		Duck	Stimulus assessment	FFT	SEP	0	Spectral analysis suggests appropriate stimulation characteristics	(Beysen2004)

		Rat	Gamma activity with anaesthetic dose	PSD	ERG, VEP	0	Gamma band power is observed before and after halothane administration	(Guarino2004; Imas OA2004)
		Rat	Corrections for spectral leakage	FFT	AEP (SS)	0	Time-interpolation / coherence coefficient calculations	(Felix L.B.2009; Felix LB2005)
		Macaque	Instrumentation: single unit & LFP comparison	Power spectra	vLFP	0	Spectral analysis separated frequency bands from spike / noise contamination	(Asher I2007)
		Dolphin	Instrumentation: Stimulus amplitude & carrier frequency investigated	FFT	AEP (SS)	ECoG ⁵	Power content in ECoG; SEP morphology only	(Finneran JJ2007)
	Patho-physiological	Cat	Drug effects: phenytoin & Phenobarbital	FFT	SEP	√	EEG spectral changes; EP – amplitudes	(Kaplan BJ1977)
		Dog	Bremazocine sedation	PSD	SEP	√	Dose related θ and δ power changes; EP – amplitude & latency (late component) changes	(Freye E1983)
		Pig	Induced hepatic encephalopathy	PSD	VEP, BAEP	√	EEG δ - power increases with HE ₀₋₃ ; EP latency and morphology only studied	(De Groot GH1985)
		Cat	Naloxone precipitated epileptic seizures	PSD	VEP	√	VEP & LFP spectral dose related changes	(Fernandez-Guardiola A1988)
		Rabbit	Acute & Chronic spinal chord injury	Power spectra	MEP	0	MEP spectral power alters with degree of injury & indicates level of residual function	(Simpson RK Jr1993a; Simpson RK Jr1993b)

		Rat	Colchicine & kainic acid injection to hippocampus	Spectral analysis	Olfaction	0	Odour stimulation produces peak at 15-20Hz; abolished by granule cell elimination	(Heale VR1995)
		Rat	Midazolam drug therapy for experimental HBO ³	FFT/ CSA PSD	SEP	√	Power band alterations in EEG; SEP used for latency measurements	(Russell GB1995)
		Cat	Surgical monitoring (in hypoxic injury)	Time-Frequency	SEP	0	Peak energy / frequency and peak frequency change during hypoxia – earlier than time-domain recording.	(Brauna JC1996)
		Rat	Cadmium treatment in experimental diabetes	Amplitude spectral analysis (TFRC) ⁴	fVEP	0	Amplitude decrement observed on lower frequency band.	(Yargicoglu P1998)
		Mouse	Cholinesterase inhibitors & EEG activity	Spectral EEG (FFT)	fVEP	√	EEG changes in θ -band; latency / amplitude studies for EP	(Tebano MT 1999)
		Rat	Effects of organic toxins (solvents / organophosphates)	FFT	VEP	0	Main harmonics (peaks) are identified; dose-related frequency domain changes demonstrated for solvents.	(Boyes W.K. 2009; Boyes WK 2005; Boyes WK 1999)
Human Studies	Normal	Effects of stimulus intensity, rate and barbiturates on slow oscillations		FFT	SEP	0	Specific harmonics can be enhanced by stimulus rate. Barbiturates demonstrate a redistribution of energy – away from lower frequency components.	(Rush JL1976)
		Effects of plasma levels of chlorpromazine		FFT	pVEP, fVEP, AEP	√	Decrease in θ & α EEG band power; plasma level dependant changes in EP amplitude & latency measurements.	(Laurian S Le PK1981)

