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Pathological Patterns of Hippocampal Sclerosis May Predict Post surgical Seizure Outcome in Intractable Temporal Lobe Epilepsy

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0.0 ABSTRACT

Medically intractable temporal lobe epilepsy is associated with hippocampal sclerosis. Following surgical removal, the hippocampi of patients suffering from intractable temporal lobe epilepsy can be pathologically evaluated. The surgical outcome of the epilepsy surgery can also be assessed. Whether or not there are patterns of hippocampal sclerosis (HS) that predict a better or worse surgical outcome is the question explored in this study. We found that using a qualitative method of HS pattern determination, that typical patterns of classical HS and total HS had better post surgical seizure outcome then more atypical patterns. The atypical patterns of HS included end folium sclerosis and CA1 predominant neuronal loss. We could not confirm this finding with a second quantitative HS pattern method, using the Histometrix analyzer. The quantitative method did confirm the better outcomes with the typical HS patterns, however. This quantitative method required manual sketching of each HS subfield that was followed by a laborious manual neuronal count of 10 high power microscopic fields from within each HS subfield drawn. From this count, a neuronal density was determined by the Histometrix analyzer. We also evaluated granule cell dispersion by measuring the width of the granule cell layer as well as by identifying nine different qualitative patterns. Severity of amount of GCD did not seem to predict seizure outcome.

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1.0 INTRODUCTION

Epilepsy is the tendency to have recurrent epileptic seizures (Shorvon 2005). In this paper, we hope to examine one small part of the field of epilepsy research. We will focus upon the syndrome of intractable temporal lobe epilepsy associated with hippocampal sclerosis and attempt to further understand this disorder by performing a careful clinicopathological correlation. We plan to correlate clinical outcome following epilepsy surgery with pathological changes that we find in hippocampal specimens of surgical resections. We will assess patterns of neuronal loss in various subfields in hippocampus, both quantitatively and qualitatively, as well as patterns of granule cell dispersion in the hippocampus. We will then evaluate if any of these pathological changes are predictive of post surgical seizure outcome.

This paper will begin with an historical overview of the treatment of epilepsy. This review of epilepsy history will then be followed by a discussion of the more modern brain based view of epilepsy. The modern view of epilepsy is best advanced via quality outcomes research. What is outcomes research in epilepsy will be discussed, followed by a description of the problem of medically intractable epilepsy. The management of medically intractable epilepsy with surgery will then be reviewed. The pathological association of hippocampal sclerosis with medically intractable epilepsy will then be discussed, as well as the research on patterns of hippocampal sclerosis. The additional pathological changes of granule cell dispersion associated with hippocampal sclerosis will also be reviewed. The study aims, methods and results will then be presented, followed by a discussion of our findings.

1.1 Historical Overview of the Treatment of Epilepsy

The first medical description of epilepsy is in a very old Babylonian medical text. The medical text is made up of 40 tablets. The tablets describe different types of seizures each caused by a different spirit (WHO,2007). The spirits were not considered friendly and the person experiencing the seizure was considered to be under demon attack. The tablets are at least 4,000 years old (WHO, 2007).
This view of epilepsy as due to demon possession has persisted for thousands of years. It competes throughout history with an alternative position that views epilepsy as a brain disease.

An Ancient Indian text from 400 BC describes epilepsy as resulting from a loss of consciousness, and views epilepsy as a brain disease (WHO, 2007). Hippocrates, writing during the same time period, also believed that epilepsy was a disorder of the brain (WHO, 2007).

During the Middle Ages, epilepsy was called the "falling sickness" and prescribed treatment consisted of the wearing of a special religiously blessed ring (WHO, 2007). The prominent belief that epilepsy is a form of spirit possession has shown remarkable persistence over historical time and across diverse cultures. It remains the present day cultural belief of the Hmong people of Laos, even when they become immigrant-refugees and relocate to America (Fadiman 1998). The book, "The Spirit Catches You and You Fall Down" describes a Hmong family dilemma that results from the clash of these two different theories of epilepsy. The book title comes from the translated Hmong word for epilepsy. Conflict between epilepsy as demon possession and epilepsy as brain disorder severely impairs the care of a frail infant child with epilepsy (Fadiman, 1998).

The historical belief that persons with epilepsy were possessed by spirits

and that epilepsy was contagious similar to a cold, produced enormous discrimination and led to profound societally sanctioned abuse. Up until the third decade of the twentieth century, they were forcibly confined in separate wings of mental institutions and forcibly sterilized in the United States. This mistreatment coincided with the growth of the American eugenics movement which viewed them as inferior humans and a potential source for a weakening and "dilution" of the gene pool (Cold Spring Harbor Laboratories 2007). American eugenics is a shameful and humbling reminder of how often science is factually, as well as morally, wrong.

American eugenics was widely and enthusiastically embraced by the medical and legal establishment of the time. Buck v. Bell is the (in)famous U.S. Supreme Court decision authorizing state police power to involuntarily sterilize a 17 year old teenager confined to a state institution. Carrie Buck lived in the Lynchburg Colony for Epileptics and the Feebleminded in Virginia (Buck v. Bell 1927).

The American eugenics movement inspired German ideas about the need for a superior human race in the Third Reich. The German Lenz called for widespread forced sterilization of disabled or ill Germans in the 1920s to improve racial stock, a view that Hilter embraced (Lifton,1986). Supportive ideas for removal of a "defective gene pool" found its first scientific and legal justification in America. Gene pool manipulation (to produce a superior genetic race) began with forcible confinement and restriction of reproduction but progressed to actual killing of patients judged genetically "inferior". The Germans enacted these ideas and epileptics were murdered by their government *en masse* as part of the Nazi "racial cleansing" campaign (Lifton 1986). Widely underrecognised is the predominant role of scientists and medical doctors in this ultimate patient mistreatment (Weber 1996; The Doctors Trial 1946).

Mistreatment of persons with epilepsy until mid-20th century reflected societal prejudice based on concepts of spirit possession but also physician attitudes of eugenics and social Darwinism. These ideas were also embraced by geneticists. Eugenics held sway for many decades in America and in Europe during the development of seizure therapy and during the same period that neuroscience was beginning to unravel the brain basis of epilepsy. It is probable that when eugenics and the racial cleansing that it inspired were discredited after WWII that this produced a societal counterreaction in favor of human rights that led to better care of persons with epilepsy (Nuremberg Code 1949, Universal Declaration of Human Rights 1948).

1.2 Modern Treatment of Epilepsy

Lest one believe that this discussion of the rights of persons with epilepsy is irrelevant today, one has only to peruse the latest report of the Joint Epilepsy Council of the UK and Ireland for contrary evidence (JEC, 2007). While epilepsy is the most common neurological disease in England today, the overall quality of care provided to epilepsy patients remains suboptimal according to this report. Epilepsy affects nearly 400,000 people in England. But epilepsy misdiagnosis rate remains high. 20-31% of patients are either misdiagnosed with epilepsy they do not have, or there is failure to diagnose epilepsy, with a resultant delay in specific antiepileptic treatment (JEC 2007). Both misdiagnoses reflect a need for higher quality epilepsy care in England. In England alone, an estimated 70,000 patients are suffering from unnecessary and not properly treated seizures (Kelly, 2007). JEC sadly recounts the 400 lives lost each year in England and the associated costs of nearly 200 million pounds per year resulting from deficiencies in the care of epilepsy. These lost lives

have all been determined as avoidable deaths (Kelly, 2007). Journalist attitudes in the UK about epilepsy are still frighteningly prejudicial and outdated (Gerard 2007). Such attitudes reflect fear of seizures as "Exorcist"-like. This suggests the persistence of demon possession in the public mind as a major societal determinant of attitudes towards persons with epilepsy (Gerard 2007).

The JEC report is good quality evidence that better outcomes research is needed to help improve care of persons with epilepsy in England right now. Outcomes research is a core feature of the worldwide move to improve the quality of health care (Elliott 2004, Institute for Healthcare Improvement (IHI) 2007,Wallner 2006). Outcomes research requires a basic understanding of the extent of the problem to be studied followed by measurement of how treatments for a given problem actually work in patient populations. Within the field of epilepsy research, outcomes research requires problem definition and subsequent measurement of outcomes of individual treatments (see for example, Engel 2003, Leppik 2006).

While the JEC report describes basic statistics of the extent of the problem of epilepsy in England now, it is probably more helpful for future research to consider epilepsy as the "epilepsies" and consider separate syndromes in isolation (Duncan 2002). Of the different epilepsies, the most common adult epilepsy is temporal lobe epilepsy (Schmidt 2005). A significant number of patients with poorly controlled seizures have temporal lobe epilepsy due to hippocampal sclerosis (Shorvon 2005). It remains unresolved whether the hippocampal sclerosis is causing the medically intractable state (Schmidt 2005). Why this syndrome is associated with antiepileptic drug resistance and develops into medically intractable epilepsy is not known (Schmidt 2005). Further pathological research into hippocampal sclerosis may shed some light on this problem.

The field of epilepsy research does not have an agreed upon definition of what medically intractable epilepsy means. The difficulty this presents is that the proportion of patients developing such a condition varies with the definition chosen. In a good quality longterm prospective study of this question in 613 children who were followed for a median of 9.7 years, 13.8% were intractable if definition required failure of 2 drugs and 1 seizure per month for 18 months. If intractable was defined less stringently as failure of 2 drugs only, then the percentage of "intractable" increased to 23.2% (Berg 2006). Differing definitions of what is medically intractable epilepsy exist and add to variation in results and make it difficult to compare across studies. Such differing definitions also makes it risky to pool data across multiple medical institutions.

One route to improved outcomes requires a more detailed understanding of the pathophysiology of specific epileptic syndromes. As Deming, the father of the worldwide quality improvement and outcomes field, has said, "There is no substitute for knowledge" (Deming as quoted by Nolan 2007). Such scientific understanding of epilepsy began during the same historical period that eugenics was in vogue. Evidence for the brain disease theory of epilepsy first emerged in the modern era from the careful observations in the 19th century of the great Queen Square neurologist, John Hughlings Jackson (WHO, 2007). Regarded by the many clinicians that he trained as the "Father of English Neurology", Jackson helped create the subspeciality of neurology (Critchley 1998).

Jackson described core features of temporal lobe epilepsy (Hogan 2003). He wrote about many examples of auras. He described ictal signs of temporal lobe epilepsy of sweating, pallor, repetitive lip smacking and chewing movements. While his prose can be somewhat challenging to understand, his "dreamy state"

encompassed both ictal and postictal states in medial temporal epilepsy (Hogan 2003).

Jackson also recognized that different signs and symptoms accompanied different epilepsy syndromes and that each localized to a specific abnormally discharging focal region of cerebral cortex (Hughlings Jackson 1888). In addition to his descriptions of auras and "dreamy state" was his observation that this type of partial complex epilepsy has its origin in the medial temporal lobe of hippocampus. This focus on neurological localization moved the field of epilepsy research forward and set the stage for Penfield's later development of neurosurgical therapies for epilepsy. Jackson's long study of epilepsy fundamentally altered the accepted understanding of the pathophysiology of epilepsy. Later discoveries in animals linking electrical discharges to seizures, the development of the electroencephalograph (EEG) and pharmacological treatments and neurosurgical development of surgical treatments for epilepsy performed by others, finally began to change the way persons with epilepsy were treated (WHO, 2007).

The modern era of surgical management of epilepsy belongs to Wilder Penfield (Rasmussen 1977). Penfield confirmed the location of seizure foci in hippocampus in some patients with intractable epilepsy. He described improved seizure control with removal of hippocampus in patients with medically intractable temporal lobe epilepsy (Penfield 1952). He introduced electrical stimulation of the awake patient's cortex which added to understanding of the human homunculus as well as to improved outcomes of resections near motor and speech areas (Penfield 1937). Much recent medical and surgical research into epilepsy has built upon the shoulders of these two epilepsy giants. One area of intense research involves medically intractable epilepsy and its associations with hippocampal sclerosis and

the potential for curing the epilepsy with surgical resection of temporal lobe.

1.3 Hippocampal Sclerosis and Surgical resection of Temporal lobe

Hippocampal sclerosis, also called Ammon's horn sclerosis, is an incompletely understood syndrome of neuronal cell loss and gliosis occurring in different areas of the hippocampus in patients with medically intractable temporal lobe epilepsy (Blumcke 1999). Typical hippocampal sclerosis begins with loss of pyramidal neurons in the CA1 subfield of the hippocampus which progresses to involve CA3 and CA4 subfields with relative sparing of the CA2 pyramidal neurons (Bruton 1988). More severe typical hippocampal sclerosis affects all subfields of the hippocampus and may involve cell loss of the dentate granule layer with or without granule cell dispersion (Margerison 1966, Thom 2005a). Whether hippocampal sclerosis drives the clinical disorder of intractable temporal lobe epilepsy is unknown.

