THE TREATMENT OF STATUS EPILEPTICUS:
EXPERIMENTAL AND CLINICAL ASPECTS

BY

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This work is dedicated to my wife and family.

*And differing judgements serve but to declare*

*That truth lies somewhere, if we knew but where.*

William Cowper (1782)
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ABSTRACT
This thesis is concerned with the treatment of status epilepticus (SE), a prolonged epileptic state, which has a high morbidity and mortality.

1) Knowledge about the pharmacokinetics of drugs used in SE at their point of action (brain) is necessary for the optimisation of treatment. Using a rat model, I was able to determine: (i) the temporal interrelationship between serum and brain extracellular fluid concentrations of phenytoin, a drug used in SE, and lamotrigine, a drug with a putative role in SE, and (ii) the regional specificity of these drugs. In addition, I was able to confirm the hypothesis that with repeat dosing there is potentially dangerous peripheral and central nervous system accumulation of diazepam.

2) It has been proposed that seizures result in rises in brain extracellular fluid concentrations of glutamate, and that these rises in prolonged seizures can result in neuronal death. Using an in vivo dialysis biosensor in a rat model of serial seizures, I was able to demonstrate a dissociation between electrographic activity and rises in extracellular glutamate. There also appeared to be effective mechanisms following seizures that increase glutamate uptake, and release ascorbate, a putative neuroprotectant.

3) Neuroprotectants given early in SE prevent neuronal death, but little is known about their effects in the later stages. Having established an animal model of SE, I determined that the putative neuroprotectants, phenytoin, lamotrigine and MK-801, were not neuroprotective or anticonvulsant in the late stages of SE. However, diazepam and pentobarbitone, drugs that act on the GABA system, were effective in halting SE at this stage.

4) In an audit of Intensive Care Physicians in the UK and of patients referred to a specialist neurological intensive care unit with a diagnosis of refractory SE, I identified ways in which our present management of SE can be improved.
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1. INTRODUCTION

1.1 Definition and Classification of Seizures, Epilepsy and Status Epilepticus

The description and classification of epilepsy are not modern phenomena, but have developed over the ages to fulfil the needs, theories and capacity of the time. Epilepsy has, since antiquity, been defined as the propensity to have seizures, paroxysmal events that occur within the brain. The magicians of ancient Greece attributed different seizure types to individual gods so that it was clear to whom any sacrifice need be made (Temkin, 1971). "Mother of the Gods" was responsible if the patient imitated a goat, Poseidon if the cries were more violent so that the patient resembled a horse, Enodia for the passing of faeces and Hekate for twisting of the mind and falling. Hippocrates cast scorn upon these ideas and considered epilepsy as one disease, "the so-called sacred disease" (Temkin, 1971).

Galen in AD375 recognised that all epilepsy came from the brain but divided epilepsy into idiopathic (the brain was primarily affected) and sympathetic (the brain was healthy but had been affected by a disease originating in another part of the body). These terms persisted into the 19th century with the addition of "symptomatic epilepsy" referring to epilepsy originating in the brain due to an underlying cerebral lesion (Temkin, 1971). "Symptomatic" and "idiopathic" are terms still used in present classifications, but it is important to realise that their origins are from a different physiological viewpoint. Indeed with the advances in modern imaging and genetics, the distinction made by these terms are becoming less meaningful.

In 1873 John Hughlings Jackson, one of the founding fathers of modern neurology, defined epilepsy as 'the name for occasional sudden, excessive, rapid and local discharges of grey matter' (Jackson, 1958). Indeed a seizure can be seen as the excessive and synchronous discharge of a group of cortical neurons, and epilepsy can be seen as the occurrence of these discharges. The seizure possibly results from an imbalance in the excitatory and inhibitory systems; the main neurotransmitters involved in these systems are glutamate and γ-aminobutyric acid (GABA), respectively. The manifestation of a seizure depends upon which neurons are involved and how far and how quickly the discharge spreads or
extends. A seizure occurring simultaneously in both hemispheres is referred to as a generalised seizure, and conversely a seizure that begins in a specific group of cortical neurons, which may then spread, is referred to as a partial seizure.

In 1969 Gastaut proposed that epilepsy should be classified according to clinical and EEG manifestations, anatomical substrate, age, aetiology, interictal neuropsychiatric changes, response to treatment and pathophysiology (Gastaut, 1969). In 1981 as its first step towards classifying epilepsies, the ILAE proposed a classification of seizure type in order to rationalise and simplify the various classifications that were in common use (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). This classification was based upon clinical form, interictal and ictal EEG. The classification had one underlying hypothesis - the differentiation of seizures into generalised and partial seizures - but in the main it was a descriptive classification. Thus it enables distinctions to be drawn between different seizures and for seizure type to be communicated in a universal fashion. It is pragmatic and easily applied, although a number of problems do exist (for example how far does consciousness have to be impaired for a simple partial seizure to become a complex partial seizure) and it is not always certain under which category a particular seizure should be included (for example many atonic seizures could be classified as complex partial seizures).

It suffers from many limitations - it does not describe aetiology, prognosis or pathophysiology. These problems were realised and in 1985 the ILAE proposed a classification of epilepsies and epilepsy syndromes (revised in 1989) (Commission on Classification and Terminology of the International League Against Epilepsy, 1985; 1989). This was a bold attempt to classify epilepsy in terms of anatomy, aetiology, EEG, seizure type, precipitation and syndromic features. It had as its basis the same hypothesis that underlay the seizure classification; that is the differentiation of partial from generalised seizures which became the differentiation of partial from generalised epilepsies.
The differentiation of partial epilepsies, in which the seizure activity commences in one part of the cerebrum, from generalised epilepsies, in which the epileptic activity involves wide areas of both cerebral hemispheres simultaneously from the onset of the attack with no evidence of an anatomic or functional focus, seems clear and simple. A third category, however, had to be introduced for epilepsies and syndromes which are undetermined as to whether focal or generalised (usually because both types of seizure are present). This uncertainty, however, is not confined to the epilepsies described in the classification, as cases of absence epilepsy have been associated with frontal and temporal lobe lesions and these cases are thus probably partial epilepsies (Fish, 1995). Indeed, the distinction between partial and generalised epilepsy which is mainly an electroclinical one will possibly have to be redefined in the light of modern imaging techniques. There is also a fourth category of special syndromes, which, although epileptic in nature, do not usually carry the diagnosis of epilepsy. This is either because there is an acute precipitating factor (e.g. metabolic or toxic events) or the seizure is an isolated event. Within each category the epilepsies are divided into idiopathic, symptomatic and cryptogenic (an underlying lesion is presumed but not discovered). These divisions are becoming less helpful. Syndromes that were classified as idiopathic are now having underlying structural and genetic aetiologies revealed.

Seizures are for the most part self-terminating. However, on occasions, seizures of any type can continue unabated and they are then considered as a separate entity, status epilepticus. In the 19th century, the entity of status epilepticus was clearly distinguished amongst the epilepsies. Calmeil (1824) used the term 'etat de mal' and later the term status epilepticus appeared in Bazire's translation of Trousseau's lectures on clinical medicine. From that time status epilepticus was, on the whole, a term used to describe solely convulsive status epilepticus. It was not until the Marseilles conference in 1962 that status epilepticus was recognised to include all seizure types and that its definition was based solely on the persistence of the seizure rather than its form (Gastaut et al., 1967). Status epilepticus was defined as 'a condition characterised by epileptic seizures that are sufficiently prolonged or repeated at sufficiently brief intervals so as to produce
an unvarying and enduring epileptic condition'. This is of great importance as it is now apparent that a persistent seizure may result in neuronal damage irrespective of any physiological compromise. The necessity of differentiating status from other seizure types is not solely clinical, but relates most importantly to the high morbidity and mortality characterised by this condition. The term status epilepticus included three entities: generalised status epilepticus, partial status epilepticus and unilateral status epilepticus (Gastaut et al., 1967). This classification is, however, both incomplete and too broad to be clinically useful, and further more detailed electroclinical classifications have been proposed. Shorvon (1994) classified status epilepticus according to the age at which it occurred (table 1.1), but once again this classification fails in that it does not describe the underlying aetiology, which in many cases is probably the major determinant of prognosis.

Table 1.1: Revised classification of status epilepticus after Shorvon (1994)

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1.2 Prognosis and epidemiology of status epilepticus

Status epilepticus is a condition that has existed and been recognised throughout history. One of the earliest references to status epilepticus can be found in a Babylonian treatise on epilepsy from the middle of the first millennium BC in which the grave prognosis of this condition was first described: 'If the possessing demon possesses him many times during the middle watch of the night, and at the time of his possession his hands and feet are cold, he is much darkened, keeps opening and closing his mouth, is brown and yellow as to the eyes.....It may go on for some time, but he will die' (Wilson & Reynolds, 1990). However in the ensuing years there were few references to status epilepticus; indeed Hippocrates made no reference to such a condition in his writings on epilepsy 'On the sacred disease'. Why this may be so is a matter of speculation; Hunter (1959) noted that status epilepticus was rare before the advent of powerful antiepileptic drugs, and the consequent risk of drug withdrawal. This is undoubtedly a significant cause of status epilepticus, but cannot account for the large number of drug-naive patients presenting with status epilepticus in present times.

Figures for the frequency of all types of status epilepticus are scant, but it has been estimated that the incidence in the UK alone (population of approximately 50 million persons) may be as high as 25,000 people per year of which 9,000-14,000 (45,000-70,000 in the USA) are convulsive status epilepticus (Shorvon, 1994; Hauser, 1990). Status epilepticus is now recognised as a form of epilepsy with very serious consequences, including focal neurological deficits, intellectual deterioration and chronic epilepsy (Shorvon, 1994; Hauser, 1990). As much as 12% of newly diagnosed epilepsy presents with status epilepticus and this figure is almost certainly much higher among children - a figure of 40% is often quoted for all epilepsies presenting in the first year of life (Shorvon, 1994). The mortality for convulsive status epilepticus is dependent on the underlying aetiologies and the age of the patients, and thus varies from study to study; it is probably of the order of 10-20 % (Shorvon, 1994; Hauser, 1990), although much higher figures have been quoted. The mortality directly attributable to convulsive status epilepticus (not including deaths attributed to the underlying condition) has been estimated at 1-2 % (Shorvon, 1994; Hauser, 1990). Recently a prospective
A population-based study has been carried out in Richmond, Virginia (DeLorenzo et al., 1996). Cases of status epilepticus presenting to the hospitals in the study area were ascertained; this may, however, exclude cases dying at home and patients with milder forms of status epilepticus such as some cases of absence or complex partial status epilepticus which may have been treated at home. Furthermore, a retrospective validation revealed that approximately 10% of cases at the main hospitals, and 67% of cases at the community hospitals may have been missed. The data set was thus incomplete. Nevertheless, broad estimates were made, and the overall incidence was estimated as 41-61 per 100,000 person-years, which is consistent with the previous high estimates. Overall 13.3% had recurrent attacks, 58% had no previous history of status epilepticus and the mortality was 22% (higher than previously reported). Infections with fever were the major cause of status epilepticus in children accounting for 52% of cases, whilst in adults low antiepileptic drug levels (34%), CVA (22%), hypoxia (13%), metabolic (15%) and alcohol (13%) represented the main acute causes. Remote symptomatic (a neurological insult >7 days before the status epilepticus) accounted for 24% of the causes in adults, and these mainly consisted of CVA. The elderly had the highest incidence of attacks with the greatest mortality (38%) and the least history of prior epilepsy (30%). Although the prognosis of status epilepticus was related to aetiology, the prognosis of certain conditions such as stroke appeared to be very much worse if they were associated with status epilepticus (32%) than if they were not (12%).

1.3 Complications of convulsive status epilepticus

Convulsive status epilepticus carries the highest morbidity and mortality of all the types of status epilepticus; it is also the commonest (although this may partly be due to the better reporting of a potentially fatal condition than of milder, possibly inconsequential, conditions). The poor prognosis of this condition is in part due to the underlying acute aetiologies many of which carry their own poor prognosis. Half the patients in status epilepticus, however, have acute antiepileptic drug withdrawal as the cause without the coexistence of a serious underlying
pathology. The poor prognosis of status epilepticus is probably mainly due to the severe physiological compromise that occurs in the condition. The systemic effects of convulsive status epilepticus can be divided into early and late stages. The initial consequence of a prolonged convulsion is a massive release of plasma catecholamines (Benowitz et al. 1986), which results in an increase in heart rate, blood pressure and plasma glucose (Meldrum & Horton, 1973; Meldrum et al., 1979; Posner et al., 1968). During this stage cardiac arrhythmias are frequently seen, and may be fatal (Boggs et al., 1993). Cerebral blood flow is greatly increased and thus glucose delivery to active cerebral tissue is maintained (Posner et al., 1968; Ingvar & Siesjö, 1983). As the seizure continues, there is a steady rise in the core body temperature, and prolonged hyperthermia above 40°C may be related to cerebral damage and a poorer prognosis (Meldrum & Brierley, 1973; Meldrum et al., 1973; Aminoff & Simon, 1980). Acidosis also commonly occurs, and in one series 25% of the patients had an arterial pH below 7.0 (Aminoff & Simon, 1980). This acidosis is mainly the result of lactic acid production, but there is also a rise in CO₂ tension which can in itself result in a life threatening narcosis (Aminoff & Simon, 1980; Wasterlain, 1974). The acidosis can increase the likelihood of life threatening cardiac arrhythmias, hypotension and in conjunction with the cardiovascular compromise may result in severe pulmonary oedema.

The status epilepticus may then enter a second phase. The main characteristics of this phase are: a fall in blood pressure; a loss of cerebral autoregulation resulting in the dependence of cerebral blood flow on systemic blood pressure, and hypoglycaemia due to the exhaustion of glycogen stores and the increased neurogenic insulin secretion (Wasterlain, 1974; Meldrum & Horton, 1973; Benowitz et al., 1986; Meldrum et al., 1979). Further complications may occur, including rhabdomyolysis leading to acute tubular necrosis, hyperkalaemia and hyponatraemia (Singhal et al., 1978). Hepatic compromise is not uncommon, and rarely there may be disseminated intravascular coagulation with its subsequent complications (Fisher et al., 1977).

In addition to these potentially treatable and preventable complications, neuronal damage also occurs that is independent of the physiological compromise.
1.4 Status-induced neuronal damage and the hippocampus

1.4.1 The hippocampus (Williams and Warwick, 1980)

The hippocampus has a two-fold interest in the study of epilepsy. As I will describe, it is the area which is particularly susceptible to status epilepticus-induced neuronal damage. The second area of interest is that in many patients a damaged, sclerosed hippocampus is the substrate of their epilepsy. This dichotomous role of hippocampal damage, as the cause and result of seizures, stems possibly from its physiological role in memory formation and neuronal plasticity.

The hippocampus (literally sea horse) is part of the limbic lobe - a structure originally defined by Broca as a ring of grey matter on the medial aspect of each hemisphere. The hippocampal formation consists of: the indusium griseum, a thin layer of grey matter covering the superior aspect of the corpus callosum, which diverges posteriorly to form the gyrus fasciolaris, which then curves downwards, forwards and laterally to merge with the dentate gyrus; the hippocampus proper, and the subiculum, part of the parahippocampal gyrus.

The hippocampus proper (figure 1.1) is also referred to as the Cornu Ammonis (CA) or literally the horn of Ammon, who is an Egyptian deity with the head of a
ram. The hippocampus is separated from the lateral ventricle by a thin layer of white matter, the alveus, which consists of fibres from the hippocampus that are directed towards the medial border of the hippocampus forming the fimbria; this continues as the crus of the fornix, the major efferent pathway of the hippocampus. Alvear fibres also project back to the subiculum and retro-hippocampal regions. The hippocampus proper is divided into 4 zones (CA1-4); the cell somata run from CA1 at the end of the subiculum to CA4 between the blades of the dentate granule cells. The cornu ammonis is a trilaminar structure consisting of molecular, pyramidal and polymorphic layers. At the beginning of the subiculum, the structure changes into a four-layered structure and then into a modified six-layered structure, which is contiguous with the six-layered structure of the entorhinal cortex. The trilaminate structure of the cornu ammonis is usually subdivided into 5 strata: stratum oriens, stratum pyramidalis, stratum radiatum, stratum lacunosum and stratum moleculare (figure 1.1). Stratum oriens is the layer through which the axons of the pyramidal cells pass. Basal dendrites of the pyramidal cells also occasionally penetrate this layer. The soma and dendrites of basket cells, small irregularly shaped neurons with extensive arborisation, that are inhibitory interneurons also occupy this layer. Basket cells receive afferent input from afferent collaterals to the hippocampus, and also from collaterals from efferent hippocampal fibres. These mediate feed forward and feed back inhibition. They form many axosomatic efferent connections with the pyramidal cells. Stratum pyramidalis contains the excitatory pyramidal cell soma. The apical dendrites of the pyramidal cells form the stratum radiatum. The dendrites terminate in the stratum lacunosum and moleculare. The deepest layers also contain the dendrites, axonal arborisation and soma of small interneurons. The dentate gyrus is also a trilaminar structure with a superficial molecular layer, a granule cell layer and a deep polymorphic cell layer. The hippocampal formation has been considered as a trisynaptic pathway. The afferent input comes primarily from the neurons of the entorhinal cortex as the medial and lateral perforant paths. The majority of these fibres synapse on dentate granule cells, a few (especially from the lateral perforant path) synapse on CA3 and CA1 pyramidal cells in the stratum moleculare. The dentate granule cells project mossy fibre axons to mainly
CA3 pyramidal cells, which in turn project Schaffer collateral axons that synapse with CA1 pyramidal cells in the stratum lacunosum. This circuit is completed by the projection of axons from CA1 to the subiculum and onto the entorhinal cortex.

1.4.2 Hippocampal sclerosis

In 1825, Bouchet and Cazauvielh presented their findings on 18 autopsied patients in a thesis that attempted to establish the relationship between epilepsy, "l'épilepsie, and insanity, "l'aliénation mentale". They noted that in a number of cases there were changes in the Cornu Ammonis, four had induration and one had softening. Sommer (1880) further described in detail the neuropathological finding of hippocampal sclerosis in the brains of patients with chronic epilepsy. He noted gliosis and pyramidal cell loss in predominantly the CA1 region of the hippocampus, and he proposed that these lesions were the cause of the epilepsy. In the same year, Pfleger (1880) described haemorrhagic lesions in the mesial temporal lobe of a patient dying in status epilepticus, and concluded that neuronal necrosis was the result of impaired blood flow or metabolic disturbances that occurred during the seizure. Since that time the debate as to whether hippocampal sclerosis is the cause or result of epilepsy has continued. Norman (1964) described post-mortem findings in 11 children dying from status. In 10 cases, acute neuronal necrosis and, in one case, old lesions were found in the hippocampus in CA1 and also frequently in the end folium, CA3-4. CA2 was preserved in every case and was described as the "resistant sector". More recent studies have also demonstrated significant acute neuronal loss in the hippocampus of patients dying in convulsive status epilepticus (DeGiorgio et al., 1992; Corsellis and Bruton, 1983). DeGiorgio et al. (1992) compared the hippocampi from 5 patients dying in status epilepticus, 5 patients with epilepsy who had a similar degree of physiological compromise (e.g. hypoglycaemia, hypotension and hypoxia), and 5 controls. The neuronal densities were least in those dying with status epilepticus.
That convulsive status epilepticus could cause hippocampal damage was thus well established, and it is this status-induced neuronal damage that is one of the factors that distinguishes status epilepticus from amongst the epilepsies, and leads to its consideration both as a symptom of an underlying disease as well as a disease entity in itself. This status-induced neuronal damage provided the impetus for much research. For many ethical and practical reasons detailed studies of this problem are difficult in patients. As an alternative, investigators have successfully turned to animal models.

1.5 Animal models of status epilepticus and status epilepticus-like damage

1.5.1 Chemically induced status epilepticus

The most notable experiments were those carried out by Meldrum and co-workers in the 1970's in adolescent baboons using systemically administered bicuculline (Meldrum and Brierley, 1973; Meldrum and Horton, 1973; Meldrum et al., 1973). Bicuculline is a GABA antagonist, and as such causes convulsive seizures through inhibition of the inhibitory system. These initial experiments demonstrated a correlation between the neuronal damage and the duration of the status epilepticus, the duration of the hyperpyrexia, severe hypotension and profound hypoglycaemia. Later experiments showed that paralysed and artificially ventilated baboons, in whom the hypotension, acidosis, hypoxia and hypoglycaemia were prevented, still sustained significant neuronal damage. Similarly flurothyl (a volatile convulsant agent that produces seizures by diffusely opening neuronal sodium channels) induces generalised seizures in ventilated rats producing widespread lesions in the pars reticulata of the substantia nigra, neocortex (layers 3 and 4), amygdala, thalamus as well as CA4 and CA1 hippocampal pyramidal cells (Nevander et al., 1985).

Both these models involved the induction of generalised status epilepticus, but similar damage has been seen in models involving limbic status epilepticus, a model of partial status epilepticus. Limbic seizures have been classified in relation to kindling. Kindling is the repetition of stimuli which initially evoke afterdischarges but not seizures (McNamara et al., 1993).
Table 1.2: Development of kindled seizures after Racine (1972)

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mouth and facial movements</td>
</tr>
<tr>
<td>2</td>
<td>Head nodding</td>
</tr>
<tr>
<td>3</td>
<td>Forelimb clonus</td>
</tr>
<tr>
<td>4</td>
<td>Rearing</td>
</tr>
<tr>
<td>5</td>
<td>Rearing and falling</td>
</tr>
</tbody>
</table>

Repetition of the same stimuli results in a gradual lengthening of the afterdischarges eventually leading to progressively more severe seizures; these are staged from 1 to 5 (table 1.2), where 1 is facial clonus and 5 is rearing and falling (Racine, 1972). There is also an earlier stage during which the seizures are accompanied by exploratory behaviour, and immobility (Racine, 1972).

Once an animal has been kindled, the heightened response to the stimulus seems to be permanent and indeed spontaneous seizures may occur (McNamara et al., 1993). Limbic status epilepticus consists of prolongation of similar seizure types.

Early work producing limbic status epilepticus-induced neuronal damage was accomplished with models using kainic acid that produced seizures when either given systemically or injected into the brain (Collins et al., 1983). Kainic acid is a powerful excitant and excitotoxin, which when injected directly into brain not only produces a local lesion, but also a seizure-linked pattern of disseminated lesions in brain regions far from the injection site (Olney et al., 1974). The administration of anticonvulsants blocks seizure activity as well as the distant neuronal damage, but has no effect on the local lesion (Ben Ari et al., 1979). From these experiments certain conclusions can be drawn:

1) both generalised and limbic status epilepticus result in neuronal damage;

2) even without the systemic disturbance associated with status epilepticus, neuronal damage still occurs (although it is less severe);

3) the neuronal damage is not necessarily due to the administration of the convulsant per se, but to the resultant seizure activity.
Similar results have been obtained with models that use other chemoconvulsants including neurotoxins that act in a similar fashion to kainate such as domoic acid (Tryphonas et al., 1990). A separate chemoconvulsant approach has been the use of drugs that act on the cholinergic system in particular the anticholinesterase inhibitors, pilocarpine and soman (Olney et al., 1983; Turski et al., 1983); the effectiveness of these convulsants can be increased by the preadministration of lithium (Honchar et al., 1983; Jope et al., 1986).

1.5.2 Electrically induced status epilepticus

The use of a chemoconvulsants has a number of problems. Some, such as kainic acid, are potent neurotoxins and thus may themselves contribute to any pathological changes that occur. Also interventions that specifically target the chemoconvulsant may be neuroprotective and prevent status epilepticus, but these model-specific interventions do not have a wider application. Thus, for example, atropine given prior to pilocarpine administration prevents the status epilepticus and the resultant neuronal damage (Jope et al., 1986), but atropine's antiepileptic and neuroprotective effects are specific for status epilepticus induced by cholinomimetics. Lastly, neurochemical changes (see later) and, to some extent, the resultant pathology are chemoconvulsant dependent.

These difficulties have been overcome to some extent by the use of electrically induced status epilepticus and status epilepticus-like damage. There are a number of these models which are distinguishable by the stimulation protocol used and the brain area stimulated. They can also be classified according to whether they are performed in anaesthetised or unanaesthetised animals, and whether the animals have been kindled prior to the induction of the status epilepticus or not.

**Anaesthetised animals**

One of the best characterised models of status epilepticus-induced neuronal damage is that of Sloviter (Sloviter, 1983; 1987). This model depends upon stimulation of the perforant path in urethane anaesthetised rats. Two distinct
models were developed by Sloviter, both involving stimulation of the perforant path and recording from the dentate granule cells. One involved intermittent stimulation of the perforant path over a period of 24 hours, and the other consisted of constant stimulation over a period of 2 hours (Sloviter, 1983). The former resulted in a more consistent pattern of damage and was the model on which most subsequent work was performed (Sloviter, 1983; 1987; 1991). These models resulted in neuronal damage in CA3-4 and CA1 (similar to the damage observed in humans and other animal models of status epilepticus). In neither model was self-sustaining status epilepticus induced, and thus these models are useful for determining anatomical and physiological changes that occur with excessive, synchronous discharges in the hippocampus, but are not models of status epilepticus itself.

Unanaesthetised animals

Early models of electrically induced status epilepticus in unanaesthetised animals involved prolonged, 1 hour, stimulation to a kindled focus in the hippocampus (McIntyre et al., 1982; 1986); this provoked limbic status epilepticus. Following partial or generalised status epilepticus considerable bilateral damage was observed in the hippocampus, basolateral amygdala, and pyriform cortex (McIntyre et al., 1982; 1986). These models suffered in that they required prior kindling, which can itself result in extensive pathological changes and neuronal cell loss (Cavazos & Sutula, 1990; Spiller & Racine, 1994). Thus models were developed in which status epilepticus was induced de novo in previously unstimulated rats. A number of stimulating protocols exist (Vicedomini & Nadler, 1987; Milgram et al., 1985; Lothman et al., 1989; Handforth & Ackermann, 1992; Cain et al., 1992), some being an extension of Sloviter's perforant path model to unanaesthetised animals (Rogers et al., 1989; Ylinen et al., 1991a). Most of these protocols involve intermittent stimulation with short interstimulus intervals or continuous stimulation of limbic structures; this eventually leads to self-sustaining status epilepticus that can last for hours.
1.6 Glutamate

In the 1980's the role of glutamate as the major cause of this seizure-mediated neuronal damage was studied, with particular emphasis on the N-methyl-D-aspartate (NMDA) receptor. Glutamate is a nonessential amino acid that does not cross the blood brain barrier, but is readily synthesised by various biochemical pathways from different precursors including alpha-ketoglutarate (an intermediate of the Krebs cycle), glutamine, ornithine and proline (McGeer et al., 1987). It is present in abundance in brain tissue, and is the major excitatory transmitter in the central nervous system. When distinguishing glutamate's role in various neural processes, it is necessary to consider three compartments: intracellular, extracellular and synaptic. Glutamate is present in the brain in large concentrations (10mM), but this is predominantly intracellular glutamate (McGeer et al., 1987). The extracellular glutamate is maintained at concentrations 5,000 times lower than this (approximately 2μM) (Lerma et al., 1986). Glutamate concentrations in the synaptic cleft can reach values of approximately 1mM from vesicular release during synaptic activity (Clements et al., 1992). Although, the fluid within the synaptic cleft freely communicates with the extracellular fluid, these local concentration rises are not directly translated into rises in the extracellular fluid because of a rapid, high affinity glutamate uptake mechanism and because of diffusion from the small volume within the synaptic cleft into the much larger volume of the extracellular space. Synaptic glutamate concentrations are the main determinants of glutamate's neurophysiological effects, but measurement of synaptic glutamate concentrations is difficult, and cannot be accomplished in vivo. Originally glutamate measurements were made in whole brain homogenates, but this is predominantly intracellular glutamate representing the metabolic pool and gives little insight into glutamate's role as a neurotransmitter. More recently various techniques have been developed to measure changes in extracellular glutamate, and it has been proposed that these are a reflection of changes in glutamate concentration within the synapse and thus an indirect measure of synaptic release. Furthermore, extracellular glutamate has direct access to the synaptic space, and thus changes in extracellular glutamate could have effects at synaptic receptors. Measurements of extracellular glutamate
in animal models of epilepsy have, however, been obfuscating rather than illuminating. Various studies draw conflicting and contradictory conclusions, yet few attempt a synthesis to include all experimental results.

1.6.1 Glutamate as a neurotransmitter

Glutamate's excitatory potential was first noted in 1951 following intracarotid injections of glutamate in studies of epileptic phenomena (Okamoto, 1951). Hayashi, a year later, injected small amounts of glutamate directly onto the cortex via a small metal tube and demonstrated that a local application of glutamate could induce the firing of rat neocortical neurons (Hayashi, 1952). Later studies using iontophoretic techniques confirmed the excitatory potential of glutamate and related compounds on mammalian neurons (Curtis & Watkins, 1963). Glutamate was not immediately accepted as a neurotransmitter for two reasons. Glutamate's ubiquity resulted in problems in differentiating the neurotransmitter pool from other metabolic pools, and thus there were difficulties in identifying a unique subpopulation of neurons that released glutamate. Also there were problems in identifying a system for inactivating glutamate within the synapse. Later work, however, demonstrated a high affinity glutamate uptake mechanism that was unique to a synaptosomal fraction (Wofsey et al., 1971); this high affinity uptake was subsequently used as a marker of glutamatergic synapses. Thus the main objections were answered. Glutamate is now generally recognised as the predominant excitatory neurotransmitter in the mammalian brain. Glutamate has at least three distinct post-synaptic receptor types: N-methyl-D-aspartate (NMDA), non-NMDA (consisting of alpha-amino-3-hydroxy-5-methylisoxazole [AMPA] and kainic acid [KA] sensitive receptors) and metabotropic glutamate receptors. These receptor subtypes have very different properties (table 1.3).

Non-NMDA receptors are mainly associated with channels that are permeable to sodium ions (table 1.3). The receptors consist of 4 subunits; receptors comprising GluR1-4 subunits are the AMPA receptors and those comprising GluR5-7 and KA1-2 are the Kainate receptors (Hollmann & Heinemann, 1994).
### Table 1.3: Properties of ion channel associated glutamate receptors

<table>
<thead>
<tr>
<th>Subunits</th>
<th>Non-NMDA receptors</th>
<th>AMPA</th>
<th>NMDA receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kainate</td>
<td>AMPA</td>
<td></td>
</tr>
<tr>
<td>GluR5</td>
<td></td>
<td></td>
<td>NMDAR1</td>
</tr>
<tr>
<td>GluR6</td>
<td></td>
<td></td>
<td>NMDAR2A</td>
</tr>
<tr>
<td>GluR7</td>
<td></td>
<td></td>
<td>NMDAR2B</td>
</tr>
<tr>
<td>KA1</td>
<td></td>
<td></td>
<td>NMDAR2C</td>
</tr>
<tr>
<td>KA2</td>
<td></td>
<td></td>
<td>NMDAR2D</td>
</tr>
</tbody>
</table>

**Associated ion channel**

- AMPA receptors lacking the GluR2 component are permeable to calcium ions. Relatively large concentrations of glutamate result in sodium channel opening and a rapid depolarisation. The concentration that gives half the maximum response (EC50) for non-NMDA receptors is of the order of 500μM glutamate (Patneau & Mayer, 1990). The excitatory post synaptic potential (EPSP) is rapid and rises in less than 1ms and decays with a time constant of 0.2-8 ms (Edmonds et al., 1995). Prolonged exposure (4-12ms) to high glutamate concentrations results in receptor desensitisation (i.e. the receptor becomes resistant to activation by glutamate) (Edmonds et al., 1995). It is likely, however, that the shape of the EPSP is rarely determined by this desensitisation as the receptors are only briefly exposed to high glutamate concentrations because of rapid clearance of glutamate from the synaptic cleft (time constant = 1.2ms) due a combination of diffusion and glutamate uptake (Clements et al., 1992). In instances in which clearance is reduced or receptor-transmitter contact is increased by, for example, simultaneous transmitter release from adjacent sites the non-NMDA receptors can be desensitised (Trussell et al., 1993). This can occasionally shape the decay of the EPSP, and can also influence responses to sequential synaptic stimuli (Trussell et al., 1993). In addition to the desensitising, non-NMDA receptor response, there is also a non-desensitising non-NMDA current with an EC50 of 16μM (Patneau &
Mayer, 1990). This is, however, accountable for only small tonic depolarisations that are unlikely to be a major contributor to synaptic responses.