	Instrumentation: spectral & phase shift comparisons with filter applications	PSD	ABR	0	Trials of zero-shift filters reduce power alterations of power main bands.	(Kavanagh KT1988)
	Instrumentation: improving signal to noise ratio for threshold detection	PSD	AEP	0	Coherence analysis improves sensitivity at lower stimulus intensity.	(Dobie RA1989)
	Instrumentation: optimum passband & filtering methods investigated.	Power spectral analysis	VEP	0	PSD used to compare analogue and digital effects on energy within varying bandwidths	(Skuse NF1990)
	Instrumentation: contrast sensitivities investigated.	(ME) ⁶ spectral analysis	VEP (SS)	0	Frequency spectrum demonstrated energy changes at different spatial frequencies for dominant bands. Different energy for mono- & binocular stimulation.	(Spileers W.1992)
	Responses isolated for stimulus intensity and monochromatic sensitivity.	FFT	VEP	0	Dominant harmonic identified and demonstrated as a function of irradiance and stimulus wavelength.	(Frederiksen J.L.1993)
	Instrumentation: effects of coil size & intensity, state of alertness and muscle contraction	FFT	MEP	0	No consistent dominant frequency identified;	(Kiers L1993)
	Brainstem, middle latency & slow vertex: frequency analysis	PSD (Time-frequency)	BAEP	0	Time-frequency analysis demonstrates frequency content at specific latencies, e.g. at waves I – V.	(Ehara Y. 1994)
	Quantitative evaluation of brain maturation	PSD	fVEP	√	Median & maximum power charted; discrimination improves with VEP data – latencies only.	(Manas S1997)

	Relationship of EEG & ERP activity; also S:N investigation. (Acoustic reaction time task)	Spectral & coherence analysis	aERP	√	δ -power correlates with reaction time; faster reaction times correlate with higher S:N power and amplitude of ERP in time-domain	(Winterer G1999)
	Instrumentation: study of multiple pass-band filtering.	FFT, PSD	fVEP	0	Power in bands >20Hz unaffected with the exception of γ -band (intra-individual variation)	(Shimoyama I.2000)
	Effect of Olanzapine on cerebral activity	qEEG	P300	√	Nine spectral [EEG] bands analysed – dose dependant α -and β -power decrease, θ -power increase. P300 time-domain changes studied only.	(Hubl D2001)
	Role of cholinergic circuits in generation of high frequency oscillations	FFT	SEP	0	Narrow band (HF) changes observed; not reflected in N20 latencies	(Restuccia2003)
	Effects of antivertiginous medications on vigilance	FFT (α -band)	AEP P300	√	Spectral changes on EEG reported; only latency & amplitude measurements studied for EPs	(Schneider2003)
	Effect of stimulus frequency on power and spatial distribution.	Laplacian, Spectral	VEP (SS)	0	Peaks in power (α , δ) are stimulus frequency dependant; band maxima also have spatial distributions.	(Srinivasan R2006)
	To determine the contribution of β and γ oscillations to SEP components.	PSD, intertrial coherence	SEP	0	Power analysis determined extent of phase-locking over several bands; coincides with latency of N30	(Cheron2007)
	Cross-modal (cortical) association: pairing a visual with tactile stimulus – topographic investigation.	ICA, coherence analysis	SEP / vERP	0	Independent [SEP] components are enhanced by expectation (cingulated cortex).	(Ku Y. 2007)