Carefully screened patients at tertiary referral centers with surgical epilepsy programs require evaluation for concordance between the EEG findings, videotelemetry and brain imaging studies (Scott 1999, Shorvon 2005). When investigations and clinical presentation are consistent with unilateral hippocampal seizure focus then the patient is potentially curable with temporal lobectomy (Cohen-Gadol 2006, Jutila 2002). Guidelines exist to assist in this clinical decision making process (Engel 2003). Existing guidelines are based on the medically intractable nature of the patient's temporal lobe epilepsy (Benadis 2006). Guidelines are promulgated after being written by a consensus of medical experts (Ossemann 2006). The difficulty with relying on published guidelines for determining clinical practice is that other research has shown that only a minority of clinicians follow

published guidelines (see for example, Institute of Healthcare Improvement 2007). The Engel criteria require that patients with "disabling complex partial seizures fail appropriate trials of first-line antiepileptic drugs" before surgical consideration (Engel 2003).

The introduction of multiple new antiepileptic drugs in the last 15 years has added to the complexity of what should be considered as failure of firstline therapy (Duncan 2002). Firstline agents have historically included carbamazepine, valproate, phenytoin, and phenobarbital (Duncan 2002). These agents are being replaced with lamotrigine, levetieracetam and oxcarbazepine (Dichter 1996, Shorvon 2005). Levetiracetam is particularly important as it can be effective in patients showing resistance to antiepileptic drugs (Shorvon 2005). Actual treatment decisions occur in the privacy of a clinician-patient relationship and are likely to vary from published guidelines.

While it is estimated that at least 25% of patients with epilepsy have medically intractable seizures, at what point in their clinical history this becomes evident is not defined (Lachhwani 2003). Such medically intractable epilepsy has many increased associated costs and risks associated with it (Begley 2000). Kwan and Brodie have recently published descriptions of their practice experience. They found 47% of their patients with epilepsy became seizure free after introduction of the first antiepileptic drug (Kwan 2001). In the remaining subgroup, only 11% eventually become seizure free with medical management that involved serial antiepileptic drug trials. Failure of first drug may identify early a group of patients destined to become "medically intractable" (Kwan 2000). This research may have important implications for the care of persons with epilepsy. It may lead clinicians to try surgery earlier in the disease course of temporal lobe epilepsy, before other

antiepileptic drug trials (such as with levetiracetam) have been performed. The time required to conduct careful serial drug trials of antiepileptic agents needs to be balanced against the risks associated with uncontrolled epilepsy and the risks and costs of surgery. This risk assessment must incorporate the patient as ultimate decisionmaker.

Some of these epilepsy risks include sudden death (SUDEP), injury or death from accidents, social stigma and prejudice leading to impaired social functioning, impaired quality of life, impairment of work potential, loss of driving, cognitive decline, costs of antiepileptic drugs and medical care, and the risk of becoming a "professional patient" (Chin 2006, Langfitt 2007) . The SUDEP risk alone decreases the survival of patients with medically intractable temporal lobe epilepsy. One study of sustained cardiac monitoring in 20 patients with focal epilepsy found 20% developed episodes of bradycardia or even asytolic episodes that required pacemaker insertion (Rugg-Gunn 2004). Some of the surgical risks include morbidity and mortality and costs from the surgery, post-surgical psychosis and post-surgical brain damage especially memory impairment (Behrens 1997, Langfitt 2007).

Carefully screened patients with medically intractable temporal lobe epilepsy are potentially curable with surgical resection of their seizure focus, with much neurosurgical experience confirming Penfield's original observations (Engel 2003). The epilepsy literature makes many estimates of 70% of patients with intractable temporal lobe epilepsy that are surgical candidates will have a good response or become seizure free after surgery (Foldvary 2000, Sperling 1996, Engel 1996). This broadly quoted figure is supported by a recent systematic review of seizure surgery outcomes publications. This review found a median of 70% seizure free using Engel criteria across all of the studies examined (McIntosh 2001).

Controlled clinical trial data suggest the surgical response rate is lower than 70% "curable", however. Definitions of what is a good surgical outcome vary. Some studies use "freedom from disabling seizures" and others use no seizures. The only trial data, to be discussed below, measured a good outcome as freedom from disabling seizures. The longterm surgical success rate falls to 40-50% if the stricter definition is used (Chin 2006, Jutila 2002). Part of the complexity of analyzing this outcomes literature is very little controlled trial evidence exists to provide an evidence base.

Two attempted American trials were unable to enroll sufficient numbers of patients (Langfitt 2007). A Canadian study provides the only controlled data. This trial randomized patients with disabling and intractable temporal lobe epilepsy to either surgical treatment with anterior temporal lobe resection or best medical management with delayed surgical treatment (Wiebe 2001). 58% of patients in the surgical arm became free of disabling seizures at one year as compared with 8% of patients in the best medical treatment arm. These data support the use of anteromesial temporal lobe resection for disabling complex partial seizures.

A longterm uncontrolled evaluation of a large number of epilepsy patients operated on at the Mayo clinic found that seizure status one year following surgery was predictive of longterm surgical outcome. In this outcomes study of 399 epilepsy patients treated with surgery, Engel Class 1 was used to define seizure free status which includes seizure free, auras or seizures with medication withdrawal. Not all of the operated upon patients had mesial temporal lobe sclerosis. Using this criterion of outcome, at one year, 78 % of their patients had Engel 1 response to surgery (Cohen-Gadol 2006). Another retrospective outcomes study in younger patients found a less robust postsurgical response (Smyth 2007). In a multicenter evaluation

of outcomes of epilepsy surgery, 77% of operated patients were in remission at one year as defined as Engel Class 1 (Spencer 2003). When a subgroup of operated upon patients with temporal lobe epilepsy are identified as having hippocampal sclerosis on pathological specimen examination, the longterm outcome improves to 83.3% Engel Class 1 (Lowe 2004).

While no treatments in medicine are expected to be 100% effective, it is perplexing why patients with clinically similar seizure type and severity react so differently to surgery. What is not known is why a subgroup of carefully screened and selected patients do not become seizure free after surgery. It is possible that detailed neuropathological evaluation of the hippocampus from these surgical specimens may uncover pathophysiological mechanisms or patterns of neuronal loss that may explain this variability in clinical outcomes.

1.4 Patterns of Hippocampal Sclerosis

Neuropathological evaluation of hippocampus following surgical resection for temporal lobe epilepsy suggests that certain patterns of hippocampal cell loss predict a better post surgical outcome (Wyler score, Davies et al., 1996, Hermann 1992). Lanerolle and colleagues evaluated 151 surgical specimens of hippocampi from one institution collected over ten year period for cell loss and immunohistochemistry for dynorphin. 43 of their specimens had mass lesions in the temporal lobe as a cause of the epilepsy and so can not be considered as representative of hippocampal sclerosis (Lanerolle 2003). They found their surgical specimens fell into distinct patterns of neuronal loss (Lanerolle 2003).

Patterns of typical and atypical hippocampal sclerosis were identified as well as a group indistinguishable from autopsy controls (Lanerolle 2003). One group of

atypical hippocampal sclerosis showed qualitative loss of cells confined to CA1 area of hippocampus. The group of surgical patients with typical hippocampal sclerosis neuronal loss had a good postoperative outcome (Lanerolle, 2003).

Blumcke and colleagues have recently added to the research on patterns of hippocampal pathology in patients undergoing surgical treatment of intractable temporal lobe epilepsy (Blumcke 2007). They confirmed Lanerolle's findings of a CA1 pattern and Bruton's earlier described end folium sclerosis pattern as two types of atypical hippocampal sclerosis (Bruton 1988). They found patients with atypical patterns had worse postsurgical outcomes(Blumcke, 2007). They recognized two types of typical hippocampal sclerosis that they called MTS1a and 1b which both had good surgical outcomes (Blumcke 2007).

This paper suffers from several potential methodological weaknesses. Blumcke and colleagues used Hematoxylin and Eosin (H & E) stain to count neurons for the autopsy controls despite using a NeuN stain for counting neurons in the pathological specimens. The use of different stains undermines the validity of the "population mean" used to calculate z-scores on their specimens. The calculation of z-scores relies on an approximation of a population mean, done in the Blumcke study with autopsy controls (Blumcke 2007). A neuronal count based on H & E will potentially count glia as well as neurons as the stain is not neuron-specific and it will not pick up interneurons seen with NeuN stain.

Another potential weakness in the Blumcke paper comes from the researchers' inclusion within the group of "normals" specimens of tumors or cortical dysplasia. This is misleading in a paper that claims to be discussing hippocampal sclerosis as a solitary pathology. Follow-up in the Blumcke paper was six months for some patients while others were assessed additionally at one year.

Six months is probably too short for adequate postsurgical outcomes assessment of this disorder (Blumcke 2007, Lachhwani 2003, Wass 1996) Seizure free percentages of 73% at 3 months, decreased to 47% at 24 months post surgery, in one multicenter cohort study (Chin 2006). Additionally, the method of assessing granule cell dispersion is not defined so as to be reproducible, in the Blumcke paper (Blumcke 2007).

1.5 Granule Cell Dispersion (GCD)

Granule cell dispersion (GCD) is a cellular abnormality found in the dentate gyrus in a subgroup of patients with hippocampal sclerosis (Wieser 2004, Thom 2005a). GCD is defined as widening of the granule layer of the dentate gyrus of the hippocampus (Houser 1990, Fahrner 2007). Its pathological significance remains unclear. It is not known whether the migration of cells from the granule layer is another representation of the underlying insult causing hippocampal sclerosis, or whether it is a second independent response to intractable temporal lobe seizures. There is also a view that it may represent a developmental anomaly in humans (Thom 2005a). Animal studies have shed some light on its underlying mechanisms.

In a kainic acid rodent model of temporal lobe epilepsy, many of the characteristics of human temporal lobe epilepsy are reproduced. This model shows persistent and unprovoked partial seizures as well as granule cell dispersion (Kralic 2005). In this rodent model of epilepsy, the granule cells hypertrophy and disperse from day 7 after kainic acid, and this granule cell dispersion progresses over weeks (Kralic 2005).

Granule cells of the dentate gyrus arise from the subgranular zone from a

population of adult neural stem cells (Kralic 2005, Ming 2005). In acute experimental seizures in rodents, neurogenesis is increased following an acute seizure. Whether this neurogenesis is protective against the development of further seizures, or whether it is pathogenic in the development of chronic epilepsy, is unknown (Kralic 2005). Even whether the granule cell hypertrophy and dispersion is due to neurogenesis is not known. One hypothesis is that neurogenesis induces new granule cells that then abnormally migrate to produce GCD (Fahrner 2007). GCD in human hippocampal sclerosis has been incompletely studied.

Blumcke and colleagues found some correlation with GCD and the severity of typical hippocampal sclerosis. GCD was found in 50% of MTS 1a and in 60% of MTS 1b. Both these patterns correspond to different degrees of severity of typical classical and more severe hippocampal sclerosis. Atypical hippocampal sclerosis, including the CA1 predominant cell loss pattern, and end-folium sclerosis, were uncommonly associated with GCD (Blumcke 2007).

Fahrner and colleagues found that Wyler grade 3 and 4 hippocampal sclerosis correlated with a significant increase in the width of the granule cell layer (Fahrner 2007). Much more detailed neuropathological evaluation of GCD is required before its significance in intractable temporal lobe epilepsy is clarified. When markers of cell proliferation and neurogenesis are immunolabeled and evaluated in patient hippocampal sclerosis specimens no increase in either were found (Fahrner 2007). This argues against neurogenesis or cell proliferation as a cause of granule cell dispersion. Other work using a more potent marker of cell cycling and proliferation, Mcm-2, has found evidence for both cell proliferation and/or neurogenesis, however (Thom 2005a).

What is not known is whether the granule cell layer of the dentate gyrus

passes through stages of GCD that precede or predict neuron loss and/or postsurgical seizure outcome.

1.6 Proposed Study

We will attempt to replicate the important findings of de Lanerolle and Blumcke as well as attempt to address some of the shortcomings identified in the literature in the following study. We will only examine specimens from patients with identified temporal lobe seizures and a preoperative diagnosis of hippocampal sclerosis. The surgical specimens of hippocampus will be compared with autopsy controls with both sets of hippocampi stained with NeuN and neuronal counts will be made of various hippocampal subfields. The specimens represent the experience of one institution. One investigator will evaluate the clinical records. Another investigator will evaluate the hippocampal neuronal counts in the hippocampal subfields, and an additional investigator will measure granule cell dispersion in the pathological specimens.