NMDA receptors are associated with channels that are permeable to calcium and sodium ions (figure 1.2). There are high affinity sites for both glycine and glutamate as well as sites for polyamines and zinc.

![Diagram of NMDA receptor complex](image)

*Figure 1.2: NMDA receptor complex showing co-activation by glutamate and glycine, calcium and sodium ion channel, voltage-dependent magnesium block. Also present but not shown are modulatory sites for polyamines and zinc.*

Relatively low concentrations of glutamate are necessary to activate the receptor. NMDA receptors typically have an EC$_{50}$ for peak response of the order of 2-3µM glutamate (Patneau & Mayer, 1990). NMDA receptor currents rise to a peak in about 20ms and then decay bi-exponentially with time constants of the order of 40ms and 200ms (Edmonds et al., 1995). The slow rise in synaptic current is due to a prolonged latency (possibly 20-30ms) from glutamate binding to the receptor to actual activation of the receptor (Edmonds et al., 1995). NMDA activation by glutamate does not necessarily result in any detectable current flow, because at negative potentials the ionic pore is tonically blocked by magnesium. This block is released by depolarisation. During normal synaptic activity, the time course of
the non-NMDA EPSP is substantially shorter than the latency for NMDA receptor activation. Even if activation of non-NMDA receptors should result in a sufficient depolarisation to release the magnesium block, by the time most NMDA receptors are activated by glutamate, most neurons will have repolarised to such an extent that the magnesium block will be in place and no current will flow through the NMDA receptors (Edmonds et al., 1995). If, however, the NMDA receptor is activated by glutamate prior to the non-NMDA mediated EPSP, then the resultant depolarisation will result in removal of the magnesium block and current flow. Thus, to some extent, NMDA receptors can be viewed as memorising, amplifying receptors which are activated by depolarisation provided that they have been previously primed with glutamate. The resultant influx of calcium through NMDA receptors has secondary consequences, affecting the phosphorylation of proteins that can produce long-term synaptic potentiation, modulation of other receptors, and even cell death.

Metabotropic receptors are not ion channel receptors but are G-protein linked receptors. Activation of these results in G-protein mediated phosphorylation which has a multitude of prolonged effects on cellular function; these receptors are not directly responsible for synaptic currents (Schoepp & Conn, 1993).

1.6.2 Glutamate as a neurotoxin

It had been known for some years, that in addition to being a neuronal excitant, glutamate has marked neurotoxic properties. In 1957, it was reported that subcutaneous administration of glutamate to suckling mice resulted in degeneration of neurones in the inner layers of the retina (Lucas & Newhouse, 1957). Experiments on glutamate and its analogues demonstrated: that there is a correlation between their excitatory and toxic actions; that specific antagonists of their excitatory activity protect against their neurotoxicity, and that they are toxic to dendrites and cell bodies (dendrosomatotoxic) and axon-sparing. These observations led to the hypothesis that the toxic action was mediated through depolarisation of dendrosomal synaptic receptors, the excitotoxic hypothesis (Olney et al., 1986). Application of large concentrations (100μM and above) of
glutamate result in cell death in cell cultures (Choi et al., 1987), and in vivo (McBean and Roberts, 1984). Excitotoxicity has been proposed as the process underlying neuronal damage not only in status epilepticus, but also in ischaemia (Siesjo & Wieloch, 1986). Neuronal death varies in different animal models of status epilepticus, but tends to be maximal in CA1, CA3 and CA4 regions of the hippocampus, amygdala, layers III and IV of the cortex, the globus pallidus, the substantia nigra pars reticulata, medio-dorsal thalamus and olfactory bulb (Siesjo & Wieloch, 1986; Meldrum, 1991). The pattern of neuronal death is not specifically correlated with the density of glutamate receptor subtypes (Meldrum, 1991), and thus neuronal vulnerability is not purely a function of the presence of NMDA receptors. This may in part be due to the mechanisms underlying excitotoxic neuronal death. It had been known that extracellular calcium concentrations fell during seizures (Lux et al., 1986), and that intracellular calcium accumulation could result in cell death (Farber, 1981). Following 2 h of status epilepticus induced by bicuculline or L-allylglycine, cellular pathology was accompanied by a marked increase in the amount of calcium pyroantimonate deposits in swollen and disrupted mitochondria of CA1 and CA3 basal dendrites, and in selected neuronal cell bodies in the CA1 and CA3-4 regions (Griffiths et al., 1983). It was proposed that enhanced calcium entry into neurons and consequent overloading of the capacity of mitochondria for calcium sequestration was part of the cytotoxic mechanism leading to selective neuronal loss in the hippocampus in status epilepticus (Griffiths et al., 1983). Further evidence of the importance of the role of calcium in excitotoxic neuronal damage came from a study in which the presence of calcium binding proteins in specific neuronal areas in the hippocampus correlated with their relative resistance to seizure-induced neuronal damage (Sloviter, 1989). However in a subsequent study, pyramidal cells in the CA3 region containing neither of the calcium binding proteins, parvalbumin and calbindin, were more resistant to overexcitation than CA1 pyramidal cells, most of which do contain calbindin (Freund et al., 1992). Thus no simple relationship exists between neuronal vulnerability in status epilepticus and neuronal calcium binding protein content, and it is likely that a number of factors, including calcium binding protein and glutamate receptor density,
determine the vulnerability of hippocampal neurons. This is further evidenced by a study in which the intracellular calcium concentrations of neurons in cell culture exposed to high glutamate concentrations corresponded poorly with eventual survival (Michaels & Rothman, 1990). Nevertheless, the in vitro neurotoxicity of glutamate is dependent upon the presence of extracellular calcium (Choi, 1987), and chelation of intracellular calcium confers protection against the effects of prolonged stimulation on vulnerable neurons in hippocampal slices (Scharfman & Schwartzkroin, 1989). There are, however, two forms of excitotoxicity in cortical cell cultures: 1) a rapidly triggered excitotoxicity resulting from activation of NMDA receptors, and 2) a slowly triggered excitotoxicity induced by activation of non-NMDA receptors (Choi, 1992). The non-NMDA mediated cell death is probably via receptors lacking GluR2 subunit, which are thus permeable to calcium. Rises in intracellular calcium during status epilepticus start a cascade of reactions which result in the expression of genes coding for proteins such as c-fos, c-jun, jun-B and p53, which regulate cell growth and neuronal plasticity; these proteins may also contribute to programmed cell death (Morgan & Curran, 1991; Le Gal La Salle, 1988; Dragunow et al., 1993; Morrison et al., 1996).

1.6.3 Extracellular glutamate

The extracellular concentration of glutamate is determined by three processes - diffusion of glutamate from the synaptic cleft following vesicular release, diffusion from the intracellular pool and a high affinity glutamate uptake mechanism. The kinetics of a transporter can be described by the maximum velocity (Vm) and the concentration of substrate at which half the maximum velocity is reached (Km). The high affinity glutamate uptake can be divided into at least three subtypes (table 1.4).

<table>
<thead>
<tr>
<th>Cellular localisation</th>
<th>EAAC1</th>
<th>GLAST</th>
<th>GLT-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neurons</td>
<td>Glia</td>
<td>Astrocytes</td>
</tr>
<tr>
<td>Km for L-glutamate</td>
<td>12μM</td>
<td>77μM</td>
<td>2μM</td>
</tr>
</tbody>
</table>

Table 1.4: High affinity glutamate uptake (adapted from Kanai et al., 1993)
The differences in affinity and tissue distribution of glutamate transporters have functional importance. It is predominantly the GLT-1 subtype that maintains the extracellular environment, EAAC1 only significantly contributing to glutamate uptake when high glutamate concentrations occur during synaptic activity. Indeed, during synaptic activity it is possible that low affinity glutamate transporters (Km > 500μM) play a significant role in glutamate uptake.

High-affinity glutamate uptake has a number of other important properties; it is a carrier mechanism with a unique ion coupling pattern. This has been determined for high affinity glutamate transport in salamander retinal glia, but is likely to be more widely applicable (Bouvier et al., 1992; Barbour et al., 1988; Attwell et al., 1993). Coupled to the uptake of glutamate is the co-transport of two Na\(^+\) ions and the countertransport of one K\(^+\) ion and one OH\(^-\) ion (figure 1.3).

![Glutamate Uptake Mechanism](Figure 1.3: Glutamate uptake mechanism showing co-transport of 2 sodium ions and counter transport of a potassium and a hydroxide ion. The pump is electrogenic with a net positive charge being transported in. Reversal of this uptake mechanism can take place with rises in extracellular potassium, extracellular hydroxide, intracellular sodium or with cell depolarisation.)

Glutamate transport is thus dependent on ionic gradients, and since the pump is electrogenic (it pumps a net positive charge in) then the minimum maintained glutamate is a play off between osmotic pressure and electrical work. The
minimum maintained glutamate can thus be described by a Hill equation (Attwell et al., 1993):

\[ [\text{Glu}]_o = [\text{Glu}]_i ([\text{Na}^+]_i ([\text{K}^+]_o ([\text{Na}^+]_i ([\text{K}^+]_o ([\text{OH}^-]_o ([\text{OH}^-]_i \exp \{ VF/(RT) \}) \right) \right) \right) \right) \]

Where \([X]_o\) is the concentration of \(X\) outside the cell, \([X]_i\) is the concentration of \(X\) inside the cell, \(V\) is the cellular potential, \(T\) is the absolute temperature, \(R\) is the gas constant and \(F\) is the faraday constant. Calculating \([\text{Glu}]_o\) in physiological conditions ([Na\(^+\])_o = 145mM, [Na\(^+\])_i = 25mM, [K\(^+\])_o = 2.5mM, [OH\(^-\])_i = 100nM, [OH\(^-\])_o = 251nM, [Glu]_i = 10mM, V = -80mV) gives a minimum glutamate concentration of approximately 0.7\(\mu\)M (similar to that actually measured).

During ischaemia ionic gradients run down (extracellular potassium increases and extracellular sodium decreases) and neurons depolarise. These are the conditions that would tend to reverse the uptake mechanism leading to extrusion of glutamate into the extracellular fluid (Szatkowski et al., 1990; Attwell et al., 1993). This may explain the large rises in extracellular glutamate that occur in ischaemia (Szatkowski & Attwell, 1994). It is, however, unlikely that such a process plays a part in synaptic transmission because the time-course of rises due to reverse glutamate uptake is too slow (Attwell et al., 1993).

The uptake mechanism can also be modulated by other compounds such as arachidonic acid; the release of which inhibits glutamate uptake (Barbour et al., 1989). Indeed, it has been postulated that NMDA mediated release of arachidonic acid decreases glutamate uptake, potentiating synaptic glutamate rises, and thus leading to long-term potentiation (Barbour et al., 1989).

The final determinant of extracellular glutamate concentration is the size of the extracellular space. The extracellular space can expand and shrink depending on ionic concentrations. During seizures, for example, sodium and with it water enter cells causing cellular expansion and concomitant shrinkage of the extracellular space (Lux et al., 1986). This in itself could lead to rises in brain extracellular amino acid concentrations.
1.6.3.1 Methods and problems with measurement of extracellular glutamate

A variety of techniques exist to study the constituents of brain extracellular fluid; each has its particular application and limitations (Benveniste, 1989). Microelectrodes have been developed to measure specific ions, but are unable to measure other substances. Carbon electrodes and voltammetry have been used to measure electroactive compounds. The carbon electrode is kept at a positive potential which results in the oxidation of surrounding compounds, and the loss of the electrons by these compounds to the electrode results in a measurable and quantifiable current. Selectivity is achieved by choosing a low enough potential that only a few compounds are oxidised at this potential. The main application has been in the measurement of ascorbate which has a low oxidising potential (it is a powerful reducing agent), and is present in abundance in the central nervous system. Other compounds such as uric acid and dopamine are oxidised at a similar potential and thus act as interferents; they are, however, present in much lower concentrations in the brain than ascorbate. Although this method cannot directly measure glutamate, it has been indirectly used for this purpose in combination with microdialysis techniques as a 'dialysis electrode' (see below). Also ascorbate has been used as an indirect measure of glutamate rises; changes in extracellular ascorbate may be dependent on a glutamate/ascorbate heteroexchange (O'Neill et al., 1984; Fillenz & Grunewald, 1984; Cammack et al., 1991; 1992).

Early methods of measuring glutamate were the cortical cup and the push-pull cannula. The cortical cup consists of placing a cup over the surface of the cortex through which fluid can be perfused - this method is limited to epileptogenic zones on the cortex. This push-pull cannula technique involves the implantation of two either concentric or adjacent small bore tubes (total diameter approximately 1mm). Two pumps are used; one to push fluid down one tube, and the other to suck the fluid from the other tube. The extracted fluid can then be analysed by a variety of techniques to determine the concentration of the compound of interest. This method can be used for foci within the brain substance (e.g. in the hippocampus). There are, however, a number of problems. The large diameter of the cannula, and the removal of extracellular fluid cause significant
perturbations to the area in which the measurements are being made. Also enzymes are extracted along with substances of interest and can result in enzymatic degradation of the substances under investigation. These problems are to some extent overcome by microdialysis probes.

Classical microdialysis consists of a small diameter (less than 500μm) tube of dialysis membrane into which perfusate (artificial CSF) is passed. The substance of interest diffuses across the dialysis membrane and can then be detected in the dialysate from the outflow. Importantly substances with large molecular weights such as enzymes and protein cannot diffuse through the membrane. This technique allows relatively good temporal and spatial resolution without significant perturbation to the system measured (the method does not rely upon the extraction of fluid from a compartment of interest), and importantly the blood-brain barrier is intact shortly following slow implantation of microdialysis probes.

Microdialysis is thus suited for studies of both brain amino acids and drug neuropharmacokinetics. An important consideration with microdialysis is that equilibrium is not achieved and thus the concentration of the substance of interest will be lower in the dialysate than in the surrounding fluid; the ratio of the concentration in the dialysate to the concentration in surrounding fluid is known as the recovery, and it is usually assumed (possibly incorrectly) that the recovery in vivo is concentration independent. Microdialysis for the measurement of brain amino acids has a number of inherent, but often ignored, difficulties. The flow of dialysate through microdialysis probes changes the environment around the probe by diffusion across the membrane of substances such as calcium, adenosine, and ascorbate (Benveniste, 1989). In addition it will tend to buffer changes in local ion concentrations, and since increases in extracellular potassium may increase extracellular glutamate levels then microdialysis may itself buffer extracellular glutamate rises (Obrenovitch et al., 1995). Furthermore, the constant and substantial removal of the amino acid being measured can lead to a 'zone of depletion', which may dampen the microdialysis response to transient changes in amino acid concentrations (Justice Jr, 1993). In addition, microdialysis probes have been shown to change brain metabolism during kainic acid-induced seizures, but not in controls (Chastain Jr et al., 1990). These and the time resolution
(sampling intervals of 5 minutes or greater are used) of classical microdialysis mean that transient and small glutamate rises will not be detected by this method.

1.6.3.2 Measurement of extracellular glutamate during seizures and status epilepticus

Since glutamate is the major excitatory transmitter, and activation of NMDA receptors is a major determinant of neuronal death in status epilepticus, then rises of extracellular glutamate in status epilepticus and seizures would seem a logical consequence. Over 20 years ago, Koyama reported an increase in the glutamate concentration in a superfusate over a cobalt induced epileptogenic cortical focus during the period of epileptiform activity (Koyama, 1972). This finding was later confirmed and a long-lasting (8 day) increase in glutamate concentration over a cobalt-induced epileptogenic region was observed (Dodd & Bradford, 1976). In order to understand more fully the role of glutamate, attempts have been made to measure extracellular glutamate concentrations in specific brain regions using microdialysis techniques in animal seizure models. These, however, have resulted in conflicting results with many showing no significant rise in glutamate during seizures (table 1.5).

These negative results have been attributed to high affinity glutamate uptake in synapses and glia, yet inhibition of glutamate uptake has still resulted in inconsistent findings (Millan et al., 1991; 1993). These inconsistencies could be model dependent, and indeed a comparison of glutamate rises in two animal models (kainic acid induced seizures and soman induced seizures) found a significant seizure-associated glutamate rise in only the soman-induced seizure model (Wade et al., 1987). These animal data are in contrast to human microdialysis studies in which consistent large glutamate rises have been seen both during seizures and just prior to seizure onset (Carlson et al., 1992; Ronne Engstrom et al., 1992; During & Spencer, 1993).
Table 1.5: Glutamate rises measured by microdialysis during seizures in different species and models of epilepsy

<table>
<thead>
<tr>
<th>Species</th>
<th>Inducing agent for seizure</th>
<th>Glutamate rise during seizure</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>spontaneous</td>
<td>⬤ ⬤ ⬤ large</td>
<td>(Carlson et al., 1992; Ronne Engstrom et al., 1992; During &amp; Spencer, 1993)</td>
</tr>
<tr>
<td>Rat</td>
<td>pilocarpine</td>
<td>none (rise detected during seizure following concomitant administration of glutamate uptake blocker, PDC)</td>
<td>(Millan et al., 1993)</td>
</tr>
<tr>
<td></td>
<td>kainic acid</td>
<td>none</td>
<td>(Wade et al., 1987; Lehmann et al., 1985; Bruhn et al., 1992)</td>
</tr>
<tr>
<td>Rat</td>
<td>soman</td>
<td>⬤ ⬤ moderate</td>
<td>(Wade et al., 1987; Lallement et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>bicuculline</td>
<td>none (not even if glutamate uptake is blocked with DHK)</td>
<td>(Lehmann et al., 1985; Millan et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>picrotoxin</td>
<td>none (not even if glutamate uptake is blocked with DHK)</td>
<td>(Millan et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>quinolinic acid</td>
<td>none</td>
<td>(Vezzani et al., 1985)</td>
</tr>
<tr>
<td></td>
<td>electroconvulsive shock</td>
<td>none</td>
<td>(Korf &amp; Venema, 1985)</td>
</tr>
<tr>
<td></td>
<td>kindling</td>
<td>⬤ ⬤ moderate, (dependent on stage of kindling)</td>
<td>(Zhang et al., 1991; Minamoto et al., 1992; Ueda &amp; Tsuru, 1994)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>folic acid</td>
<td>↑ small</td>
<td>(Lehmann, 1987)</td>
</tr>
<tr>
<td></td>
<td>bicuculline</td>
<td>none (unless seizure was preceded by a short period of hypoxia)</td>
<td>(Young et al., 1992)</td>
</tr>
</tbody>
</table>

The matter is further complicated by the glutamate rises seen in kindling models of epilepsy. A number of studies have shown that glutamate rises occur following stimulation and that these rises are greater following stimulations further on in the kindling protocol (Zhang et al., 1991; Minamoto et al., 1992; Ueda & Tsuru,
1994). In a further study, basal glutamate concentrations were observed to increase approximately 2 fold following kindling to stage 5 seizures (Kaura et al., 1995). In addition, it was observed that depolarisation-induced release of glutamate in amygdala slices from kindled animals was twice as great as from sham operated controls (Kaura et al., 1995). Thus, it is likely that kindling results in a long-term decrease in glutamate uptake.

1.7 The anatomical and physiological consequences of status epilepticus

The patterns of cell loss have been described previously, but the question arises as to what are the consequences of this cell loss. Not surprisingly status epilepticus in animals is associated with learning difficulties and behavioural deficits (Milgram et al., 1988; Rogers et al., 1989). Status epilepticus in animal models is also associated with the development of chronic epilepsy often after a quiescent period (Leite et al., 1990; Lothman et al., 1990; Mello et al., 1992) that may progress for up to three months (Bertram & Cornett, 1994).

The mechanisms underlying the development of chronic epilepsy are uncertain. Sloviter (1983; 1987; 1991) used paired pulse stimulation of the perforant path to study the changes in physiology of the dentate granule cells during the development of status epilepticus-like neuronal damage. Paired pulses (40ms) were given at 2Hz, and in normal dentate granule cells, the response to the first pulse is greater than the response to the second pulse. This inhibition is probably due to feed forward and feed back inhibition mediated by local inhibitory interneurons. After the stimulation protocol, this paired pulse inhibition was lost. Immunocytochemical staining revealed that the gamma-aminobutyric acid (GABA)-containing neurons, thought to mediate inhibition in this region had survived. There was, however, loss of adjacent somatostatin-containing interneurons and mossy cells that may normally activate inhibitory neurons. These results suggested that the seizure-induced loss of a basket cell-activating system, rather than a loss of inhibitory basket cells themselves, caused disinhibition and was thus involved in the pathophysiology (Sloviter, 1987), and this was referred to as the "dormant basket cell" hypothesis. More recently, in a
similar model application of NBQX, an AMPA antagonist, was shown to inhibit the loss of these interneurons, but to have no effect on the loss of paired pulse inhibition (Penix & Wasterlain, 1994). Similarly in the tetanus toxin model, disinhibition occurs in the absence of hilar cell loss (Whittington & Jefferys, 1994). Other factors must thus play a large part in the loss of paired pulse inhibition such as loss of affinity and activity of GABA_A receptors in the hippocampus (Kapur et al., 1994). Support for the "dormant basket cell" hypothesis as a mechanism underlying temporal lobe epilepsy came from hippocampal slice work from an animal model of chronic epilepsy (Bekenstein & Lothman, 1993). IPSPs were elicited in CA1 pyramidal cells by activation of basket cells. IPSPs evoked indirectly by activation of terminals that then excited basket cells were reduced in the epileptic tissue, whereas IPSPs evoked by direct activation of basket cells were not different from controls (Bekenstein & Lothman, 1993). Thus status epilepticus appears to result in a loss of inhibition that may result in chronic epilepsy. There is, however, considerable evidence that the converse occurs, that there is a potentiation of excitation, in both chronic models of epilepsy and following status epilepticus. This takes the form of mossy fibre sprouting. Mossy fibres are zinc containing axons from the dentate granule cells which project to neurons in the dentate hilus and to CA3. It had been noted that in a kindled animal model, the mossy fibres, projected back through the granule cell layer to form a terminal field in the region of the granule cell proximal dendrites (Sutula et al., 1988; Cavazos et al., 1991). It was later confirmed that a similar histological picture occurred in human temporal lobe epilepsy (Sutula et al., 1989; Babb et al., 1991). Inhibition of mossy fibre sprouting following kainic acid seizures by the concomitant treatment with phenobarbital prevented the expected facilitation of subsequent kindling development (Sutula et al., 1992). This suggested a correlation between mossy fibre sprouting and the potentiation of excitation. Mossy fibre sprouting has been observed in models of status epilepticus and appears to correlate with the appearance of late seizures (Mello et al., 1992; 1993). The hypothesis that mossy fibre collaterals form granule cell-granule cell synapses has been confirmed by visualising dentate granule cells and their mossy fibres after terminal uptake and
retrograde transport of biocytin (Okazaki et al., 1995). The physiology of mossy fibre sprouting has revealed some unexpected findings (Cronin et al., 1992). Stimulation of the hilus or perforant path of hippocampal slices with mossy fibre sprouting evoked relatively normal responses. However, in the presence of the GABA_A-receptor antagonist, bicuculline, low-intensity hilar stimulation evoked delayed bursts of action potentials and spontaneous bursts of synchronous spikes occurred that were not seen in normal controls. These data suggest that mossy fibres form excitatory circuits that are normally suppressed, but which can result in epileptiform activity when synaptic inhibition is blocked (Cronin et al., 1992). More recently Buhl et al. (1996) have found that during kindling the GABA_A receptor in dentate granule cells becomes zinc sensitive so that zinc reduces GABAergic inhibition. They proposed the hypothesis that zinc released from mossy fibres could thus inhibit GABAergic inhibition. The mechanism by which this zinc sensitivity occurs, and whether this has any direct bearing on human epilepsy is unknown.

Status epilepticus thus results in both enhancement of excitatory circuits and loss of inhibition, and these may be the underlying mechanisms that result in the consequent chronic epilepsy.

1.8 Preventing neuronal damage in status epilepticus

The degree of neuronal damage is dependent upon the length of time that the animal spends in status epilepticus (Sloviter, 1983; Nevander et al., 1985; Meldrum and Brierley, 1973). Thus, rapid and effective termination of status epilepticus is an important aim of treatment. Since NMDA receptor activation plays a large part in status epilepticus-induced neuronal damage, then NMDA antagonists, irrespective of their effects on electrographic seizure activity, would be expected to ameliorate this damage. Confirmation of this hypothesis has come from a number of groups that have observed no change or an increase in electrographic seizure activity in animals in status epilepticus who had been pre-treated with NMDA antagonists, yet the NMDA antagonists still result in significant neuroprotection. Intraventricular application of 2-amino-7-
phosphonoheptanoic acid (AP5), a selective competitive NMDA antagonist, decreased the CA1 neuronal damage induced by kainic acid seizures, but did not significantly reduce the seizures (Lason et al., 1988). Similarly, N-[1-(2-thienyl)cyclohexyl]-piperidine (TCP), ketamine, phencyclidine and MK-801, all non-competitive NMDA antagonists, prevent seizure-related damage in several brain regions without suppressing seizure activity in kainate induced status epilepticus (Clifford et al., 1990; Lemer Natoli et al., 1991), and bicuculline induced focal status epilepticus (Clifford et al., 1989). Indeed, in one study (Fariello et al., 1989), there was a dose-dependent increase in the severity of the kainate-induced electrographic manifestations of epilepsy after MK-801. This consisted of an earlier appearance and higher number of EEG seizures, longer time spent in seizures, and an earlier onset of status epilepticus (Fariello et al., 1989), yet MK-801 administration still resulted in considerable neuroprotection.

In these studies, NMDA antagonists prevented seizure-related brain damage in the amygdala, piriform cortex, thalamus, and CA1 region of the hippocampus but often conferred little or no protection in the CA3 region of the hippocampus. This regional selectivity suggests that NMDA receptors may play a more dominant role in seizure-related brain damage in some brain regions than in others. Some status epilepticus-induced damage may be due to activation of non-NMDA receptors that are associated with calcium permeable ion channels (see above). Further evidence for this comes from a study in which NBQX, a non-NMDA receptor antagonist, protected against neuronal damage in an electrical stimulation model of status epilepticus-induced damage (Penix & Wasterlain, 1994).

The apparent dissociation between seizure activity and neuronal death can be explained by the hypothesis that it is not the seizure activity per se that results in neuronal death, but the activation of NMDA receptors. In addition, it appears that seizure activity in status epilepticus can continue without the participation of NMDA receptors. Indeed, separate roles for NMDA receptors have been suggested in the initiation and maintenance of status epilepticus (Young & Dragunow, 1993; 1994). Thus, in an electrical stimulation model of status epilepticus, MK-801 can prevent the development of status epilepticus, but once
the status epilepticus is established MK-801 can suppress the motor activity, but
not terminate the electrographic activity (Young & Dragunow, 1993; 1994).

Other studies of NMDA antagonists in animal models of status epilepticus have
demonstrated a protective effect of these agents against the long-term sequelae of
status epilepticus including, memory and behavioural problems and chronic
epilepsy (Rogers et al., 1989; Ylinen et al., 1991a; Stafstrom et al., 1993).

Other agents given before induction of status epilepticus have also been found to
reduce the neuronal damage, but many of these modulate the seizure activity.
Thus, diazepam given prior to kainic acid blocks the seizures and neuronal
damage (Ben Ari et al., 1979), and a similar effect is seen with antiepileptic drugs
on pilocarpine-induced status epilepticus (Turski et al., 1987).

The question arises as to whether such drugs given after the status epilepticus has
become well-established also ameliorate the resultant neuronal damage. Treatment
with glutamate antagonists is neuroprotective in a number of models of
ischaemia many hours after the ischaemic episode has taken place (Obrenovitch
& Richards, 1994; Szatkowski & Attwell, 1994). The mechanism of this delayed
effect is not known. Whether neuroprotective drugs used in status epilepticus can
have such a delayed effect is of great clinical importance; if neuroprotective drugs
are going to be given in the clinical situation, then they must have some
neuroprotective potential once the status epilepticus has become established. CGP
40116, a competitive NMDA antagonist, and ketamine given 15 minutes after the
onset of lithium-pilocarpine induced status epilepticus still have a considerable
neuroprotective effect (Fujikawa et al., 1994; Fujikawa, 1995). Similarly,
felbamate, a newly developed antiepileptic drug that may have some action on
NMDA receptors, administered to 30-day-old rats 1 h after they received a
convulsant dosage of kainic acid protected against the behavioural effects of
status epilepticus and the later decrease in seizure threshold (Chronopoulos et al.,
1993). This study was confounded by the observation that the felbamate-treated
group had less severe seizures.
1.9 The treatment of status epilepticus

The treatment of status epilepticus depends upon its type and the underlying cause. The type with the greatest consequences is convulsive status epilepticus, which is a medical emergency. I will thus concentrate on the treatment of this particular form. I will also discuss briefly the treatment of non-convulsive status epilepticus, because, although the consequences are not as severe, status epilepticus restricted to the limbic lobes still results in extensive neuronal damage in animal models (see above).

1.9.1 Convulsive status epilepticus

The aim of treatment in convulsive status epilepticus is to: 1) halt seizure activity; 2) to maintain homeostasis, and 3) to treat complications. It is the latter two that I will discuss first. General measures are integral to the treatment of convulsive status epilepticus. As in all medical emergencies, it is essential first to secure the airway and resuscitate if necessary. Oxygen should be given as hypoxia is common during convulsions. The subsequent treatment can be divided into two stages, related to the physiological compromise previously described.

Stage 1 (0-60 minutes)

1) Emergency investigations: Blood should be taken for measurement of arterial blood gases, glucose, renal and liver function, calcium and magnesium, full blood count, clotting screen and anticonvulsant levels. Serum and urine (if possible) should be saved for possible future analysis. An ECG should be performed.

2) Monitoring: Regular neurological observations, pulse, blood pressure and temperature need to be taken. Continuous ECG monitoring, and oximetry are necessary, and regular glucose, electrolytes, arterial blood gases and pH should be performed.

3) Emergency drug treatment should be started (see below).
4) *Intravenous glucose and thiamine:* 50ml of 50% glucose should be given intravenously if there is any suspicion of hypoglycaemia. 250mg of thiamine should be administered intravenously in anyone with a history of poor nutrition or alcoholism. This is especially important if intravenous glucose is going to be given, as glucose infusions can precipitate Wernicke's encephalopathy.

5) *Correction of metabolic abnormalities:* correction of severe metabolic abnormalities is best managed on the intensive care unit. Acidosis is a common complication of status epilepticus, and is usually corrected through the rapid control of respiration and the abolition of motor seizure activity. In severe acidosis, however, bicarbonate infusion should be given. Hyperthermia may be treated by successful abolition of the motor seizure activity.

**Stage 2 (0-90 minutes)**

It is at this stage (failure of the first line treatments) that the patient should be transferred to the intensive care unit, as the second phase of systemic effects will occur.

1) *Hypotension and cardiac arrhythmias:* In addition to the hypotension resulting from the status epilepticus, most of the drugs used in the treatment of status epilepticus compound this problem. As the cerebral blood flow at this stage is dependent on systemic blood pressure, and may be compromised further by raised intracranial pressure, maintenance of the blood pressure is paramount and pressor agents are indicated (this is especially so if barbiturate anaesthesia is used). Continuous ECG monitoring is mandatory.

2) *Respiratory compromise:* Again this results not only from the continued seizure, and pulmonary oedema, but also from the drugs used. There should be a low threshold for intubation and ventilatory support. The use of subanaesthetic doses of anaesthetic agents without ventilatory support is not to be recommended. Aspiration pneumonia is not an uncommon problem and if suspected, then broad spectrum antibiotics should be started.
3) Hypoglycaemia: This is common in the later stages of status epilepticus. It should not, however, be routinely corrected with glucose, unless severe. There is evidence to suggest that hyperglycaemia in the later stages of status epilepticus may result in an increase in cerebral damage, and that mild hypoglycaemia may be neuroprotective (Meldrum et al., 1973; Blennow et al., 1978).