	Comparison of Fractal & Fourier Transforms of EEG data during odorous stimulation	FFT / FD ⁷	Olfact	√	FFT does not highlight elicited potentials but FD does for a range of concentrates.	(Murali S.2007)
	Co-variation of cortical and cutaneous responses: emotional stimulation	FFT	VEP (SS)	0	Main spectral power band related to amplitude of skin conductance.	(Keil A.2008)
	Development of auditory sensory system	FFT	AEP	0 (hi-density record)	Spectral bands identified – evolve to adult profile with maturation; α -power peaks migrate to β and γ bands	(Lippe S.2009)
Patho-physiological	Assessment of prolonged dialysis & post transplant improvements.	PSD	VEP SEP	√	Power levels of main EEG frequency components reduced <i>cf</i> normals; EP latencies [only] prolonged. Findings normalise post-transplant.	(Lewis EG1978)
	Unilateral cerebral (post CVA) lesions – deep & superficial; comparison of brain activity.	PSD/ SWI ⁸	SEP	√	Alterations in SWI power specific for location of lesion; latency changes only for SEP (similar pattern of alteration).	(Iwayama K.1979)
	Slow oscillations in cerebral lesions; effect of stimulus frequency.	PSD	SEP	0	Spectral distributions different from normals; stimulus frequency drives specific bands in normals only.	(Kusske JA1980)
	Hemispheric asymmetries in pre-term neonates.	Auto-regression (AR spectral analysis)	fVEP AEP	0	Main AEP power band increases L > R, wks 40-42. Main VEP power band increases R > L, wks 34-36.	(Ogawa T. 1980)
	Investigation of electrophysiology in the aged. Effects of neurotropics	PSD	VEP P300	√	Spectral analysis of EEG; EPs studied for amplitude.	(Zimmermann P.1982); (Gallois2002)

	MS and glaucoma investigated.	FFT	ERG / VEP	√	Second main frequency component absent in glaucoma; diagnostic yield for MS higher with latency studies	(Bobak P1983)
	Assessment of cerebral function in schizophrenia.	PSD	VEP	0	Reduced power in α -band; asymmetric power in β -band (temporal lobe).	(Jutai JW1984)
	Assessment of MS: frequency & latency comparison.	PSD	VEP	0	Loss of power in high frequency components; does not correlate with latency due to deformed morphologies.	(Trick GL1984)
	Electrophysiological study of hypoglycaemia on visual function.	PSD	VEP	√	QEEG demonstrates main power shift from α to θ to δ ; correlates to amplitude and latency changes [only]	(Harrad RA1985)
	Assessment of analgesia by study of cortical responses to pain stimuli	Spectral analysis (ME)	SEP (single trial)	√	Frequency transformed post-stimulus activity shows a band (2 – 4Hz) with consistent variability	(Bromm1987)
	Spectral alterations in MS and Head Injury.	PSD/ME	BAEP	√	Three consistent frequency peaks identified in all subjects (170Hz, 520Hz & 950Hz); ME-power in these peaks is reduced.	(Kamath MV1987)
	Spectral content of EPs in epilepsy; focal abnormalities, seizure onset and post-operative recovery.	PSD Coherence	P300 limbic	√ (partial seizures)	Spectral power reduced in foci / hemisphere; normalised post-surgery. Limbic PSDs (high and low bands) altered in abnormal hemisphere. PSD alterations lateralised to abnormal side in TLE.	(Meador KJ1988a; Meador KJ1988b; Nuwer MR1988)

	Detection of subclinical encephalopathy in cirrhotics.	n/a	EEG*	√	Baseline reactivity from *EEG feedback arousal are inversely correlated to trail-making performance.	(McLaughlin TJ 1989)
	Spectral analysis of VEPs in Alzheimer's; technical approaches	FFT	VEP	0	Comparisons of windowing effects; lo-sinh & Gaussian recommended - cosine & rectangular may cause misclassifications.	(Moody EB Jr1989)
	Neurophysiology in children with autism	Spectral EEG (AR)	BAEP	√	Alterations in brainstem functions. AEP – latency measurements.	(Ogawa1989)
	Neurophysiology in children with reading and writing difficulties.	Power spectra	AEP	√	All EEG spectral bands - power increase; AEPs - amplitude changes in late components.	(Pinkerton F1989)
	Evaluation of neuromonitoring in diagnosis of brain death.	PSD CSA	SEP BAEP	√	CSA (EEG) aids diagnosis; EP latency changes studied.	(Shiogai T1989; Shiogai T1993)
	Neurophysiology of delirium in chronic liver disease.	Spectral-EEG	SEP BAEP	√	Mean power in α and θ range reduced; EP – latency studies only	(Trzepacz PT1989b)
	Serial neurophysiological studies in children with ALL.	PSD	VEP BAEP	√	Main spectral EEG peak reduces in frequency; EP - latency only (VEP not significant).	(Korinthenberg R 1990)
	Evaluation and prediction of coma outcome.	Spectral EEG CSA	BAEP SSEP	√	Frequency analysis can classify groups which match underlying pathology and prognosis; EP latency studies only (alterations concur with EEG findings)	(Liesiene R2006; Tsubokawa T1990)