A priori, atypical and typical hippocampal sclerosis patterns will be defined (see Methods) and we will look to see if our specimens fall into one of our predetermined patterns when neurons are quantitatively counted, and whether such patterns are associated with subsequent outcomes. We will also conduct a qualitative evaluation of each specimen and make a more global determination of hippocampal sclerosis pattern as a second part of the HS pattern study. An additional part of the study will examine each NeuN specimen for both quantitative and qualitative evidence of granule cell dispersion (GCD) and identify patterns of GCD and whether or not such patterns correlate with total neuronal loss, patterns of neuronal loss, as well as clinical outcomes. We predict that typical hippocampal sclerosis, both

classical, and more severe, will have a better outcome then atypical hippocampal sclerosis patterns. We also hope to identify new patterns of GCD that predict postsurgical outcome.

2.0 METHODS

2.1 General Approach

a. 144 surgical cases. 3 PM controls-both right and left hippocampi from Brain Bank specimens, the first obtained from a patient aged 85 years at time of autopsy with cause of death pulmonary emboli. Specimens 2 and 3 were aged 63 and 49 and cause of death was myocardial infarction.

b. Pathological specimens from hippocampal resections for intractable temporal lobe epilepsy from the National Hospital for Neurology and Neurosurgery were used in this study. Institutional ethics approval has been obtained and the research has been approved by the Joint Research Ethics Committee of the Institute of Neurology and National Hospital for Neurology and Neurosurgery.

2.2 Details of Hippocampal Sclerosis Pattern Methods

a. The neuronal counts will be done by Dr. Kathryn Elliott

b. Single NeuN section per case

c. All HS cases (original sections from file) will be reviewed from 1993-2006. Cases will be included that have representation of more than 3 subfields (including hilus and CA1) or where an atypical pattern of HS had been reported (ie. not classical HS). From the review a single block will be selected from the

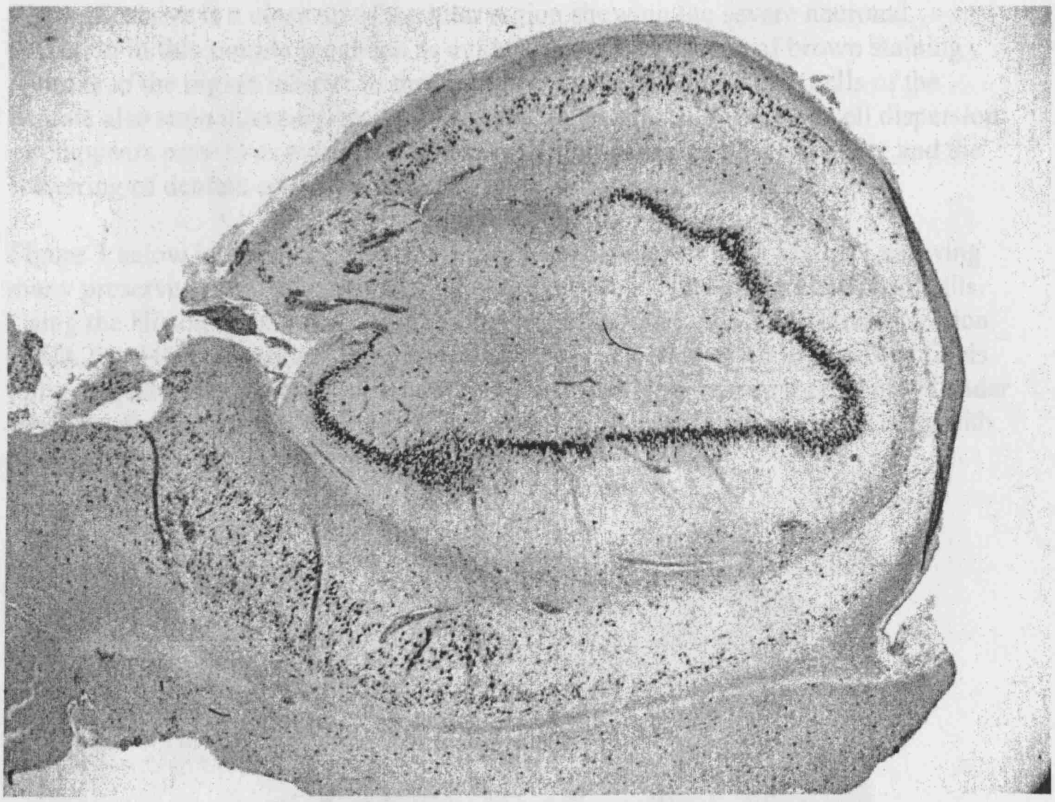
hippocampal body, and immunohistochemistry for NeuN carried out (1:100, Chemicon).

d. Neurons will be identified with the neuronal marker NeuN. A Histometrix Image analyser will be used for cell counts. Using the Histometrix image analyser, neurons stained brown with NeuN, where a nucleus could be identified, will be counted (Thom 2005b). The Histometrix stereological analysis utilizes a Zeiss Axioskip microscope (Kinetic Imaging, Nottingham, UK).

e. The whole area of CA4(hilum) will be drawn out manually at 2.5 magnification. Areas of approximately similar size will be selected from CA2, 3 and 1. The entire subfields will not drawn out manually in these sections. The section drawn out manually will approximate the size of CA2. Similar sized samples will be outlined from CA2 and CA3, and two comparable regions of CA1 will be drawn out manually. A CA1 (a) block will be selected with most neurons and a second CA1 block (b) will be selected that shows least neurons at 2.5 magnification. These areas to be outlined are from stratum pyramidalis (Duvernoy, 1988). 10 randomly selected high power fields with 40x objective lens of each hippocampal subfield CA1, CA2, CA3 and hilar region will then be manually counted for neurons by the investigator. The computer will randomly select 10 fields from within the manually outlined region of each subfield. CA1, CA2, CA3 and hilar region will be selected based on anatomical sources (Duvernoy, 1988, Lanerolle 2003). It is anticipated that each specimen neuronal

count will be labor intensive. Time to count subfields will also likely shorten with experience in use of the Histometrix analyzer.

Figure 1. NeuN specimen with a classical pattern of hippocampal sclerosis. There is marked neuronal loss in CA1 and CA4 (hilus) with cell loss also present in CA3 and granule cell dispersion present on gross inspection as evidenced by the widened granule cell layer. The NeuN stain colors neurons brown. While the larger neurons are presumed to be pyramidal neurons in the stratum pyramidalis, NeuN also stains smaller sized interneurons.



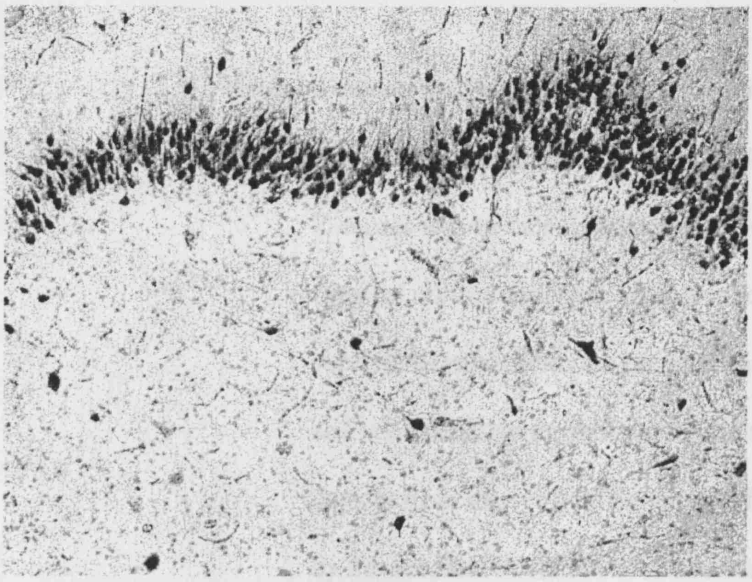
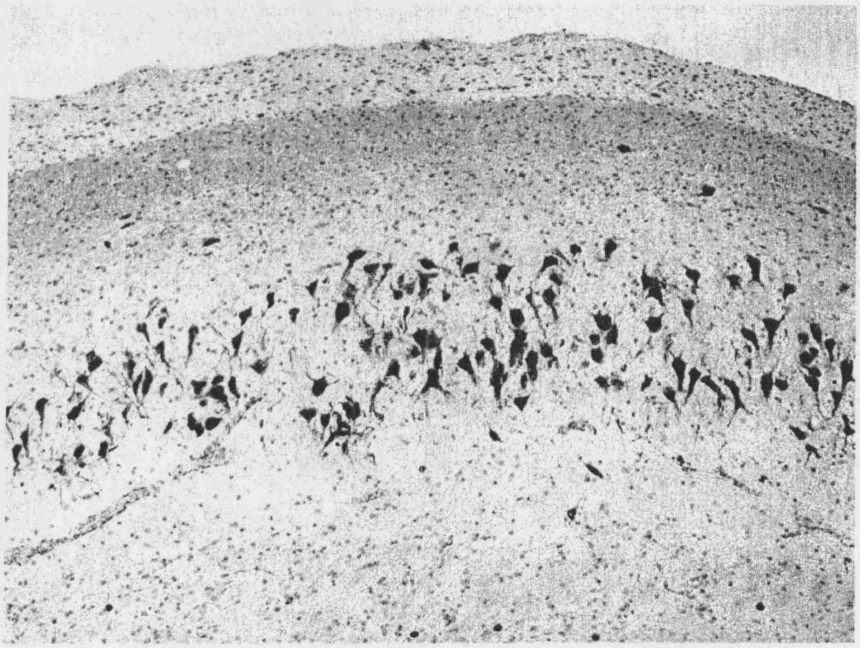


Figure 2 above is a close up of the hilar region showing the severe neuronal depletion in this sample specimen as evidenced by the absence of brown staining neurons in the region inferior to the granule cell layer. The granule cells of the dentate also stain intensely brown. On gross inspection mild granule cell dispersion also appears present as evidenced by the widening of the granule cell layer and the scattering of dentate cells outside of the layer.

Figure 3 below is a higher power view of the CA2 subfield from Figure 1 showing many preserved pyramidal neurons staining with NeuN in the stratum pyramidalis. Using the Histometrix image analyzer, the investigator selects a handdrawn section of CA2 and then requests a computerized random selection of 10 high power fields to be presented for neuronal counts to be performed. Neurons are then counted under 40x objective lens magnification. Neurons are defined as brown-labelled cells with clearly recognizable nuclei present.



f. Neuronal counts will be obtained with the Histometrix image analyzer and *a priori* pattern categories will be defined as follows:

   i. Z-scores will be calculated for each subfield of each NeuN surgical section analysed. Z-scores reflect the distance a subfield deviates from the predetermined population mean and are calculated by subtracting the subfield neuronal density from the subfield density of the mean and dividing by the standard deviation of the mean. We will define population mean as the average neuronal counts obtained from our "normal" controls. We will obtain this from the 3 PM series. Z-scores are expected to be negative in this study and reflect the number of standard deviations each sample and each subfield, is from the arbitrarily predetermined population mean. Only negative z-scores will be considered meaningful in this study. Positive z-scores will be interpreted as overlapping with the autopsy normal controls, and therefore not considered different from normal. The magnitude of the negative z-score will be used to construct our arbitrary categories. For ease of discussion, the absolute value of the negative z-score value will be used. Descriptive statistics will be done using SPSS (SPSS Inc, Chicago, IL, USA), hand calculator, and Excel graphs.

ii. Normal. Defined as (absolute) z-score less than 1.5 in each hippocampal subfield. Z-score of 1.5 as an arbitrary "Z-score cut-off" between normal and abnormal is a widely used method. Psychometric tests commonly use this cut-off value, as do many metabolic tests such as Body Mass Index (BMI) (Kirk 2005, Visser 2002). Temporal lobe epilepsy outcome research using PET has also used this technique (Wong 1996). Z-score cut-off of 1.5 will be used to in this study to create categories of hippocampal neuronal loss to identify HS patterns.

iii. Classical Hippocampal Sclerosis (HS). Defined as z-score less then 1.5 in CA2 but abnormal (1.5 or more) in all other subfields. If only one block of CA1 is abnormal, then will be called focal (f) (same for other patterns below).

iv. More severe total HS (THS). Abnormal "cut-off z-scores" in all subfields (z-score 1.5 or more).

v. CA1 predominant loss. Abnormal z-score in CA1 only.

vi. End folium sclerosis. Abnormal z-score confined to hilar region with or without CA3 involvement as well.

vii. Undetermined. Group of specimens not falling into above mentioned patterns.

g. In addition, qualitative evaluation will be performed as follows:

i. Individual NeuN specimens will be graded as either normal, atypical or typical Hippocampal sclerosis.

ii. Typical HS will be labelled Classical HS (CHS) or more severe HS, labelled total HS (THS).

iii. Atypical patterns will be labelled as end folium sclerosis or CA1 predominant loss. Specimens that can not be readily classified will be grouped as indeterminant. Within each group, if a focal region of CA1 is found to be affected, it will be labelled (f) for focal.

iv. Qualitative pattern definitions: Normal pattern with no cell loss in any subfield. EFS pattern with cell loss affecting CA4 with other subfields normal. CA1predominant with cell loss in CA1 and other subfields normal. CHS pattern with cell loss in CA1, CA4 and CA3 and CA2 relatively preserved. THS will have more severe cell loss overall as well as CA2 involvment.