4) Hyperthermia and lactic acidosis: These are usually controlled by halting the motor seizure activity, and if this persists despite adequate treatment, then paralysing agents may be indicated. Sodium bicarbonate may need to be used in severe cases of acidosis, but it is important to realise that this represents a large sodium load and may compound the problems of cerebral and pulmonary oedema. Cooling is indicated if the temperature rises above 40°C.

5) Rhabdomyolysis and renal failure: These are not uncommon. In the early stages there may be benefit in giving mannitol and a dopamine infusion. Electrolytes and renal function should be closely monitored.

6) Cerebral oedema: This may be a complication in the later stages, and continuous intracranial pressure monitoring is advisable, especially in children. Depending on the underlying cause, active therapy with dexamethasone or mannitol may be necessary, and occasionally neurosurgical decompression may be required (Shorvon, 1994).

7) Seizure and EEG monitoring: A patient may enter a drug-induced coma with little outward sign of convulsions with on-going electrographic epileptic activity. In addition, if a patient is inadequately treated or has a severe underlying encephalopathy, then electromechanical disassociation may occur such that the patient may enter a stage of subtle generalised convulsive status epilepticus characterised by profound coma, bilateral EEG ictal discharges and only subtle motor activity, regardless of the presence or absence of sedating drugs or paralysing agents (Treiman, 1993; Treiman et al., 1984). The titration of the dose of anaesthetic agents in their use in status is commonly based upon burst suppression on the EEG or cerebral function monitor (CFM) with interburst intervals of 2-30 seconds as an acceptable end-point (Yaffe & Lowenstein, 1993; Rashkin et al., 1987; Lowenstein et al., 1988). Both CFM and continuous EEG
are suitable, and if continuous monitoring is not available then at the very least some form of intermittent EEG should be used. A patient with intractable status should have at least one EEG to exclude the commonplace, but often misdiagnosed condition of pseudo-status (Leis et al., 1992; Howell et al., 1989).

8) Establish aetiology: This is of prime importance, and depending on circumstance, CSF examination and CT scanning are usually necessary. If the status has been precipitated by drug withdrawal, then immediate reintroduction of the withdrawn drug may terminate the status even at low doses. It is important to realise that status epilepticus is symptomatic of an underlying condition, and this should be identified and treated appropriately.

1.9.1.1 Drug treatment

Convulsive status epilepticus can be temporally divided, both clinically and electrographically, into a series of progressive stages (Shorvon, 1994; Treiman, 1993). As the status epilepticus continues so it may become harder to treat. This progression, the advent of status-induced neuronal damage and the physiological effects described above have resulted in the development of temporal regimens in the drug treatment of convulsive status epilepticus.

There are in essence two stages in the drug treatment of status epilepticus: the use of drugs in order to uniquely halt seizure activity, and the use of drugs to depress cerebral activity and consequently ictal activity (the induction of general anaesthesia). It is important to differentiate these stages as, although they have a common aim, they seek to achieve this through different means. This is especially important in the consideration of certain compounds which in low doses are anticonvulsant, but which at higher doses may be primarily anaesthetic (e.g. thiopentone, benzodiazepines). Choosing the most appropriate drug treatment for status epilepticus is difficult, because of the lack of good comparative trials, the contradictory anecdotal reports of the use of various agents and the diversity of advice in the numerous reviews on the subject. The choice of a particular agent must at present be based primarily upon theoretical considerations. The ideal treatment should be efficacious, fast-acting, should have a persistent action,
should not cause respiratory or circulatory problems, and if coma does result this should be easily reversed.

Premonitory stages

In both animal models and in human convulsive status epilepticus, there is a prodromal period. During this there is a gradual increase in the frequency of tonic-clonic seizures until they become confluent or there is no recovery in-between. During this premonitory stage, acute administration of antiepileptic drugs may abort the progression to status epilepticus (Shorvon, 1994). Plans can certainly be made for those who have frequent or prolonged seizures to receive acute treatment at home or in the institution in which they live. Rectal diazepam and intramuscular midazolam are easily administered, and have a rapid action (Remy et al., 1992; Ghirlain et al., 1988). Repeat doses, however, can lead to accumulation and cardiorespiratory collapse, and so should not be encouraged. Rectal paraldehyde is difficult and unpleasant to use, but causes less severe sedation, and fewer respiratory problems. It is necessary for carers to have formal training in the preparation and administration of rectal paraldehyde, and if this is available it may be preferable to the benzodiazepines (Whiting et al., 1993).

Early stages

If a patient is transferred to hospital before the status epilepticus is established or if the status epilepticus occurs in the hospital setting, then appropriate treatment may abort the status epilepticus.

In these initial stages intravenous benzodiazepine, intramuscular or rectal paraldehyde may be given. There is little to choose between clonazepam, diazepam and lorazepam at this stage (Treiman, 1990). If respiratory compromise is a consideration (patients with concomitant respiratory disease), then intravenous lignocaine is an alternative (Pascual et al., 1988).
Established stage

Once the seizures have continued for 30 minutes then the stage of established status epilepticus is entered. It is practical to also define this period as failure of treatment in the early stages or a patient in which an accurate estimate of the duration of seizures cannot be made. The choice of treatments at this stage is dependent on previous treatments. Three randomised comparative trials of: 1) phenobarbitone against phenytoin and diazepam (non-blinded); 2) lorazepam against diazepam (blinded), and 3) lorazepam against phenytoin (blinded) have resulted in three equally effective regimes (Shaner et al., 1988; Leppik et al., 1983a; Treiman et al., 1983). Additionally there have been reports of the successful use of lignocaine (and phenytoin) in convulsive status epilepticus although there has been no comparative study of its use (Pascual et al., 1988). The following alternatives are thus available at this stage:

i) diazepam or lorazepam with intravenous phenytoin;
ii) phenobarbitone;
iii) lignocaine (and intravenous phenytoin). (This should be reserved for patients in whom there is respiratory failure).

If the seizures continue then the patient should be transferred to the ICU as the stage of refractory status has been reached.

Refractory status epilepticus

At this stage there are two important considerations:

1) the second stage of physiological compromise will be occurring;
2) further antiepileptic drug (AED) treatment is likely to cause respiratory and circulatory compromise.

Further drug treatment

Second line therapies can be tried at this stage and these include any of the first-line therapies not already given, chlormethiazole, intravenous paraldehyde or
benzodiazepine infusion. Although there have been favourable reports of the efficacy of clonazepam, diazepam and midazolam infusions in status epilepticus, there are theoretical reasons why long term use of these should not be advised. Diazepam and clonazepam given acutely have substantially shorter distribution half-lives than their elimination half-lives, which are of the order of 30 hours (Schmidt, 1989; Greenblatt et al., 1987). Thus, although the blood concentrations may drop precipitously following a single bolus as a result of redistribution, infusions and repeat boluses may lead to accumulation and so persistent action. This often results in sudden hypotension, respiratory and circulatory collapse. Unfortunately this problem is compounded by the difficulties, in most centres, of monitoring benzodiazepine plasma concentrations. Midazolam, however, may be preferable in this instance as its elimination half-life is 1.5-3.5 hours - this may be prolonged in the elderly, in hepatic disease and with repeated doses given on the intensive care unit (Dundee et al., 1984). Nevertheless it has a large volume of distribution, and with prolonged use accumulation is a potential problem, which has not yet been fully evaluated. Furthermore tolerance to the antiepileptic action of benzodiazepines occurs with prolonged administration, requiring higher doses that are potentially dangerous. There may also be recurrence of seizures on drug withdrawal.

Benzodiazepine infusions should therefore perhaps not be used in the treatment of status epilepticus, especially if ventilatory and circulatory support are not immediately available. If a benzodiazepine infusion is necessary, then midazolam is probably the drug of choice, although clinical experience of long-term midazolam infusions (over a period of days rather than hours) is limited. Other agents, at this stage, also present particular difficulties. Chlormethiazole, which is not licensed in the USA, is an easily administered agent. Infusions, however, can be complicated by hypotension, cardiac depression, respiratory arrest and like benzodiazepines, this drug shows a propensity for dangerous accumulation with prolonged administration (Browne, 1983; Pentikainen et al., 1976; Robson et al., 1984). Paraldehyde is difficult to use as an intravenous preparation, decomposes in light and in overdose there are potentially serious toxic side-effects. It may be useful in premonitory stages per rectum, but in established SE where an
intravenous infusion is indicated, its use is limited (Browne, 1983; Burstein, 1943). Alternatively general anaesthesia (drug-induced coma) can be used.

**General anaesthesia**

It is important to differentiate the anticonvulsant effects of a drug used in status epilepticus from its anaesthetic effects. Only the barbiturate anaesthetic agents have been shown to have anticonvulsant activity in addition to their anaesthetic activity. Indeed, most non-barbiturate agents have been shown both to produce epileptogenic activity on the EEG, and to induce seizures (Poulton & Ellington, 1984; Makela et al., 1993).

i) The barbiturate anaesthetics have the advantage of long experience in the treatment of status, and potent anti-epileptic activity (Brown & Horton, 1967; Opitz et al., 1983). The main disadvantages, however, are in their pharmacokinetics. Thiopentone has saturable kinetics, with a resultant long half-life at high concentrations. It has a tendency to accumulate, and thus recovery times may be protracted in prolonged therapy (Turcant et al., 1985). It can result in profound hypotension, and concomitant inotropes are often required. The favourable reports of subanaesthetic doses of thiopentone being used without ventilatory support should be interpreted with much caution, and cannot be recommended (Dundee & Gray, 1967). Pentobarbitone, which is one of the major metabolites of thiopentone, has a shorter half-life, but has received less favourable reports in the literature, and is subject to many of the same problems as thiopentone (Yaffe & Lowenstein, 1993; Van Ness, 1990).

ii) The non-barbiturate anaesthetics have more suitable kinetics, but less is known about their anti-epileptic activity, and there has been less experience in their use in status epilepticus. Propofol is easy to use, fast acting, and recovery is quick. There have been a number of favourable reports of its use in status (Mackenzie et al., 1990; Campostrini et al., 1991), and it has demonstrated some anti-epileptic activity in animal seizure models (De Riu et al., 1992). It has, however, been reported to precipitate seizures and status epilepticus (Makela et al., 1993). In intracortical recordings during epilepsy surgery, it has activated epileptiform
discharges on the electrocortigram (Hodkinson et al., 1987). Further study is required before it can be routinely recommended for the treatment of status epilepticus. Isoflurane has the disadvantage of being an inhalational anaesthetic. There have been reports of isoflurane having some epileptogenic activity, and one report of a seizure induced with isoflurane (Poulton & Ellington, 1984). The evidence, however, for its epileptogenicity is less convincing than in the case of propofol. There have, nevertheless, been very favourable reports of its use in status epilepticus resistant to benzodiazepines, phenytoin and barbiturates (Kofke et al., 1989). The other inhalational anaesthetics, enflurane and halothane, are not suitable for the treatment of status; enflurane is a potent epileptogen, and halothane in prolonged use may cause severe side-effects. Etomidate may cause adrenal suppression, and thus is no longer licensed in the UK for prolonged infusion in anaesthesia. It has been reported to cause both myoclonus, and activation of the EEG in epileptic patients. It has, however, been shown to have anticonvulsant properties and its effectiveness in the treatment of refractory status has been well documented (Yeoman et al., 1989; Wauquier, 1983). It has low cardiovascular toxicity and is especially safe in patients with cardiovascular disease. Benzodiazepine infusions can be used in anaesthetic doses, and midazolam infusion may possibly be a suitable alternative to barbiturate anaesthesia (Rivera et al., 1993; Kumar & Bleck, 1992). In summary, non-barbiturate anaesthetics have more favourable pharmacokinetics than barbiturate anaesthetics, but less definite anticonvulsant action. Whether the intrinsic anticonvulsant properties of an anaesthetic agent are of importance in the treatment of status epilepticus has not been investigated, and thus which anaesthetic agent should be used remains unclear.

1.9.2 Complex partial status epilepticus

The correct diagnosis of complex partial status presents one of the major difficulties with this condition. Not only does it have to be differentiated from other forms of non convulsive status epilepticus, but also from post-ictal states, and other neurological and psychiatric conditions. EEG may be helpful, but often
the scalp EEG changes may be non-specific and the diagnosis is very much clinical in nature (Shorvon, 1994; Cascino, 1993). Shorvon (1994) defined complex partial status epilepticus as 'a prolonged epileptic episode in which focal fluctuating or frequently recurring electrographic epileptic discharges, arising in temporal or extratemporal regions, result in a confusional state with variable clinical symptoms'.

How aggressively complex partial status epilepticus is treated depends upon: 1) the prognosis of the condition; 2) if treatment improves the prognosis. As in all epilepsies the prognosis relates to the prognosis of the underlying aetiology and any concomitant medical conditions. Animal models of limbic status show neuronal damage similar to that seen in convulsive status (see above). Human data, however, are less convincing. There have been reports of prolonged memory problems, hemiparesis and death occurring following complex partial status epilepticus, although in many of these cases, the outcome relates to the underlying cause (Cascino, 1993; Treiman & Delgado-Escueta, 1981; Sung et al., 1993). Furthermore, in many of these cases the complex partial status epilepticus is treated aggressively with barbiturate anaesthesia, and the treatment itself could have resulted in the morbidity observed. In addition, there have been reports of prolonged complex partial status epilepticus with no neurological sequelae (Williamson et al., 1985; Cockerell et al., 1994). A recent study of 8 patients demonstrated that, despite the absence of an acute neurological insult, complex partial status epilepticus resulted in significant rises in serum neuron-specific enolase, a marker for acute neuronal injury (DeGiorgio et al., 1996). In this study there was no correlation between duration of seizure and the serum neuron-specific enolase, although this may be the result of the small sample size as larger studies in status epilepticus have found such a correlation (DeGiorgio et al., 1995). The degree to which serum neuronal specific enolase correlates with neurological and cognitive disability in complex partial status epilepticus, and whether the results of this pilot study are true for the majority of patients (especially those with recurrent complex partial status epilepticus) is unknown. At present, early recognition and treatment with oral or rectal benzodiazepines should be used. For more persistent complex partial status epilepticus,
intravenous therapy as with convulsive status epilepticus should be used, but whether general anaesthesia is justified remains a matter for speculation; since most complex partial status epilepticus is self-terminating often without any serious neurological sequelae (Cockerell et al., 1994), then such aggressive therapy should perhaps be avoided.

1.9.3 Absence SE

This entity needs to be distinguished from complex partial status epilepticus and atypical absences seen in mental retardation (Shorvon, 1994; Cascino, 1993).

This term should perhaps be reserved for prolonged absence attacks with continuous or discontinuous 3 Hz spike and wave occurring in patients with primary generalised epilepsy (Shorvon, 1994; Cockerell et al., 1994). The EEG, however, may include irregular spike/wave, prolonged bursts of spike activity, sharp wave or polyspike and wave, and whether to include such cases as absence status epilepticus is uncertain (Porter & Penry, 1983; Shorvon, 1994). Conversely the EEG during complex partial status epilepticus may show runs of 3 Hz spike and wave.

Although absence epilepsy has its peak in childhood and commonly remits in adolescence, absence status epilepticus commonly occurs in later life. Absence status epilepticus can be divided into childhood absence status epilepticus (those usually already receiving treatment), late-onset absence status epilepticus with a history of primary generalised history (often a history of absences in childhood) and late-onset absence status epilepticus developing de novo (usually following drug or alcohol withdrawal) (Shorvon, 1994).

There is no evidence that absence status induces neuronal damage, and thus aggressive treatment is not warranted (Porter & Penry, 1983; Shorvon, 1994). Treatment can either be intravenous or oral. Absence status epilepticus responds rapidly to intravenous benzodiazepines, and these are so effective that the response is almost a sine qua non. The effect may only be transient and a longer acting AED may need to be given. If intravenous treatment is required, but either
benzodiazepines are ineffective or contraindicated then iv acetazolamide, valproate, or chlormethiazole can be given. In cases of primary generalised epilepsy treatment should be continued with ethosuxemide, valproate or clonazepam, although lamotrigine may be equally effective. If a precipitating factor can be identified in late-onset de novo cases, then long term therapy is not usually indicated (Cascino, 1993; Porter & Penry, 1983; Shorvon, 1994).

1.10 Drug pharmacokinetics in status epilepticus

In the rational drug treatment of status epilepticus, an understanding of the pharmacokinetics of acutely administered drugs is needed. In particular, it is important to realise three fundamental points:

1) drugs in status epilepticus are given acutely, and thus it is the acute, single-dose pharmacokinetics of a drug that are relevant;
2) the drugs are given to a seizing animal;
3) the pharmacokinetics that are relevant are the pharmacokinetics of the drug at its point of action (i.e. in the brain).

1.10.1 Acute drug pharmacokinetics

The pharmacokinetics of a drug are a description of the "movement" of a drug, and embrace three processes: 1) the absorption of the drug, 2) the distribution of the drug through the body and 3) the elimination of the drug from the body. The pharmacokinetics of a drug are often described by a number of quantifiable terms. Oral bioavailability is the fraction of an oral dose that reaches the systemic circulation. \( T_{\text{max}} \) is the time elapsed from administration to the maximum concentration being achieved in plasma or serum. \( \text{AUC}_{a-b} \) is the area under the concentration versus time curve from time=\( a \) to time=\( b \). The apparent volume of distribution (Vd) is the volume of a compartment necessary to contain all the drug present in the body if it were present in the same concentration found in plasma/serum; if the drug distributes through the body in negligible time, then \( \text{Vd}=\text{dose/concentration at time}=0 \). Since this is rarely the case, and back
calculation of the concentration at time=0 is often inaccurate then an alternative method (given below) using clearance and elimination rate constant is usually used to calculate the Vd. First order kinetics are when the rate of fall of plasma concentration is proportional to the concentration, and the constant of proportionality is referred to as the elimination rate constant (k). Half-life ($T_{1/2}$) is the time for the plasma/serum concentration of a drug to decline by a half. Clearance (Cl) is the rate of removal of a drug from plasma, expressed as the volume of plasma that is cleared of the drug in unit time.

Fast drug absorption is essential in the treatment of status epilepticus, and thus most drugs need to be administered parenterally. Intravenous administration means that all of the dose given enters the plasma. Clearance, and AUC are model independent variables.

Below: $C_t$ is the concentration of drug at time=$t$, and $V_t$ is the volume of distribution of the drug at time=$t$.

Then $\text{AUC} = \int_{t=0}^{t=\infty} C_t \, dt$ (1)

and $\text{dose} = -\int_{t=0}^{t=\infty} V_t \, dC$ (2)

and $k = -(dC/dt)/C_t$ (3)

At $t+\delta t$, $C_t$ would change by $\delta C$ so that $C_{t+\delta t} = C_t + \delta C$

The amount of drug thus removed from the body = $-V_t \times \delta C$

so the volume of plasma cleared = $-(V_t \times \delta C)/C_t$

so the volume of plasma cleared per unit time (Cl) = $-(V_t \times \delta C)/(C_t \times \delta t)$

as $\delta t \to 0$, so $\delta C/\delta t \to dC/dt$

thus $\text{Cl} = -(V_t/C_t) \times (dC/dt)$

so $V_t = -\text{Cl} \times C_t \times (dt/dC)$ (4)

Substituting $V_t$ in equation (2) with (4) gives $\text{dose} = \int_{t=0}^{t=\infty} \text{Cl} \times C_t \, dt$

thus $\text{dose} = \text{Cl} \times \int_{t=0}^{t=\infty} C_t \, dt$
thus dose=Cl×AUC \ (5)

thus Cl=dose/AUC

and substituting for (dC/dt)/C in equation (3) from equation (4)
gives: \( V_t = \frac{Cl}{k} \) \ (6) when calculated in this fashion \( V_d \) is referred to as \( V_{darea} \)

**First order kinetics**

By definition:

\[
k = -(\frac{dC}{dt})/C_t \text{ where } k \text{ is constant}
\]

thus \[
\int k \ dt = -\int \frac{1}{C_t} \ dC
\]

thus \( [kt] = \ln(C_0/C_t) \)

thus \( C_t/C_0 = e^{-kt} \)

when \( C_t/C_0 = 1/2, t = \ln(2)/k \) \ (7) this is the half-life

Also \( AUC_{a-b} = \int_{a}^{b} C_0 \times e^{-kt} \ dt \)

so \( AUC_{a-b} = [C_0 \times e^{-ka} - C_0 \times e^{-kb}] / k \)

so \( AUC_{a-b} = (C_a - C_b) / k \) \ (8), when \( b = \infty, C_b = 0 \), so \( AUC_{a-\infty} = C_a / k \)

**1.10.1.1 Two compartment models**

During parenteral administration, a drug directly enters the central compartment (blood and extracellular fluid of highly perfused organs) from here it is distributed to peripheral compartments, in particular fat and muscle. The degree to which this occurs depends upon the lipid solubility of the drug. Drugs which are highly lipid soluble have a large volume of distribution and are rapidly redistributed into the peripheral compartment from the central compartment. This leads to an initial drop in plasma concentrations, which can be quantified as a redistribution half-life. In addition the drug may be eliminated from the central compartment either through renal excretion, hepatic metabolism (the major route of elimination for the majority of antiepileptic drugs) or exhalation, and this can be quantified as the
elimination half-life. This leads to two phases to the concentration versus time curve, an $\alpha$ and a $\beta$ phase; the concentration versus time curve can be modelled by a biexponential decay:

$$C_t = A e^{-\alpha t} + B e^{-\beta t}$$

$\beta$ approximates the elimination rate constant $k$, and $\alpha$ represents a redistribution rate constant. Half-lives for each of these constants can also be calculated as in equation (7). Clearance can be calculated as before, equation (5) as this is a model independent variable. $Vd_{area}$ can be approximated by assuming that $k \approx \beta$ and using equation (6).

1.10.1.2 Acute kinetics of drugs used during status epilepticus

Drugs used in status epilepticus need to have a rapid onset of action. In order to accomplish this, the drugs need to cross the blood brain barrier readily. Antiepileptic drugs achieve this either by being lipid soluble or by having an active transport mechanism (Loscher & Frey, 1984). This has led to many drugs that are effective in status epilepticus, having a high lipid solubility, and thus a large volume of distribution and a biphasic concentration versus time curve; in addition, these drugs often have a much shorter distribution half-life than their elimination half-life.

1.10.1.3 Kinetics of drugs during seizures

Seizures (especially convulsive seizures) may affect both blood and central nervous system pharmacokinetics of drugs. During convulsive seizures, there is a greater fall in the pH of the blood resulting in a change in the degree of ionisation (and thus lipid solubility) of drugs in plasma. This will affect their distribution half-lives, their ability to cross the blood brain barrier, and their protein binding. In addition, the pH in blood decreases to a greater degree than in brain; this pH gradient facilitates the movement of a weakly acid drug from blood to brain. This effect can be seen with phenobarbitone (Simon et al., 1985; 1987; Walton & Treiman, 1989). Other peripheral pharmacokinetic effects are also apparent.
during status epilepticus. These may result from increased blood flow to muscle, and hepatic and renal compromise (often resulting in a prolongation of the elimination half-life of anticonvulsant drugs). In addition to these peripheral effects, there is also a direct effect of status epilepticus on the brain compartment. There is a break down in the blood brain barrier during convulsive seizures, which again results in more effective brain penetration of anticonvulsant drugs (Petito et al., 1977; Simon et al., 1987). During seizures there is increased blood flow to seizing brain; thus drugs in which the cortical blood flow determines the rate at which the drug crosses the blood brain barrier (e.g. phenobarbitone) will concentrate in seizing brain.

1.10.2 Neuropharmacokinetics

Although serum concentration monitoring of antiepileptic drugs (AEDs) is widely and effectively used in the management of patients, its primary purpose is as an index of brain concentrations (i.e. the concentration at the site of action) during chronic administration. Serum pharmacokinetics may, however, be a very poor index of brain pharmacokinetics following acute administration of AEDs such as are used in the treatment of status epilepticus (Sechi et al., 1989; Wilder et al., 1977). An understanding of the neuropharmacokinetics of such drugs is critical for: optimisation of therapy; determining the value of serum concentration monitoring, and ascertaining their modes and mechanisms of action. Additionally it is not enough to picture the brain as a single compartment. The brain consists of extracellular, intracellular, and cerebro-spinal fluid compartments and depending on where a drug acts depends on which compartment's kinetics are of most relevance. The constitution of and access to these compartments are different (Davson et al., 1987). The pharmacokinetics of antiepileptic drugs in whole brain are determined by non-specific binding to brain lipids and proteins; they are thus unlikely to represent the pharmacodynamically relevant compartment. Receptors on neurons, and ion channels on axons are surrounded by extracellular fluid, and it is likely that the pharmacodynamics of drugs that act on these receptors and ion channels are determined by the unbound concentration of drugs in the
extracellular fluid (Sechi et al., 1989). Although, the ECF and CSF are produced independently, they are in direct communication with one another so that changes in the composition of one are reflected in changes in the composition of the other (Davson et al., 1987). CSF drug concentrations could thus be an indirect index of ECF concentrations; since they are produced by different mechanisms, there may be circumstances when the ECF pharmacokinetics could differ from the CSF pharmacokinetics.

In order to study the pertinent pharmacokinetic compartments, direct sampling of cerebrospinal fluid with simultaneous blood sampling has been successfully used (Semba et al., 1993; Patsalos et al., 1992; Lolin et al., 1994). The limited accessibility of cerebrospinal fluid and the impracticability of repeated sampling in humans have meant that most of these drug studies have been carried out in animal models.

Various methods have been used to study drug pharmacokinetics in brain ECF; ECF phenytoin has been measured in the left temperoparietal region using implanted polypropylene balls in dogs (Sechi et al., 1989). The technique of microdialysis has recently enabled the temporal measurement of extracellular fluid (ECF) drug concentrations in discrete brain areas following peripheral administration (Alavijeh et al., 1993; Bradberry et al., 1993; Dubey et al., 1989; Hurd et al., 1988; Lunte et al., 1991; Scott et al., 1991; Stahle et al., 1991). This technique has a temporal and spatial resolution suitable for the study of drug neuropharmacokinetics, without significant perturbation to the system measured (the method does not rely upon the extraction of fluid from a compartment of interest). Importantly the blood-brain barrier (BBB) is intact shortly following slow implantation of microdialysis probes (Allen et al., 1992; Benveniste, 1989; Tossman and Ungerstedt, 1986). Microdialysis is, thus, highly suited for studies of drug neuropharmacokinetics (Patsalos et al., 1995; de Lange et al., 1994). The further advantage of the microdialysis technique is that it enables simultaneous measurement in two or more brain areas (de Lange et al., 1995).
1.11 Drugs investigated in this thesis

In this thesis, I looked at 4 drugs: phenytoin, an established drug in the treatment of status epilepticus; lamotrigine, a new antiepileptic drug; MK-801, an NMDA antagonist, and diazepam, an established drug in the treatment of status epilepticus. The neuropharmacokinetics of lamotrigine, phenytoin and diazepam were investigated, and phenytoin and lamotrigine were examined for their neuroprotective properties late in status epilepticus and compared to MK-801.

1.11.1 Phenytoin

It could be argued that the discovery of the anticonvulsant effect of phenytoin by Merritt and Putnam (1938) marked the modern age of antiepileptic drug development. It was their use of an animal model of epilepsy (maximal electroshock model in cats, which is predicative of anticonvulsant efficacy against generalised tonic-clonic seizures) that led the way to the modern screening of compounds for anticonvulsant activity. Phenytoin (diphenylhydantoin) was one of a number of barbiturate derivatives tested by Putnam and Merritt; its structure, however, is distinct from the substituted barbituric acids such as phenobarbital, and it consequently has very different mechanisms of action. Indeed, it became one of the mainstays of antiepileptic drug treatment, as it possessed good efficacy without the troublesome sedative effects of the barbiturates. Phenytoin has many sites of action, and it interacts with numerous processes that regulate neuronal excitability. Phenytoin has effects on the sodium-potassium ATPase, voltage-dependent sodium channels, calcium channels, calmodulin target enzymes, chloride permeability of GABAA receptors, and cyclic nucleotide metabolism (DeLorenzo, 1995). However, it is likely that the action most pertinent to its antiepileptic action is its use-dependent inhibition of voltage-dependent sodium channels, an action which it shares with a number of other antiepileptic drugs such as carbamazepine, valproic acid and lamotrigine (MacDonald & Meldrum, 1995). Phenytoin is a well-established antiepileptic drug for the treatment of partial and generalised epilepsies. It has also been well established as a first-line treatment in status epilepticus.
Neuroprotective effects

Phenytoin has been found to have neuroprotective effects in hypoxic-ischaemic insults in gerbils (Taft et al., 1988) and neonatal rats (Hayakawa et al., 1994). In this last study (Hayakawa et al., 1994), phenytoin (50 mg/kg) administered intraperitoneally 1 hr before the hypoxia, reduced hypoxic-ischaemic infarction in the cerebral cortex and striatum, and attenuated neuronal necrosis in the hippocampus; however, when phenytoin was administered immediately after the hypoxia, there was no difference between vehicle-injected controls and phenytoin-treated animals. The mechanism of this protective action is unknown, but it has been found that compounds such as phenytoin, carbamazepine, lamotrigine or RP 66055, all of which act at sodium channels to prevent depolarisation, prevent neuronal damage induced by focal ischaemia (Rataud et al., 1994); this they may achieve through inhibition of the release of neurotransmitters such as glutamate by both pre- and post-synaptic inhibition of abnormal neurotransmission (Rataud et al., 1994).

Neuropharmacokinetics

Intravenously administered phenytoin rapidly enters the central nervous system, and consequently results in prompt and effective treatment for status epilepticus (Leppik et al., 1983b; Wilder et al., 1977). The distribution and binding of phenytoin in the brain has been extensively studied in both humans and animals (Wilder et al., 1977; Baron et al., 1983; Firemark et al., 1963; Geary et al., 1987; Goldberg, 1980; Houghton et al., 1975; Kemp and Woodbury, 1971; Nakamura et al., 1966; Ramsay et al., 1979; Sechi et al., 1989; Vajda et al., 1974), and also in animals in status epilepticus (Sechi et al., 1987). In most of these studies, however, total brain phenytoin concentrations were measured, which may not necessarily reflect the pharmacodynamically active component of phenytoin (Ramzan and Levy, 1989). The pharmacokinetics of CSF phenytoin has been well characterised in rats following intraperitoneal administration (Lolin et al., 1994).
1.11.2 Lamotrigine

Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is a phenyltriazine derivative, which was developed as an AED because of its putative antifolate properties. Its mechanism of action, however, is independent of this, and is probably mediated through inhibition of voltage-activated sodium channels (Cheung et al., 1992; Lees & Leach, 1993). It has been licensed in many countries for the treatment of refractory partial epilepsy and more recently for generalised tonic clonic seizures. It may also be of use in both typical and atypical absences (Yuen, 1994). Because of its anticonvulsant and neuroprotectant properties (see below), lamotrigine has been proposed as a possible treatment for status epilepticus. Indeed, there has been a report of its successful use in one case of refractory tonic status epilepticus (Pisani et al., 1991).

Neuroprotective effects

Lamotrigine has also been shown to be neuroprotective in ischaemia. Pretreatment of rats with lamotrigine (8-16mg/kg) protected against lesions produced by microinjections of kainate into the rat striatum (McGeer & Zhu, 1990). At high doses (20mg/kg), lamotrigine given 1 hour before or after middle cerebral artery occlusion reduced cortical infarct volume (Smith & Meldrum, 1993). The mechanisms underlying its neuroprotective properties are unknown, but, similar to phenytoin, lamotrigine acting on voltage-dependent sodium channels may inhibit abnormal neurotransmission and the excessive synaptic release of glutamate (Rataud et al., 1994).