	Physiological changes in Alzheimer's and non-specific cognitive decline.	Spectral EEG	CNV P300	√	EP latency studies only; CNV demonstrate with treatment / clinical improvement.	(Zappoli R1991; Zappoli R.1991)
	Study of main frequency components in patients with optic neuritis	FFT	VEP	0	Pathological changes can be observed in different frequency harmonics - reversal rate dependant	(Hasegawa S 1992)
	Objective evaluation of pain-relief surgery (DREZ) ⁹	PSD Coherence	VEP SEP	√	spectral EEG changes ($<\theta$), EP latency & morphology normalisation coincide with clinical improvement.	(Arkhipova NA 1993)
	Treatment and monitoring of mild hepatic encephalopathy in cirrhotics.	EEG power spectrum	P300	√	EEG changes with treatment α -power increases, δ -power decreases; P300 latency only [improvement].	(Higuchi K1994)
	Effect on brain function of tyrosine treatment in adults with phenylketonuria.	Spectral analysis	VEP	√	EP latencies studied only, EEG β -power reduced in patients cf controls; (treatment ineffective).	(Pietz J1995)
	Physiological effects of substance abuse in psychiatric patients.	QEEG	AEP VEP P300	√	Significant power changes in EEG; latency changes only for EPs	(Braverman ER1996)
	Electrophysiological diagnosis of hepatic and portosystemic encephalopathy.	Spectral EEG	pVEP mVEP	√	EEG spectrum shifts towards lower frequencies; mVEP more sensitive than pVEP (latency measurements)	(Kuba M1996)
	Depth of anaesthesia monitoring.	FFT ARX ¹⁰	AEP	√	Both EEG & EP spectra alter during transitions through consciousness.	(Capitanio L1997)
	Induction of anaesthesia and moderation of inadequate anaesthesia.	FFT PSD	AEP	√	AEP amplitude changes studied only; Ketamine reduces α -power [EEG] amplitude of AEP, remifentanyl exerts no effects.	(Bischoff P1998; Plourde G1997)
						(de Tommaso

	Investigation of migraine attacks.	FFT	VEP	√	Main frequency component reduced during attack phase	M1998)
	Repeated periods of conscious – unconsciousness mediated by Propofol infusion.	PSD FFT	AEP	√	AEP frequency index varies significantly during transitions.	(Gajraj RJ 1998)
	Spectral study of fusiform gyrus (pharmaco-resistant epileptics).	PSD	ERP Word / face	√	Spectral changes are anatomically distinct and vary with stimulus type.	(Klopp J 1999)
	Neurophysiological investigation of lead absorption.	FFT PSD	VEP SEP BAEP P300	√ (auto- nomic)	(Review article); spectral postulography, EPs studied for latency.	(Araki S2000)
	A preliminary demonstration of a continuous wavelet transforms (CWT).	Wavelet	VEP	0	First order wavelet distinguishes norms from patients with fewer trials. The spectral analysis has improved S:N ratio <i>cf</i> signal averaged EPs.	(Drissi H. 2000)
	A study of chronic fatigue syndrome in workers from the Chernobyl incident.	QEEG	SSEP	√	Lateralised decrease in α -power, lateralised increase in θ -power EEG; SEP alterations in amplitude & latency.	(Loganovsky K.N.2000)
	Spectral power estimates in patients with vertigo or deafness.	FFT	BAEP	√	Frequency components are identified in controls that are absent in patient group.	(Nagaraj HC 2000)
	Spectral comparisons in juvenile patients with headache (migraine and tension-type)	FFT	VEP	0	P100 latencies not [stat.] altered but main frequency components show differences.	(Marrelli2001)
						(Canafoglia L. 2003)