2.3 Details of of granule cell dispersion (GCD) measurement methods

a. The GCD measurements will be performed by Dr.Maria Thom.

b. Single NeuN section per case.

c. Image Pro plus software will be used(media cybernetics). Four images at x20 objective lens of the granule cell layer (GCL) will be captured where the dispersion or width of the granule cell layer appears maximal, avoiding the curvatures.

d. In each image the 10 most distal granule cells at the border with the molecular layer will be tagged and a best fit line between these points will be drawn. The 10 most basal cells (the border with the

hilus) will be also tagged and a best fit line drawn. The average distance between these lines will be measured and calibrated in microns(μm). The average and maximal measurement of granule cell dispersion (GCD) will then be recorded.

e. In addition a qualitative evaluation of the GCL will be made as follows:

   i. dGCL-depletion of the GCL (Group 1)
   ii. Normal (Group 2)
   iii. Mild GCD + dGCL = mild GCD with focal depletion (Group3)
   iv. Mild GCD (group 4)
   v. Moderate GCD + dGCL = moderate GCD with regions of depletion (group 5)
   vi. Moderate GCD (Group 6)
   vii. Severe GCD with dGCL (Group7)
   viii. Severe GCD (Group 8)
   ix. Controls (Group9)

2.4 Details of record review methods

Jane de Tisi, The Departmental Personal Assistant of the DCEE, has already retrospectively reviewed paper records of the NHNN to create a patient database. Available data includes date of birth, the operated side, sex and seizure outcome at 1 year. The ILAE classification system for seizure outcome post surgery will be used in this study and groups patients from 1-6 based on seizure frequency post surgery. 1 is seizure free with no

auras while 2 is only auras, for example (Wieser 2001). Dr. Luis Caboclo, a post-doctoral neurologist and fellow of the DCEE, will also perform a more extensive clinical chart review, but his data set will not be available for use in this study.

3.0 RESULTS

3.1 HIPPOCAMPAL SCLEROSIS PATTERNS

Figure 1.
Graph of Qualitative HS pattern and good post surgical outcome

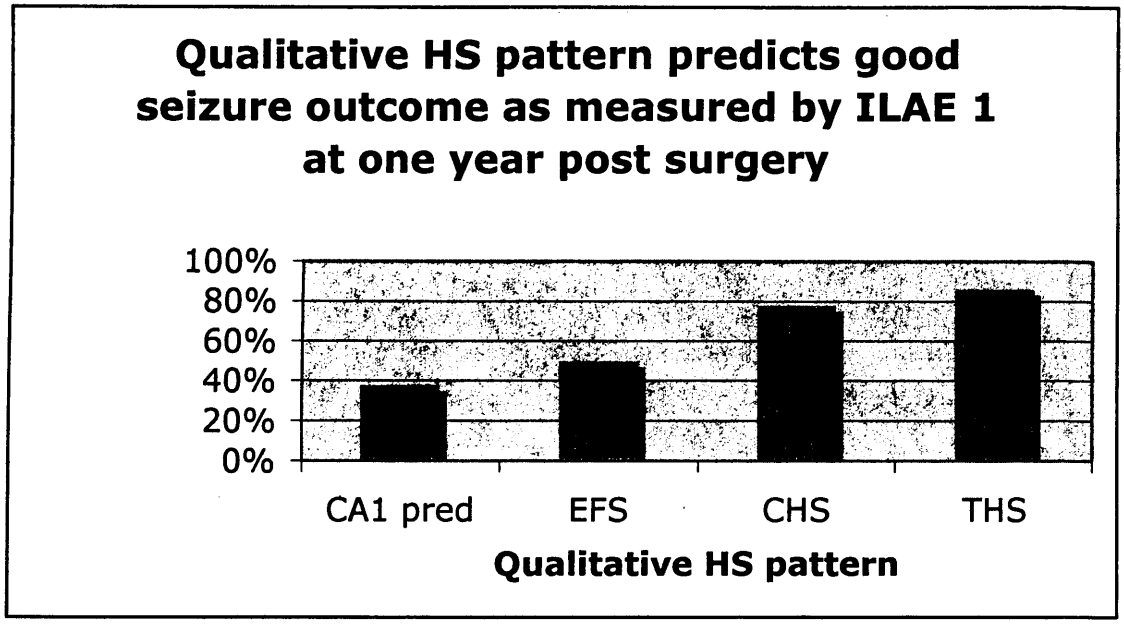


Fig. 1.As the above graph demonstrates, the qualitative HS patterns have different post operative seizures outcomes. ILAE 1 is used as definition of a good surgical outcome. ILAE is defined as no seizures following anterior lobectomy (unlike Engel 1 which permits auras, and larger seizures if occurring during medication withdrawal). Only data available at one year post surgery is included in the above figure. The normal group was not included due to very low numbers. The data show that CA1 pred (38%). and EFS (50%) have a less favourable post surgical outcome then the more typical HS patterns of CHS(78%) and THS(86%).

The numbers in each qualitative HS pattern were as follows: CA1 =13, EFS=8, CHS =78, and THS=37 (see Appendix 1 for raw data). Of these 144 specimens, 94 had 1 year outcomes data as follows: CA1=8, EFS=4, CHS=51, THS=27, Normal =3 (see Appendix 2).

3.1 HIPPOCAMPAL SCLEROSIS PATTERNS

Figure 2.
Quantitative HS pattern does not predict seizure outcome very well

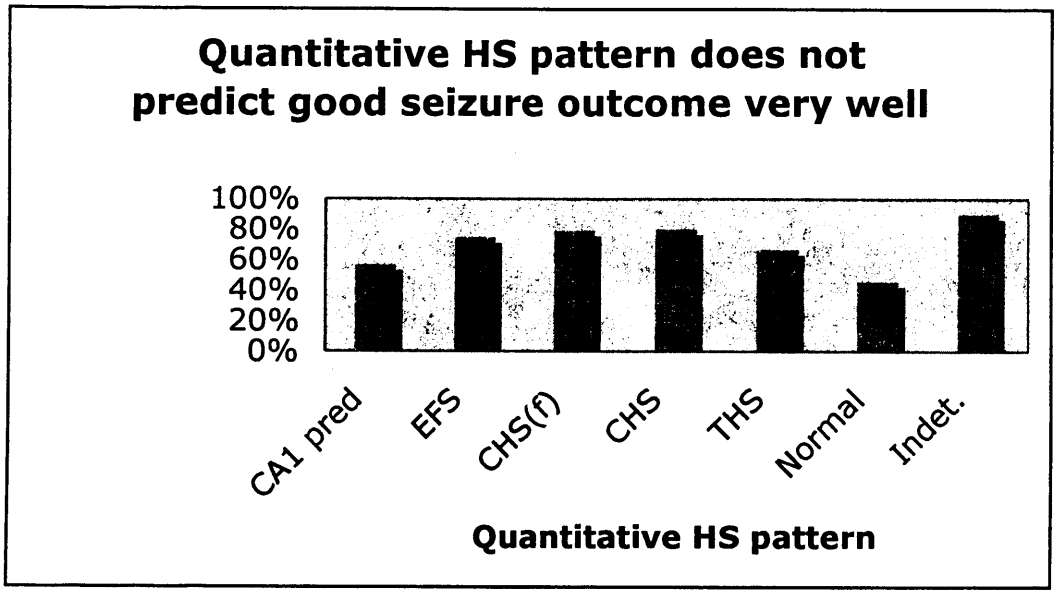


Figure 2.
Quantitative HS pattern method produced CA1 with 57% (4/7), EFS 75% (6/8), indet. 90% (9/10), Normal 46% (5/11), CHS(f) 79% (15/19), CHS 80% (24/30), and THS 67% (6/9). This method produced similar good outcomes for CHS and THS as qual. Method but EFS is not associated with a poor outcome. CA1 has a lower percentage of good outcomes then typical HS but not as low as qual. HS method.

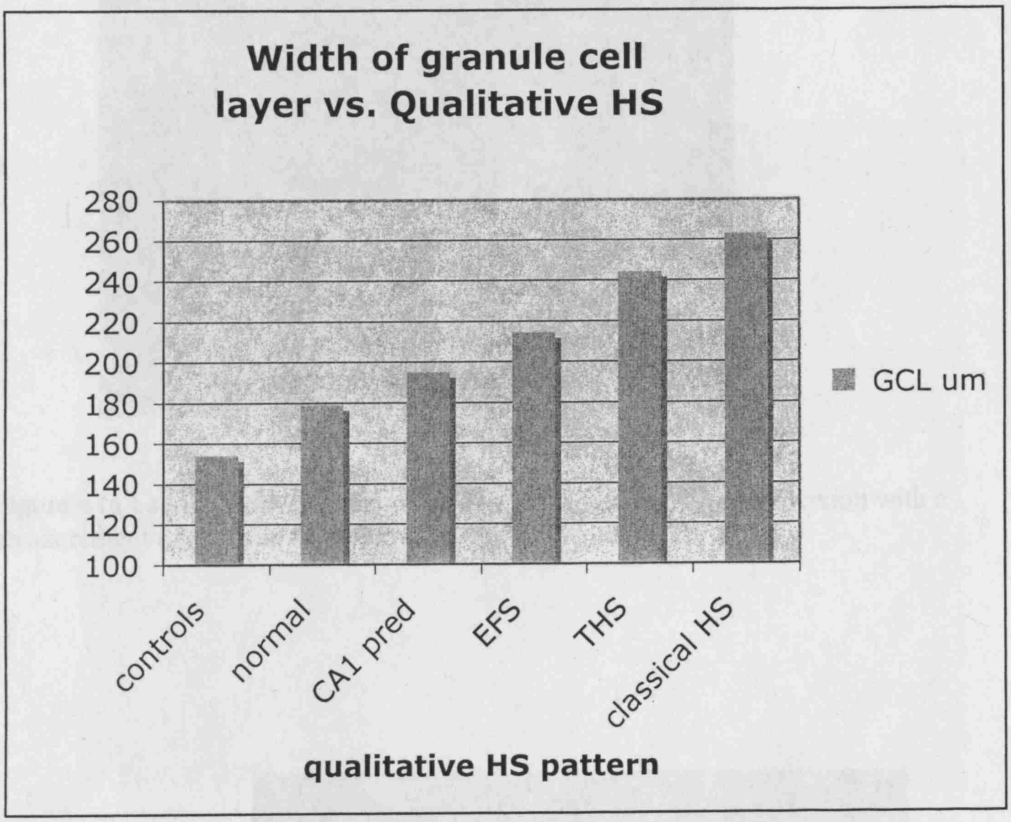
The numbers in each quantitative pattern were as follows: CA1=12, EFS=9, CHS=46, CHS(f)=31, Normal=20, indet.=14, THS=12. Of these 144 specimens, 94 had 1 year outcomes data as follows: CA1=7, EFS=8, CHS=30, CHS(f)=19, Normal=11, indeterminant=10, THS=9. Average total z-scores (score for each subfield summed for complete specimens only) were as follows: CA1=-4.4 (S.D=2.1), EFS=-7.6 (2.1), CHS=-11.8(1.3), CHS(f)=-9.4(1.8), Normal=-.8(.9), indet= -7.2(2.1) THS= -12.9(1.5).

Quantitative HS pattern was compared to the qualitative HS pattern of the same patient (indeterminant group not included and focal groups collapsed into main group, eg. CHS(f)=CHS). Two tailed Pearson correlation was .732 (significant at 0.01). This suggests a moderate agreement between the qualitative HS pattern method and the quantitative HS pattern method we used in this study.

One data observation that emerged while using the Histometrix image analyzer, was that there were often smaller NeuN stained neurons in subfields that on gross inspection appeared neuronally depleted. These smaller neurons are most likely interneurons. They were counted as per our Methods section.

3.2 GRANULE CELL DISPERSION

Figure 3. Qualitative HS patterns vary with GCL Max.



The graph above shows increasing Max GCL with different qualitative HS patterns. The width of GCL is a very gross global impression of extent of granule cell dispersion. The qualitative HS patterns were determined by a global impression and inspection of the hippocampus and subfields, but no neuronal counts were made. As the graph suggests, as the qualitative HS patterns progress from controls, normals, atypical CA1 predominant and EFS to more typical hippocampal sclerosis, there is a corresponding increase in GCL width. This data suggest there may be a relationship between the two phenomena.