Neuropharmacokinetics

Little is known about lamotrigine's neuropharmacokinetics. In one post-surgical specimen, total brain concentration of lamotrigine was found to be higher than the unbound plasma concentration (Remmel et al., 1992).
1.11.3 Diazepam

Diazepam was originally developed as a sedative and anxiolytic, but soon after its first clinical use, its potency in treating status epilepticus was realised (Gastaut et al., 1965) and it has now become one of the first-line treatments for this condition (Shorvon, 1994). Because of its high lipophilicity, it rapidly crosses the blood-brain barrier resulting in a fast onset of action (Greenblatt et al., 1980; Arendt et al., 1983; Friedman et al., 1986; Schmidt, 1995). Given as an intravenous bolus, it halts status epilepticus in approximately 80% of patients in a matter of minutes (Schmidt, 1995). Diazepam, however, has a short redistribution half-life of less than 1 hour and a large volume of distribution of 1-2 l/kg (Schmidt, 1995). This results in a rapid fall in serum concentration and a concomitant fall in brain concentration, leading to recurrence of seizure activity (Greenblatt & Sethy, 1990; Schmidt, 1995). Within 2 hours of successful treatment with diazepam, over half the patients with status epilepticus relapse (Prensky et al., 1967). For this reason, repeat intravenous boluses or a continuous infusion of diazepam are commonly used. There is, however, a risk of accumulation. This would lead to a decrease in the volume of distribution, and clearance of the drug would thus become dependant upon elimination, which in humans has a half-life of over 30 hours (Schmidt, 1995). Thus, although the blood concentrations may drop precipitously following a single bolus as a result of redistribution, infusions and repeat boluses may lead to accumulation and so persistent action (Shorvon, 1994). This could result in sudden hypotension, respiratory and circulatory collapse. Despite these theoretical risks, repeat boluses and infusions of diazepam are still used. Furthermore, it is the concentration of the drug at the site of action (i.e. the brain) that is of greatest relevance, and serum pharmacokinetics may be a poor index of brain pharmacokinetics following acute administration of AEDs. In the case of diazepam, there is a possibility that repeat boluses could lead to accumulation of diazepam in brain resulting in a maintenance of cerebrospinal fluid (CSF) and brain extracellular fluid diazepam concentrations despite falling serum concentrations.
1.11.4 MK-801

MK-801 (\((+)-10,11\)-dihydro-5-methyl-5H-dibenzo [a,d]cycloheptene-5,10 imine maleate) was developed as an antiepileptic drug, and was found to be effective in rodent models including maximal electro-shock, pentylenetetrazol- and bicuculline-induced seizures (Troupin et al., 1986). It was initially suggested that it was an indirect catecholamine releasing agent (Troupin et al., 1986), but subsequently, it was found to be a potent and selective non-competitive antagonist of NMDA receptors (Wong et al., 1986).

MK-801 has lost favour as a potential antiepileptic drug, principally because of the unacceptable side-effects associated with non-competitive NMDA antagonists (MacDonald & Meldrum, 1995). It is, however, a potent neuroprotectant, and protects against the excitotoxic effects of excitatory amino acids (Olney et al., 1987). Indeed, it exhibits a protective effect in vitro when given 1-4 hours after the administration of endogenous glutamate-related toxin to rat retinal ganglion cell neurons (Levy & Lipton, 1990). This late protective effect has also been noted in animal models of ischaemia, and MK-801 (1-10mg) has been effective up to 24 hours after the ischaemic insult (Gill et al., 1988). Administration of MK-801 prior to the induction of status epilepticus has been shown in numerous models to have a neuroprotective effect; 1mg/kg in rats is an effective dose (Clifford et al., 1989; 1990; Fariello et al, 1989; Rogers et al., 1989; Sparenborg et al., 1992). In these models MK-801 predominantly protects CA1 neuronal loss.

1.12 Aims of this thesis

In this thesis, I have looked at several aspects relevant to the treatment of status epilepticus. I wished to:

1) explore the pharmacokinetics of acutely administered drugs given during status epilepticus;

2) determine the role of extracellular glutamate in seizure production and neurotoxicity;
3) determine the neuroprotective and antiepileptic effects of drugs given late on in status epilepticus;

4) survey and audit the present treatment of status epilepticus.

Pharmacokinetics

In investigating the pharmacokinetics of acutely administered antiepileptic drugs, I set out to answer questions germane to the treatment of status epilepticus. First line therapy is very effective and stops status epilepticus in most patients. The problems that occur are usually with seizure recurrence, and thus repeat boluses of drugs (especially benzodiazepines) are given. There is anecdotal evidence to suggest that repeat doses of diazepam result in respiratory and cardiac arrest. I wished to test the hypothesis that this is a pharmacokinetic consequence and that: 1) repeat boluses of diazepam lead to accumulation in the peripheral compartment resulting in a significant decrease in the volume of distribution and clearance of diazepam, and 2) repeat boluses lead to accumulation of diazepam in the brain compartment resulting in a relative persistence of diazepam in the CSF despite falling serum concentrations. Furthermore I wished to examine the role of serum sampling of phenytoin, a first-line drug in status epilepticus, in guiding dosing during this phase by looking at the relationship between serum and brain pharmacokinetics, and lastly I wished to determine if lamotrigine, a new antiepileptic drug with potential neuroprotective properties, had advantageous pharmacokinetics and neuropharmacokinetics for its use in the treatment of status epilepticus. I thus developed animal pharmacokinetic models in order to address these questions.

Glutamate

It has been proposed that at the later stages of status epilepticus the excessive release of glutamate results in neuronal damage, and that methods of modulating extracellular glutamate could be neuroprotective. I wished to examine this
hypothesis, and as a first step I wished to examine the relationship between extracellular glutamate and seizure activity.

**Drugs late on in status epilepticus**

I also wished to examine the role of phenytoin, lamotrigine and MK-801 late on in status epilepticus and to compare their neuroprotective and antiepileptic effects. This involved first developing an animal model of late status epilepticus and then testing and comparing these drugs.

**Clinical work**

Lastly, having examined possible methods of improving the treatment, I wished to explore how well our present treatment regimes are followed. In order to accomplish this, I carried out a survey of the intensive care treatment of refractory convulsive status epilepticus in the UK, and also an audit of patients transferred to the National Hospital for Neurology and Neurosurgery for further treatment of their status epilepticus.
2 METHODS

2.1 Animals

Male Sprague-Dawley rats (Charles River, Margate, Kent) were used in all experiments. Rats were individually housed under a 12 hour light-dark cycle with free access to water and to a normal laboratory diet (SDS R and M number 1 expanded, Scientific Dietary Services, Witham, Essex, UK). All surgical procedures were performed under general anaesthesia according to Home Office regulations. For most experiments, the rats were first placed in an induction chamber (4% halothane in O₂), when unresponsive to foot pinch, they were transferred to a stereotaxic frame (Stoelting Co. IL, USA) and given 1-2% halothane in O₂ at 2l/min via a specially constructed facemask. For the phenytoin pharmacokinetic experiments, pentobarbitone anaesthesia (60mg/kg intraperitoneally; Sagatal, BDH Chemicals, Poole, Dorset, UK) was used.

2.2 Procedures used for pharmacokinetic experiments

2.2.1 Blood catheterisation

The procedure for inserting the blood catheter was essentially as previously described (Patsalos et al., 1992). In brief the rats were placed on their back, and their forelegs were fixed to a surgical board with tape. The skin between the right clavicle and sternum was shaved, and a 1-2 cm incision was made in the skin rostral but parallel to the clavicle. Using blunt dissection the internal jugular vein was exposed, and three cotton threads were passed under the vein. The vein was ligated using the rostral-most thread. Using fine scissors, a slit was made in the vein between the other two threads. The blood catheter consisted of a 10cm length of silastic tubing (o.d. 1.09 mm, i.d. 0.63 mm and the end cut at 45°; Dow, Corning, MI, USA) that was attached via a short piece of polythene tubing (i.d. 0.58 mm; Portex Ltd, Hythe, Kent, UK) to a 23-G needle attached to a 1ml Syringe. The catheter and syringe were primed with 5U/l heparinised saline. Using a pair of fine tweezers, the catheter was inserted 3-4 cm so that the tip was lying in or just above the right auricle, and blood flowed in and out of the catheter
easily. The caudal-most thread was tied around the vein thus holding the catheter within the vein, the other two threads were tied around the catheter to keep it in place. A cut was then made just behind the right ear and the catheter was passed under the skin and through this incision. The wounds were then sutured. The catheter was further flushed with 5U/l heparin and the end was cut so that 2-3 cm protruded out of the skin, and the end of the catheter was tied.

### 2.2.2 CSF catheterisation and catheter construction

The procedures for construction and insertion of CSF cannulae are adapted from Patsalos et al. (1992).

![Figure 2.1: CSF cannula with freely moving inner tube, and copper wire introducer](image)

The catheters were constructed from intersliding polythene tubing (all tubing was from Portex Ltd, Hythe, Kent, UK unless otherwise stated) with the outer tubing (i.d. 0.76 mm) having the internal diameter decreased at one end by the formation of a bouton from gently heating the tubing with a soldering iron (figure 2.1). This resulted in a tight fit around the inner polythene tubing (i.d. 0.28 mm, o.d. 0.61 mm).
mm). A 37-G enamelled copper wire (Scientific wire Co., London, UK) was used as an introducer.

**Insertion**

The cannulae were inserted into the cisterna magna using the following protocol. The rats were placed in a stereotaxic frame (Stoelting Co. IL, USA), and the head was shaven. A midline incision was made in the scalp, and lambda and the external occipital crest were identified. Two burr holes either side of lambda were made for placement of stainless steel anchor screws. A burr hole was made at 45° to the skull pointing down caudally in the midline just caudal to the occipital crest for insertion of the catheter into the cisterna magna. The end of the catheter was bent at 45° with the copper wire protruding approximately 1mm. The catheter was then slowly inserted, and the protruding wire punctured the dura. Once the catheter was in up to the bouton, it was kept in place with dental cement (De Trey, Surrey, UK). With the dental cement dry, the copper wire was withdrawn. Adjustments were then made to the inner tube of the catheter, until the CSF flowed easily up the tube, with a slight negative pressure applied by a connected syringe. The end of the catheter was then heat sealed with a soldering iron.

**2.2.3 Microdialysis probe construction and insertion**

Concentric dialysis probes with Filtral 12 (Hospal, Rugby) dialysis membrane 4 mm long, 200μm diameter were prepared as previously described (Hutson et al., 1985) except that the internal glass capillary tubes were replaced by vitreous silica tubing (SGE, Milton Keynes). In brief, two silica tubes were held in place in a 15 mm 24-G steel tube with epoxy resin such that at one end 1 cm of each silica tube protruded and at the other end (the end for the dialysis membrane) one silica tube protruded 4 mm, and the other was approximately 5 mm within the steel tube. Dialysis membrane was placed over the 4 mm protruding silica tube and passed up into the steal tubing, it was then fixed in place with epoxy resin and the end was sealed with a small amount of epoxy resin 1 mm from the end of the
silica tubing. Two 27-G steel tubes (8 mm long) inserted into polythene tubing (i.d. 0.28mm) were placed over the silica tubing at the other end and held in place with epoxy resin. These were then sealed with hot melt adhesive (figure 2.2).

Figure 2.2: Microdialysis probe construction

In vitro recovery

On the day of surgery the in vitro recovery for each probe was calculated by placing the probes in either a 8 μM solution of sodium phenytoin (Parke, Davis & Co, Gwent, UK) or lamotrigine (Glaxo-Wellcome, UK) dissolved in artificial cerebrospinal fluid (CSF; composition mM: NaCl 125, KCl 2.5, MgCl₂ 1.18 and CaCl₂ 1.26), and then perfusing the probes with artificial CSF at 2 μl/min. Samples (40 μl) were collected every 20 min for 80 min, and stored at -70°C until analysis.

Insertion
The rats were placed in a stereotaxic frame (Stoelting Co. IL, USA), and the head was shaved. A midline incision was made in the scalp, and two burr holes were made either side of the midline for placement of stainless steel anchor screws. Two further burr holes were made for dialysis probe insertion, and the probes were slowly implanted in either the hippocampus (from bregma 5.6 mm posterior, 5 mm lateral, 8.2 mm ventral) or the frontal cortex (from bregma 2.5 mm anterior, 1.5 mm lateral, 5.5 mm ventral) or both according to the atlas of Paxinos and Watson (1986). The probes were held in place with dental cement (De Trey, Surrey, UK).

2.2.4 Blood, microdialysate and CSF collection

Two days after surgery when the animals were fully recovered (Patsalos et al., 1992), the jugular vein catheter, dialysis probes and CSF catheters were checked for patency. This time difference from implantation to experimentation (24-48 hours) has minimal effect on the ability of microdialysis to determine drug pharmacokinetics (de Lange et al., 1994). All samples were stored immediately at -70°C until HPLC analysis as described below.

**Blood**

A baseline sample of blood (100 µl) was taken, and following administration of drug, further 100 µl blood samples were taken. After each blood sampling, the catheter was flushed with 100 µl of 5 U/l heparinised saline. Blood samples were collected in 1.5 ml polyethylene tubes (Treff AG, Switzerland), and sera were separated by centrifugation and clot retraction.

**Dialysate**

Artificial CSF was perfused through the microdialysis probes at 2µl/min. Three baseline dialysate samples (40 µl) were taken in the first hour. Dead space was estimated by calculating the volume of tubing from the microdialysis probe to the collection point. After allowing time for dead space, there was continuous
collection of dialysate samples following drug administration. Dialysate samples were collected in 250 µl polyethylene tubes (Elkay, MA, USA).

**CSF**

Baseline CSF (20µl) samples were collected at 30 minute intervals for 1 hour. Following drug administration, CSF samples were collected every 20-30 minutes to allow time for CSF reconstitution (Patsalos et al., 1992). CSF samples were collected in 250 µl polyethylene tubes (Elkay, MA, USA).

**2.2.5 HPLC Analysis**

**2.2.5.1 Phenytoin**

The concentrations of phenytoin in sera or dialysates were determined by high performance liquid chromatography (HPLC) with ultraviolet detection using a modification of the method of Ratnaraj et al. (1990). Sera (50µl) and acetonitrile (50 µl) containing 20 µM 5-p-toly-5-phenylhydantoin (Aldrich, Dorset, UK) as the internal standard were pipetted into a 1.5 ml polyethylene tube (Treff AG, Switzerland), vortex mixed and then centrifuged for 5 min at 9,500g (Abbott Micro-Centrifuge, Abbott, Maidenhead, UK). 20 µl of the supernatant extract were injected into the HPLC system. As dialysate contains no protein, 5 µl were directly injected into the HPLC system without acetonitrile extraction. The HPLC system comprised a Spectra-Physics SP 8750 equipped with a Spectroflow 783 UV detector and an SP 4270 integrator/printer plotter with LABNET data system (Spectra-Physics, Maidenhead, UK). Chromatograms were run at ambient temperature on a Merck HI-BAR column (250 X 4.0 mm) packed with Lichrosorb RP select B, 5 µm (BDH, Poole, UK). A mobile phase of acetonitrile: 0.05 M phosphate buffer, pH 5.6 of 48:52 by volume with a flow rate of 1.3 ml/min was used. The column effluent was monitored at 215 nm with a sensitivity range of 0.03 absorption units full scale. Area under the peak corresponding to phenytoin was calculated (for serum this was expressed as a ratio compared to the area under the peak for the internal standard), and was
compared to a standard curve. The phenytoin standards for dialysate were: 2, 4, 8, 12, 16 and 20 μmol/l, and for serum were: 20, 40, 60, 80 and 100 μmol/l. Typical standard curves for dialysate and serum are presented in figure 2.3, showing linearity over the standard concentration range.

Figure 2.3: Typical phenytoin standard curves for (A) serum and (B) dialysate. Dotted lines are the least mean squares regression lines.

The lower limit for detection for phenytoin was 0.5 μmol/l. For this method over the concentration range used, the within batch coefficient of variation was 1-2%, and the between batch coefficient of variation was approximately 18%.

2.2.5.2 Lamotrigine
The concentrations of lamotrigine in sera or CSF were determined by high performance liquid chromatography (HPLC) with ultraviolet detection using a method devised by Mr A.A. Elyas. Sera (50μl) and acetonitrile (50 μl) containing 3.75 μg/ml 10-methoxycarbamazepine as the internal standard were pipetted into a 1.5 ml polyethylene tube (Treff AG, Switzerland), vortex mixed and then centrifuged for 5 min at 9,500g (Abbott Micro-Centrifuge, Abbott, Maidenhead, UK). 50 μl of the supernatant extract were mixed with 25μl of mobile phase and 10μl were injected into the HPLC system. The HPLC system comprised a Spectrasystem AS3000 autosampler, a Spectrasystem P4000 pump, a Spectrasystem UV2000 detector and an SP 4270 integrator/printer plotter with LABNET data system controller (Spectra-Physics, Maidenhead, UK). For CSF and dialysate analysis, 20 μl were directly injected into the HPLC system without acetonitrile extraction. Chromatograms were run at ambient temperature on a Merck LiChroCART column (125 X 4.0 mm) packed with Lichrosorb RP-8, 5 μm (BDH, Poole, UK). Mobile phases of 0.045M phosphate buffer with 15% acetonitrile (buffer A) and 0.0225M phosphate buffer with 37.5% acetonitrile (buffer B) were run on a gradient (table 2.1).

Table 2.1: Table of gradient of buffers with flow rate during the HPLC run

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Buffer A</th>
<th>Buffer B</th>
<th>Flow rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>73</td>
<td>27</td>
<td>0.7</td>
</tr>
<tr>
<td>12</td>
<td>73</td>
<td>27</td>
<td>0.8</td>
</tr>
<tr>
<td>26</td>
<td>67</td>
<td>33</td>
<td>0.9</td>
</tr>
<tr>
<td>28</td>
<td>67</td>
<td>33</td>
<td>1.4</td>
</tr>
<tr>
<td>31</td>
<td>67</td>
<td>33</td>
<td>1.5</td>
</tr>
<tr>
<td>35</td>
<td>73</td>
<td>27</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The column effluent was monitored at 215 nm with a sensitivity range of 0.02 absorption units full scale. Area under the peak corresponding to lamotrigine was calculated (for serum this was expressed as a ratio compared to the area under the peak for the internal standard), and was compared to a standard curve. The
Lamotrigine standards for dialysate and CSF were: 1.95, 7.9 and 15.8 μmol/l, and for serum were: 4, 15 and 64 μmol/l. Typical standard curves are shown in figure 2.4. This HPLC method has a lower limit of detection of 0.2 μmol/l. For this method over the concentration range used, the within batch coefficient of variation was 2-12%, and the between batch coefficient of variation was 5-14%.

![Graph A](image1)

![Graph B](image2)

Figure 2.4: Typical lamotrigine standard curves for (A) serum and (B) CSF or dialysate. Dotted lines are the least mean squares regression lines.

The procedures for determining the non-protein bound, serum lamotrigine concentration were similar to that for sera, except that the sera were first filtered through an Amicon Centrifree Micropartition System (Amicon, Stonehouse, UK) at a temperature of 25°C using a Sorvall RC-5B refrigerated centrifuge (Du Pont, Stevenage, UK), and 50μl of the ultrafiltrate were used in the analysis.
2.2.5.3 Diazepam and desmethyldiazepam

The concentrations of diazepam and desmethyldiazepam in sera or CSF were determined by high performance liquid chromatography with ultraviolet detection using a method devised by Ms S Brown. 50 μl sera, 10 μl NaOH, 25 μl internal standard (30 μg/ml desmethyldiazepam in methanol) and 500 μl dichloromethane were pipetted into a 1.5 ml polyethylene tube (Treff AG, Switzerland), vortex mixed and then centrifuged for 5 min at 10,800 rpm (Abbott Micro-Centrifuge, Abbott, Maidenhead, UK). The aqueous layer was discarded and the organic layer was evaporated in a Gyro-vap centrifuge set to 60°C for 45 minutes. The dried extracted sera were reconstituted with 50 μl acetonitrile and 10 μl of this was injected into the HPLC system. The HPLC system comprised a Spectrasystem AS3000 autosampler, a Spectrasystem P4000 pump, a Spectrasystem UV2000 detector and an SP 4270 integrator/printer plotter with LABNET data system (Spectra-Physics, Maidenhead, UK). 20 μl CSF, 100 μl internal standard (20 μg/ml in acetonitrile) were pipetted into a 1.5 ml polyethylene tube (Treff AG, Switzerland), vortex mixed and then centrifuged for 5 min at 10,800 rpm (Abbott Micro-Centrifuge, Abbott, Maidenhead, UK). The samples were evaporated in a Gyro-vap centrifuge set to 60°C for 45 minutes. The dried extracted sera were reconstituted with 15 μl acetonitrile and 10 μl of this was injected into the HPLC system. Chromatograms were run at ambient temperature on a Merck LiChroCART column (125 X 4.0 mm) packed with Lichrosorb RP-8, 5 μm (BDH, Poole, UK). A mobile phase of 0.030 M phosphate buffer (pH 3) with 30% acetonitrile was pumped isocratically at a constant flow of 0.8 ml/min. The column effluent was monitored at 215 nm with a sensitivity range of 0.02 absorption units full scale. The ratios of areas under the peak corresponding to diazepam and desmethyldiazepam to the internal standard were calculated, and were compared to standard curves. The diazepam standards for CSF were: 200, 400 and 800 ng/ml, and for serum were: 125, 250, 1000, 2000 and 4000 ng/ml. The desmethyldiazepam standards for CSF were: 100, 200, 400 and 800 ng/ml, and for serum were: 250, 500, 1000, 2000 and 8000 ng/ml. Typical standard curves for diazepam are shown in figure 2.5.
Figure 2.5: Typical diazepam standard curves for (A) serum and (B) CSF. Dotted lines are the least mean squares regression lines.

The lower limit for detection of both diazepam and desmethyldiazepam was 10ng/ml. For this method over the concentration range used, the within batch coefficient of variation for diazepam was 2-4% and for desmethyldiazepam was 5-8%, and the between batch coefficient of variation for diazepam was 5-6%, and for desmethyldiazepam was 3-10%. The procedures for determining the serum free diazepam concentration was similar to that for sera, except that the samples were first filtered through an Amicon Centrifree Micropartition System (Amicon, Stonehouse, UK) at a temperature of 25°C using a Sorvall RC-5B refrigerated centrifuge (Du Pont, Stevenage, UK) set at 4000rpm for 15 minutes.
2.2.6 Pharmacokinetic analysis

*Lamotrigin and phenytoin*

The data for these two drugs were modelled using one-compartment models. Where appropriate, terminal half-lives were calculated assuming first-order kinetics. Area under the curves (AUCs) were calculated using the trapezoid rule. The maximum serum concentration ($C_{\text{max}}$) and the time to maximum serum concentration ($T_{\text{max}}$) were estimated from the concentration against time plots. Ratio of CSF concentration to serum concentration was calculated for each individual animal at each time point for which there were concurrent data. For comparisons, AUC and $C_{\text{max}}$ were corrected for dose.

*Diazepam*

In analysing single dose data three assumptions were made: 1) that the bioavailability of a drug given i.p. was 100%; 2) that the data could be modelled using a two compartment model where $C(t) = Ae^{-\alpha t} + Be^{-\beta t}$, and 3) that $\beta$ and $B$ could be approximated from the log-linear regression analysis for a single exponential function of the terminal concentrations.

$\alpha$ was calculated from the log-linear regression analysis for a single exponential function of $C(t) - Be^{-\beta t}$ (i.e. by curve stripping). Area under the curve (AUC) was calculated using the trapezoid rule for $t=0-x$ (where $x$=last time point), and was extrapolated to infinity by calculating $\text{AUC}_{x-\infty} = \frac{C(x)}{\beta}$. Total serum clearance (Cl) was calculated as $\text{Dose} / \text{AUC}_{0-\infty}$ and volume of distribution ($Vd_{\text{area}}$) was calculated as $\text{Cl} / \beta$.

For repeat doses, kinetic analysis was performed on the concentration data following the last (third) dose. $\alpha$, $\beta$ and $\text{AUC}_{0-\infty}$ were calculated as for the single dose data using the same assumptions. Since on giving the third dose there is still some diazepam present from the first two doses, I calculated an AUC that was specifically due to the administration of the third dose, AUC(3). This was calculated by subtracting the $\text{AUC}_{1h-\infty}$ for the second dose from $\text{AUC}_{0-\infty}$ for the third dose. In calculating $\text{AUC}_{1h-\infty}$ for the second dose, I assumed that $\beta$ was...
dose independent (an assumption that was confirmed from single dose and repeated dose data) and used the formula: $AUC_{1h-\infty}$ for the second dose = $C(1h \text{ following the second dose})/\beta$. Clearance could then be calculated as Third Dose/AUC(3) and $Vd_{area}$ as $C/\beta$.

2.3 Procedures for stimulation and recording

2.3.1 Electrode construction

All parts, unless otherwise specified, were purchased from Plastics One Inc via Semat Technical, Herts, UK. Wire was purchased from Advent Research Materials, Suffolk, UK. Bipolar stimulating electrodes were constructed from two 125 μm teflon coated stainless steel wires twisted together which were then attached to gold female connectors either by franking or soldering. The gold connectors could be mounted in a six holed plastic pedestal along with the connectors for the recording electrode and earth. The tips of the electrode were cut such that they were separated by 0.5mm. The construction of the recording electrode was identical except that only one wire was used for unipolar recording, the other wire gave the electrode stability. Later experiments improved this set up by using a single 250 μm teflon coated stainless steel wire as the recording electrode. The recording electrode in the dialysis probe experiments was attached to the outside of the probe (see below). Earth was a subcutaneous silver wire, which in the freely moving experiments was connected to an anchor screw. Care was taken to control any bleeding prior to electrode implantation. For the freely-moving experiments the electrodes were kept in place using dental cement (De Trey, Surrey, UK), and three anchor screws.

2.3.2 Stimulator

Constant current stimuli were delivered from a Neurolog stimulator (NL304 period generator, NL301 pulse generator, NL505 flip-flop, NL800 stimulus isolators: Digitimer Ltd, Welwyn Gdn City, UK) as shown in figure 2.6.
Figure 2.6: Stimulator arrangement. The pulse width could be set at 50, 150 or 500ms; the frequency of pulses could be set from 0-10kHz; the amplitude could be set from 0-10mA, and using the alternating switch the polarity of the pulses from the constant current generators could be alternated.

The pulse generator could be gated so that the pulses could be synchronised with the computer recording. This gating also enabled fixed length trains to be generated, and using a purpose built series of flip-flop circuits, it was possible to generate fixed length trains that occurred at fixed intervals.

2.3.3 Recording equipment

Potentials were amplified and filtered (0.1 Hz to 5 kHz band pass) via a Neurolog amplifier (NL100A pre-amp, NL104A AC amplifier, NL125 filter: Digitimer Ltd, Welwyn Gdn City, UK) onto a storage oscilloscope (figure 2.7) and then via an NB-MIO-16 interface (National Instruments, Berkshire, UK) into VAST an early version of A/DVANCE (FST, Foster City, CA, USA).
**Figure 2.7: Amplifier arrangement.** The amplifier was a differential AC amplifier with B connected to earth, and a built in high pass filter set at 0.1 Hz. A low pass filter set at 5 kHz was also necessary to reduce high frequency interference that seemed to be due to computer equipment.

### 2.3.4 Perforant path stimulation and the dentate granule cell layer

In a number of experiments stimulation of the perforant was used with simultaneous recording from the dentate granule cells. Implantation of electrodes would take place under 1-2% halothane anaesthesia.

The dialysis electrode or recording electrode was implanted stereotaxically into the hippocampus (co-ordinates 2.5 mm lateral, 4 mm posterior from bregma). A bipolar stimulating electrode was advanced into the angular bundle (co-ordinates 4.4 mm lateral, 8.0 mm posterior to bregma) to stimulate the perforant path. The stimulating electrode was advanced 3-4 mm ventral from the dural surface. Test shocks consisted of 1-2 mA, 50 μs monopolar pulses every 10 seconds.
Figure 2.8: (a) Normally the inside of dendrites, soma and axons are kept at a negative potential with respect to the outside as the cells are permeable to potassium and the intracellular potassium concentration is greater than the extracellular. (b) The perforant path forms axodendritic synapses on the dentate granule cells. Activation of these synapses makes the dendrites permeable to sodium and potassium, which causes sodium to enter the dendrites causing dendritic depolarisation. The intracellular fluid in the dendrites is in communication with the intracellular fluid of the soma, and thus the intracellular somatic space also depolarises. This results in positive charge being extruded through the soma and axons, resulting in current as indicated. A voltage gradient thus exists from the extracellular space surrounding the soma to the extracellular space surrounding the dendrites.

The dialysis electrode or recording electrode was lowered until the electrode entered the granule cell layer of the dentate. During field EPSPs, positive charge flows: into dendrites resulting in a decrease in potential of the fluid surrounding dendrites, and out of soma and axons resulting in an increase in the potential surrounding soma and axons (figure 2.8). Thus in the dendritic region the field potential from neuronal depolarisation is negative with respect to surrounding tissue, whilst in the cell layer the field potential is positive with respect to surrounding tissue. If the intracellular depolarisation is large enough to result in an action potential, then the axon and soma become very permeable to sodium,
sodium ions flow in and the circuit is reversed. This results in the typical dentate granule cell field potentials seen (figure 2.9).

![Graph showing field potential](image)

**Figure 2.9: Field potential recorded from dentate granule cell layer following single pulse stimulation of the perforant path**

Once the recording electrode was positioned in the dentate granule cell layer, the depth of the stimulating electrode was adjusted to maximise the slope of the population excitatory post synaptic potential (EPSP) as described by Errington et al. (1987). The current was then adjusted in the range 1-3 mA to produce a population spike (amplitude 10 mV) and the test shock frequency was reduced to every 30 seconds.

### 2.4 Measurement of extracellular glutamate

* Dialysis electrode construction:

The biosensors used in this work were either purchased from Sycopel International (6 Hutton St, Boldon, Tyne & Wear, UK) or made on site by Dr P. Galley (Galley, 1992) and were similar to Sycopel’s “SPF 10-10-2-2” model.
Figure 2.10: Dialysis electrode, consisting of an electrochemical detector (platinum and silver electrodes) contained within a microdialysis probe. There is no flow of perfusate during measurements, but there is the ability to change the environment surrounding the electrodes.

The biosensor consisted of an electrochemical cell (Albery et al., 1987) inside a microdialysis probe (see figure 2.10). The working electrode was a 50 μm teflon-coated platinum wire (all wire came from Goodfellow, Cambridge, UK and Advent, Suffolk, UK) with its insulation exposed for the final 2 mm. A plain silver and chloridised silver wire were used as counter and reference electrodes respectively. Two 125 μm diameter polyester-coated stainless steel electrodes
were glued parallel to the dialysis fibre, one as far down as the tip, the other 1mm above the tip, to measure field potentials. An inflow and outflow tube permitted the removal and replenishment of solution in the biosensor, thus controlling the environment around the electrode.

For measurement of glutamate, the probe was filled with L-glutamate oxidase (0.05 U/μl), ascorbate oxidase (1 U/μl) and Ringer's solution containing (mM): NaCl 136, KCl 2.54, KH₂PO₄ 1.18, Mg₂SO₄ 1.18, NaHCO₃ 16, glucose 10. The L-glutamate oxidase was a generous gift from Dr Kusakabe of Yamasa Shoyu Ltd (Japan). Ascorbate oxidase was purchased from Boehringer Mannheim and all other chemicals used in these experiments were from Sigma Chemical Ltd, Poole, UK (unless stated).

As seen in figure 2.11, L-glutamate is oxidised by L-glutamate oxidase within the dialysis membrane to produce hydrogen peroxide, which was detected on the platinum electrode amperometrically at +650mV. 1,2-diaminobenzene (Aldrich, Dorset, UK) was electropolymerised onto the bare platinum; this cuts down interference from electroactive compounds (Sasso et al., 1990). Ascorbate, however, is present in such large concentrations that it still causes significant interference (1,2-diaminobenzene only reduces the ascorbate signal by 95-99%). Ascorbate oxidase was thus included within the dialysis membrane in order to further attenuate the ascorbate signal. This enzyme is a copper centred-protein which produces water instead of hydrogen peroxide, when ascorbate is oxidised. To measure ascorbate, the probe was filled with enzyme-free Ringer solution. As figure 2.12 illustrates, the ascorbate is oxidised directly on the platinum surface and does not require an FADH oxidase enzyme.