	Myoclonus in HIV-encephalopathy	Spectral (AR)	SEP	√	SEP latency only; EEG / EMG coherence similar to cortical myoclonus.	
	Diagnosis of minimal hepatic encephalopathy.	Spectral analysis	P300	√	Dominant frequencies established for EEG; P300 latency only.	(Amodio2005)
	Effects [acute] of solvents on the nervous system (toluene & actone)	QEEG	VEP	√	Insignificant spectral EEG changes; P100 latencies unchanged.	(Muttray 2005)
	Contralateral cortical reorganisation following capsular stroke.	EEG spectral coherence	MEP	√	No EP analysis – TMS fails to elicit response. Coherence analysis suggests a an activity shift to contralateral hemisphere	(Gerloff2006)
	Neurophysiological monitoring during carotid endarterectomy.	Spectral analysis	SEP	√	SEP changes before EEG in reduced (TCD) blood flow; spectral analysis of EEG only, SEP – N20 amplitude	(Stejskal L 2007)
	Deficits in gamma band in patients with schizophrenia	Time-frequency analysis PSD	AEP (SS)	0	Schizophrenic patients demonstrate reduced power and phase-locking in γ -band	(Krishnan G.P.2009)

Appendix IV

Fig: 7.3 (and overleaf): Confirmation of ethics approval.

Royal Free Hampstead 
NHS Trust

Royal Free Local Research Ethics Committee
Chief Executives office
Pond Street
London
Hampstead
London
NW3 2QG

21 November 2002
Dr Marsha Y Morgan
Centre for Hepatology
Royal Free Hampstead NHS Trust
Pond Street
Hampstead
London
NW3 2QG

Dear Dr Morgan

To develop a sensitive and specific grading system for the diagnosis and monitoring of hepatic encephalopathy in cirrhotic patients

Ethics Reference: 6115 (Please quote on ALL correspondence)

I refer to your recent application to the Ethics Committee regarding the above project and am pleased to inform you that the project was approved at the committee meeting on 20.11.2002.

This approval is for one year from the date of this letter. We also require to be notified of the completion of the project and to be sent a copy of any subsequent publication. Extension of this period will be dependent on the submission of a brief synopsis of the progress of the project together with an estimation of the time required for its ultimate completion.

In addition we require that:

(a) You inform the committee immediately of any information received by yourself or of any information of which you become aware which would cast doubt upon, or alter, any information contained in the original application, or any amended later application, submitted to the committee which would raise questions about the safety and/or continued contact of the research. This would include the reporting of all "serious adverse events" of which you become aware and "adverse events" that happens on Royal Free site. These "adverse events" should also be reported to the person who provided independent review of the original application.

(b) All those involved in the study appreciate the importance of maintaining confidentiality and that they comply with the Data Protection Act 1998.

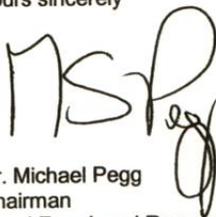
(c) All proposed amendments to the protocol, that have a bearing on the treatment or investigation of patients or volunteers, are submitted to the committee for approval.

(d) The conduct of the study complies with good clinical research practice as outlined in the ICH GCP guidelines.

(e) A copy of the patient consent form and information sheet be lodged in the clinical notes.

Please note that ethical committee approval does not mean that the study may commence. The study may only commence following approval by the Trust through the office of the Director of Research & Development (please contact Sjokvist Garcia-Stewart on extrn. 8304).