3.2 GRANULE CELL DISPERSION

Figure 4.
Examples of Granule cell layer measurements of Dispersion

a

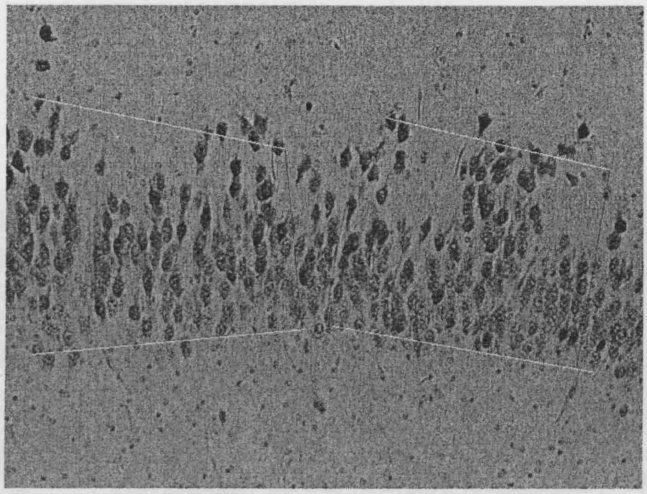


Figure 4 (a) above is an example of moderate granule cell layer dispersion with a measurement of the granule cell layer width of 205 um.

b

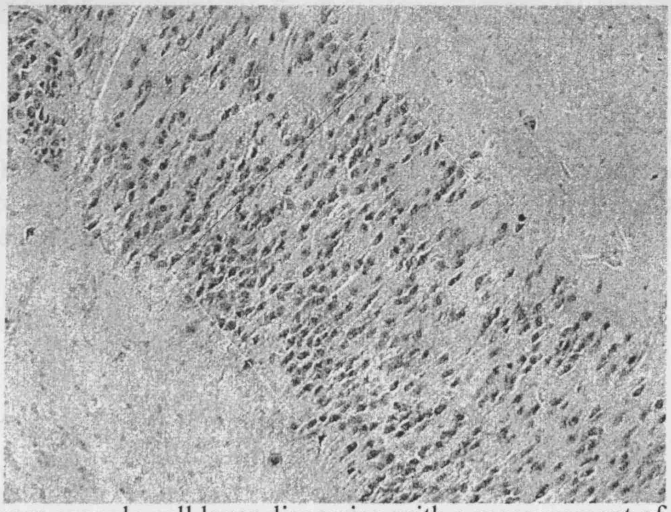


Figure 4 (b) shows severe granule cell layer dispersion with a measurement of the most maximal measurement of the granule cell layer of 277 um.

3.2 GRANULE CELL DISPERSION

Figure 4.
Examples of Granule cell layer measurements of Dispersion

c

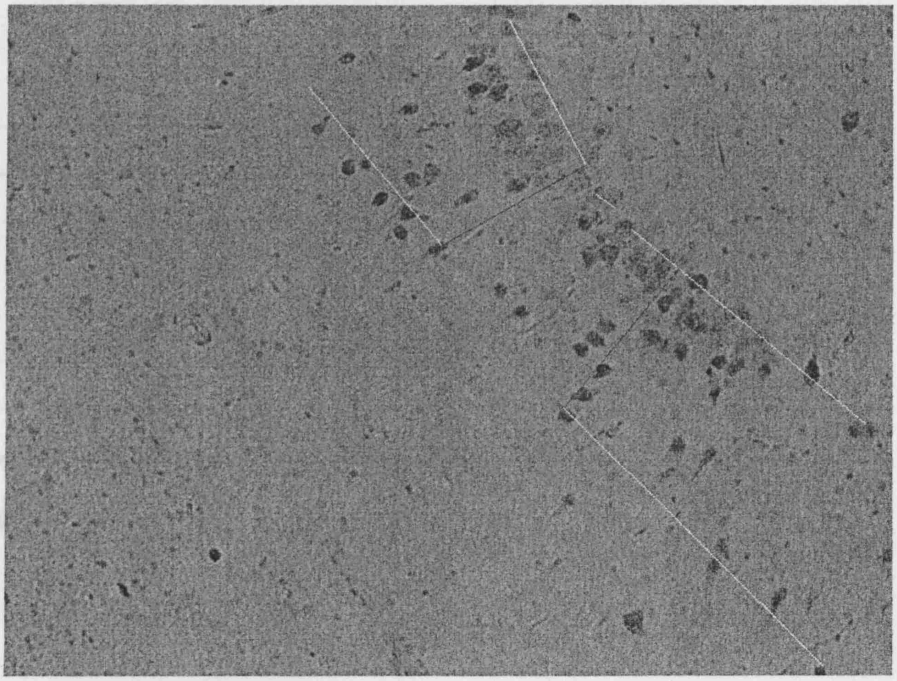
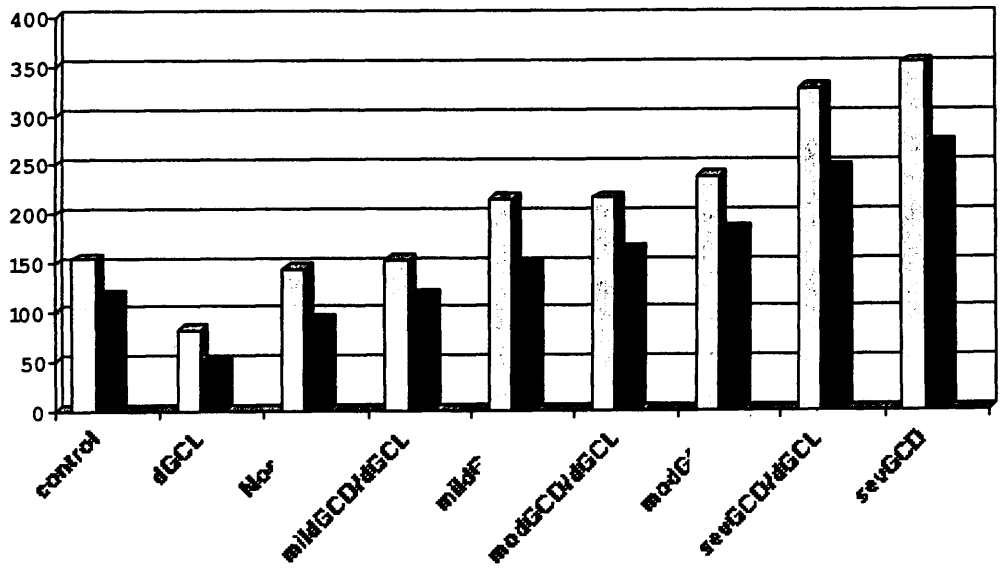


Figure 4 (c) is an example of a bilayer granule cell layer, another pattern of granule cell dispersion (GCD). What is uncertain about this pattern of dispersion is whether the cells have migrated to this position or whether this pattern develops because of granule cell loss in the medial portion of this layer.

3.2 GRANULE CELL DISPERSION

Figure 5.
Graph of GCD measurements in Qualitative descriptive groupings



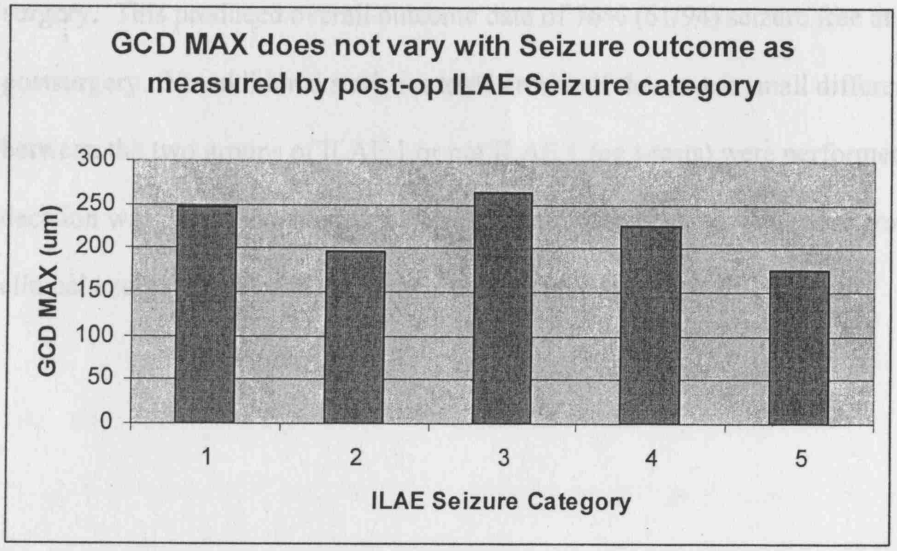
Light blue = mean max GCL thickness measured in um,
Dark blue = mean average thickness

More severe GCD qualitative groups trend towards a larger GCL max. Linear regression does not suggest a strong association. R^2 is .408 (adjusted R^2 is .404)

3.2 GRANULE CELL DISPERSION

Figure 6.

Post-op Seizure Outcome as Measured by ILAE Category
shows no relationship to GCD MAX measurement..



The overall width of the granule cell layer, as assessed here by the GCD MAX measurement, does not appear to vary with the post-operative ILAE Seizure score.

3.3 SEIZURE OUTCOMES DATA

Of the 144 pathological specimens, 94 had 1 year outcome data available to be used for outcomes assessment.

Of these 94, 23 had a poor surgical outcome and 71 had a good post-surgical outcome, defined as ILAE 1 classification (seizure free) at one year following surgery. This produced overall outcome data of 76% (61/94) seizure free at one year postsurgery. No additional statistics to determine if there were small differences between the two groups of ILAE 1 or not ILAE 1 (eg t-tests) were performed. This decision was based on amount of missing data. A future study with more complete clinical and pathological data comparisions is planned.

4.0 DISCUSSION

This research is the beginning of the type of research the Joint Epilepsy Council of 2007 states is needed to advance knowledge of epilepsy (JEC 2007). This study of one institution's experience in patients undergoing anterior temporal lobectomy for medically intractable temporal lobe epilepsy from hippocampal sclerosis, is also an example of outcomes research embraced by the new healthcare quality movement. We assume that such clinicopathological evaluation will provide some of the improved knowledge that Deming claims is required for improved outcomes research (Nolan 2007).

4.1 Study Strengths

A study strength is the evaluation of hippocampal pathology patterns, clinical data and epilepsy management of one institution. This potentially removes sources of variance when data from multiple institutions are grouped together. We assume that clinicopathological evaluation from one institution will show less variability amongst clinicians medically treating and diagnosing "medically intractable" epilepsy then a similar study that groups data from multiple institutions. Major differences exist in clinician treatment preferences across many neurological treatments and that this clinician variability in treatment occurs across medicine (Institute for Healthcare Improvement 2007). Such variable clinical practice contributes to major differences in outcomes because patients get tried on different medications and get referred for surgery at different points in their disease course. The recent Kwan clinical practice experience is illustrative (Kwan 2000).

An additional strength of this one institution experience is that potential variability in surgical technique is also lessened. Just as there is variability in

clinician practice, so there is variability in surgical practice across both individual neurosurgeon and across institutions. The clinicopathological study of Blumcke evaluated NeuN specimens obtained from the surgical experience across four centres (Blumcke 2007). Our patients underwent the same surgical procedure of anterior temporal lobectomy, performed by two surgeons.

Additional study strengths are that a single investigator evaluated NeuN specimens for patterns of neuronal counts, a single separate investigator evaluated NeuN specimens for granule cell dispersion, and a separate researcher evaluated all of the patient clinical records. This homogenous assessment is assumed to produce less variance than might result from interinvestigator differences in technique.

We also believe that our sample size is representative of the broader population of patients undergoing temporal lobe resection for hippocampal sclerosis and intractable epilepsy. We believe this because our clinical analysis revealed post surgical outcomes at one year using the ILAE classification criteria that broadly agree with other published experience. We found 76% were seizure free with ILAE 1 at one year post surgery. Our results are very similar to the Cohen-Godal data of 399 patients followed at the Mayo clinic (Cohen-Godal 2006). The Mayo clinic investigators also used ILAE 1 as the definition of good post surgical outcome and found 78% of their 399 patients had ILAE 1 one year post surgery for intractable temporal lobe epilepsy. This 78% 1 year outcomes data is very similar to the 76% found in our data set, and suggest that our sample is broadly representative of the larger population of patients with intractable epilepsy who undergo temporal lobectomy. This is despite much missing data in our study as compared with the Mayo electronic dataset.

4.2 Results Interpretation

We identified patterns of hippocampal sclerosis, using our qualitative HS pattern method, that predict a better or worse post surgical outcome. Both patterns of typical hippocampal sclerosis showed better one year seizure control then the two atypical patterns of CA1 predominant and End folium sclerosis. While we found that end folium sclerosis had a worse postsurgical outcome then typical HS, interpretation of these findings is limited by the small numbers of these specimens. While Blumcke found six month outcomes of 28% with EFS as defined by Engel 1, we found that at one year using ILAE 1, 50% of patients with pathological HS pattern of EFS, were seizure free. We found very low numbers of EFS, with 4 out of the 94 specimens (with one year outcome data available), showing this pattern, limiting this finding. The lower postsurgical good outcome figure would change to close to the mean of the entire study with the change in the result of just one of the EFS specimens. This is why the finding needs to remain speculative, at present, until it can be replicated (or not) in a larger, more complete study.