The dialysis electrodes were connected to a low current potentiostat (EMS, Newbury, Berkshire, UK). The results were recorded using an NB-MIO-16 interface board (National Instruments, Berkshire, UK) into VAST. The real-time response from the biosensor was sampled at 100Hz and averaged over a five second period.
**In vitro calibration**

Prior to all experiments the dialysis electrodes were calibrated in vitro by placing the tip in a small receptacle containing 2 ml Ringer solution mounted on a magnetic stirrer. Aliquots of analyte were then added from a concentrated solution.

**Figure 2.11: Glutamate electrode: the enzymes ascorbate oxidase and glutamate oxidase are contained within the dialysis membrane. Glutamate oxidase converts glutamate to 2-oxo glutamate forming hydrogen peroxide which is measured amperometrically at +650 mV on the platinum electrode. Ascorbate is oxidised to dehydro-ascorbate with the release of water. A coating of 1,2-diaminobenzene on the platinum electrode repels possible electroactive interferents.**
Figure 2.12: Ascorbate electrode: no enzymes are present in this biosensor. A coating of 1,2-diaminobenzene on the platinum wire repels possible electroactive interferents. Ascorbate, however, is present in such large concentrations in brain that although 95-99% is repelled and only 1-5% is oxidised on the electrode; this is still detectable.

2.5 Perfusion and Histology

In the experimental status epilepticus, 14-17 days after stimulation, animals were deeply anaesthetised with pentobarbitone. The heart was exposed and a cannula was placed in the aorta via the left ventricle; an incision was also made in the right auricle. Saline was administered via the cannula at a pressure of 1-2 bar until the animal had been exsanguinated (usually 1-2 minutes). Then freshly made, cold 4% paraformaldehyde was administered via the cannula at a pressure of 1-3 bar for 10-15 minutes. The head was then removed and the brain was exposed and placed in 4% paraformaldehyde for 12-24 hours before undergoing further dissection freeing the brain from the skull. The olfactory bulbs and cerebellum were removed, and the brain was cut coronally through the optic chiasm, so
giving a consistent reference point. The brains were then placed in 4% paraformaldehyde for 18-24 hours before being embedded in paraffin wax. 16μm coronal sections were then taken using a Leitz sledge microtome, and two sections every 320μm were dewaxed in xylene, placed in alcohol, washed and then stained with cresyl fast violet for 10 minutes at 60°C. Cresyl fast violet stains Nissl granules, and cell nuclei through its bonding with anionic nucleic acids. Neurons were distinguished from other cell types on the basis of their size, the presence of Nissl granules in the cytoplasm, and a large pale staining nucleus with one or more nucleoli.

When neuronal damage was assessed, it was done so in a blinded fashion so that the observer was unaware of what treatments the rat had received or whether it was a treated or control rat. Neuronal damage was assessed in a semi-quantitative fashion as described by Kelly and McIntyre (1994). Neurons and glia were counted in a 50μm x 60μm grid. For each rat, two sections 320μm apart were selected such that the sections were either side of the recording electrode. The counts were averaged from two randomly chosen locations for each structure (CA1, CA3 or hilus) on each section. Neuronal loss and gliosis were scored as 0-no damage, 1-slight damage, 2-moderate damage and 3-severe damage. These were defined as: slight damage - 50% increase of glial cells and/or a few necrotic neurons in the cell field beyond the grid; moderate damage - 50-200% increase in glial cells and/or a 50% reduction of neurons, and severe damage - more than 200% increase of glial cells and/or a 90-100% loss of neurons.

In situations where no damage was obvious, this was further confirmed using detailed cell counts. An optical dissector microscope (Zeiss) with a digital length gage (Heidenhaim, Illinois) was used. Neurons in three dimensional boxes 70μm x 70μm x 5μm for the hilus, 20μm x 20μm x 5μm for CA1 and CA3 were counted according to the rules described by Williams & Rakic (1988):

1) Cell nuclei completely inside the box were counted;
2) Cell nuclei completely outside the box were not counted;
3) Cell nuclei that touch the bottom, front and left planes of the counting box were not counted;
4) Cell nuclei that touch top, back or right plains were counted.
Rows of boxes that spanned from one blade of the dentate fascia to the other were used to count the neurons within the hilus (figure 2.13).

Figure 2.13: Section from rat hippocampus showing approximate positions of CA1, CA2, CA3, hilus and dentate granule cells. Section was stained with Nissl stain.

Every other row was missed out, resulting in approximately 100 boxes being used per hilus. The hilus contains cells from CA3 and CA4. The rest of CA3 was counted using 4 boxes (dimensions above) across the cell layer every 70μm so that 32 boxes were counted in all. The change from CA2 to CA1 was distinguishable by a change in the density and width of the cell layer. Neurons were counted in CA1 using 3 boxes (dimensions above) to span the cell layer, every 70μm and a total of 24 boxes were counted. The number and size of boxes were chosen to decrease inter-observer variability. Using the method above the repeat measure variability for one observer on two occasions was <5%. The same observer was used for all subsequent counting. For each rat, three sections 320μm apart were selected such that the sections were either side of the recording electrode.
The area between the blades of the dentate fascia, and the lengths of CA1 and
CA3 were also calculated using a Kontron mini-MOP in order to determine if
there was significant shrinkage accounting for difference in cell density between
cell groups.

Neuronal cell counts were expressed as three dimensional cell densities. Results
for cell density and area and length measurement for each region were compared
using a multifactorial analysis of variance (ANOVA). Main effects were section
number, side, and stimulation. The data were modelled using main effects, and
the interactions between stimulation and side, and between stimulation and slice
position.

2.6 Audit methods

2.6.1 UK survey

A questionnaire was sent to 694 members of the Intensive Care Society working
in the UK. The questionnaire asked for the grade and speciality of the respondent,
the type of intensive care unit (ICU) on which they worked, and whether there
was a protocol on their ICU for the treatment of SE. They were then given a
scenario of a patient in casualty with SE who had not responded to initial therapy
comprising intravenous diazepam and phenytoin, and had been transferred to the
ICU still in SE. They were asked:

1) which drug they would use next, and which other drugs they would consider
alternatives;

2) if the patient continues to fit despite the above lines of action, after what period
of time since seizures began do they consider anaesthetising the patient;

3) which anaesthetic agent they would prefer to use and which others they would
consider suitable alternatives;

4) how they would monitor the effectiveness of their treatment in the
anaesthetised patient given their present facilities.
If they were not involved in the treatment of SE, they were asked to return the questionnaire uncompleted.

2.6.2 Intensive care unit audit

The National Hospital for Neurology and Neurosurgery (NHNN) is a tertiary referral centre and does not have a casualty department. The intensive care unit accepts most of its referrals from other hospitals. I reviewed the records of 26 patients who had been transferred from other hospitals to the ICU at NHNN with a diagnosis of intractable SE between 1 March 1993 and 31 October 1995. From the records, I determined the most likely diagnosis at time of admission to the ICU. The patients were divided into: those who were still in SE; those who had had SE which had resolved, and in whom the EEG and clinical findings were consistent with drug-induced coma; those who had had SE which had resolved, and in whom the EEG and clinical findings were consistent with an encephalopathy; those who were likely to have had or were in pseudostatus epilepticus, and those who had a different diagnosis. As far as possible from the records, I determined the treatment in the referring hospital, and AED levels on arrival at NHNN ICU before any treatment at NHNN. In those with a diagnosis of SE or probable SE, I identified the probable aetiology of the episode of SE. For all patients, I identified the treatments that were instigated, the complications and the outcomes which were: discharge from NHNN; transfer back to the referring hospital conscious; transfer back to the referring hospital unconscious, and death.

2.7 Statistical methods

Data for two groups were compared using student's t-test, and for more than two groups, analysis of variance was used. When more than one factor needed to be assessed, multifactorial analysis of variance was used. For ordinate data, non-parametric rank analysis, the Kruskal-Wallis one-way ANOVA, was used. P<0.05 was taken as the level of significance. All statistics were carried out using SPSS for windows (release 6.0).
3. PHARMACOKINETICS AND NEUROPHARMACOKINETICS

3.1 Introduction

This chapter deals with the determination of the pharmacokinetics and neuropharmacokinetics of lamotrigine, phenytoin and diazepam in rat. The detailed study of the serum pharmacokinetics of a drug in a particular species has its own relevance. There are species specific modes of elimination, often making the extrapolation of animal serum pharmacokinetic data to humans and vice versa inappropriate. The full characterisation of serum pharmacokinetics in a particular species is thus of paramount importance in understanding the pharmacodynamics of a particular drug in that same species. Indeed, poor kinetic characterisation of antiepileptic drugs has led to incorrect assessments of their effectiveness in epilepsy models (McNamara et al., 1989).

Determination of the neuropharmacokinetics is of importance as the critical drug concentration is the concentration at the point of action (e.g. synapses, receptors etc.) Plasma concentrations may be a poor index of this concentration and this is especially so with acute administration.

I set out to characterise the plasma pharmacokinetics and neuropharmacokinetics of acutely administered antiepileptic drugs, lamotrigine and phenytoin, in rats in order to evaluate 1) the results from rat pharmacodynamic studies; 2) the speed of brain penetration and thus potential for use in status epilepticus, 3) the role of therapeutic drug monitoring following acute administration, and 4) the regional variations in neuropharmacokinetics.

Using similar techniques to those used for the experiments above, I wished to test two hypotheses regarding the acute administration of diazepam: 1) that repeat boluses of diazepam lead to accumulation in the peripheral compartment resulting in a significant decrease in the volume of distribution and clearance of diazepam, and 2) that repeat boluses lead to accumulation of diazepam in the brain compartment resulting in a relative persistence of diazepam in the CSF despite falling serum concentrations. These two phenomena would tend to increase the magnitude and length of action of diazepam with repeated dosing, which could lead to potentially fatal consequences.
3.2 Serum and brain extracellular fluid pharmacokinetics of phenytoin

3.2.1 Experimental protocol

CSF pharmacokinetics of phenytoin were not determined by me as they had already been described in detail by Lolin et al. (1994) from the same laboratory.

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 330-460g were used to determine the brain ECF concentrations. Venous catheter and microdialysis probes were implanted as described. Two days after surgery, baseline samples were taken and the rats were then injected intraperitoneally with 50 or 100 mg/kg ready mixed parenteral phenytoin (Sodium Phenytoin 250 mg in 5 ml; Parke, Davis & Co, Gwent, UK). Venous blood samples (100 µl) were withdrawn at 20, 40, 60, 90, 120, 150 and 180 min after phenytoin administration. Dialysate samples (10 µl) were collected every 5 min for 40 min and then every 20 min (40 µl) for a further 140 min.

3.2.2 Results

Blood pharmacokinetics

The serum concentration versus time profiles of phenytoin (figure 3.1) demonstrate rapid absorption following intraperitoneal injection with peak concentrations achieved at 0.33-0.67 h. The pharmacokinetic constants for individual rats are contained in table 3.1. $C_{\text{max}}$ values were 62.4 ± 4.5 µmol/l following phenytoin 50 mg/kg, and 112.5 ± 10.7 µmol/l following phenytoin 100 mg/kg. Since $T_{\text{max}}$ and $C_{\text{max}}$ values were determined from the graphs, and the first time point was at 20 min, these values may have been overestimated and underestimated respectively. The mean $T_{1/2}$ value appeared higher at the higher phenytoin dose (2.4 ± 0.6 h compared to 1.3 ± 0.3 h), but this difference was not statistically significant at $p<0.05$. 

93
Table 3.1: Pharmacokinetic constants for serum for 50 mg/kg and 100 mg/kg i.p. phenytoin

<table>
<thead>
<tr>
<th>Rat no</th>
<th>$C_{\text{max}}$ (µmol/l)</th>
<th>$T_{\text{max}}$ (h)</th>
<th>AUC (µmol h/l)</th>
<th>$T_{1/2}$ (h)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
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</tr>
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</tr>
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<td>± SEM</td>
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<td>17.8</td>
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<td></td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
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</tr>
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<td>± SEM</td>
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<td>12.8</td>
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</tr>
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</table>
Brain neuropharmacokinetics

The mean ± SEM phenytoin in vitro relative recovery for the 18 microdialysis probes was 17 ± 1 % at a dialysate flow rate of 2 µl/min. These data were used to adjust the in vivo concentration data.

Following a dose of 50 mg/kg phenytoin, the phenytoin was rapidly observed in the dialysate of both hippocampus and frontal cortex (figure 3.2a). The phenytoin concentrations plateaued at approximately 15 min and then declined exponentially after approximately 60 min. In hippocampus in all animals, two phenytoin peaks were discernible, one at 15 to 25 min and the other at 60 min.

Similarly, following a dose of 100 mg/kg phenytoin (figure 3.2b), phenytoin was rapidly detectable in both hippocampal and frontal cortex dialysates. The phenytoin concentrations plateaued at 30 min, and then began to decline.
exponentially after 80 min. In hippocampus, there were, once again, two peaks discernible over this time period in most animals.

Figure 3.2: Phenytoin versus time profiles in hippocampus (closed squares) and in frontal cortex (open circles) after an intraperitoneal injection of phenytoin (A) 50 mg/kg and (B) 100 mg/kg. Values are mean ± SEM of 4-5 rats.
The pharmacokinetic constants are contained in table 3.2. AUC corrected for dose was dependent upon position with AUC for hippocampus being larger than that for frontal cortex ($F_{(1,14)}=12.4, p<0.01$). $T_{1/2}$ was independent of position, but was dose dependent, with the half-life being greater at the higher dose ($F_{(1,14)}=8.54, p<0.05$). The ratios of AUC brain ECF to AUC serum at 50 mg/kg and 100 mg/kg respectively were 0.11 and 0.13 for hippocampus, and 0.08 and 0.07 for frontal cortex. The half-lives in brain ECF did not significantly differ from that in serum.

Table 3.2: Pharmacokinetic constants for brain extracellular fluid (ECF) in hippocampus and frontal cortex for 50 mg/kg and 100 mg/kg i.p. phenytoin

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<th>Rat no</th>
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<th>Frontal Cortex</th>
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</tr>
</thead>
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<td>AUC (µmol h/l)</td>
<td>$T_{1/2}$ (h)</td>
<td>AUC (µmol h/l)</td>
</tr>
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<td></td>
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<tr>
<td>1</td>
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<td>4.4</td>
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</tr>
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<td>4</td>
<td>11.8</td>
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</tr>
<tr>
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<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>9.3</td>
<td>1.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Mean</td>
<td>9.1</td>
<td>1.2</td>
<td>6.6</td>
</tr>
<tr>
<td>± SEM</td>
<td>1.1</td>
<td>0.1</td>
<td>1.2</td>
</tr>
<tr>
<td>B. 100 mg/kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>3.7</td>
<td>8.9</td>
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<td>± SEM</td>
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</table>
3.3 Serum and CSF pharmacokinetics of lamotrigine

3.31 Experimental protocol

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 260-360g were used. Jugular vein and CSF catheterisation were performed as described. Two days after recovery from surgery, baseline samples were collected and then the rats were given lamotrigine (Glaxo-Wellcome, Cheshire) in propylene glycol by intraperitoneal injection (10 or 20mg/kg). Venous blood samples (100 µl) were withdrawn at 10, 20, 30, 40, 60 minutes, then hourly to 12 hours and then 3 hourly to 30 hours after lamotrigine administration. CSF samples were taken at 20-30 minute intervals for the first hour, then hourly to 12 hours and then 3 hourly to 30 hours after lamotrigine administration.

3.32 Results

Blood pharmacokinetics

Figure 3.3: Serum lamotrigine concentration-time profile following intraperitoneal injection of lamotrigine 10 mg/kg (open circles) and 20 mg/kg (closed circles). Values are mean ± SEM of 6 and 5 rats respectively.
The serum concentration versus time profiles of lamotrigine (figure 3.3) demonstrate rapid absorption following intraperitoneal injection with peak concentrations achieved at 0.2-1.0 h for 20mg/kg and 0.2-0.5 h for 10 mg/kg (table 3.3). Thereafter there is a fall in sera concentrations over an 8 h period with a dose dependent half-life (p=0.004 for the difference at the two doses) of 9.6±0.6 h for 20 mg/kg and 6.1±0.7 h for 10 mg/kg: AUC was calculated for this period (table 3.3). Over the next 20 hours, the concentration remains essentially constant following the 20 mg/kg dose and declines very slowly following the 10 mg/kg dose so that $T_{1/2}$ was not calculable. $C_{\text{max}}$ is dose related with the $C_{\text{max}}$ for 20 mg/kg representing approximately twice the $C_{\text{max}}$ for 10 mg/kg.

The lamotrigine non-protein bound/total serum concentration ratio was 0.39±0.01 (n=27) and was not dose or time dependent (figure 3.4).

![Figure 3.4: Serum lamotrigine free/total concentration ratio at differing total lamotrigine concentrations demonstrating no concentration effect on lamotrigine serum protein binding. Dotted line indicates mean value of ratio.](image-url)
Table 3.3: Serum pharmacokinetic constants following intraperitoneal injection of lamotrigine

<table>
<thead>
<tr>
<th>Dose</th>
<th>Rat</th>
<th>$T_{1/2}$ (h)</th>
<th>AUC$_{0-8}$ (µmol h/l)</th>
<th>$T_{max}$ (h)</th>
<th>$C_{max}$ (µmol/l)</th>
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</thead>
<tbody>
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<td>7.7</td>
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</table>

$T_{1/2}$ = half-life for first 8 hours post dose, AUC$_{0-8}$ = area under concentration versus time curve from time=0 to time=8h post dose

**CSF neuropharmacokinetics**

Lamotrigine was detectable in the CSF at the first time point, 10 minutes post-dose (figure 3.5). At both doses the concentration then rose over a period of 0.5-1 h up to a maximum, and then slowly declined.
Figure 3.5: Cerebrospinal fluid lamotrigine concentration-time profile following intraperitoneal injection of lamotrigine 10 mg/kg (open circles) and 20 mg/kg (closed circles). Values are mean ± SEM of 6 and 5 rats respectively.

The slow rate and variation of this decline precluded accurate determination of a decay constant. The calculated neuropharmacokinetic constants are contained in table 3.4. At 20 mg/kg there was a plateau from 1-7 h, and at this dose, $T_{\text{max}}$ ranged from 0.5-7 h (median 2 h).

The CSF: serum concentration ratio rises to approximately steady state value in 0.5-1 h (figure 3.6). There is, however, a large inter-rat variability and the range varies from 0.5 to 5 hours. During the period of declining serum concentrations, there is a rise in the CSF/serum concentration ratio (figure 3.6). At approximately 15 h post-dose, the ratio attains a plateau value of approximately 0.56 which is marginally greater than the free:total concentration ratio in serum.
Table 3.4: CSF neuropharmacokinetic constants following intraperitoneal injection of lamotrigine

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Rat</th>
<th>( \text{AUC}_{0-8} ) (( \mu \text{mol h/l} ))</th>
<th>( T_{\text{max}} ) (h)</th>
<th>( C_{\text{max}} ) (( \mu \text{mol/l} ))</th>
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<td>22.3</td>
</tr>
<tr>
<td></td>
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<td>114</td>
<td>7.0</td>
<td>20.6</td>
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<tr>
<td></td>
<td>3</td>
<td>139</td>
<td>5.0</td>
<td>26.7</td>
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<td></td>
<td>4</td>
<td>188</td>
<td>1.0</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>180</td>
<td>0.5</td>
<td>29</td>
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<tr>
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<td></td>
<td>150</td>
<td>3.1</td>
<td>27.0</td>
</tr>
<tr>
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<td></td>
<td>15</td>
<td>1.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

\( \text{AUC}_{0-8} \) = area under concentration versus time curve from time=0 to time=8h post dose, \( T_{\text{max}} \) = time to maximum concentration, \( C_{\text{max}} \) = maximum concentration
3.4 Brain extracellular concentrations of lamotrigine

3.4.1 Experimental protocol

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 260-370g were used to determine the brain ECF concentrations. Venous catheter and microdialysis probes were implanted as described. Two days after surgery when the animals were fully recovered, baseline samples were collected and the rats were then injected intraperitoneally with 20 or 40 mg/kg lamotrigine (Glaxo-Wellcome, Cheshire) in propylene glycol; 40mg/kg was higher than that used in the CSF experiments, because following 10 mg/kg i.p. lamotrigine, the concentrations of lamotrigine in dialysate would have been below the detectable limit of the HPLC analysis. Venous blood samples (100 µl) were withdrawn every 20 minutes to 120 minutes and thereafter hourly until 300 minutes after lamotrigine administration. Dialysate samples (20 µl) were collected every 10 mins for 120 mins and then every 20 mins (40 µl) for a further 180 mins.
3.42 Results

Due to the short duration of sampling, descriptive rather than quantitative pharmacokinetics were used. The maximum serum concentration ($C_{\text{max}}$) and the time to maximum serum concentration ($T_{\text{max}}$) were estimated from the concentration against time plots.

The serum concentration values are comparable to those in the CSF/serum pharmacokinetic study (figure 3.7).

![Figure 3.7: Serum lamotrigine concentration-time profile following intraperitoneal injection of lamotrigine 20 mg/kg (open circles) and 40 mg/kg (closed circles). Values are mean ± SEM of 6 rats respectively.](image)

The serum $T_{\text{max}}$ and $C_{\text{max}}$ for 20 mg/kg are not significantly different from those determined over the first 8 hours in the CSF/serum pharmacokinetic study of 20 mg/kg (table 3.5).
Table 3.5: Serum pharmacokinetic constants following intraperitoneal injection of lamotrigine

<table>
<thead>
<tr>
<th>Dose</th>
<th>Rat</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µmol/l)</th>
</tr>
</thead>
<tbody>
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<td>0.4</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.4</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.4</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.4</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.1</td>
<td>4</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>1</td>
<td>0.7</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.7</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.5</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.7</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.8</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>SEM</td>
<td>0.2</td>
<td>11</td>
</tr>
</tbody>
</table>

AUC<sub>0-8</sub> = area under concentration versus time curve from time=0 to time=8h post dose

The neuropharmacokinetics of lamotrigine in hippocampus and frontal cortex were indistinguishable (figure 3.8). The recovery corrected concentrations for the ECF were a half to one third of those in CSF. From the graphs, lamotrigine appears rapidly in brain ECF and reaches its approximate peak value at both doses after 30-40 minutes (i.e. at approximately the same time as the serum concentrations peak)
Figure 3.8: Lamotrigine versus time profiles in hippocampus (open circles) and in frontal cortex (closed circles) after an intraperitoneal injection of lamotrigine (a) 20 mg/kg and (b) 40 mg/kg Values are mean ± SEM of 6 rats.
3.5 CSF and serum pharmacokinetics of repeated dose diazepam.

3.51 Experimental protocol

Male Sprague-Dawley rats (Charles River, Margate, Kent) weighing 260-340g were used. Jugular vein and CSF catheterisation were performed as described. Two days after recovery from surgery, baseline samples were collected.

Two protocols were used, one to assess single dose pharmacokinetics and the other to assess repeat dose. In the single dose paradigm, the animals were given diazepam by intraperitoneal injection (20 or 30mg/kg). Venous blood samples (100 µl) were withdrawn at 10, 20, 30, 40, 60 minutes and then half hourly to 180 minutes. CSF samples were taken at 10, 30 minutes and then half hourly to 180 minutes.

In the repeat dose protocol, the animals were given diazepam by intraperitoneal injection (10 or 20mg/kg) at time 0, time 60 minutes, and time 120 minutes. Venous blood samples (100 µl) were withdrawn at 10, 30, 60, 70, 90, 120, 130, 150 minutes, then half hourly to 360 minutes. CSF samples were taken at 10, 30, 60 minutes, then half hourly to 360 minutes.

3.52 Results

Serum data

Following a single intraperitoneal injection, diazepam is rapidly absorbed with a time to maximum concentration of approximately 10 minutes (figures 3.9a,b). Following this the serum concentrations show a biexponential decline.

The first rapid decline represents distribution from the peripheral to central compartment and is characterised by a T₁/₂(α) of 9.0±2.1 minutes for 20 mg/kg, and 10.2±1.4 minutes for 30 mg/kg (table 3.6). These were not significantly different. The subsequent decline is predominantly due to elimination and is characterised by a T₁/₂(β) of 72.0±10.5 minutes for 20 mg/kg, and 73.5±8.7 minutes for 30 mg/kg (table 3.6).
Figure 3.9: Single dose serum concentration versus time data for diazepam (closed circles) and desmethyldiazepam (open squares) following i.p injection of (a) 20mg/kg diazepam and (b) 30 mg/kg diazepam. Data are given as mean±SEM for 4 and 5 animals, respectively.
Table 3.6: Pharmacokinetic constants for single dose diazepam data

<table>
<thead>
<tr>
<th></th>
<th>$T_{1/2(\alpha)}$</th>
<th>$T_{1/2(\beta)}$</th>
<th>$AUC_{0-\infty}$</th>
<th>Cl</th>
<th>$Vd_{area}$</th>
</tr>
</thead>
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<tr>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(min mg/l)</td>
<td>(l/min/kg)</td>
<td>(l/kg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.7</td>
<td>102.1</td>
<td>86.0</td>
<td>0.232</td>
<td>34.2</td>
</tr>
<tr>
<td>2</td>
<td>8.8</td>
<td>63.3</td>
<td>102.9</td>
<td>0.194</td>
<td>17.8</td>
</tr>
<tr>
<td>3</td>
<td>6.4</td>
<td>69.0</td>
<td>104.4</td>
<td>0.191</td>
<td>19.1</td>
</tr>
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<tr>
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<td>4.3</td>
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<td></td>
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<tr>
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<td>291.2</td>
<td>0.130</td>
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<tr>
<td>5</td>
<td>6.2</td>
<td>102.9</td>
<td>164.9</td>
<td>0.182</td>
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<td>73.5</td>
<td>216.4</td>
<td>0.146</td>
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<tr>
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<td>8.7</td>
<td>25.8</td>
<td>0.016</td>
<td>2.9</td>
</tr>
</tbody>
</table>

$T_{1/2(\alpha)}$=redistribution half-life, $T_{1/2(\beta)}$=elimination half-life, $AUC_{0-\infty}$=area under concentration versus time curve from time 0 to infinity, Cl=clearance, $Vd_{area}$=volume of distribution

For the repeat dose data, peak concentrations similarly occur rapidly after each intraperitoneal injection (figures 3.10a,b). The maximum concentration increases substantially from the first to the second to the third injection (figures 3.10a,b). This is in part due to residual diazepam being present in serum just prior to the subsequent injection.
Figure 3.10: Repeat dose serum concentration versus time data for diazepam (closed circles) and desmethyldiazepam (open squares) following i.p injections, given at 0, 60 and 120 minutes, of (a) repeat dose 10mg/kg diazepam, and (b) repeat dose 20 mg/kg diazepam. Data are given as mean±SEM for 5 animals.
Table 3.7: Pharmacokinetics for third dose of repeat dose diazepam data

<table>
<thead>
<tr>
<th></th>
<th>$T_{1/2}$(alpha)</th>
<th>$T_{1/2}$(beta)</th>
<th>AUC$_{0-\infty}$</th>
<th>AUC(3)</th>
<th>Cl</th>
<th>Vd$_{area}$</th>
</tr>
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<tr>
<td></td>
<td>(min)</td>
<td>(min)</td>
<td>(min mg/l)</td>
<td>(min mg/l)</td>
<td>(l/min/kg)</td>
<td>(l/kg)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>NC</td>
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<td>47.0</td>
<td>43.3</td>
<td>0.231</td>
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</tr>
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</tr>
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<td>8.2</td>
</tr>
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<td>20 mg/kg</td>
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<td></td>
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<td>428.7</td>
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<td>5.7</td>
</tr>
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<td>417.0</td>
<td>339.6</td>
<td>0.063</td>
<td>6.5</td>
</tr>
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<td>SEM</td>
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<td>34.1</td>
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<td>0.010</td>
<td>1.2</td>
</tr>
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</table>

AUC(3) is AUC$_{0-\infty}$ corrected for residual diazepam from the second dose and represents the AUC due solely to the third dose, NC=not calculable

Pharmacokinetic analysis was carried on the concentration data following the third dose (table 3.7). In most animals no alpha phase was discernible and the data were best modelled monoexponentially. There was no significant effect of dose or repeated dosing on $T_{1/2}$(beta) ($F_{(2,15)}$=1.98, p=0.172 and $F_{(1,15)}$=0.04, p=0.851 respectively). Thus AUC$_{1h-\infty}$ for the second dose was calculated from the decay constant derived from the third dose (see methods). The AUC values could thus be adjusted for the presence of diazepam in the serum as described in the methods.
section, and clearance and volume of distribution could thus be calculated for the third dose. Volume of distribution was significantly affected by repeated dosing (F(1,15)=16.43, p=0.001), but not by the actual dose (F(2,15)=1.63, p=0.228). Clearance, however, appeared to be both dose and repeat dosing dependent (F(2,15)=6.79, p<0.01 and F(1,15)=25.40, p<0.001 respectively).

The free to total diazepam serum concentration ratio was 0.17±0.01 and was not dose or time dependent (figure 3.11).

![Figure 3.11: Serum diazepam free/total concentration ratio at differing total diazepam concentrations demonstrating no concentration effect on diazepam serum protein binding. Dotted line indicates mean value of ratio.](image)

The pharmacokinetic effects of repeat dosing on diazepam are reflected in the pharmacokinetics of diazepam’s major active metabolite, desmethyldiazepam. So comparing the $T_{1/2}$ and estimated terminal $C_{\text{max}}$ for desmethyldiazepam following
single dose 20 mg/kg (figure 3.9a), and following the third dose 20 mg/kg (figure 3.10b) gives values of 51±1 mins and 813±49 ng/ml, and 153±23 mins and 2223±336 ng/ml, respectively. There is thus both a 3 fold increase in C_max ($F(1,7)=13.5, p<0.01$), and a 3 fold increase in $T_{1/2}$ ($F(1,7)=15.2, p<0.01$), both of which would contribute to an increased and persistent action following administration of diazepam.

_Cerebrospinal fluid data_

CSF samples were analysed following 30mg/kg single dose, and 20 mg/kg repeat dose, as at the lower doses the CSF concentrations fell below the limits of detection. These data are presented as absolute concentrations (figure 3.12a,b), and CSF to total serum concentration ratios following single 20 mg/kg, and following the third repeat dose of 20 mg/kg (figure 3.13). Following 30 mg/kg i.p., the CSF to total serum concentration ratio reached the equilibrium value by 10 minutes (figure 3.12). The CSF to total serum concentration ratio was approximately equal to the free to total serum concentration ratio over the 180 minutes. For the first 90 minutes, however, the CSF to total serum concentration ratio was marginally greater than the free to total serum concentration ratio.

The 20 mg/kg repeat dose data (figure 3.12b) demonstrate an increase in the concentration following the third dose that would be predicted from the serum free levels (figure 3.13). However, there is a relative persistence of both diazepam and desmethyldiazepam in the CSF despite falling serum concentrations following the third dose (figure 3.12b) This is reflected in a rise in the CSF to serum concentration ratio from approximately 0.3 to approximately 0.8 (figure 3.13). This represents a relative persistence of the CSF concentrations compared to the single dose data, and a relative persistence despite falling serum concentrations.
Figure 3.12: CSF concentration versus time data for diazepam (closed circles) and desmethyldiazepam (open squares) following i.p injection of (a) single dose 30 mg/kg diazepam, and (b) Repeat dose 20 mg/kg. Data are given as mean±SEM for 5 and 4 animals, respectively.
3.6 Discussion

The rat models that I used were chosen so that: interindividual variability (as occurs when composite values of individual rats killed at different time points after drug administration are used) was minimised and thus the number of experimental animals needed to obtain detailed kinetic data was reduced, and long sampling protocols could be used.