Yours sincerely



Dr. Michael Pegg
Chairman
Royal Free Local Research Ethics Committee

Documents received:

Application form received	Yes	
Consent form	Yes	
Patient information sheet	Yes	healthy volunteers, Disease control subjects, subjects with Hepatic impairm
Protocol	Yes	
GP letter/ Consultant informat	Yes	
Other	Yes	Assessment Results

Appendix V

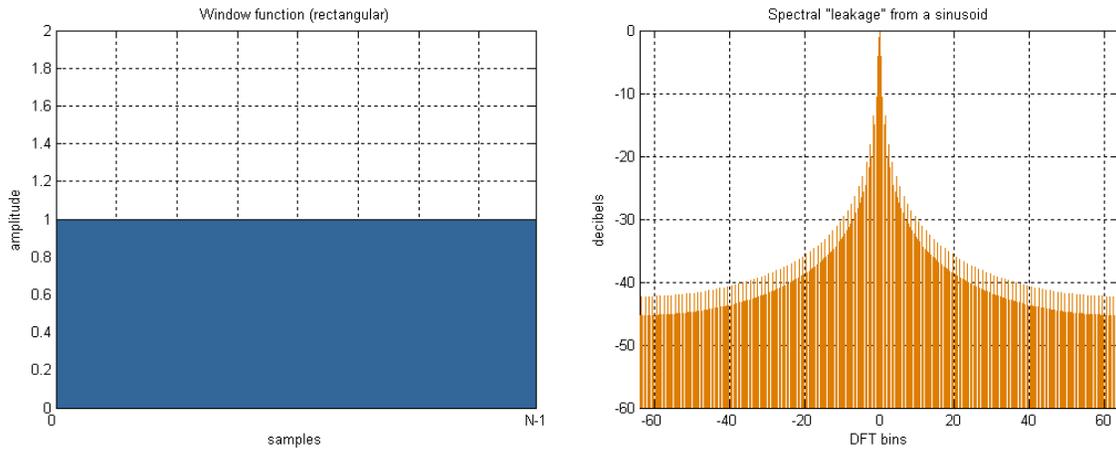
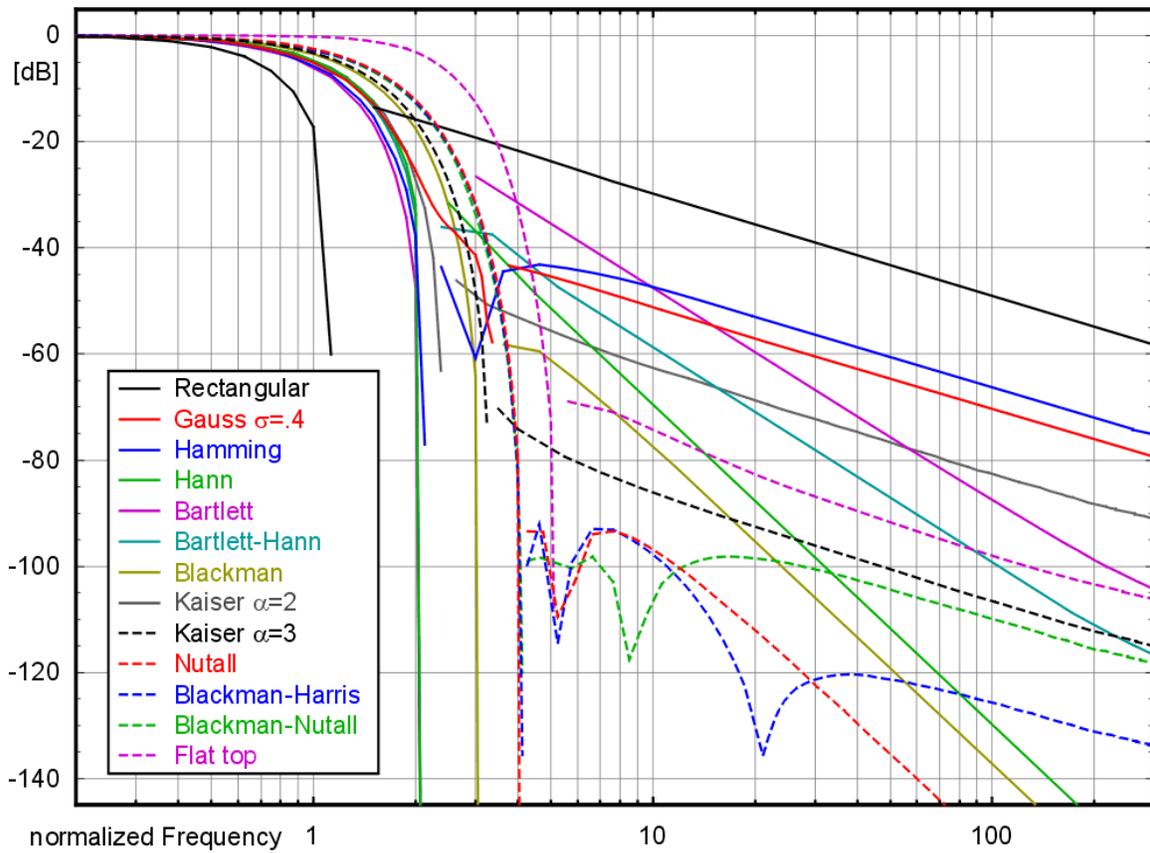