Despite these limitations, our data broadly agree with Blumcke's finding that EFS has a worse postsurgical outcome. This is despite the use of different outcome measures (ILAE vs. Engel 1) (Blumcke 2007). We also confirmed the Blumcke finding that CA1 predominance is a HS pattern also associated with a poorer postsurgical seizure outcome then more typical HS. We found very similar results to Blumcke with 38% as compared with his 50% good outcome (Blumcke 2007). We also confirmed the better results with the more typical HS patterns of CHS and THS, with 86% good outcome with THS and 78% good outcome with CHS.

Our finding of an association between qualitative HS pattern of EFS with poor postsurgical outcome was not confirmed with the quantitative HS pattern method, however. We found no association between quantitative pattern and seizure outcome for the EFS pattern. We found CA1 pattern with 57% good outcome which is less then typical HS pattern outcomes. We confirmed with this method that the typical HS patterns have a better outcome with CHS(f) with 79% ILAE 1 at one year and CHS of 80%. These results are very similar to the qualitative HS method results for typical HS seizure outcome. Our inability to find similar results with EFS and CA1 may reflect the small numbers of these patterns. This may also reflect "faulty methods' such as the inclusion of many NeuN staining interneurons that "artificially" increase the neuronal count. This presumes that pyramidal neuronal loss is the feature that is most predictive of outcome. There was moderate agreement between the two methods of HS pattern classification as evidenced by a Pearson coefficient of 0.7. Additionally, both methods detected the majority of the patterns as typical HS. The qualitative method found 115 of 144 as CHS or THS and the quantitative method found 128 of 144. These results broadly agree with the literature (see Blumcke 2007, de Lanerolle 2003). These methods diverged in their ability to predict postsurgical seizure outcome with the atypical patterns. It is not unique to this study to find differences between quantitative neuronal counts measured by neuronal density and qualitative HS patterns.

Other researchers have commented on the difficulty of correlating neuronal counts with qualitative HS patterns. de Lanerolle suggests that this difficulty reflects much interindividual specimen variability in neuron loss (de Lanerolle 2003). Babb found it difficult to match his cell counts with the patterns of typical HS and EFS identified by Bruton (Babb 1991). If the pyramidal cell loss is what is important for

outcome, then the qualitative method will prove superior, but this remains hypothetical. Blumcke performed a post hoc cluster analysis of z-scores and not an *a priori* comparision of neuronal counts with qualitative assessment as we have, so the studies are not directly comparable (Blumcke 2007). It also remains hypothetical that there is a correct way to estimate neuronal count so as to predict seizure outcome. These divergent data may also reflect a lack of association between atypical HS pattern and outcomes and a larger study with better clinical data is needed to answer this.

4.3 Implications for Future Research

NeuN is used routinely in the pathological postsurgical evaluation of temporal lobe resections for hippocampal sclerosis. Potential future studies of interest may be to utilize our qualitative patterns of both dentate granule cell dispersion and neuronal pattern of cell loss to predict, in a prospective manner, future patient seizure behavior. More detailed investigator conducted studies of computerized high power neuronal counts in hippocampal subfields and dentate granule layer for patterns also has a potential future role in identifying as yet unknown pathophysiological factors underlying intractable seizures. It has been recognized for some time, and it is quite puzzling, why a more global neuronal loss in hippocampus appears to be associated with a better postsurgical outcome then a more patchy "atypical" pattern of neuronal loss. Something about the inherent wiring of the hippocampus as well as the hippocampal response to injury must be relevant here. If the postsurgical outcome had nothing to do with hippocampal pattern and neuronal loss, then it would be believable that the cause of intractable seizures after surgery must lie outside the hippocampus and possibly involve a

"network" type perturbation in the rest of the brain. But this cannot be the entire explanation for failure of seizure control postsurgery if a repeated research finding is that the most severe hippocampal sclerosis has the best operative outcome.

If these findings that patients with more atypical neuronal loss have worst outcomes are real (ie. they are reproducible in a larger dataset), then these findings may have implications for clinical care of patients with temporal lobe epilepsy, in the future. If these findings can be replicated, then it may be possible with better MRI imaging in the future, to view the subfields of the hippocampi and then possibly, to identify these atypical hippocampal patterns of neuronal loss prior to epilepsy surgery. New higher strength magnet MRIs will be able to evaluate hippocampal anatomy in great detail. It may soon be possible to identify atypical hippocampal sclerosis patterns of cell loss predictive of poor postsurgical outcome, such as CA1 loss or end folium sclerosis, prior to surgery. It is possible if such findings of poorer surgical outcome are upheld with future research, that such data will be incorporated into clinical decisionmaking about medical management versus surgery. Such patterns may also need to be incorporated into the informed consent between clinician and patient as our data tentatively support the recent findings that the atypical HS patterns predict a worse postsurgical outcome (Blumcke 2007). Another pathological process associated with intractable temporal lobe epilepsy that we evaluated in this study was quantitative and qualitative patterns of granule cell dispersion.

4.4 Granule Cell Dispersion

GCD has been assessed by others by measurement of the width of the granule cell layer of the dentate gyrus (Haas 2002, Fahrner 2007). In our study,

maximal widths were measured by accurate best fit measurements obtained with Image Pro software. Fahrner and colleagues have found width of granule cell layer correlated with the severity of hippocampal sclerosis as determined by the Wyler score method (Fahrner 2007). We confirmed Fahrner's findings by finding that GCL MAX as a measurement of maximal layer width, varied with HS pattern and both types of typical classical hippocampal sclerosis, the classical and total HS, had the highest average values of GCL MAX (see figure 3). These findings suggest that a shared process may explain both typical HS and widening of GCL in our patients.

We have evaluated GCD in more detail then previous studies. In this study, the extent of GCD was further defined by creating nine qualitative categories of GCD. The nine qualitative categories of GCD grouped granule cell loss and dispersion from mild, moderate to severe. These qualitative categories of GCD varied in the same direction as the semiquantitative measures of GCD obtained with the image Pro software (see Figure 5).

GCD has been previously reported as being present only in a minority of post-surgical hippocampal specimens from temporal lobectomy for treatment of intractable temporal lobe epilepsy due to hippocampal sclerosis (Thom 2005a). Previous work has demonstrated that GCD varies with amount of principal hippocampal neuron loss (Thom 2002). The cell cycle marker Mcm2 is a marker of cell turnover as it measures cycling cells as well as cells with proliferative potential (Thom 2005a). In pathological specimens with severe hippocampal sclerosis, higher numbers of Mcm2 positive cells in the dentate gyrus are found (Thom 2005a). Whether these cells are small neuronal precursors or proliferating radial glia, is not known (Thom 2005a). Other researchers have not found any evidence of cell proliferation or neurogenesis using a different marker, Ki-67 (Fahrner 2007).

Cystatin C expression may be a marker for both neurodegeneration as well as neurogenesis. It appears to be upregulated in both a chronic rodent model of epilepsy and in the molecular layer of the dentate gyrus in human epilepsy specimens (Pirttila 2005). It appeared to localize to glia and to GCD (Pirttila 2005). This is suggestive that it may have a role in guiding new granule cells that derive from the subgranular zone into the dentate gyrus aberrantly, in part explaining GCD in the presence of chronic epilepsy. The GCD evaluation in this study is a work-in-progress, and the evaluation of GCD is with a more continuous assay then prior research has used. It is too early to determine the significance of some of the GCD patterns without better seizure outcomes data.

A limitation of these GCD studies are that we have not proven that there is a direct causal relationship between hippocampal subfield neuron loss and granule cell dispersion. While it is possible that ongoing intractable seizures can cause both phenomena, it is also possible that no relationship actually exists. Intriguing new research into the pathophysiology of depression is illustrative. Rodent work suggests that a mechanism by which antidepressant agents may reverse depression is via the induction of neurogenesis in the dentate layer of the hippocampus (Malberg 2000). This neurogenesis in the dentate is considered the primary mechanism of antidepressant action (Santarelli 2003). Primate research has replicated this work in a monkey model (Perera 2007). The time course of the neurogenesis in the rat mirrored the time course required for therapeutic effect of the antidepressant therapy (Malberg 2000) The primate study used electroconvulsive shock therapy as the specific antidepressant "agent" used to induce neurogenesis. The neurogenesis occurred from increased precursor cell proliferation in the subgranular zone of the dentate gyrus of the hippocampus. Most of these new cells became neurons (Perera

2007). ECT is the therapeutic induction of a generalized seizure while under muscular paralysis, and while Perera used it as a model of antidepressant therapy, it can also be considered a model of recurrent seizures. It is not known yet the significance of the GCD patterns we have described. Our study of HS patterns and GCD also suffers from multiple study limitations.

4.5 Study Limitations

The first study limitation arises from the weaknesses of retrospective review. There may be unidentified sources of bias that we were not able to uncover that somehow explain our results. Medical records are notoriously weak as a source of clinical information and unfortunately the records of the NHNN are both paper and handwritten which make them often difficult to read. Data is often lost, misplaced, or difficult to decipher in such untyped paper records. This makes the clinical data collected subject to error. Such sources of error are evident by the database extraction for this project, which revealed many missing and incomplete clinical records to correlate with the pathological evaluation. The absence of a robust electronic record system for managing clinical records is a major limitation of this study that is beyond the scope of this research to address.

Other sources of bias may result from the potential selection of the patients that come to the NHNN for epilepsy advice and medical care. These patients may have more severe epilepsy then the general population of patients with medically intractable epilepsy considered for surgery.

Other technical limitations include the small number of autopsy controls (3 with both left and right hippocampi sampled) this may have over or undercounted what is actually just an estimate of the "population mean" from which the z-scores

were calculated. It is difficult to get appropriate autopsy controls, and the time limitations of this study produced a small autopsy control sample from which to estimate hippocampal neuronal counts.

Other technical limitations result from the evaluation of surgical specimens. Missing or damaged subfield data were a problem. An electrode or surgical artifact often produced hemmorrhage throughout CA2 or CA3 subfields. Occasionally a subfield was missing entirely. These specimens were evaluated anyway given the pilot study"explorative" nature of this research. A "best fit" was made for both the qualitative arm and within the quantitative study. This is a serious weakness of our study as compared with the more complete surgical specimens in the Blumcke study (Blumcke 2007).

The most severe limitation of this study is that the phenomenon we are studying- outcome after anterior temporal lobe resection for epilepsy management- must be more complex than can be explained by specimen neuropathological patterns. Many variables impact an individual patient's seizure frequency from mood,stress, alcohol use, unknown genotypes, unmeasured transporter proteins, and intercurrent illnesses and medications, for example. We are unable to control for all of these variables in an uncontrolled retrospective review.

Alternative reasons for why surgical resection of seizure focus fails in some patients is that the epilepsy in such patients results from perturbation of a network, with abnormal neuronal excitability spread outside the hippocampus (Moran 2001, Ryvlin 2005). The existence of such a network may explain the temporal "plus" epilepsies that may not respond to temporal lobectomy (Barba 2007). Such temporal plus disorders involve hippocampus but also additional nearby structures of orbitofrontal cortex, insula and operculum in the maintenance of the seizure disorder

(Barba 2007). Such temporal plus epilepsy patients may be identified with invasive depth EEG recordings (Kahane 2004). The clinical phenomenology of temporal plus epilepsy has been recently reviewed.

Barba and colleagues retrospectively re-evaluated 80 patients from an epilepsy surgical cohort with a diagnosis of hippocampal sclerosis preoperatively (Barba 2007). Ictal symptoms and scalp EEG were able to differentiate patients with temporal plus epilepsy from more typical temporal lobe epilepsy. Epigastric aura localized to temporal lobe, while gustatory, vestibular and auditory aura were more often seen in temporal plus patients. Temporal plus patients were also more likely to have ipsilateral tonic or versive motor changes (Barba 2007). Another difference between temporal and temporal plus epilepsy patients is likely to be variability in postsurgical outcomes. It would be anticipated that temporal plus patients will not become seizure free after surgery.

Patients who do not become seizure free after epilepsy surgery have been recently evaluated (McIntosh 2006). The majority of patients having one seizure after surgery went on to have more seizures and one half of this group had seizures regularly (McIntosh 2006). This data support our use of ILAE 1 at one year as a measure separating good from bad outcome post surgery (McIntosh 2006, Cohen-Gadol 2006). It is possible that some of the patients that do not become seizure free after seizure surgery have a temporal plus syndrome. Maybe the atypical patterns of hippocampal sclerosis we have tentatively identified, represent aberrant connections to extratemporal seizure foci that help lay the foundation for temporal plus syndrome.