3.6.1 Lamotrigine pharmacokinetics and neuropharmacokinetics

Although, as part of the preclinical development of lamotrigine, many studies were undertaken to determine its blood (plasma) pharmacokinetics in a variety of species, there is a sparcity of published data (Parsons et al., 1995). Furthermore, there are no published data describing the central neuropharmacokinetics of
lamotrigine and this is the first report on the temporal inter-relationship of lamotrigine serum pharmacokinetics and CSF neuropharmacokinetics in the rat. The pharmacokinetics of lamotrigine I observed could not have been predicted from sampling of less than 8 hours.

After intraperitoneal administration, lamotrigine rapidly appeared in serum (mean T_{max} = 0.5h) suggesting ready penetration from the peritoneal cavity. As might be expected, T_{max} values for the CSF compartment are somewhat longer and represent the time of lamotrigine penetration from serum to brain and finally the CSF compartment. The dose dependent increase in both serum and CSF lamotrigine after lamotrigine administration (10 and 20 mg/kg) suggests that transport across the blood brain barrier is not rate limiting over the concentration range observed in the present study. The major findings of my study relate to the serum pharmacokinetics of lamotrigine. A number of interesting features are observed in this respect. The kinetics are biphasic: the first phase lasts approximately 8 hours and is associated with a dose dependent decrease in T_{1/2}, and the second phase was associated with a much prolonged T_{1/2}, which was not quantifiable even though sampling of up to 30 hours was undertaken. It is not possible from this study to determine the mechanism underlying this effect, but it is likely that the first phase represents metabolism, elimination and redistribution, and that the second phase is due to redistribution from peripheral tissues back into the blood compartment or from entero-hepatic recirculation. There is evidence to suggest that both these effects play a part. Lamotrigine accumulates in the kidney of male rats such that the kidney to plasma concentration ratio can be up to 300:1 (Parsons et al., 1995). This effect is both gender and species specific, and could explain the maintenance of serum concentrations in my study after 8 hours post-dose. Although there is no evidence of entero-hepatic recirculation in rats, it is noteworthy that following intravenous and oral administration of single doses to humans multiple concentration peaks were apparent (Yuen & Peck, 1988; Mikati et al., 1989). These kinetic effects would predict a prolonged action of lamotrigine in rat models of epilepsy, and could be important in the determination of lamotrigine's effects in chronic animal models (e.g. kindling), and in comparing lamotrigine with other AEDs in rat models.
The \( T_{1/2} \) values obtained for serum lamotrigine in this study after a single intraperitoneal administration of 10 and 20 mg/kg were respectively 6.1±0.7 h and 9.6±0.6 h. It is somewhat difficult to compare these values with values previously reported since different doses (2.7 and 4 mg/kg) and administration routes (intravenous or oral) were used (Parsons et al., 1995). A further complication is that different lamotrigine formulations were used (free base or isethionate and mesylate salts). In the present study the free base was used. The dose related increase in the initial mean serum \( T_{1/2} \) has been previously seen in male rats with relatively high doses of lamotrigine (> 25mg/kg) (Parsons et al, 1995), but was attributed to a rodent-specific delaying effect upon absorption following oral ingestion; this effect cannot explain my results.

The mean±SEM serum free fraction of lamotrigine as determined by ultracentrifugation was 0.39±0.01% and was not concentration dependent over the concentration range of 5-52 \( \mu \)mol/l. This is somewhat different to a previously reported value of 0.46 for rat plasma determined by equilibrium dialysis (Parsons et al., 1995). The free fraction (0.39) was also of the same order of magnitude as the CSF: serum concentration ratio at equilibrium (figure 4). Since lamotrigine is a weak base with a pKa of 5.5, at physiological pH it is highly undissociated (a feature it shares with most AEDs). Thus, as with other AEDs, the major determinant of rate of entry of lamotrigine into the CSF compartment is likely to be its lipid solubility.

Accurate determination of rates of entry into the CSF compartment can be derived from data following bolus intravenous administration. Although I used intraperitoneal administration, the concomitant sampling of serum and CSF concentrations gives an index of the rate of CSF penetration. From my data, equilibration between the CSF and blood compartments occurs within 30-60 minutes (figure 4) and is compatible with the observed \( T_{\text{max}} \) values. Thereafter the ratio rises, and then decreases back to the equilibrium value. The transient rise of the CSF:serum concentration ratio can probably be explained by the relatively rapid decline in serum concentrations at that time along with a delay in passage of lamotrigine from the CSF to the blood compartment. A similar effect in humans has been described for viloxazine, a drug that passes slowly across the blood brain barrier.
barrier (Elwan & Adam, 1980). Another explanation is that during the acute phase the hypothesis that the free serum drug concentration is the tissue exchangeable drug concentration is incorrect; indeed, a number of studies have demonstrated that plasma protein bound drug is often available for transport into the brain (Urien et al., 1987; Pardridge et al., 1983; Cornford et al., 1992; Lolin et al., 1994).

The delayed entry into the CSF is not reflected in a delay in lamotrigine entering the brain ECF, and this is consistent with the passage of lamotrigine into these two compartments via separate mechanisms with passage into brain ECF being faster than passage into CSF. The microdialysis concentrations are substantially smaller than the CSF concentrations even after correcting for in vitro recovery. This is partly due to the in vitro recovery being a poor index of in vivo recovery. There are a number of factors that may contribute to this. The in vitro recovery is performed in a solution of the substrate, which is very different from the in vivo situation in which the microdialysis probe is surrounded by brain tissue, which may inhibit free diffusion. Also the microdialysis probes are in place for two days before the experiment takes place; this is to allow recovery of the animals from surgery (Patsalos et al., 1992). During this time, however, proteins bind to the dialysis membrane and are likely to substantially decrease the permeability of the membrane; in vivo microdialysis probe recoveries after a similar period of time after implantation have been shown to be a maximum of 2 fold lower than the in vitro recoveries (de Lange et al., 1994). Correction by in vitro recovery does, however, allow for correction for individual differences between microdialysis probes. The indistinguishable pharmacokinetics between the frontal cortex and the hippocampus and the equivalent lamotrigine concentrations for these two areas suggest that factors that may interfere with probe recovery are not area specific. Despite the above explanation, others who have used direct sampling of brain ECF have found that ECF concentrations are generally lower than those in the CSF, and so the difference between CSF and corrected ECF concentrations may partly be due to a physiological phenomenon (Sechi et al., 1989).
3.62 Phenytoin pharmacokinetics and neuropharmacokinetics

The total phenytoin concentration in various brain areas has been measured in both animals and humans following both chronic and acute administrations. Following administration, phenytoin because of its high lipid solubility rapidly enters the brain (Woodbury, 1989), where it is substantially bound to subcellular fractions of neurones (Kemp & Woodbury, 1989) more so than glia (Yanagihara & Hamberger, 1971).

In chronic studies, the total phenytoin concentration found in white matter regions often equals or exceeds that found in grey matter. The concentrations of phenytoin obtained from a human post-mortem specimen demonstrated twice as much phenytoin in white matter as compared to grey matter (Sherwin et al., 1973). A less impressive difference has been seen in specimens from patients undergoing epilepsy surgery (Sironi et al., 1980) where the ratio of white matter/plasma concentrations (1.33) exceeded that of grey matter/plasma concentrations (1.13). This is perhaps due to the higher lipid content of white matter, and the high affinity of phenytoin for protein and phospholipids (Goldberg, 1980). Thus, in neonates given phenytoin there is a higher concentration in grey matter perhaps due to the lack of myelin in the new-born brain (Painter et al., 1981). However this disparity is possibly also due to a yet unidentified mechanism, since in physiologically active brain slices the phenytoin accumulates to a greater degree in white matter, whilst in frozen brain slices the phenytoin accumulates to a greater degree in grey matter (Geary II et al., 1987).

Contrasting results have been obtained for the comparative brain concentrations within white or grey matter between differing regions. Sironi et al.(1980) found the concentration to be greater in the temporal and parietal lobes than in the frontal lobes, whilst Geary II et al.(1987) found that the phenytoin concentration was uniform within white and grey matter regions. Preferential accumulation of phenytoin has been seen in the superior colliculus, inferior colliculus, amygdala and hippocampus of chronically treated dogs and cats (Nakamura et al., 1966). In chronic human and animal studies the total plasma concentration of phenytoin
bears a significant relationship to brain and CSF phenytoin concentrations (Houghton et al., 1975; Sherwin et al., 1973; Vajda et al., 1974). Furthermore recent microdialysis studies in humans (Scheyer et al., 1994), have shown that with chronic administration the brain ECF concentration of phenytoin closely corresponds to unbound serum concentrations.

These findings, however, are in stark contrast to the pharmacokinetics of acutely administered phenytoin. In cats, given phenytoin acutely, autoradiography of radiolabelled phenytoin demonstrated an initial preferential accumulation of phenytoin in the grey matter (Firemark et al., 1963). A similar result was seen for PET scanning of labelled phenytoin given acutely to humans (Baron et al., 1983). Indeed in this last study the initial uptake resembled PET maps of cerebral blood flow. Thus the initial high uptake of phenytoin by grey matter could be due to the higher blood flow in these regions; because of phenytoin's high lipid solubility, blood-flow may be the rate limiting step in its passage across the blood-brain barrier (Baron et al., 1983; Firemark et al., 1963). In both these studies the white matter/grey matter concentration ratio slowly rose as phenytoin accumulated in the white matter.

All these studies, however, measured the total phenytoin, and in brain this is predominantly bound phenytoin (Goldberg, 1980). The binding of phenytoin is not determined by receptor concentration, but is non-specific binding and is dependent on protein and phospholipid concentrations (Goldberg, 1980). The bound phenytoin is thus unlikely to be pharmacologically active, and intuitively it is perhaps the free interstitial fluid phenytoin that surrounds neurones that is of greater pharmacological importance. This free extracellular phenytoin has been measured using implanted polypropylene balls in dogs (Sechi et al., 1987); this study, however, only looked at one brain area in the left temperoparietal region. The advantage of the microdialysis technique used by me is that it enables simultaneous measurement in two or more brain areas without withdrawal of fluid from the brain. The two areas I investigated were the frontal cortex (predominantly a white matter region) and the hippocampus (predominantly a grey matter region) with concomitant serum monitoring.
In the present study, the phenytoin serum pharmacokinetic parameters, and inter-rat variability at the two phenytoin doses (50 and 100 mg/kg) were similar to those found by Lolin et al. (1994). Dose dependency of T1/2 seen in both studies can be attributed to product inhibition of the major metabolite of phenytoin (±)-5-(4-hydroxyphenyl)-5-phenylhydantoin (Vicuna et al., 1980). This is in contrast to humans where non-linear pharmacokinetics are due to saturation of hepatic enzymes (Woodbury, 1989). In this study, I have shown that: 1) phenytoin rapidly enters brain ECF; 2) in the initial period serum pharmacokinetics are a poor index of brain ECF pharmacokinetics, and 3) the AUC is greater for brain ECF phenytoin concentrations in hippocampus than in frontal cortex.

The rapidity of phenytoin entry into the brain ECF is in accordance with studies of total brain phenytoin (Woodbury, 1989). An equal or shorter T_{max} for brain phenytoin pharmacokinetics compared to CSF phenytoin pharmacokinetics has led to the suggestion that diffusion of phenytoin into extracellular brain fluid is via a mechanism independent from diffusion of phenytoin into the CSF (Wilder et al., 1977; Ramsay et al., 1979; Woodbury 1989). In my study, phenytoin was administered intraperitoneally making rate of penetration of phenytoin difficult to evaluate. However, the use of concomitant plasma concentration monitoring and the similarity between my study and that of Lolin et al. (1994) permits certain comparisons to be made. Phenytoin enters the brain ECF faster in my study than it enters CSF in the study of Lolin et al.(1994) in which phenytoin, similarly administered, had a T_{max} of 1.2-1.4 h. This is consistent with pharmacokinetic and pharmacodynamic data in humans (Wilder et al., 1977). The plateau observed in the brain ECF phenytoin concentrations in the present study is similar to that seen in previous studies in which there were persisting or rising brain concentrations of phenytoin despite falling serum concentrations (Wilder et al., 1977; Sechi et al., 1989). This may be due to rapid diffusion of phenytoin across the BBB and then a delayed diffusion front from the CSF compartment, which maintains extracellular phenytoin concentrations despite falling serum concentrations (Sechi et al., 1989). This phenomenon may also explain the double peak that I observed in hippocampal ECF phenytoin concentrations in a number of animals. This phenomenon could be explained by rapid diffusion of phenytoin across the BBB.
resulting in the first peak, followed by rapid binding and redistribution through brain parenchyma resulting in the initial fall. The second peak could be explained by a delayed diffusion front from the CSF compartment. Indeed the timing of the second peak is similar to the $T_{\text{max}}$ observed in brain CSF (Lolin et al., 1994). Furthermore, the concentration of phenytoin at the first peak appeared to be independent of dose, possibly implying that the immediate passage of phenytoin across the BBB may be via a saturable mechanism in contrast to its penetration into CSF. The fact that the magnitude of this peak is related to phenytoin dose, and its occurrence is specific to only certain brain areas could explain differences in the delay in action of phenytoin observed in different models (Ensor et al., 1959; Leppik & Sherwin, 1979). Thus different ECF phenytoin profiles observed in frontal cortex and hippocampus may not only be a reflection of their different constitutions, but also a reflection of the difference in the degree of CSF permeation into these areas.

The larger AUC in hippocampus compared to frontal cortex could be the result of a combination of higher blood flow in grey matter (hippocampus), and the extensive non-specific binding of phenytoin to white matter (frontal cortex). The possibility, however, that the difference observed in my study might be partly due to the methodology used must also be considered. The in vivo phenytoin dialysate concentrations were adjusted by the in vitro phenytoin recovery of the microdialysis probes in order to give an estimate of absolute ECF concentrations. As explained in the lamotrigine discussion, this is probably a crude estimate as in vitro microdialysis recoveries do not accurately represent the in vivo situation. It is possible that the difference observed between the hippocampus and frontal cortex phenytoin concentrations could be the result of the effects of different regional brain densities on the recoveries of the probes in vivo. Such an effect, however, has not been observed in studies of other drugs such as milacemide and antipyrine (Patsalos et al., 1993) and in my study of lamotrigine, and also it is unlikely that this effect could be large enough to explain the differences observed in the present study.
3.6.3 Repeated dose diazepam

Previous reports of diazepam pharmacokinetics deal with single dose kinetics in systemic blood or plasma. The kinetics of repeat doses such as may be used in status epilepticus has not been previously established. Moreover, the simultaneous monitoring of CSF concentrations may give a more accurate indication of the concentrations at the receptor site (Greenblatt & Sethy, 1990). The main aim of my study was to determine whether repeat administration of diazepam may significantly alter its serum and CSF kinetics leading to dangerous accumulation.

My single dose pharmacokinetic constants for rats are comparable to that found in other studies; using doses of 5-10mg/kg, Vd\_area ranged from 7.84L/kg to 20.25L/kg and clearance ranged from 0.132L/kg/min to 0.255L/kg/min in those studies (Raymond & DeGennaro, 1980; Klotz et al, 1976; Friedman et al, 1986). That the total serum clearance was to some extent dose dependent in my study is not surprising, as with larger doses there may have been accumulation of diazepam within the peripheral compartment. It is, however, noteworthy that the elimination time constant appears to be independent of dose.

Following repeat doses of diazepam, I observed a significant decrease in both the volume of distribution and clearance. Comparing the single dose pharmacokinetics for 20mg/kg with the pharmacokinetics for the third 20mg/kg dose, I found a 3 fold decrease in the volume of distribution and clearance. This is almost certainly due to accumulation in the peripheral compartment. Indeed, by the third dose it was not possible in most animals to distinguish the alpha and beta phases.

The free to total serum concentration ratio for rat in my study (0.17) is similar to that found in other studies using equilibrium dialysis (0.14-0.20) (Klotz et al., 1976; Friedman et al., 1986). Since this is the component that can readily diffuse into the brain compartment, it is not surprising that the CSF to total serum concentration ratio is similar following a single dose. The rapid attainment of equilibrium following intraperitoneal injection between the brain and serum (within 10 minutes) has been similarly observed in other studies, following
intraperitoneal injection of 5mg/kg diazepam in rats, in which equilibrium between brain and peripheral blood was reached within 5 minutes from time of injection (Arendt et al., 1983; Friedman et al., 1986).

The initial rise in the CSF to serum concentration ratio above the free to total serum concentration ratio may be due to the fact that during the acute phase the hypothesis that the free serum drug concentration is exclusively the tissue exchangeable drug concentration is incorrect; indeed, a number of studies have demonstrated that plasma protein bound drug is often available for transport into the brain (Urien et al., 1987; Pardridge et al., 1983; Cornford et al., 1992; Lolin et al., 1994). The repeat dose data, which is the main consideration in my study, demonstrate a relative persistence of CSF diazepam following the third dose so that the ratio of CSF to serum concentrations rose as high as 0.8 (i.e. 4 times the ratio for the single dose data).

Compared to human data, the volume of distribution is larger in rats; $T_{1/2(\beta)}$ is substantially shorter in rats ($T_{1/2(\beta)}=30$ h in humans), clearance is more rapid in rats and the free to total serum concentration is greater in rats (Klotz et al., 1976). In fact, it has been previously noted that the clearance in rats is greater than hepatic blood flow, indicating that extrahepatic elimination probably also occurs (Klotz et al., 1976). Although, the absolute values of the pharmacokinetic constants differ, there are qualitative similarities between the pharmacokinetics of diazepam in rats and humans. They both have biphasic serum concentration-time profiles with the redistribution half-life being much shorter than the elimination half-life. In both species, diazepam has a relatively large volume of distribution and a high clearance following single dose administration.

The effects that I observed with repeated dosing could thus have important consequences. A decrease in the volume of distribution means that following repeated administration of diazepam, the consequent serum concentrations would be greater than that predicted from single dose data. A three fold decrease in the volume of distribution such as observed by me would mean that the serum concentrations would be those predicted from a single dose three times as large. The substantial decrease in clearance would lead to a persistence of diazepam in
the serum compartment and thus a persistence of action. Indeed, the greater discrepancy between alpha and beta elimination constants in humans would amplify this effect. Furthermore, following the third diazepam dose there is a relative persistence of CSF diazepam leading to a rise in the CSF to serum concentration ratio. This is almost certainly due to accumulation and sequestration of diazepam in the brain from non-specific binding to brain lipids; this may serve as a diazepam 'pool' maintaining the CSF concentration despite falling serum concentrations. This could lead to a further persistence of action following repeated dosing that cannot be predicted from serum data alone.

The effects that I have observed of repeat dosing of diazepam upon its pharmacokinetics would explain many of the problems encountered in the use of diazepam in the treatment of status epilepticus, and can lead to a potentially fatal situation. Furthermore, as with all benzodiazepines, persistence in the CSF can lead to problems of tolerance and rebound seizures (Shorvon, 1994; Schmidt, 1995). Due to these considerations, the use of repeat dose and infusions of diazepam in status epilepticus is potentially hazardous, and should not be recommended.
4 EXTRACELLULAR GLUTAMATE

4.1 Introduction

Excitatory amino acids have been proposed to play a critical role in the development and maintenance of epileptic seizures. Additionally, there is a substantial body of evidence that glutamate plays an important part in the development of neuronal damage following a variety of brain insults including status epilepticus, hypoglycaemia and ischaemia (Siesjo & Wieloch, 1986; Meldrum, 1991).

Attempts have thus been made to measure the extracellular glutamate concentration using microdialysis techniques in animal seizure models. These, however, have resulted in conflicting results with the majority of these studies showing no significant rise in glutamate during seizures (Wade et al., 1987; Vezzani et al., 1985; Millan et al., 1991; Lehmann, 1987; Lehmann et al., 1985; Lallement et al., 1991; Korf & Venema., 1985; Bruhn et al., 1992; Millan et al., 1993). These negative results have been purported to be due to high affinity glutamate uptake in synapses and glia, yet inhibition of these has still resulted in a failure to measure these putative glutamate rises (Millan et al., 1991). These animal data are in contrast to human microdialysis studies in which glutamate rises have been seen both during seizures and just prior to seizure onset (Carlson et al., 1992; During & Spencer, 1993).

I wished to measure glutamate in an animal model designed to circumvent many of the problems inherent in previous studies. Implantation of probes was performed with stereotaxic and neurophysiological localisation - offering greater precision (Errington et al., 1987). Electrographic seizure activity was induced by stimulation of the perforant pathway, thus avoiding the interpretative difficulties caused by the use of convulsant compounds which initiate seizures by often unknown mechanisms. Measurement of glutamate was performed using a newly developed dialysis biosensor (Albery et al., 1992; Galley, 1992), which does not involve the flow of perfusate through the probe and which can measure real time glutamate changes. Furthermore, using the same technique, it is possible to measure changes in extracellular ascorbate (Albery et al., 1992; Galley, 1992)
which may be dependent on a glutamate/ascorbate heteroexchange (Grünewald, 1993).

I wished to examine the relationship between extracellular glutamate and seizure activity, and then further to consider the role of modulating extracellular glutamate during status epilepticus.

4.2 Experiments

The experiments were performed acutely in fully anaesthetised animals (1-2% halothane in O₂) without recovery. Preliminary experiments were carried out in unanaesthetised animals using perforant path stimulation as described below. The seizure behaviour that the animals exhibited (especially wet dog shakes) resulted in movement of the dialysis probe despite it being fixed with skull screws and dental cement. This resulted in two confounding phenomena; first, there were large current artefacts from the probe, and secondly, the movement of the brain relative to the skull meant that the probe could be causing damage to the brain and the blood brain barrier which would make the results difficult to interpret. Thus, I chose to use anaesthetised animals, whose skulls were kept stationary in a stereotaxic frame (Stoelting Co. IL, USA). This enabled me to get good recordings with no movement artefact, and in addition reduced any distress caused to the animals. Unfortunately, I was unable to induce status epilepticus-like damage in halothane anaesthetised animals using electrical stimulation (see chapter 5) and so I concentrated on the relationship between repeat seizures and rises in extracellular glutamate. Seven male Sprague-Dawley rats, 290-340 g body weight, were used. The rats were kept anaesthetised using 1-2% halothane and body temperature was measured with a rectal probe and kept at 37±1°C. Three had a glutamate biosensor implanted, and 4 had an ascorbate biosensor implanted. A stimulating electrode was implanted in the perforant path and the dialysis electrode was implanted stereotaxically so that the upper electrode was in the dentate granule cell layer as described (figure 4.1). At this point the exposed dialysis membrane would straddle CA1 and the dentate gyrus - the area where
most damage is seen following prolonged stimulation of the perforant path (Sloviter, 1987).

Figure 4.1: Whilst the biosensor was lowered into the hippocampus, the perforant path was stimulated with test pulses and the response of the hippocampus was recorded by the recording electrodes attached to the side of the biosensor. Typical electrical responses to a 1 mA, 50 μs monopolar pulse of the CA1 cell layer, synaptic layer and dentate cell layer are shown. By these means precise localisation of the biosensor was possible.

Once in position, the electrodes were then left untouched for 4-5 hours in order to allow full recovery of the blood-brain barrier (Benveniste, 1989). The perforant path was then stimulated with twice the test current (2-6 mA), 500 μs monopolar pulses at 20 Hz for 10 seconds. This resulted in a prolonged afterdischarge. After 3-7 minutes when the EEG had returned to its pre-stimulation pattern, the stimulus was repeated. After 5-13 minutes when the EEG had again returned to its
pre-stimulation pattern, the stimulus was repeated a third time. In some rats, the above stimulation protocol was repeated at one and two hours after the initial stimulus. The magnitudes of the glutamate and ascorbate changes were expressed as a percentage of the base-line currents.

4.3 Results

4.3.1 In vitro calibration

Figure 4.2: (a) In vitro response of a glutamate biosensor to 1-2 μM steps in glutamate concentration. 40 μM glutamine gave no response. The addition of ascorbate to the solution resulted in no change in the current measured or the response to glutamate (not shown). (b) plot of glutamate biosensor current against glutamate concentration (c) In vitro response of ascorbate biosensor to 20 μM steps in ascorbate concentration. (d) plot of ascorbate biosensor current against ascorbate concentration.
The dialysis biosensor filled with glutamate oxidase and ascorbate oxidase responded to 1 and 2 µM steps in glutamate in a linear fashion (figure 4.2 a, b). Furthermore, addition of ascorbate to the test solution had no effect on this response even though ascorbate has been reported to reduce the sensitivity of hydrogen peroxide sensors (Lowry & O'Neill, 1992). In addition, there was no response to glutamine (glutamine oxidase is a possible enzyme contaminant). Similarly the dialysis biosensor filled with enzyme-free Ringer solution showed no response to glutamate, but a linear response to 10 and 20 µM steps of ascorbate (figure 4.2c, d). In both instances, the response to analyte occurred within 10-15 seconds of its addition to the bulk solution (some of this delay was due to mixing).

4.3.2 Glutamate and ascorbate rises following the first stimulus

Figure 4.3 shows the results from one glutamate experiment. In this experiment, a sequence of 3 trains of stimuli, minutes apart, were given, and this was repeated 1 and 2 hours later. Following the first train in the sequence, there is an almost immediate rise in glutamate peaking after 15 seconds and lasting approximately 30 seconds. There is then a late longer glutamate rise which was not apparent in all animals. This late rise is less obvious in the averaged glutamate response to the first 10 second train of 20 Hz monopolar pulses to the perforant path (figure 4.4a). The glutamate rise is attenuated from the first to the second, and from the second to the third train in the sequence. By the third train in the sequence, the glutamate rise was indistinguishable from background noise. This protocol repeated at 1 and 2 hours produced similar results (figure 4.3). Indeed, in all animals, the sequence repeated at one and two hours showed comparable changes in ascorbate and glutamate, so responses to each train in the sequence repeated one or two hours later were included in the average.

There were 3 rats (7 stimuli) in the glutamate group, and 4 rats (7 stimuli) in the ascorbate group. Following the first train in the sequence, there was a rapid rise and fall of extracellular glutamate (peak value ± SEM, 119.5 ± 4.5 %), during which there was an initial fall in extracellular ascorbate. There was then a delayed
rise and fall in the extracellular ascorbate concentrations (peak value ± SEM, 111.0 ± 4.4 %). The ascorbate peak coincided with the glutamate trough.

Figure 4.3: Typical experiment protocol in which the perforant path was stimulated by a sequence of three 10 second trains of 20 Hz monopolar pulses (500 µs duration, 2-6 mA amplitude) applied 3-13 minutes apart (shown by arrows), and this was repeated after 1 and 2 hours. Shown is the glutamate response in one experiment recorded by the biosensor positioned in the dentate gyrus. The response of the glutamate biosensor to the stimuli sequence was similar after 1 and 2 hours. The response to the first train in the sequence was larger than that to the second train, which was larger than that to the third train (the response to the third train in the sequence was not detectable above background noise). The response to the first train showed an immediate glutamate rise lasting 30 seconds, followed by a later, longer rise (see text).

4.3.3 Glutamate and ascorbate following the two subsequent stimulations

The glutamate and ascorbate peaks are attenuated from the first to the second (3-7 minutes after the first train in the sequence; peak value glutamate ± SEM, 105.7 ± 2.8 %; peak value ascorbate ± SEM, 102.0 ± 1.3 %) and from the second to the third train (5-13 minutes after the second train; peak value glutamate ± SEM, 102.9 ± 2.8 %; peak value ascorbate ± SEM, 99.9 ± 1.5 %) (figures 4.4b, c). These include the averaged responses to the sequence repeated one and two hours later. This attenuation in the glutamate and ascorbate response from the first to the third train was significant at P<0.05 using one-way ANOVA. Despite this no
difference was detectable in the electrographic response to stimulation or in the duration (range 6-12 seconds) of afterdischarges (see figure 4.5) from one train to the next.

Figure 4.4: Mean SEM of 7 glutamate and 7 ascorbate responses to: (a) the first 10 second train of 20 Hz monopolar pulses (500 μs duration, 2-6 mA amplitude) in the sequence. (b) the second train in the sequence. (c) the third train in the sequence.
Figure 4.5: Typical EEG response to each 10s train of 20Hz monopolar pulses in the sequence. Shown is 20s of baseline, followed by the stimulation period (10s), and then the resultant afterdischarge. In this, as in other experiments, there was no detectable difference in the afterdischarge from one train to the next.

4.4 Discussion
I have shown: 1) a rise in glutamate following an epileptic afterdischarge; 2) a concomitant initial fall and then a later rise in ascorbate, and 3) progressive
dwindling of this effect when afterdischarges are repeated within minutes, despite similar EEG responses.

The power of the dialysis biosensor can be seen here by the successful measurement of a transient rise in glutamate in the hippocampus following stimulation of the perforant path, which has not been measured with previous methods.

The first train in the sequence resulted in a rapid rise of extracellular glutamate, followed by a later rise in ascorbate, the peak of which coincided with the trough in extracellular glutamate. Furthermore, smaller rises in extracellular glutamate in subsequent trains were associated with smaller or no rises in extracellular ascorbate. This is consistent with the suggested extracellular ascorbate/glutamate heteroexchange in which glutamate uptake results in a release of ascorbate (O'Neill et al., 1984; Fillenz & Grünewald, 1984; Cammack et al., 1991; 1992; Grünewald, 1993). This release of ascorbate may protect against the neurotoxic effects of rising extracellular glutamate via ascorbate's antioxidant effects, and via its modulation of NMDA receptor function (Grunewald, 1993).

Using this experimental paradigm, I was unable to measure glutamate rises that occurred during status epilepticus or status epilepticus-induced damage, but I was able to examine the relationship of extracellular glutamate to seizure discharges.

4.4.1 What is the cause of glutamate rises in seizures?

A rise in extracellular glutamate can come from three sources: 1) an increase in synaptic release; 2) a decrease or reversal of glutamate uptake, and 3) a shrinkage of the extracellular space. If glutamate rises in seizures were purely a result of increased synaptic release, then a rise would be expected in all animal models of seizures which is not the case. Increased synaptic release almost certainly results from seizure activity, but this rarely translates into an increase in extracellular glutamate probably because of the high affinity glutamate uptake mechanism. In order to investigate this further, studies have been carried out in which the glutamate transporter has been blocked. Such a study has been conducted in a
model in which seizures are induced by pilocarpine. Pilocarpine seizures do not result in rises in extracellular glutamate (Millan et al., 1993). If, however, the glutamate uptake mechanism is blocked with 1-trans-pyrrolidine-2,4-dicarboxylic acid (PDC) prior to the administration of pilocarpine then substantial rises in extracellular glutamate are detected during the seizures (Millan et al., 1993). Blocking the glutamate uptake mechanism with dihydrokainate (DHK) during bicuculline or picrotoxin induced seizures, however, did not uncover such rises in extracellular glutamate (Millan et al., 1991). These apparent anomalies are almost certainly due to the glutamate uptake blocker chosen. DHK effectively blocks the GLT-1 glutamate transporter, resulting in a rise in basal glutamate levels. Concentrations of DHK in excess of 1mM are, however, necessary to block the EAAC1 transporter. In the above studies, the brain was exposed to concentrations of DHK well below this threshold. Thus failure to measure glutamate rises could have resulted from an increase in extracellular glutamate leading to an increase in the activity of the EAAC1 transporter (Km for glutamate of 12 μM), and this prevented further rises in the extracellular glutamate during seizures.

Could a reversal or decrease in glutamate uptake contribute to increases of extracellular glutamate during seizures? During seizures extracellular potassium rises to approximately 10mM, extracellular sodium falls by at least 10mM to 135mM and the neurons depolarise by at least 20mV to -60mV (Lux et al., 1986). Assuming that the extracellular volume is approximately 20% of the intracellular volume, then intracellular potassium would be expected to fall to 143mM and intracellular sodium would rise to 27mM. Assuming that the hydroxide gradient remains unchanged then the Hill equation for the high affinity glutamate uptake transporter would predict a minimum extracellular glutamate level of 7.6μM during seizures; this represents an 11-fold rise in minimum maintained extracellular glutamate. It is thus theoretically possible that inhibition or reversal of glutamate uptake during seizures could lead to a significant increase in extracellular glutamate. The evidence that this plays a role is indirect; initial rises in glutamate during epileptic afterdischarges in my experiments were accompanied by a fall in ascorbate which would be consistent with reversal of the glutamate/ascorbate heteroexchange. Further evidence, however, has come from
hippocampal and amygdala slices from kindled animals which have an enhanced potassium- or depolarisation-evoked glutamate release (Kaura et al., 1995). These potassium evoked glutamate releases are predominantly due to reversal of glutamate uptake. Thus the progressive seizure-evoked extracellular glutamate rises that occur during kindling might result from a decrease in glutamate uptake. This could be indirectly mediated by NMDA receptor activation, which increases arachidonic acid inhibition of glutamate uptake (Barbour et al., 1989).