Fig 7.5 (above): a rectangular window function (left) and its effect on a sinusoid waveform (right).

Fig 7.6 (below): a comparison of window functions in the frequency domain.

Data is generated by Matlab™ code (Oppenheim & Schaffer 1989)



Appendix VI

(Table 7.5): EP latency measurements for *combined* hemispheres, by neuropsychiatric status.

Evoked Potential	Controls (n = 96)	Unimpaired (n=53)	Minimal (n= 25)	Overt (n=59)
VEP (ms)				
N75	73.1±5.2	79.4±8.4*****	78.7±8.7***** ⁺	81.0±6.9*****
P100	101.7±4.8	105.7±5.4***** ^o	109.3±6.9*****	107.5±7.5*****
N145	137.2±11.2	137.0±10.2 ^{oo}	144.112.4*	141.8±10.7
SEP (ms)				
N9	9.5±0.7	10.8±1.1*****	10.5±1.4**** ⁺	11.2±0.8*****
N13	13.2±0.8	14.6±1.4*****	14.5±1.7 ⁺⁺⁺	15.0±1.0*****
N20	19.1±1.0	20.9±1.5*****	20.3±2.0*	21.1±1.3*****
BAEP (ms)				
Wave I	1.5±0.2	1.6±0.2	1.6±0.1*	1.6±0.1
Wave III	3.7±0.2	3.8±0.2***	3.8±0.2**	3.8±0.2*****
Wave V	5.6±0.2	5.7±0.2* ⁺⁺⁺	5.8±0.3***	5.9±0.3*****
Auditory Cognitive (ms)				
P300	306.5±19.4	354.3±55.4** ⁺	351.8±44.9** ⁺⁺⁺	401.5±42.4*****

Data are expressed as (mean ± SD) for the combined right and left hemispheres.

Significance of the difference from controls: *p < 0.05, **p < 0.01, *** p < 0.005, ****p < 0.001, *****p < 0.0001.

Significance of the differences from patients with overt HE: +p < 0.05, ++p < 0.01, +++p < 0.005.

Significance of the differences from patients with minimal HE: ^op < 0.05, ^{oo}p < 0.01.

Acknowledgments

I am extremely grateful to Professor Richard Bayford and Dr. Richard Billings for their support and encouragement over these many years, and without which I would have neither remained a neurophysiologist or sane.

I am deeply indebted to Dr. Marsha Morgan, for her guidance and (above all) patience, throughout the research and during the preparation of this thesis.