Our study suggests that it may be possible to explain a small part of the complexity of medically intractable epilepsy by cellular neuronal patterns of loss and dispersion in dentate layer and neuronal loss in subfields of hippocampus.

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Appendix 1. Table 1 Raw Data.
Each pathological specimen is identified by a number from 1-144 in the lefthand column, followed by data from the 6 controls. Each neuronal density measurement from each subfield in μm E^{-04} follows. The Qualitative HS pattern is then followed by the quantitative HS pattern. The GCL max measurement is in the righthand column.

specimen	CA1 (a)	CA1 (b)	CA2	CA3	Hilar	Qual. pattern	Quant pattern	GCL max
1	1.68	1.66	n/c	.731	.188	chs	Indet.	255
2	1.1	.526	2.46	n/c	.307	chs	chs(f)	329.7
3	1.1	.044	2.26	n/c	.131	ths	chs (f)	154.8
4	.922	.569	1.85	n/c	.965	chs	Indet.	121.2
5	.438	.351	2.06	.526	.57	chs	chs	174.9
6	5.21	5.38	2.74	.834	.049	efs	efs	221.6
7	.263	.263	n/c	n/c	.131	ths	chs	201
8	2.24	.439	1.18	1.27	.789	ths	chs(f)	345.4
9	1.27	.483	2.02	.833	.834	chs	chs(f)	256.6
10	.175	.659	1.62	n/c	.175	chs	chs	145.6
11	.746	1.01	n/c	1.93	2.44	CA1 pred	CA1 (f)	130.2
12	.395	.746	1.4	.394	.088	ths	chs	428.8
13	.921	.35	2.19	1.85	1.89	CA1	CA1	183.4
14	.57	.79	n/c	1.27	1.01	chs	indet	220.7
15	1.14	1.14	3.55	1.01	.438	chs	efs	363.8
16	1.36	.39	1.36	.613	.35	chs	chs(f)	342.7
17	.833	1.27	2.24	1.27	.0877	ths	chs(f)	21.7
18	1.1	.35	.934	1.0	.702	ths	chs(f)	42.4
19	1.14	.789	1.84	.79	.833	chs	chs(f)	105.5
20	1.97	1.31	1.58	.658	.658	ths	efs	126.7
21	2.85	2.32	n/c	n/c	.088	efs	efs	176.9
22	.438	.438	2.11	.657	.57	ths	chs	293.7
23	1.23	.921	2.15	.701	.613	chs	Indet.	272.9
24	2.54	.483	n/c	.376	.176	ths	chs(f)	371.7
25	1.54	1.1	4.21	.146	.789	chs	efs	409.1
26	1.18	.352	.832	.263	.701	ths	chs(f)	132.7
27	.702	.088	1.89	n/c	.088	ths	chs	120.9
28	3.07	2.72	n/c	n/c	1.4	normal	normal	177.8
29	3.41	4.34	n/c	n/c	1.81	efs	normal	178.4
30	.682	.307	3.23	.340	.351	chs	chs	318.3
31	2.28	2.46	n/c	.747	.614	chs	efs	217.4
32	1.31	.614	1.27	n/c	.383	chs	chs (f)	330.4
33	2.68	1.7	2.67	2.19	1.49	chs	normal	187.9
34	.482	1.05	1.93	1.43	.966	chs	chs (f)	166.6
35	5.05	1.89	2.39	2.45	2.49	chs	normal	189.4
36	1.01	.964	2.32	.482	.482	ths	chs (f)	167.3
37	3.46	.634	n/c	n/c	.536	chs	chs(f)	123
38	1.8	1.36	2.11	2.37	.921	chs	normal	276.9
39	1.32	.614	1.01	1.4	.438	chs	chs (f)	178.6
40	.57	.351	1.4	n/c	.097	chs	chs	336.7
41	1.58	.878	n/c	n/c	.615	chs	indet	387.1
42	.658	.57	4.24	.92	.263	chs	chs	276.8
43	1.54	1.01	1.75	2.06	1.1	chs	normal	351.1
44	.603	.833	n/c	n/c	.702	chs	chs	293.
45	1.31	.73	2.3	n/c	.921	chs	chs (f)	155.3
46	2.72	1.45	n/c	1.41	1.31	efs	indet.	220.5
47	2.19	1.84	n/c	2.63	2.67	normal	normal	156.3
48	.701	.73	2.39	2.98	2.1	CA1	CA1	131.1
49	3.2	2.11	2.06	1.49	1.12	normal	indet	212.9

50	3.38	1.18	1.31	2.44	1.85	chs	normal	126.7
51	.828	.834	.702	.351	.536	chs	ths	111.8
52	.219	.063	n/c	n/c	.614	chs	chs	355.8
53	5.31	5.04	7.33	n/c	4.09	normal	normal	103.2
54	3.54	4.34	1.4	2.76	1.89	efs	normal	343
55	1.53	1.18	1.71	2.81	2.14	CA1	normal	244.8
56	.833	.11	.921	.745	.274	ths	chs	218.7
57	.92	.526	n/c	.351	.78	chs	chs	298.9
58	1.14	.702	1.1	1.32	.974	chs	chs (f)	361.6
59	.747	.921	1.18	.657	.702	chs	chs(f)	349.8
60	.439	.175	.833	.877	.585	chs	chs	79.6
61	.92	.044	2.63	.745	.682	chs	chs	105.1
62	.307	.351	n/c	n/c	2.24	CA1	CA1	200.2
63	.438	.571	1.23	.571	.0973	chs	chs	268.1
64	1.23	.922	.965	.219	.39	ths	efs	263.2
65	2.89	2.67	2.68	2.19	1.18	normal	normal	101.1
66	1.62	.789	.744	.351	.0876	ths	ths (f)	512.4
67	1.58	.39	n/c	n/c	.263	chs	chs(f)	442.3
68	1.14	.482	.789	n/c	.088	chs	chs(f)	413.5
69	3.29	.394	n/c	n/c	.828	chs	chs(f)	375.2
70	.438	.175	.833	.088	.438	chs	chs	126.9
71	1.27	.395	3.07	.585	.175	chs	chs (f)	344.1
72	1.67	.351	4.97	.219	.389	chs	chs(f)	151.4
73	.483	.746	n/c	n/c	.195	ths	chs	194.
74	4.95	5.31	1.36	n/c	1.67	efs	normal	194.8
75	.613	.219	1.36	.438	.351	ths	chs	416.3
76	.789	.307	1.88	n/c	.175	chs	chs	202.5
77	.833	.175	.92	.613	.921	chs	chs	310.1
78	.35	.175	1.71	.22	.307	ths	chs	123.9
79	1.1	.57	.828	1.71	.175	chs	Indet.	668.6
80	1.27	.835	.965	.395	.341	ths	chs(f)	149.4
81	.657	.834	3.12	2.19	2.06	CA1	CA1	286.8
82	1.05	.701	1.93	1.71	1.05	chs	CA1	135.3
83	.57	.526	2.85	2.24	1.9	CA1	CA1	194.3
84	1.01	.482	2.32	2.81	1.88	chs	CA1	259.8
85	5.52	.658	n/c	n/c	.35	chs	chs(f)	241.2
86	.877	.57	.615	.57	.131	ths	ths	266.9
87	.263	.307	1.49	.394	.351	chs	chs	160.2
88	3.29	2.06	n/c	n/c	1.41	chs	Normal	113.5
89	6.36	1.27	4.86	2.58	3.46	CA1	normal	253.4
90	.657	.439	n/c	.921	.131	chs	chs	289.3
91	.097	.175	.682	.263	.219	ths	ths	353.5
92	.745	.219	.352	n/c	.263	ths	ths	427.4
93	1.14	.702	1.85	1.1	.483	chs	chs (f)	349.9
94	.923	.926	1.22	.702	1.13	ths	indet	95
95	2.02	.131	.965	.162	.292	chs	chs(f)	121.2
96	.195	.307	.395	n/c	.483	ths	ths	175.6
97	.746	.614	1.18	.615	.341	ths	chs	198
98	.877	.131	.275	.351	.351	ths	ths	259
99	.614	.614	1.92	.658	.125	ths	chs	247.5
100	.439	.132	.657	.351	.659	chs	ths	275.3
101	.438	.394	1.71	1.1	.658	chs	chs	296.5
102	.351	.439	.483	.876	.746	ths	ths	184.4
103	1.75	.701	2.45	1.14	.176	efs	chs (f)	204.2
104	3.86	2.59	3.33	3.11	2.41	normal	normal	212.1
105	.659	.73	2.85	n/c	.482	chs	chs	130.4
106	2.89	2.72	n/c	n/c	.493	efs	efs	175.1
107	3.24	2.02	n/c	2.09	1.89	normal	normal	322.4
108	.615	.569	1.51	.394	.088	ths	chs	350.5
109	1.1	.614	n/c	n/c	.395	chs	chs(f)	465

110	.79	.526	2.05	.307	.131	ths	chs	422.1
111	1.1	.745	2.44	1.02	.395	ths	chs(f)	258.8
112	1.45	.964	2.37	n/c	.92	chs	normal	280.2
113	.57	.351	n/c	n/c	.439	chs	chs	280.2
114	.307	.746	2.78	2.08	1.4	chs	CA1	69.1
115	.57	.65	2.63	n/c	.307	chs	chs	187.7
116	.512	.389	.745	.219	.876	ths	ths	171
117	.483	.263	1.23	1.23	.585	chs	chs	164.
118	.878	.57	.585	.395	.395	ths	ths	284
119	1.05	.964	3.51	1.36	.351	chs	chs(f)	336.1
120	n/c	.34	.877	.483	.307	chs	chs(f)	336.1
121	.78	.351	.964	1.14	.876	chs	chs	429.6
122	.832	.263	1.1	.307	.175	ths	chs	203.2
123	.483	.351	.833	n/c	.263	chs	chs	260
124	.395	.175	n/c	.57	.176	ths	chs	164
125	.614	.263	1.49	.614	.263	chs	chs	268
126	1.49	.702	3.38	2.54	2.24	CA1	CA1(f)	143
127	.483	.351	.833	n/c	.263	chs	chs	516
128	1.62	.657	1.62	.833	.487	chs	chs(f)	589
129	.57	.834	.702	n/c	.307	chs	ths	336
130	.926	.659	1.62	.307	.482	chs	chs	380
131	.878	.351	.921	.92	.878	chs	chs	341.2
132	.66	.395	1.53	1.61	1.41	CA1	CA1	103.4
133	.482	.703	3.11	2.28	1.32	CA1	CA1	205
134	1.14	.351	.964	1.05	1.37	chs	indet	132.
135	.933	.263	1.23	1.8	.626	chs	indet	129
136	2.32	2.15	3.41	5.31	2.69	CA1	normal	206.4
137	1.05	.57	2.76	.964	.131	chs	chs	235
138	1.71	1.18	1.67	3.17	2.78	CA1	normal	248.9
139	.966	.731	1.8	n/c	.175	chs	chs	219.7
140	.877	.35	2.89	1.01	1.01	chs	Indet.	246.6
141	.615	.584	.964	.632	.307	ths	chs	259.6
142	1.27	1.01	2.15	1.45	.633	chs	efs	82.9
143	1.23	.57	1.45	.614	.131	chs	chs(f)	241
144	.966	.483	.659	n/c	.146	ths	ths	426.3
Control	2.1	1.27	2.63	2.54	1.45			118.7
Control	1.66	1.14	1.75	2.85	.966			167.3
Control	1.07	2.1	1.18	1.66	1.23			139.6
Control	1.97	1.36	1.43	1.84	1.31			172.7
Control	3.07	1.32	2.37	2.41	1.23			167.6
Control	2.41	1.07	.974	1.88	1.84			159.2

*2 specimens(#45 and #77) were labeled CHS (f) instead of indeterminant as one subfield had 1.4 (instead of 1.5) but otherwise their pattern fit most with CHS.
** If CA2 was damaged or missing, and the pattern was either CHS or THS, CHS was selected.

Appendix 2.

Table 2. Z-scores.