In my experiments, repeated stimulations of the perforant path 3-13 minutes apart resulted in dwindling extracellular glutamate and ascorbate rises. Stimulation an hour later, however, resulted in comparable rises. Furthermore, following each perforant path stimulation, there was no detectable change in the length of consequent afterdischarges. The decreased glutamate rises could be due to:

1) increased synaptic and glial uptake of glutamate resulting in a smaller overspill into the extracellular fluid;

2) decreased vesicular glutamate release perhaps consequent upon activation of presynaptic receptors, or

3) decreased non-vesicular glutamate release (reverse glutamate uptake).

Decreased vesicular glutamate release, however, would be expected to lead to a diminution in the electrographic response to perforant path stimulation - an effect that was not observed. Therefore the most likely mechanism is that, following the initial stimulation, there is either decreased non-vesicular release or a short-lived enhancement of synaptic and glial glutamate uptake mechanisms in which glutamate/ascorbate heteroexchange plays little part. Indeed, transient (40 minute) increases in glutamate uptake have been previously described during soman-induced seizures (Lallement et al., 1991). Thus there may be short-term mechanisms to prevent rises in extracellular glutamate, but with repeated stimuli such as occur with kindling a long-term decrease in glutamate uptake may occur.

Decreases in the extracellular space have been proposed as a cause of amino acid rises following seizures. Although there is undoubtedly a decrease in the extracellular space, its importance in determining glutamate rises has not been
established. In most studies in which glutamate rises with seizures are observed, there is a variable effect of the seizure on other amino acids or as in the case of ascorbate, the rise occurs after the glutamate rise. This is inconsistent with the rises being solely due to a shrinkage of the extracellular space as this would be expected to lead to a simultaneous increase in all amino acids.

4.4.2 What is the role of extracellular glutamate rises?

Even with the methodological problems of microdialysis, large glutamate rises would have been detectable in all studies and what may have been missed are small, transient rises. That large extracellular glutamate rises were not detected in all animal seizure models implies that these rises are not necessary for seizure activity. This is perhaps not surprising as seizures are synaptically driven events, and it is thus the local concentration of glutamate in the synapse that is relevant. That extracellular glutamate rises may in fact be epiphenomena of seizure activity is further confirmed by the dissociation of the size of extracellular glutamate rises and seizure activity such that both large and imperceptible rises in glutamate observed in my experiments can be associated with indistinguishable epileptic afterdischarges.

The rises in extracellular glutamate that do occur are usually too small to activate non-NMDA receptors; non-NMDA receptors have an EC$_{50}$ of approximately 500µM (i.e. far in excess of extracellular glutamate rises that are observed). Since the fast decay time course of AMPA mediated EPSPs depends upon clearance of glutamate from the synaptic cleft, then there is a theoretical possibility that increasing the extracellular glutamate concentration could impair diffusion of glutamate from the synaptic cleft, thus prolonging the EPSP. The magnitude of this effect, however, is likely to be small. However, the extracellular glutamate rises that are observed are of a sufficient magnitude to activate NMDA receptors; so that when a neuron is later depolarised either through activation of AMPA receptors or via non-synaptic mechanisms, the magnesium blockade of the activated NMDA receptor is removed and calcium flows into the cell. Indeed, this system has positive feedback in that activation of NMDA receptors can result in a
decrease in high affinity glutamate uptake. Since activation of NMDA receptors is a requisite of kindling (McNamara et al., 1993), and since rises in extracellular glutamate are associated with kindling, then it is likely that these rises in extracellular glutamate contribute to the kindling process. Indeed, kindling can be induced by the local application of glutamate (Croucher & Bradford, 1989). A further piece of confirmatory evidence of glutamate's role in kindling is the diminution in glutamate rises in the above experiments with short interstimulus intervals, and identical glutamate rises with interstimulus intervals of 1 hour; this corresponds to the greater numbers of stimulations required to kindle if the stimulation interval is less than one hour (Racine et al., 1973).

What is the role of rises in extracellular glutamate in neurotoxicity? The role of the glutamatergic system in cell death is in little doubt. Application of large concentrations (100μM and above) of glutamate result in cell death in cell cultures (Choi et al., 1987), and in vivo (McBean & Roberts, 1984). It is incorrect, however, to assume that large extracellular glutamate rises must thus be associated with neurotoxicity or even necessarily have a significant role. Activation of NMDA receptors in the presence of glycine occurs at low glutamate concentrations, and it is the association of activation with depolarisation, which relieves the magnesium block, that results in the calcium influx causing neuronal death. In order to cause neuronal death by the application of glutamate, large concentrations are necessary to induce a significant depolarisation, and to overcome glutamate uptake. In neuronal cell culture Choi et al. concluded that glutamate uptake was not a consideration because DHK did not potentiate the glutamate-induced neuronal death (Choi et al., 1987). However, DHK is a weak inhibitor of glutamate uptake in neurons (see above), and it is thus possible that the administration of DHK resulted in minimal change to glutamate dynamics in the neuronal cell culture. The extracellular glutamate accumulation that occurs during ischaemia is surplus to requirement for the activation of NMDA receptors, and although it may play some part in depolarisation, it is the disruption of ionic homeostasis that is the main contributor to ischaemic depolarisation (Obrenovitch & Richards, 1994). Indeed, there is conflicting evidence for the role of these large glutamate rises in ischaemia being directly responsible for cell death at all, as cell
death appears to be due to events that occur after the ischaemic episode. The evidence that the large glutamate rises during ischaemia do not directly contribute to cell death is as follows (Obrenovitch & Richards, 1994; Szatkowski & Attwell, 1994):

1) Neuronal death can occur several hours after the period of ischaemia; glutamate, on the other hand, is cleared from the extracellular space within minutes of reperfusion.

2) Neurons in the area surrounding the ischaemic area (the penumbra) also die, and are very receptive to protection with glutamate antagonists, yet extracellular glutamate rises in this area during ischaemia are small.

3) Treatment with glutamate antagonists is neuroprotective in a number of models many hours after the ischaemic episode has taken place.

The explanation for these findings is not immediately obvious. It has been noted that a large calcium influx into neurons occurs during ischaemia, and a smaller, but increased, calcium influx occurs post-ischaemia (Szatkowski & Attwell, 1994). Why the latter smaller calcium influx should result in cell death, whilst the former large calcium influx does not is a matter for speculation. It has been suggested that oxygen is necessary for the formation of free radicals that result in cell death and that ironically the period of hypoxia could be neuroprotective (Szatkowski & Attwell, 1994). Nevertheless, the calcium influx and the activation of NMDA receptors that occur during ischaemia lead to a widespread and large potentiation of synaptic transmission involving both non-NMDA and NMDA receptors (Obrenovitch & Richards, 1994; Szatkowski & Attwell, 1994). Indeed, after ischaemia the NMDA receptor current shows less voltage-dependent magnesium block at negative potentials. The combination of this synaptic and NMDA potentiation following ischaemia result in the increased post-ischaemic calcium influx into neurons, which is susceptible to NMDA antagonist blockade.

That large extracellular glutamate rises alone do not necessarily lead to neuronal death has been demonstrated by experiments in which prolonged PDC (a glutamate uptake blocker) induced rises of extracellular glutamate to "neurotoxic" levels do not result in cell death (Massieu et al., 1995). Comparable glutamate
rises due to DHK (a different glutamate blocker) result, however, in significant neuronal death (Massieu et al., 1995). In the same study, kainic acid which resulted in no detectable rises in extracellular glutamate caused considerable neuronal death. Why the two uptake inhibitors had different effects on cell survival is not immediately clear. DHK is a relatively poor synaptic uptake blocker that has some action on glutamate-gated ion channels. PDC is a stronger more selective glutamate blocker that affects synaptic glutamate uptake. It could be that sustained synaptic rises in glutamate caused by PDC act on presynaptic terminals inhibiting the synaptic release of glutamate, and also that these sustained rises lead to non-NMDA receptor desensitisation. Both these effects would tend to prevent large post-synaptic depolarisations from occurring, thus preventing removal of the magnesium block of NMDA receptors and the neurotoxic influx of calcium. There is certainly evidence that PDC depresses excitatory synaptic transmission via glutamate activation of presynaptic metabotropic receptors that decrease synaptic glutamate release (Maki et al., 1994). Since synaptic concentrations of glutamate are not affected to a great degree by DHK, then these effects would not occur.

Thus, looking for sustained extracellular glutamate rises during status epilepticus is a quixotic mission. Only small rises in extracellular glutamate are probably necessary to potentiate NMDA mediated cell death; depolarisation is probably the main activator of these receptors in pathological conditions. Furthermore large rises in extracellular glutamate are not in themselves a sufficient explanation for neurotoxicity.
5. TREATMENT OF LATE STATUS EPILEPTICUS

5.1 Introduction

The neuroprotective effects of NMDA antagonists given prior to or shortly after the onset of status epilepticus in animal models has been well established (Lason et al., 1988, Clifford et al., 1990; Lerner Natoli et al., 1991, Fariello et al., 1989, Fujikawa et al., 1994; Fujikawa, 1995). Yet this does not address the practical issue of giving NMDA antagonists, because in the clinical situation these agents would be given once status epilepticus has been established. The purpose of this study was to establish if NMDA antagonists or other agents are significantly neuroprotective in the late stages of status epilepticus, and the hypotheses proposed are that:

1) late administration of potentially neuroprotective drugs in status epilepticus still result in neuroprotection, and

2) the resultant neuroprotection is greater than that that occurs following administration of standard antiepileptic drugs.

In order to address these hypotheses, I needed to develop a suitable model of status epilepticus-induced neuronal damage. Due to the unknown mechanisms, the model specific treatments and the differing pathologies of chemoconvulsant models of status epilepticus, I chose to develop an electrical model of status epilepticus. There are a variety of such models described, and so as a preliminary aspect of this study I compared different rat stimulation models with respect to the neuronal damage observed, the ease of development and consistency of the models. The drugs that I then chose to investigate were phenytoin, MK-801 and lamotrigine. MK-801 is a non-competitive NMDA antagonist that has been shown to be neuroprotective in a variety of models of status epilepticus and ischaemia. Phenytoin is routinely used in the treatment of status epilepticus and has been shown to be neuroprotective in models of ischaemia. Lamotrigine is a novel antiepileptic drug, marketed in the UK in 1991, which has also been shown to be neuroprotective in animal models of ischaemia; its role in status epilepticus, however, has not yet been fully characterised.
5.2 Choosing a model of status epilepticus

I chose to look at adaptations of 3 different stimulation protocols.

5.2.1 Experimental protocols

Protocol 1 & 2

The first was described by Lothman et al. (1989), and was tried in three rats. In this the stimulating electrode was implanted in the right hippocampus (from bregma 3.6mm caudal, 4.9mm lateral; 5.0mm ventral from dura). One week later, the hippocampus was stimulated with 1 msec biphasic pulses, 400 µA peak amplitude, at 50 Hz in 10 second trains every 11 seconds for 90 minutes. After which recordings were made from the stimulating electrode.

The second protocol used the same electrode implantation in three rats, but the stimulation protocol was an adaptation of that used by Cain et al. (1992). 10 minute trains of 3Hz, monopolar 0.5ms, 400 µA (peak amplitude) pulses, were delivered, each train was followed by a period of observation to see if continuous seizure activity was occurring. This was continued for a maximum of 2 hours. Three rats were used as controls and were implanted with the electrode but not stimulated.

Third protocol

The third protocol involved continuous stimulation of the perforant path, and this was carried out in anaesthetised and unanaesthetised animals, it was an adaptation of previously used protocols (Sloviter, 1983; Rogers et al., 1989; Ylinen et al., 1991). The animals were fully anaesthetised with 1-2% halothane in O₂. Body temperature was measured with a rectal probe and kept at 37±1°C. For stimulation, the stimulating electrode was advanced into the right angular bundle (co ordinates 4.4 mm lateral, 8.0 mm caudal to bregma) to stimulate the perforant path. A recording electrode was implanted into the hippocampus (co-ordinates 2.5
mm lateral, 4 mm caudal from bregma). The depths of the electrodes were adjusted to maximise the slope of the population excitatory post synaptic potential (EPSP) as described in chapter 2.

Test shocks consisted of 1-2 mA, 50 μs monopolar pulses every 10 seconds. The recording electrode was lowered until it entered the cell layer of dentate. The current was then adjusted in the range 2-3 mA to produce a population spike (amplitude 5-10 mV). Once in position, the electrodes were then left untouched for 2 hours.

The animals were divided into 4 groups. In groups 1 and 2, the halothane anaesthesia was maintained at 1-2% by volume. In group 1 (n=9) the perforant path was stimulated with 2-3 mA, 50 μs alternating monopolar pulses at 20 Hz for 2 hours under halothane anaesthesia (1-2%). In group 2 (n=3), the animals were unstimulated, but maintained under halothane anaesthesia (1-2%) for 2 hours and thus acted as a control for group 1. Both groups were then allowed to fully recover from the anaesthetic. In groups 3 and 4, the electrodes were held in place with dental acrylic and three skull screws. Both of these groups were allowed to recover fully from anaesthesia. In group 3 (n=3), 24-48 hours after recovery from anaesthesia the perforant path was stimulated with 2-3 mA, 50 μs alternating monopolar pulses at 20 Hz for 2 hours. Group 4 (n=3) received no stimulation (i.e. acted as a control for group 3).

5.2.2 Results from protocols 1&2

One rat from protocol 1 developed self-sustained status epilepticus that lasted at least 3 hours after which the animal was no longer monitored. The spiking continued after stimulation at 1-2 Hz. 2 weeks later histology was performed on that animal as described and stage 1 damage was noted in CA1 and CA3/4 bilaterally. No animals from protocol 2 developed status epilepticus, and there was no neuronal damage observed.
5.2.3 Results from protocol 3

Anaesthetised animals

In all animals (n=12) before stimulation, a field EPSP with a population spike with an amplitude of 5-10mV was obtained (figure 5.1).

Figure 5.1: Typical field potential recorded from dentate granule cell layer, prior to stimulation, demonstrating population spike.

The pattern of response to continuous stimulation was similar in all animals in group 1 (n=9). The initial response in the first minute to continuous stimulation was a diminution in the slope and height of the field EPSP with a loss of the population spike. Thereafter a large amplitude population spike reappeared, and remained for the next 6-12 minutes (figure 5.2). Then over a period of 30 seconds to one minute, the population spike diminished in size until it was indiscernible. The field potentials remained unchanged for the rest of the stimulation (figure 5.2).

After 2 hours, the perforant path was stimulated infrequently (every 30s-1 minute) to determine if the population spike would return, and by 10 minutes the population spike had returned in all animals.

14-17 days later there was no obvious hippocampal cell loss in any animal. To confirm this, regional neuronal cell densities and morphometry were performed in
three hippocampal sections from each of 3 of the stimulated and 3 control animals (table 5.1a,b).

![Graph showing response at 3 minutes and 6-240 minutes with a 4mV amplitude and 50ms scale.]

Figure 5.2: Typical response to constant stimulation of perforant path at 20Hz in halothane anaesthetised rats, showing presence of population spike at 3 minutes, which disappears by 6 minutes and then does not reappear for the duration of the stimulation. The polarity of the stimulating electrode alternates with each pulse; this varies the efficiency of each stimulus, and so results in alternating amplitude of the field potential with each pulse.

Cell densities were not significantly different between the control and stimulated animals for pyramidal cells in the hilus, CA1 or CA3, and there was no significant difference between the right and left hippocampi. Stimulation did, however, have a significant effect on morphometry with CA1 being significantly longer in stimulated animals (F(1,31)=5.28, p<0.05) and the area of the hilus being significantly greater in stimulated animals (F(1,31)=9.19, p<0.01). Furthermore the length of the right CA1 and the area of the right hilus were significantly smaller than that on the left (F(1,31)=4.22, p<0.05 and F(1,31)=5.43,
p<0.05 respectively). There was no significant interaction between side and stimulation, or stimulation and slice position.

Table 5.1a: Neuronal cell densities

<table>
<thead>
<tr>
<th></th>
<th>Right hilus</th>
<th>Left hilus</th>
<th>Right CA1</th>
<th>Left CA1</th>
<th>Right CA3</th>
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<tbody>
<tr>
<td></td>
<td>x10⁴ neurons/mm³</td>
<td>x10⁴ neurons/mm³</td>
<td>x10⁶ neurons/mm³</td>
<td>x10⁶ neurons/mm³</td>
<td>x10⁶ neurons/mm³</td>
<td>x10⁶ neurons/mm³</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.72</td>
<td>8.12</td>
<td>7.19</td>
<td>6.99</td>
<td>2.74</td>
<td>2.72</td>
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<td>SEM</td>
<td>0.36</td>
<td>0.33</td>
<td>0.31</td>
<td>0.40</td>
<td>0.15</td>
<td>0.24</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
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<td>7.06</td>
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<td>0.21</td>
<td>0.26</td>
<td>0.21</td>
<td>0.38</td>
<td>0.16</td>
<td>0.10</td>
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</table>

Table 5.1b: Lengths and areas of hippocampal regions

<table>
<thead>
<tr>
<th></th>
<th>Right hilus</th>
<th>Left hilus</th>
<th>Right CA1</th>
<th>Left CA1</th>
<th>Right CA3</th>
<th>Left CA3</th>
</tr>
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<td></td>
<td>area (mm²)</td>
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<td>length (mm)</td>
<td>length (mm)</td>
<td>length (mm)</td>
<td>length (mm)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.58</td>
<td>0.65</td>
<td>4.2</td>
<td>4.53</td>
<td>2.38</td>
<td>2.28</td>
</tr>
<tr>
<td>SEM</td>
<td>0.04</td>
<td>0.02</td>
<td>0.31</td>
<td>0.28</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Stimulated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.66</td>
<td>0.73</td>
<td>4.57</td>
<td>4.87</td>
<td>2.36</td>
<td>2.44</td>
</tr>
<tr>
<td>SEM</td>
<td>0.02</td>
<td>0.02</td>
<td>0.26</td>
<td>0.27</td>
<td>0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

*Unanaesthetised animals*

24-48 hours after electrode implantation, stimulation of the perforant path gave a similar response to that seen in anaesthetised animals (figure 5.1). Again, the pattern of response to continuous stimulation was similar in all animals in group 3.
(n=3). Initially, the population spike diminished and the amplitude and slope of the field EPSP decreased. Thereafter, the population spike returned, but decreased and increased in amplitude in a cyclical fashion over 5-10 minute periods. After approximately 1 hour, regular, frequent, and spontaneous depolarisations occurred. After the 2 hour stimulation had stopped, regular spiking at 1Hz and above continued for at least 3 hours and on one occasion for greater than 24 hours (figure 5.3).

Figure 5.3: Prestimulation baseline EEG and EEG 30 minutes after stimulation had ceased showing regular spiking which continued for at least 3 hours in all cases.

During the period of stimulation, seizures (class 1 to 5) occurred. After stimulation, exploratory behaviour and class 1 to 2 seizures occurred.

Histology revealed moderate to severe cell loss in the hilus, CA1 and CA3 regions in all stimulated animals (figure 5.4). CA1 had the greatest amount of cell loss, then the hilus and finally CA3. A small area between CA1 and CA3, corresponding to CA2, was relatively unaffected by stimulation. No neuronal cell loss was detectable in the unstimulated animals, group 4. Thus this protocol in unanaesthetised animals was chosen for the drug assessment.
Figure 5.4: Histology of hippocampus from (A) stimulated animal and (B) unstimulated animal, and close up of CA1 of (C) stimulated animal and (D) unstimulated animal. Sections were stained with Nissl stain. (Scale: A, B ×20; C,D ×200)
5.3 Assessment of drugs in late status epilepticus.

5.3.1 Experimental protocol

Implantation and stimulation were as described for protocol 3 in unanaesthetised animals above except that the time from implantation to stimulation was 3-5 days, and monopolar pulses were used. After 2 hours stimulation, the rats (n=15) were randomised to intraperitoneal injections of either 20mg/kg lamotrigine (20mg/ml in 50% ethylene glycol), 50mg/kg phenytoin (50mg/ml in 50% ethylene glycol), 1 mg/kg MK-801 (1mg/ml in saline), or equivalent volumes of 50% ethylene glycol or saline (these last two groups were control groups). The EEG was monitored for a further 3 hours after which time if the animal was still in status epilepticus, it was given 30-40mg/kg pentobarbitone i.p. Two weeks later the animals were killed and histology was performed as described.

5.3.2 Results

In all 15 animals, the stimulation protocol resulted in self-sustaining status epilepticus. During stimulation, the rats all exhibited behavioural seizures and all rats had stage 5 seizures. The EEG pattern during stimulation was as described above. After 2 hours most animals exhibited stage 0-2 seizures often in a cyclical fashion. The EEG usually demonstrated cyclical activity of 1-3 Hz spikes with occasional fast spiking. None of the drugs given terminated the status epilepticus, and there was little to distinguish the various drugs or vehicle with respect to their effect on EEG or behavioural seizures. MK-801 did appear to lessen the motor component of the seizures and resulted in some sedation, but this observation was not quantified and was unblinded. Furthermore, MK-801 seemed to lessen the amplitude, but increase the frequency of the spikes. After 3 hours, all animals still had behavioural and electrographic seizures. Following intraperitoneal pentobarbitone, behavioural and electrographic seizures stopped within 3-6 minutes, and the animal slept for usually 2 hours. There was no recurrence of seizure activity after this time.
Histology showed stage 2-3 (moderate to severe) neuronal damage in CA1, CA3 and hilus (figure 5.5). There was no significant difference between the damage in the left and right hippocampi. There was also no significant difference between any of the treatment groups (table 5.2).

Table 5.2: Severity of damage to CA1, CA3 and hilar neurons following different treatments. Figures given are median and range

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CA1</th>
<th>CA3</th>
<th>Dentate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>3</td>
</tr>
<tr>
<td>MK-801</td>
<td>3 (2-3)</td>
<td>2 (2-3)</td>
<td>3 (2-3)</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>2.5 (2-3)</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>2.5 (2-3)</td>
<td>2 (1-3)</td>
<td>3</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>3 (2-3)</td>
<td>2.5 (2-3)</td>
<td>3</td>
</tr>
</tbody>
</table>

0=no damage, 1=mild damage, 2=moderate damage, 3=severe damage

In further experiments in 5 rats, I looked at the role of intraperitoneal diazepam (20mg/kg) at stopping the status epilepticus after the 2 hour stimulation. In all animals diazepam stopped the electrographic discharges without any recurrence of seizure activity at three hours after the stimulation had stopped. Since these animals were not randomised to a treatment, they could not be included in the statistical analysis of the histopathology. They had, however, only stage 1-2 damage in CA1, CA3 and dentate hilus, implying that stopping the status epilepticus earlier may offer some neuroprotection.
Figure 5.5: Comparison of histology of hippocampi (×20) and CA1 (×200) from animals in status epilepticus treated with (A) saline, (B) MK-801, (C) propylene glycol, (D) lamotrigine and (E) phenytoin showing no obvious effect of treatment. Sections were stained with Nissl stain.
5.4 Discussion

I chose the third stimulation protocol as protocols one and two were neither consistent nor easy to use (there was the disadvantage of recording and stimulating via the same electrode). There are a number of reasons why these models may have failed. In the original descriptions, the animals were pre-selected prior to stimulation by the ease with which afterdischarges could be obtained from the stimulating electrode (Lothman et al., 1989; Cain et al. 1992). This is a means by which electrode position and function can be tested. In neither paper is the percentage of animals discarded because of failure at this stage described. Furthermore, in these studies, the majority, but not all rats, using these stimulating protocols developed status epilepticus. Thus there is a preselection process following which not all animals develop status epilepticus. In addition in the protocol described by Cain et al. (1992), the rats were different (Victoria hooded) and the stereotaxic co-ordinates that they used were not given.

Protocol 3 has the advantage of neurophysiological localisation, so that the function and position of the electrodes are confirmed during surgery. Furthermore by having separate electrodes for stimulation and recording it is possible to record during the stimulation protocol. Initially, I wished to have an anaesthetised model of status epilepticus, as it would then have been possible to make detailed recordings of glutamate rises during status epilepticus using the protocols described in chapter 4. However, halothane appeared to prevent status epilepticus-induced neuronal damage, and so I turned to the unanaesthetised model. Constant stimulation of the perforant path in the unanaesthetised rats resulted in severe hippocampal cell loss such as been observed in other studies of perforant path stimulation in unanaesthetised rats and rats anaesthetised with urethane (Sloviter, 1983; 1987; Rogers et al., 1989; Ylinen et al., 1991a; Ylinen et al., 1991b). Sloviter described in some rats undergoing constant perforant path stimulation a cyclical change in the amplitude of the dentate granule cell population spike (Sloviter, 1983), and it was this pattern that I observed in the unanaesthetised animals. Sloviter also observed that in approximately 20% of rats anaesthetised with urethane the population spike disappeared, and in those animals no detectable hippocampal cell damage occurred (Sloviter, 1983). This is similar to
the phenomenon that I observed in all the halothane anaesthetised animals. That my observations were not due to a displacement of the stimulating electrode was confirmed by the re-emergence of the population spike after the continuous stimulation had stopped. Halothane thus appears to have a delayed effect (6-12 minutes) in preventing the dentate granule cell population spike despite, during this period, there being no detectable change in the slope or amplitude of the field EPSP. The population spike is the summation of the surrounding action potentials, and thus either desynchronisation and temporal dispersion of the action potentials or decreased numbers of action potentials must be occurring. Both these effects could be neuroprotective, since the population spikes represent activation of a system of axons which terminate on hilar neurons, and the pyramidal cells of CA1 and CA3. In chapter 4, I found that repeated, prolonged epileptic afterdischarges (6-12s) following 10s stimulations of the perforant path occur under halothane anaesthesia. This and the presence of the population spike for the first 6-12 minutes of continuous stimulation suggest that the mechanisms underlying production of afterdischarges and the initial production of excessive and synchronised discharges are different from those necessary for the later maintenance of these discharges. Halothane thus seems either to potentiate late inhibitory mechanisms or to inhibit late excitatory mechanisms which are necessary for the maintenance of excessive, synchronised discharges. That halothane is neuroprotective, either via or independent of this action, is confirmed by the absence of cell loss. The similarity in cell density between stimulated and unstimulated animals could be due to cell loss and volume shrinkage. The morphology of the stimulated animals, however, is consistent with swelling rather than shrinkage - a phenomenon that has been observed in both humans and animals following seizures. It is also noteworthy that the right hippocampus appears to be shrunken compared to the left hippocampus in both stimulated and control animals; an effect that is probably due to the recording electrode implantation in the right hippocampus.

Halothane has been found to be neuroprotective in a number of studies of ischaemia (Freund et al., 1990; Warner et al., 1993), although some of its efficacy may be due to its effects on brain temperature. Nevertheless, when brain
temperature is kept constant, halothane still has a neuroprotective effect (Warner et al., 1995). The mechanisms underlying this, and indeed the mechanisms underlying halothane's anaesthetic effect have not been established. Halothane has a number of properties which could be neuroprotective and antiepileptic. Halothane depresses synaptic transmission (Richards & White, 1975). It has a presynaptic effect decreasing calcium entry possibly through an action on specific voltage-gated calcium channels, which results in decreased glutamate release (Miao et al., 1995). Halothane has a post-synaptic effect in prolonging inhibitory postsynaptic currents through enhancement of GABA<sub>A</sub>-mediated inhibition (Gage & Robertson, 1985; Mody et al., 1991). Indeed, in a study in CA1, halothane had no effect on excitatory potentials or long-term potentiation, but it significantly prolonged paired pulse inhibition compared to urethane anaesthesia (Pearce et al., 1989). This may partly explain the different effects of urethane and halothane on the dentate granule cell responses to prolonged perforant path stimulation. Halothane also has a number of non-synaptic, possible antiepileptic effects, including closure of astrocyte gap junctions (Mantz et al., 1993), and direct hyperpolarisation of neurons possibly via an increase in potassium conductance (Nicoll & Madison, 1982).

I have thus observed a neuroprotective effect for halothane in a model of status epilepticus-like neuronal damage. This effect may be due in part to the inhibition of excessive synchronised discharges by halothane, but halothane may also have a primary neuroprotective effect. This needs to be confirmed in other models of status-induced neuronal damage, and importantly at later stages in status epilepticus. Furthermore, anaesthetics that have similar modes of action, but which may be more practical to use (e.g. propofol), should also be studied.

The resistance of status epilepticus after two hours stimulation to treatment with large doses of lamotrigine, phenytoin or MK-801, whilst responding to diazepam is of interest. Phenytoin is very effective in the initial treatment of status epilepticus in humans (Leppik et al., 1983b; Wilder et al., 1977), and in animal models is effective in preventing the onset of status epilepticus and in stopping status epilepticus shortly after its initiation (Morrisett et al., 1987; Walton & Treiman, 1988). However, once the status epilepticus has been established it
appears to be resistant to treatment with phenytoin whilst it is often responsive to
diazepam or phenobarbitone, drugs that act on the GABA<sub>A</sub> receptor (Morrisett et
al., 1987; Sofia et al., 1993; Bertram & Lothman, 1990; Handforth & Treiman,
1994). This is consistent with the idea that the initiation and maintenance of status
epilepticus have different underlying mechanisms. That drugs acting on the
GABA<sub>A</sub> receptor are effective in stopping late status epilepticus may be due to
the later stages of status epilepticus being dependent upon the disinhibition that
occurs through neuronal death and changes in the GABA<sub>A</sub> receptor (Sloviter,
1987, Kapur et al., 1994). The inefficacy of lamotrigine at this stage is a new
finding although Walton et al. (1996) have recently found lamotrigine to be
ineffective in the early stages of two animal models of status epilepticus that
responded to early treatment with phenytoin. My findings, however, are quite
distinct and the failure of lamotrigine in their model may be due to its
neuropharmacokinetics; although lamotrigine enters the brain ECF relatively
rapidly, it does not exhibit the large peak seen with phenytoin. The failure of
lamotrigine in my model is likely to be for the same reasons as the failure of
phenytoin as the action of these two drugs is similar. The failure of MK-801 to
suppress completely electrographic activity whilst possibly having an effect on
motor seizure activity is similar to findings in other studies of MK-801 given
prior to the induction of the status epilepticus (Clifford et al., 1990; Lerner Natoli
et al., 1991; Clifford et al., 1989; Fariello et al., 1989), and in the late stages of
status epilepticus (Walton & Treiman, 1991). Interestingly in this last study, MK-
801 given in combination with diazepam stopped the electrographic and
behavioural manifestations of status epilepticus, whilst either drug alone did not
(Walton & Treiman, 1991). One study did find that 1mg/kg MK-801 i.p. given
after the electrical induction of status epilepticus did suppress electrographic
discharges (Bertram & Lothman, 1990). In this study, MK-801 was given 2 hours
after the cessation of a 90 minute stimulation protocol when the EEG was
characterised by periodic epileptiform discharges (PEDs) with intermittent
superimposed bursts of fast activity or PEDs alone; this is a much later stage than
the stage at which MK-801 was given in my study. Furthermore in this same
study (Bertram & Lothman, 1990), only 5/8 animals responded to the first
injection and it may be that 1 mg/kg MK-801 is on a steep part of a dose-response curve so that slight under- or over-dosing may have a large effect on the response of status epilepticus to this treatment; indeed, in a study on soman-induced status epilepticus, pre-treatment with 0.5 or 1 mg/kg doses of MK-801 did not prevent nor delay the onset of seizure activity, whilst 5 mg/kg MK-801 completely prevented the development of seizure activity and brain damage (Sparenborg et al., 1992). Further light has been shed on the matter by Young & Dragunow (1993) who showed that MK-801 did not terminate generalised convulsive status epilepticus, but terminated limbic status epilepticus. Thus the effects of MK-801 may be dose related, model dependent and stage of status epilepticus dependent; factors that deserve further investigation.