CA1a autopsy mean neuronal density = 2.0467 μm E $^{-04}$ (SD=.67695)
CA1b=1.3767 (.37098)
CA2=1.7223 (.66086)
CA3=2.1967 (.4706)
Hilar=1.3377 (.29225)

The z-score is calculated by subtracting the specimen neuronal density from the mean and dividing by the SD. Only negative values reflecting neuronal loss are considered relevant for this study. Positive values that exceed 0 are considered 0. The z-score for each subfield for each specimen is included below. Sx outcome at 1 year is included in rightmost column. O is "not ILAE 1" and 1 is ILAE 1.

specimen	CA1 (a)	CA1 (b)	CA2	CA3	Hilar/CA4	Qual.pattern	Quant.pattern	GCL max	Sx
1	-.54	0	n/c	-3.1	-3.9	chs	indet.	255	1
2	-1.4	-2.3	0	n/c	-3.5	chs	chs(f)	329.7	
3	-1.4	-3.6	0	n/c	-4.1	ths	chs (f)	154.8	1
4	-1.7	-2.2	0	n/c	-1.3	chs	indet	121.2	1
5	-2.4	-2.8	0	-3.6	-2.6	chs	chs	174.9	0
6	0	0	0	-2.9	-4.4	efs	efs	221.6	1
7	-2.6	-3	n/c	n/c	-4	ths	chs	201	1
8	0	-2.5	-.8	-2	-1.9	ths	chs(f)	345.4	1
9	-1.2	-2.4	0	-2.9	-1.7	chs	chs(f)	256.6	1
10	-2.8	-1.9	-.2	n/c	-4	chs	chs	145.6	
11	-1.9	-1	n/c	-.6	0	CA1 pred	CA1 (f)	130.2	1
12	-2.4	-1.7	-.5	-3.8	-4.3	ths	chs	428.8	1
13	-1.7	-2.8	0	-.7	0	CA1	CA1	183.4	0
14	-2.2	-1.6	n/c	-2	-1.1	chs	indet	220.7	1
15	-1.3	-.6	0	-2.5	-3.1	chs	efs	363.8	1
16	-1	-2.7	-.6	-3.4	-3.4	chs	chs(f)	342.7	
17	-1.8	-.3	0	-2	-4.3	ths	chs(f)	21.7	1
18	-1.4	-2.8	-1.2	-2.5	-2.2	ths	chs(f)	42.4	1
19	-1.3	-1.6	0	-3.0	-1.7	chs	chs(f)	105.5	0
20	-.1	-.2	-.2	-3.3	-2.3	ths	efs	126.7	1
21	0	0	n/c	n/c	-4.3	efs	efs	176.9	
22	-2.4	-2.5	0	-3.3	-2.6	ths	chs	293.7	1
23	-1.2	-1.2	0	-3	-2.5	chs	indet.	272.9	1
24	0	-2.4	n/c	-3.9	-4	ths	chs(f)	371.7	1
25	-.8	-.8	0	-4.4	-1.9	chs	efs	409.1	0
26	-1.3	-2.8	-1.4	-4.1	-2.2	ths	chs(f)	132.7	0
27	-2.0	-3.5	0	n/c	-4.3	ths	chs	120.9	1
28	0	0	n/c	n/c	0	normal	normal	177.8	0
29	0	0	n/c	n/c	0	efs	normal	178.4	0
30	-2	-2.9	0	-3.9	-3.4	chs	chs	318.3	1
31	0	0	n/c	-3.1	-2.5	chs	efs	217.4	1

32	-1.1	-2.1	-.7	n/c	-3.3	chs	chs (f)	330.4	0
33	0	0	0	0	0	chs	normal	187.9	1
34	-2.3	-.9	0	-1.6	-1.3	chs	indet	166.6	1
35	0	0	0	0	0	chs	normal	189.4	0
36	-1.5	-1.1	0	-3.6	-2.9	ths	chs (f)	167.3	1
37	0	-2	n/c	n/c	-2.7	chs	chs(f)	123	1
38	-.4	-.1	0	0	-1.4	chs	normal	276.9	1
39	-1.1	-2.1	-1.1	-1.7	-3.1	chs	chs (f)	178.6	1
40	-2.2	-2.8	-.5	n/c	-4.2	chs	chs	336.7	1
41	-.7	-1.3	n/c	n/c	-2.5	chs	indet	387.1	1
42	-2.1	.2.2	0	-2.7	-3.7	chs	chs	276.8	1
43	-.75	-1	0	-.3	-.8	chs	normal	351.1	1
44	-2.1	-1.5	n/c	n/c	-2.2	chs	chs	293	1
45	-1.1	-1.7	0	n/c	-1.4	chs	chs (f)	155.3	
46	0	0	n/c	-1.7	-.1	efs	indet.	220.5	
47	0	0	n/c	0	0	normal	normal	156.3	0
48	-2	-1.7	0	0	0	CA1	CA1	131.1	1
49	0	0	0	-1.5	-.8	normal	indet	212.9	
50	0	-.5	-.6	0	0	chs	normal	126.7	
51	-1.8	-1.5	-1.5	-3.9	-2.7	chs	ths	111.8	0
52	-2.7	-3.5	n/c	n/c	-2.6	chs	chs	355.8	
53	0	0	0	n/c	0	normal	normal	103.2	
54	0	0	-.5	0	0	efs	normal	343	
55	-.8	-.5	-.02	0	0	CA1	normal	244.8	
56	-1.8	-3.4	-1.2	-3.1	-3.6	ths	chs	218.7	
57	-1.7	-2.3	n/c	-3.9	-1.9	chs	chs	298.9	
58	-1.3	-1.8	-.9	-1.9	-1.3	chs	indet	361.6	
59	-1.9	-1.3	-.8	-3.3	-2.2	chs	chs(f)	349.8	1
60	-2.4	-3.2	-1.4	-2.8	-2.6	chs	chs	79.6	
61	-1.7	-3.6	0	-3.1	-2.2	chs	chs	105.1	
62	-2.6	-2.8	n/c	n/c	0	CA1	CA1	200.2	1
63	-2.4	-2.2	-.75	-3.5	-4.3	chs	chs	268.1	1
64	-1.2	-1.2	-1.2	-4.2	.-3.2	ths	efs	263.2	1
65	0	0	0	-.01	-.54	normal	normal	101.1	
66	-0.6	-1.6	-1.5	-3.9	-4.3	ths	ths (f)	512.4	
67	--.7	-2.7	n/c	n/c	-3.7	chs	chs(f)	442.3	
68	-1.3	-2.4	-1.4	n/c	-4.3	chs	chs(f)	413.5	
69	0	-2.7	n/c	n/c	-1.7	chs	chs(f)	375.2	
70	-2.4	-3.2	-1.4	-4.5	-3.1	chs	chs	126.9	
71	-1.2	-2.6	0	-3.4	-4.0	chs	chs (f)	344.1	
72	-.6	-2.8	0	-4.2	-3.3	chs	chs(f)	151.4	
73	-2.3	-1.7	n/c	n/c	-3.9	ths	chs	194.1	
74	0	0	-.6	n/c	0	efs	normal	194.8	
75	-2.1	-3.1	-0.5	-3.7	-3.4	ths	chs	416.3	
76	-1.9	-2.9	0	n/c	-4.0	chs	chs	202.5	
77	-1.8	-3.2	-1.2	-3.4	-1.4	chs	chs	310.1	
78	-2.5	-3.2	-.02	-4.2	-3.5	ths	chs	123.9	
79	-1.4	-2.2	-1.4	-1	-4	chs	Indet.	668.6	
80	-1.2	-1.5	-1.2	-3.8	-3.4	ths	chs(f)	149.4	

81	-2.1	-1.5	0	0	0	CA1	CA1	286.8	
82	-1.5	-1.8	0	-1	-1	chs	CA1	135.	
83	-2.2	-2.3	0	0	0	CA1	CA1	194.3	
84	-1.5	-2.4	0	0	0	chs	CA1	259.8	
85	0	-1.9	n/c	n/c	-3.4	chs	chs(f)	241.2	
86	-1.7	-2.2	-1.7	-3.5	-4.1	ths	ths	266.9	1
87	-2.6	-2.9	-.4	-3.8	-3.4	chs	chs	160.2	1
88	0	0	n/c	n/c	0	chs	Normal	113.5	
89	0	-.3	0	0	0	CA1	normal	253.4	0
90	-2.1	-2.5	n/c	-2.7	-4.1	chs	chs	289.3	
91	-2.9	-3.2	-1.6	-4.1	-3.8	ths	ths	353.5	1
92	-1.9	-3.1	-2.1	n/c	-3.7	ths	ths	427.4	0
93	-1.3	-1.8	0	-2.3	-2.9	chs	chs (f)	349.9	0
94	-1.7	-1.2	-.8	-3.2	-.7	ths	indet	95	1
95	-.04	-3.4	-1.2	-4.3	-3.6	chs	chs(f)	121.2	1
96	-2.7	-2.9	-2.0	n/c	-2.9	ths	ths	175.6	0
97	-1.9	-2.1	-.8	-3.4	-3.4	ths	chs	198	1
98	-1.7	-3.4	-2.2	-3.9	-3.4	ths	ths	259	1
99	-2.1	-2.1	0	-3.3	-4.1	ths	chs	247.5	0
100	-2.4	-3.4	-1.6	-3.9	-2.3	chs	ths	275.3	1
101	-2.4	-2.6	-.02	-2.3	-2.3	chs	chs	296.5	
102	-2.5	-2.5	-1.9	-2.8	-2.0	ths	ths	184.4	1
103	-.44	-1.8	0	-2.3	-4.0	efs	chs (f)	204.2	1
104	0	0	0	0	0	normal	normal	212.1	
105	-2.1	-1.7	0	n/c	-2.9	chs	chs	130.4	
106	2.89	2.72	n/c	n/c	.493	efs	efs	175.1	0
107	0	0	n/c	-.23	0	normal	normal	322.4	1
108	-2.1	-2.2	-.32	-3.8	-4.3	ths	chs	350.	1
109	-1.4	-2.1	n/c	n/c	-3.2	chs	chs(f)	465	1
110	-1.9	-2.3	0	-4	-4	ths	chs	422.3	1
111	-1.4	-1.7	0	-1.8	-3.2	ths	chs(f)	258.8	1
112	-.9	-1.1	0	n/c	-1.4	chs	normal	280.2	1
113	-2.2	-2.8	n/c	n/c	-3.1	chs	chs	280.2	1
114	-2.6	-1.7	0	-.25	0	chs	CA1	69.1	1
115	-2.2	-2	0	n/c	-3.5	chs	chs	187.7	1
116	-2.3	-2.7	-1.5	-4.2	-1.6	ths	ths	171	
117	-2.3	-3	-.8	-2.1	-2.6	chs	chs	164	1
118	-1.7	-1.5	-1.7	-3.8	-3.2	ths	ths	284	
119	-1.5	-1.1	0	-1.8	-3.4	chs	chs(f)	336.1	
120	n/c	-2.8	-1.3	-3.6	-3.5	chs	chs	336.1	1
121	-1.9	-2.8	-1.2	-2.3	-1.6	chs	chs	429.6	0
122	-1.8	-3	-.9	-4	-4	ths	chs	203.2	1
123	-2.3	-2.8	-1.4	n/c	-3.7	chs	chs	260	1
124	-2.4	-3.2	n/c	-3.5	-4.0	ths	chs	164	1
125	-2.1	-3.0	-0.4	-3.4	-3.7	chs	chs	268	1
126	-.8	-1.8	0	0	0	CA1	CA1(f)	143	
127	-2.3	-2.8	-1.4	n/c	-3.7	chs	chs	516	1
128	-.6	-1.9	-.2	-2.9	-2.9	chs	chs(f)	589	
129	-2.2	-1.5	-1.5	n/c	-3.5	chs	ths	336	1

130	-1.7	-1.9	.16	-4.0	-2.9	chs	chs	380	0
131	-1.7	-2.8	-1.2	-2.7	-1.6	chs	chs	341.2	1
132	-2.1	-2.6	-.3	-1.3	0	CA1	CA1	103.4	0
133	-2.3	-1.8	0	0	-.06	CA1	CA1	205	0
134	-1.3	-2.8	-1.2	-2.4	0	chs	indet	132	1
135	-1.7	-3.0	-.7	-.8	-2.4	chs	indet	129	1
136	0	0	0	0	0	CA1	normal	206.4	
137	-1.5	-2.2	0	-2.6	-4.1	chs	chs	235	1
138	-.5	-.5	-0.1	0	0	CA1	normal	248.9	0
139	-1.6	-1.7	0	n/c	-4	chs	chs	219.7	0
140	-1.7	-2.8	0	-2.5	-1.1	chs	Indet.	246.6	0
141	-2.1	-2.1	-1.2	-3.3	-3.5	ths	chs	259.6	1
142	-1.2	-1	-.7	-1.6	-2.4	chs	efs	82.9	1
143	-1.2	-2.2	-.4	-3.4	-4.1	chs	chs(f)	241	1
144	-1.6	-2.4	-1.6	n/c	-4.1	ths	ths	426.3	
Control	0	0	0	0	0	n	n	118.7	
Control	0	0	0	0	0	n	n	167.3	
Control	0	0	0	0	0	n	n	139.6	
Control	0	0	0	0	0	n	n	172.7	
Control	0	0	0	0	0	n	n	167.6	
Control	0	0	0	0	0	n	n	159.2	