The effectiveness of pentobarbital to terminate refractory status epilepticus regardless of its resistance to other drugs is a common finding in humans (Van Ness, 1990; Rashkin et al., 1987; Lowenstein et al., 1988; Jagoda & Riggio, 1993); this finding has been further confirmed in my animal model.

I used an ordinal scoring system for determining the degree of damage following status epilepticus rather than neuronal densities. This is because changes in neuronal densities are determined both by neuronal loss and by changes in morphometry. I noted that when severe damage occurred, this was accompanied by shrinkage such that the change in cell density did not accurately reflect cell loss. Kelly and McIntyre (1984) have developed an ordinal brain damage score that depends upon both neuronal loss and glial proliferation. They have successfully used this scale to demonstrate a neuroprotective effect of kindling against kainic acid-induced status epilepticus (Kelly & McIntyre, 1994). Using this scale, I failed to find any effect of MK-801, phenytoin or lamotrigine in preventing neuronal damage at a late stage of status epilepticus. This is in contrast to the potency of neuroprotectants many hours after the insult in models of ischaemia (Obrenovitch & Richards, 1994; Szatkowski & Attwell, 1994). This implies that at the very least the temporal aspects of the mechanisms leading to neurotoxicity in ischaemia and status epilepticus differ, and it is possible that the mechanisms themselves are dissimilar. There may have been some neuroprotection which my method of analysis was not sensitive enough to detect;
however, the magnitude of such an effect would be small, and, in the face of the severe neuronal damage that occurs, it is unlikely to be clinically relevant. Although, it was not possible to compare the effect of diazepam on the histopathology, as the animals were not randomised to this treatment and the histopathology was analysed at a later date, it did appear that stopping the status epilepticus earlier conferred some neuroprotective effect. This is consistent with the finding of others that the degree of neuronal damage is dependent upon the length of time that the animal spends in status epilepticus (Sloviter, 1983; Nevander et al., 1985; Meldrum and Brierley, 1973).
6 AUDITS OF THE TREATMENT OF STATUS EPILEPTICUS

6.1 Introduction

The first-line treatment of convulsive status epilepticus is effective in 70-80% of cases, but when this fails the patient should be transferred to an intensive care unit where intensive monitoring can take place, and where general anaesthesia can be given if necessary (Jagoda & Riggio, 1993; Shorvon, 1994).

The management of convulsive SE resistant to first line therapy is controversial. In order to determine the present practice in the UK, I undertook a survey of which antiepileptic drugs and which anaesthetic agents (drug-induced coma) are favoured in the UK in the management of SE following failure of first line therapy, when intensive care treatment is necessary.

Also, I have reviewed the patients admitted to a neurological ICU with a diagnosis of refractory SE in order to determine: 1) the type of SE, and the accuracy of previous diagnoses; 2) why first-line therapy failed, and 3) the effectiveness of the therapies that were instigated.

6.2 Results

6.2.1 UK survey

There were 434 replies (62.5%) to the survey; 28 of the questionnaires were returned uncompleted. Thus there were a total of 408 completed returns: 358 were completed by Consultants, 37 by senior registrars and 13 by other grades. 346 of the respondents gave their speciality as anaesthetics, although 46 considered intensive care their main speciality and the remainder were physicians or neurosurgeons. 45 worked on an intensive care which had a specialisation in neurosurgery, and 36 on an intensive care which had a specialisation in neurology. 228 worked in an adult ICU, 16 in a paediatric ICU and 140 in a mixed paediatric and adult ICU.
1) In answer to the question "which drug would you use next, and which other drugs would you consider alternatives?"

Following phenytoin and diazepam, the preferred therapeutic option and alternatives that would be considered are shown in table 6.1

Table 6.1: Agents favoured for the treatment of status epilepticus resistant to first line treatment with phenytoin and diazepam.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Number who would consider this their preferred agent</th>
<th>Number who would consider this an alternative to their preferred agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzodiazepine infusion</td>
<td>142</td>
<td>93</td>
</tr>
<tr>
<td>General anaesthetic</td>
<td>130</td>
<td>112</td>
</tr>
<tr>
<td>Chlormethiazole</td>
<td>51</td>
<td>95</td>
</tr>
<tr>
<td>Thiopentone (subanaesthetic dose)</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>Phenobarbitone</td>
<td>26</td>
<td>57</td>
</tr>
<tr>
<td>Paraldehyde</td>
<td>8</td>
<td>63</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td>29</td>
</tr>
</tbody>
</table>

The majority would use a benzodiazepine infusion (35%) or a general anaesthetic (32%), but significant numbers would use or consider using chlormethiazole, paraldehyde, phenobarbitone or subanaesthetic doses of thiopentone. Other replies included vigabatrin, subanaesthetic doses of propofol, magnesium, sodium valproate and carbamazepine. In the subgroup working on paediatric ICUs the preferred agents in order of frequency were phenobarbitone (31%), general anaesthetic (25%), paraldehyde (19%), benzodiazepine infusion (12.5%) and thiopentone infusion (12.5%).
2) In answer to the question "if the patient continues to fit despite the above lines of action, after what period of time since seizures began do you consider anaesthetising the patient?"

Most (57%) would institute general anaesthesia within 60 minutes from the start of the SE, although a significant proportion (34%) gave no set period (see table 6.2).

Table 6.2: Time interval that was considered appropriate from onset of status epilepticus to induction of general anaesthesia

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>No set interval</td>
<td>138</td>
</tr>
<tr>
<td>Only if breathing is compromised</td>
<td>7</td>
</tr>
<tr>
<td>Within 60 minutes</td>
<td>231</td>
</tr>
<tr>
<td>60-120 minutes</td>
<td>31</td>
</tr>
<tr>
<td>Longer than 120 minutes</td>
<td>1</td>
</tr>
</tbody>
</table>

3) In answer to the question "which anaesthetic agent would you prefer to use and which others would you consider suitable alternatives?"

The details of the preferred anaesthetics and those that would be considered alternatives are shown in table 6.3.

The most popular anaesthetic agent was thiopentone; this was the preferred agent of 82% of respondents, and considered as an alternative by 68% of the remainder. A significant number (46%) would use or consider using propofol. Other anaesthetic agents were considered as alternatives by some of the respondents.
Table 6.3: General anaesthetic agents favoured for second line management of status epilepticus resistant to first line treatment

<table>
<thead>
<tr>
<th>Agent</th>
<th>Number who would consider this their preferred agent</th>
<th>Number who would consider this an alternative to their preferred agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiopentone</td>
<td>333</td>
<td>51</td>
</tr>
<tr>
<td>Propofol</td>
<td>56</td>
<td>133</td>
</tr>
<tr>
<td>Pentobarbitone</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>6</td>
<td>31</td>
</tr>
<tr>
<td>Etomidate</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

4) In answer to the question "how would you monitor the effectiveness of your treatment in the anaesthetised patient given your present facilities?"

45% monitored the patient under general anaesthesia using solely clinical criteria (in some cases this was due to EEG facilities not being available), although 30% used a cerebral function monitor (CFM or CFAM) and 23% used intermittent EEG (see table 6.4).

Table 6.4: Types of monitoring of status epilepticus

<table>
<thead>
<tr>
<th>Type of Monitoring</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent EEG</td>
<td>92</td>
</tr>
<tr>
<td>Continuous EEG</td>
<td>12</td>
</tr>
<tr>
<td>Cerebral function monitor (CFM or CFAM)</td>
<td>122</td>
</tr>
<tr>
<td>Clinical monitoring only</td>
<td>1</td>
</tr>
</tbody>
</table>

Those working on an intensive care with a specialisation in neurology or neurosurgery were much more likely to use a CFM (54%) as opposed to clinical
criteria alone (17%), and this was also true of paediatric intensive care units (63% as opposed to none).

Only 50 (12%) of the respondents had a protocol for SE on their intensive care.

6.2.2 ICU audit

Patients and diagnosis (table 6.5)

The median age was 33 years (17-73 years), and there were 15 men and 11 women. 18 had had a previous history of epilepsy. 46% of patients had been incorrectly diagnosed as having refractory status epilepticus. The diagnoses of the patients on admission to NHNN were: SE in 14; previous SE and drug-induced coma in 4; previous SE, and encephalopathy in 1; pseudostatus in 6, and post-hypoxic myoclonus in 1. Of the 14 (54%) in SE: 9 had convulsive SE, 3 had partial SE, 1 had tonic SE and 1 had non-convulsive SE.

The patients with pseudostatus tended to be younger than those with true SE (median age 21.5 compared to 34.0), and had a greater female to male ratio (4:2 compared to 7:12). A previous diagnosis of epilepsy was present in 67% of patients with pseudostatus and 73% of patients who had or had had true SE.

The aetiology of the SE in the 19 who had had or were in SE was unknown in 6 cases, and due to drug reduction or withdrawal in 5 cases of which 2 were due to poor compliance. Other causes included: encephalitis, theophylline toxicity, neurocytoma, progressive myoclonic epilepsy, alcohol, lymphoma and cortical dysplasia.

Treatment prior to transfer

A range of treatments was used in the referring hospital prior to transfer (table 6.6). Benzodiazepines (usually intermittent intravenous diazepam, although there were also instances of diazepam, clonazepam or midazolam infusions) were most frequently used followed by phenytoin and chlormethiazole. All four patients who were in drug induced coma had had chlormethiazole, often in large doses (in one
case over 10l of 0.8% solution in 4 days). Rectal paraldehyde and phenobarbitone were occasionally used.

Table 6.5: Diagnoses

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>number</th>
<th>median age and range (years)</th>
<th>sex ratio (male:female)</th>
<th>number with previous history of epilepsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>convulsive SE</td>
<td>9</td>
<td>31 (17-73)</td>
<td>4:5</td>
<td>7</td>
</tr>
<tr>
<td>partial motor SE</td>
<td>3</td>
<td>21 (19-44)</td>
<td>3:0</td>
<td>2</td>
</tr>
<tr>
<td>tonic SE</td>
<td>1</td>
<td>34</td>
<td>0:1</td>
<td>1</td>
</tr>
<tr>
<td>non-convulsive SE</td>
<td>1</td>
<td>60</td>
<td>1:0</td>
<td>0</td>
</tr>
<tr>
<td>convulsive SE/encephalopathy</td>
<td>1</td>
<td>44</td>
<td>1:0</td>
<td>0</td>
</tr>
<tr>
<td>convulsive SE/drug induced coma</td>
<td>4</td>
<td>35 (21-45)</td>
<td>3:1</td>
<td>4</td>
</tr>
<tr>
<td>pseudostatus epilepticus</td>
<td>6</td>
<td>21.5 (17-45)</td>
<td>2:4</td>
<td>4</td>
</tr>
<tr>
<td>post hypoxic myoclonus</td>
<td>1</td>
<td>61</td>
<td>1:0</td>
<td>0</td>
</tr>
</tbody>
</table>

I was able to assess the loading dose of phenytoin in 16 cases; 7 patients (44%) had not received an adequate loading dose (taken as less than 750mg), and 4 had possibly not received an adequate loading dose (taken as 750mg to less than 1000mg). Indeed in two cases the loading dose for phenytoin was 200mg or less. One of the two patients given phenobarbitone had not received an adequate loading dose.

Antiepileptic drug (AED) serum concentrations were performed on transfer and before further treatment in 14 cases for phenytoin, and 8 cases for carbamazepine in patients supposedly receiving those AEDs. 43% on phenytoin and 50% on carbamazepine fell below the lower limit of the therapeutic range for the NHNN laboratory (40 µmol/l for phenytoin and 20 µmol/l for carbamazepine). Overall the median level and range were 47.5 µmol/l (12-96µmol/l) for phenytoin and 24.5 µmol/l (0-43µmol/l) for carbamazepine.
Only 50% of patients were intubated prior to transfer. A greater proportion of patients with pseudoseizures than patients with convulsive SE were intubated prior to transfer.

Table 6.6: Treatment at referring hospital prior to transfer

<table>
<thead>
<tr>
<th>Condition</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>convulsive SE (9)</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>partial motor SE (3)</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>tonic SE (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-convulsive SE (1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>convulsive SE/drug induced coma or encephalopathy (5)</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pseudostatus epilepticus (6)</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>post hypoxic myoclonus (1)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total (26)</td>
<td>3</td>
<td>18</td>
<td>22</td>
<td>2</td>
<td>17</td>
<td>13</td>
</tr>
</tbody>
</table>

A = paraformaldehyde, B = phenytoin, C = benzodiazepine, D = phenobarbitone, E = chlormethiazole, F = intubated

Treatment after transfer.

Loading doses and adequate maintenance doses of AEDs were given as necessary. Patients in drug induced coma had their chlormethiazole stopped, were given adequate doses of AEDs and when necessary were weaned from their anaesthesia and extubated.

Ten patients in SE required intubation and anaesthesia (table 6.7). Two patients in convulsive SE did not require anaesthesia. One patient, who had received an inadequate loading dose of phenytoin, responded to a further bolus of phenytoin and one, who had been treated with chlormethiazole alone, responded to boluses of phenytoin and phenobarbitone. One patient with partial motor SE did not require anaesthesia as he responded to a bolus of phenobarbitone (700mg).
The commonest anaesthetic agent, used in 7 cases, was propofol (1-10 mg/kg/h with 5 mg/kg/h usually being sufficient). Most patients responded well to which ever anaesthetic agent was chosen. In the case of non-convulsive SE, the patient was tried on thiopentone but this resulted in difficulties with hypotension and so the patient was transferred onto propofol. In the case of partial motor SE due to cortical dysplasia, neither propofol nor thiopentone were able to control the seizures and thus ketamine (100 mg/h) was successfully used. This patient was eventually fully controlled following a subpial transection.

EEG monitoring was performed mostly using standard 10-20 system of scalp electrodes intermittently every 12-24 hours. In 3 of the earlier cases of convulsive SE a cerebral function monitor was used. Burst suppression was only achieved in 3 patients and was achieved with propofol, thiopentone and midazolam. The attainment of burst suppression was not associated with a better outcome for those patients; indeed, two of them subsequently died. In most patients adequate anaesthesia resulted in widespread slow activity with the abolishment of epileptic activity.

Table 6.7: Anaesthetic agents used

<table>
<thead>
<tr>
<th></th>
<th>intubated</th>
<th>propofol</th>
<th>midazolam</th>
<th>thiopentone</th>
<th>ketamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>convulsive SE (9)</td>
<td>7</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>partial motor SE (3)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>tonic SE (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-convulsive SE (1)</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>total (14)</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Complications

The commonest complication was chest infection which occurred in 8 patients all of whom had been ventilated (table 6.8). Four patients had cardiac arrests prior to admission to the ICU. Two of these had cardiac arrests during convulsive SE; in one this occurred in the ambulance on the way to the referring hospital before she
had received any drug treatment, and the other had a cardiorespiratory arrest in the referring hospital following treatment with phenytoin, diazepam and then a chlormethiazole infusion. One patient had a cardiac arrest in the referring hospital prior to the episode of convulsive SE; this patient was an alcoholic who was also taking oral temazepam and chlormethiazole. One patient had a cardiac arrest in the referring hospital which resulted in post-hypoxic myoclonus. The cardiac arrests in these last two were antecedent to the status epilepticus, and were thus not included in table 6.8. One patient who had been in partial motor SE for 4 months and had been ventilated for a large part of this time, regained consciousness but developed a severe critical illness neuropathy.

Table 6.8: Complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>chest infection</th>
<th>urinary tract infection</th>
<th>renal failure</th>
<th>hepatic failure</th>
<th>septicemia</th>
<th>cardiac arrest</th>
<th>neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>convulsive SE (9)</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>partial motor SE (3)</td>
<td>1</td>
<td></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>tonic SE (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-convulsive SE (1)</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>convulsive SE/drug induced coma or encephalopathy (5)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pseudostatus epilepticus (6)</td>
<td>1</td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post hypoxic myoclonus (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total (26)</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Outcome

The eventual neurological outcome for all patients is unknown; of those transferred conscious, one had developed a left hemiplegia secondary to his neurosarcoidosis and one was noted to have had a worsening of her pre-existing left pyramidal weakness.
22 patients were either discharged or transferred back to the referring hospital conscious (table 6.9). Two of the remaining 4 patients died at NHNN: one with non-convulsive SE and encephalitis died from renal failure, the other was the patient who, prior to admission to hospital, had a cardiac arrest - an event from which she never recovered. The two patients who had antecedent cardiac arrests were unconscious on transfer back to the referring hospitals.

In all cases, SE was successfully controlled.

<table>
<thead>
<tr>
<th>Table 6.9: Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>transferred conscious</td>
</tr>
<tr>
<td>convulsive SE (9)</td>
</tr>
<tr>
<td>partial motor SE (3)</td>
</tr>
<tr>
<td>tonic SE (1)</td>
</tr>
<tr>
<td>non-convulsive SE (1)</td>
</tr>
<tr>
<td>convulsive SE/drug induced coma or encephalopathy (5)</td>
</tr>
<tr>
<td>pseudostatus epilepticus (6)</td>
</tr>
<tr>
<td>post hypoxic myoclonus (1)</td>
</tr>
<tr>
<td>total (26)</td>
</tr>
</tbody>
</table>

6.3 Discussion

My survey reveals that in the UK, following initial management with intravenous phenytoin and diazepam: benzodiazepine infusion or general anaesthetic would be the preferred second lines of treatment in adult intensive care units; in paediatric intensive care units phenobarbitone was the agent of choice. General anaesthesia would be induced within 60 minutes of the start of SE; most would use thiopentone as the anaesthetic agent, and the patients would be monitored by the majority using clinical assessment only, except on paediatric intensive care units and specialist neurological or neurosurgical intensive cares where the majority would use a CFM.
From the audit it is apparent that the diagnosis and treatment of SE prior to transfer to the ICU are inadequate, and that treatment of refractory SE in a specialist ICU results in a good outcome with few serious complications.

**Diagnosis**

A high incidence of pseudostatus presenting as refractory SE has been previously reported (Howell et al., 1989), but from my audit it is also apparent that a large proportion of patients with a diagnosis of 'refractory SE' have pseudostatus. The inadequacy of diagnosis from most referring centres is partly due to absent or insufficient EEG services available at those centres. As has been found in another study (Howell et al., 1989), many patients with pseudostatus had a previous diagnosis of epilepsy which may have confounded the diagnosis. Pseudostatus is often misdiagnosed as true SE and is often refractory to initial therapy (leading to general anaesthesia and mechanical ventilation) (Howell et al., 1989). It is thus not only a dangerous misdiagnosis, but also a considerable drain on hospital facilities. Failure by admitting doctors at the referring hospital to recognise the possibility of pseudostatus was common in my audit. Pseudostatus should be considered if an episode of SE does not respond promptly to initial therapy (especially if the seizures are in any way atypical). Although in my and other studies (Howell et al., 1989), there is a higher female to male ratio, it is important to note that pseudostatus can be present in both sexes.

A significant number of patients in my audit were in drug-induced coma rather than SE. These referrals possibly result from a misunderstanding of the pharmacokinetics of chlormethiazole and benzodiazepines, which when given acutely redistribute with a short redistribution half-life, but when given as repeat boluses or as infusions accumulate. This accumulation leads to a prolongation of half-life and a diminution in the volume of distribution resulting in an increased and prolonged effect. Indeed from my audit, one cardiorespiratory arrest prior to transfer probably resulted from a prolonged chlormethiazole infusion.
Management prior to transfer to ICU

In my audit, the aetiology of the SE was AED reduction or withdrawal in 21% of those who were still in SE. This is equivalent to other studies of SE (Lowenstein & Alldredge, 1993; Barry & Hauser, 1994). Importantly reintroduction of a withdrawn AED often results in termination of the SE; however, in 2 patients this was not done at the referring hospital.

Otherwise, the types of AED treatment (except the frequent use of chlormethiazole) at the referring hospital were generally those recommended by reviews on the subject (Shorvon, 1994). The doses of AEDs given were, however, inadequate. Because of drug redistribution, large loading doses of AEDs have to be given in order to achieve rapid therapeutic blood levels. In the case of phenytoin, intravenous doses of 15-18 mg/kg (>1000mg for a 70kg adult) should be given (Delgado Escueta & Enrile Bacsal, 1983; Wilder, 1983). Similarly phenobarbitone should be given as an intravenous bolus of 10 mg/kg (Shaner et al., 1988). The inadequate loading doses and inadequate maintenance dose contributed to the generally subtherapeutic AED blood levels obtained on transfer to NHNN. The close monitoring of AED blood levels should perhaps be mandatory in unconscious patients.

Many of these problems can be overcome by easily accessible protocols, and indeed it has been frequently found in studies of SE that the use of a protocol significantly improved the prognosis (Treiman et al., 1983; Lowenstein et al., 1988).

Treatment in the ICU

Following failure of initial treatment, early referral to an intensive care unit is mandatory as the late stages of convulsive SE are associated with physiological compromise. Benzodiazepine infusion was the most popular second-line treatment in my survey, despite serious reservations expressed in recent reviews (Ramsay, 1993; Shorvon, 1993; Jagoda & Riggio, 1993). General anaesthesia should perhaps be preferred. Intubation and mechanical ventilation, not only aid
the treatment of many of these complications, but also the use of general anaesthetic agents is an effective means of halting both motor and electrographic seizure activity. From the survey, the preferred time to induction of anaesthesia from the beginning of the SE was usually within 60 minutes. The seems a sensible choice and is supported by both animal and human data, which show that it is after this period has elapsed that status-induced brain damage and the severe physiological and metabolic abnormalities associated with SE are likely to occur.

The choice of anaesthesia in SE remains a matter of debate. Non-barbiturate anaesthetics have more favourable pharmacokinetics than barbiturate anaesthetics, but less definite anticonvulsant action. How important it is for an anaesthetic agent in SE to have intrinsic anticonvulsant activity has not been investigated, and thus which anaesthetic agent should be used remains uncertain; it is an area where good comparative trials are urgently required.

**EEG monitoring of SE in the ICU**

Once general anaesthesia is induced, electrographic monitoring is necessary, as a patient may enter a drug-induced coma with little outward sign of convulsions yet on-going electrographic epileptic activity. In addition, if a patient is inadequately treated or has a severe underlying encephalopathy, then electromechanical disassociation may occur such that the patient enters a stage of subtle generalised convulsive SE characterised by profound coma, bilateral EEG ictal discharges and only subtle motor activity, regardless of the presence or absence of sedating drugs or paralysing agents (Treiman et al., 1984; Treiman, 1993). In my survey, however, patient monitoring during anaesthesia was usually clinical, although this was often determined by circumstances rather than choice.

The electrographic end-point is controversial as there are sparse published data on the subject. The titration of the dose of anaesthetic agents in their use in status is commonly based upon burst suppression on the EEG or cerebral function monitor (CFM) with interburst intervals of 2-30 seconds as an acceptable end-point (Rashkin et al., 1987; Lowenstein et al., 1988; Van Ness, 1990). Burst suppression supposedly represents disconnection of cerebral grey matter from
underlying white matter (Fischer-Williams, 1963). In the audit, it appears that burst suppression was difficult to achieve, because the dose of anaesthesia required was not usually tolerated by the patient (leading in a number of cases to uncontrollable hypotension). Aiming for a more realistic end-point such as seizure suppression, although more difficult to define, seemed to be associated with a good outcome. Aiming for this also meant that careful anaesthetic titration with continuous EEG or cerebral function monitoring was unnecessary, and indeed in most cases intermittent 12-24 hourly standard scalp EEG monitoring was all that was required.
7 GENERAL DISCUSSION AND FURTHER WORK

The aim of this thesis was to suggest ways in which the treatment of status epilepticus could be improved, and I have looked at problems posed by initial treatment, and those posed by late treatment (i.e. refractory status epilepticus). Although first line therapy is very effective, problems occur with recurrence of seizures especially after single doses of diazepam when approximately 50% of patients can have recurrence of their seizures within 2 hours (Prensky et al., 1967). Repeat boluses of diazepam are thus given. I have found that repeat boluses of diazepam have three important pharmacokinetic effects: 1) a substantial decrease in the volume of distribution leading to greater peak levels; 2) a decrease in clearance leading to a relative persistence of serum concentrations, and 3) maintenance of CSF levels despite falling serum concentrations. These pharmacokinetic effects would greatly potentiate the action of diazepam and could result in cardiorespiratory arrest such as has been reported in humans (Shorvon, 1994). If diazepam is to be used, then tapering of repeat doses would need to be used to counter these potentially lethal pharmacokinetic effects. Indeed a tapering protocol may increase the effectiveness of diazepam in treating status epilepticus by preventing relapses without substantially increasing toxicity. However, as with all benzodiazepines, problems may occur due to tolerance and rebound seizures especially with continuous infusions (Shorvon, 1994; Schmidt, 1995). Human studies with tapering protocols are necessary if we are to improve the effectiveness of diazepam in status epilepticus. Alternatively, other treatments which result in rapid treatment without the problems of recurrent seizures such as phenobarbitone or lorazepam should be used, and perhaps we should abandon the use of diazepam.

The recurrence of seizures following first-line treatment has been further examined by me in this thesis by considering the relationship between serum pharmacokinetics and brain extracellular fluid pharmacokinetics in relationship to phenytoin in order to determine the optimum time for serum monitoring and subsequent dosing. I have found that serum concentrations of phenytoin in the initial stages following administration do not reflect the neuropharmacokinetics of extracellular brain phenytoin; the subsequent decline, however, in serum
phenytoin concentrations reflects a concomitant decline in brain ECF concentrations. Thus I would recommend late monitoring (> 1 hour postdose) of serum phenytoin concentrations rather than earlier monitoring. Of interest is how the CSF pharmacokinetics of phenytoin studied by Lolin et al. (1994) differ greatly from the brain extracellular fluid neuropharmacokinetics described by me. This is not surprising as brain ECF and CSF exist as two separate although contiguous compartments. There are thus pharmacokinetic reasons why CSF drug monitoring may in certain circumstances be a poor index of brain ECF drug kinetics and thus perhaps drug pharmacodynamics. A further unexpected finding in this study was that the neuropharmacokinetics of phenytoin were region specific such that the concentrations were higher in the hippocampus than the frontal cortex (this was not observed with lamotrigine). This may have pharmacodynamic implications as acutely administered phenytoin could be more effective in treating hippocampal rather than frontal foci. Lamotrigine in my study of acute drug pharmacokinetics not only provided a contrast to phenytoin in its lack of regional variability of the brain ECF concentrations, but also demonstrated more rapid entry into brain ECF than CSF as was observed with phenytoin. The delayed passage of lamotrigine and phenytoin into and out of the CSF may be of benefit in maintaining brain concentrations in the face of changing serum concentrations. Further research needs to be done to relate these pharmacokinetic observations to pharmacodynamic effects such as EEG modification and treatment of seizures. Furthermore, further research into the effect of seizures themselves on drug pharmacokinetics would provide further information and means of assessing the potential of antiepileptic drugs for the acute treatment of seizures. Additionally, the differences of drug pharmacokinetics in rats when compared to humans (especially in the case of lamotrigine) emphasise the importance of detailed pharmacokinetic and neuropharmacokinetic studies in a particular species to determine appropriate dosing strategies to study the pharmacodynamics of a drug and also to aid appropriate interpretation of experimental data.

The second part of this thesis considered experiments that had relevance to those patients that go on to have refractory status epilepticus. It is at this late stage that
significant neuronal damage occurs probably through activation of glutamate receptors. An understanding of the relationship between extracellular glutamate, seizures and neuronal damage is thus important. My experiments provide indirect evidence, through the association of ascorbate falls with glutamate rises, that inhibition or reversal of glutamate uptake contributes to extracellular glutamate rises during seizures. I have also found that there are mechanisms that appear to limit extracellular glutamate rises in rapidly recurring seizures, possibly through changes in glutamate uptake. Lastly, there is a dissociation between seizure activity and extracellular glutamate rises, such that extracellular glutamate rises do not correlate with the extent of the seizure activity. These findings suggest that there is no direct relationship between extracellular glutamate rises and seizure activity. Furthermore, experiments of others seem to suggest that large rises in extracellular glutamate are not sufficient for seizure induction. Although, I was unable to measure glutamate rises in status epilepticus, there is now considerable evidence to suggest that large rises in extracellular glutamate are probably neither sufficient nor necessary for neurotoxicity. Small rises of extracellular glutamate may, however, play a part in these processes through the potentiation of NMDA receptor responses. Furthermore, rises in extracellular glutamate may have a role in the kindling process, and the development of epilepsy. The effects I have observed of: 1) ascorbate release following transient glutamate rises, and 2) diminution of glutamate rises following indistinguishable epileptic afterdischarges may thus be important mechanisms in preventing seizure related neuronal damage, and indeed may be important in protecting against the occurrence of seizures themselves. Further experimental work is needed to identify the role of small transient rises in glutamate in seizures, neuronal damage and epileptogenesis. Furthermore if a role is identified, then determining the mechanisms controlling these rises and leading to the diminution that I observed may provide further therapeutic targets for preventing seizures, excitotoxicity and epileptogenesis.

I also examined in this thesis the role of phenytoin, lamotrigine and MK-801 late on in status epilepticus and compared their neuroprotective and antiepileptic effects. The therapeutic implications of my findings are important as it appears
from my experiments that in status epilepticus there is little benefit in the late administration of these neuroprotectants; furthermore I found that large doses of these drugs did not terminate status epilepticus in the late stages, but subanaesthetic doses of pentobarbital were very potent, emphasising the important role of barbiturate anaesthesia in terminating refractory status epilepticus. Whether other anaesthetics are equally effective requires further investigation. During the development of my animal model of status epilepticus, I discovered that halothane has potent neuroprotective and antiepileptic properties; whether this is so with other non-barbiturate anaesthetic agents remains to be seen. Furthermore large doses of diazepam given at this late stage were also effective in terminating the status epilepticus and it may be that drugs that act on the GABAergic system are more effective in these late stages than drugs that act as membrane stabilisers (lamotrigine and phenytoin) or on NMDA receptors (MK-801). This could be because in the late stages of status epilepticus disinhibition plays an important part in the maintenance of epileptic discharges, and thus the most effective drugs at this stage are those that potentiate GABAergic inhibition. This hypothesis requires further examination, and other drugs that act on this system such as vigabatrin, tiagabine and phenobarbitone should be examined.

Lastly, having examined possible methods of improving the treatment, I wished to explore how well our present treatment regimes are followed. In order to accomplish this I carried out: 1) a survey of the intensive care treatment of refractory convulsive status epilepticus and 2) an audit of patients transferred to the National Hospital for Neurology and Neurosurgery for further treatment of their status epilepticus. From this second study, it was apparent that diagnosis of persistent SE in the referring hospital was often inaccurate with a large number of patients having pseudostatus - this was possibly due to poor EEG facilities and failure to recognise this diagnostic possibility. The use of prolonged chlorpromazine infusions in the referring hospital resulted in persistent coma and in one case cardiac arrest. AED treatment in the referring hospital frequently consisted of inadequate loading and maintenance doses, leading in some cases to subtherapeutic plasma AED concentrations. I would thus recommend the use of a
standard protocol for treatment of SE, as this simple measure will undoubtedly improve its treatment.

From the literature it is apparent that there is at present little scientific basis for the choice of treatment in intractable SE, as there are no comparative trials. From the intensive care survey, the most frequently used strategies for both second line therapy and anaesthesia may not be the best (the popular use of benzodiazepine infusions should certainly be re-evaluated), and there is at present insufficient electrographic monitoring of patients. The use of a standard protocol for treatment of SE in the ICU has been shown to reduce the mortality and morbidity of SE regardless of the treatment options chosen. Yet in my survey only 12% of those replying had a protocol on their ICU, a deficit that can be easily rectified. In order to improve the prognosis of this devastating condition randomised trials of treatments in refractory status epilepticus are needed. It may, however, be that the treatment of the status epilepticus has little influence on the ultimate prognosis, which may be largely determined by aetiology.

I have thus in this thesis been able to make a number of recommendations that may improve the treatment and the prognosis of status epilepticus. Further work, some of which I have identified, is, however, still needed.
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