EVOKED POTENTIALS IN NEOCORTICAL FOCAL EPILEPTOGENESIS IN THE RAT

by

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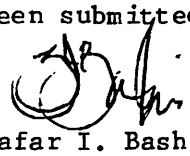
DECLARATION

This thesis comprises my own original research. No part of this work has previously been submitted as a thesis in any form.



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Summary

The aims of this work were to investigate some of the mechanisms underlying the development of epileptiform activity by studying the changes in evoked potential characteristics that occurred in the in vivo penicillin model of focal epilepsy and to use any such findings as a means of further investigation into the role of the different excitatory receptors in this abnormal activity. Experiments were performed on 93 male wistar rats anaesthetised with urethane (1.9 g/kg; intraperitoneally). The electrocorticogram (ECoG) and intracortical activity was continually monitored whilst penicillin was electrophoretically ejected into the neocortex in order to produce epileptiform activity.

Investigations into the mechanisms of epileptiform activity, using both in-vitro and in-vivo models of focal epilepsy, have on the whole, concentrated on the established focus and the characteristics of established epileptic activity, whether in the form of the extracellularly recorded epileptic spike or the intracellularly recorded paroxysmal depolarisation shift (PDS). A disadvantage of such an approach is that it cannot yield any information as to any changes in cortical activity that may be occurring before the appearance of the established epileptic focus; although the processes underlying the gross manifestations of epilepsy can be studied, those that lead to such a state cannot. In this study I have used evoked cortical activity to follow any changes that may be occurring in cortical activity before spontaneous epileptiform activity occurs. Evoked potentials can provide an indication as to the overall state of cortical excitability; any changes in

cortical excitability being reflected by appropriate changes in evoked activity.

During the development of the acute penicillin epileptiform focus in the rat neocortex two distinct and separable stages were defined by the investigation of the changing characteristics of somatosensory evoked potentials (SEPs) and potentials evoked by direct cortical stimulation (DCS).

The first indication that two separate phases of cortical hyperexcitability might exist arose from the differences between the waveforms of enhanced evoked potentials during the development of the epileptiform focus. Small concentrations of penicillin (ejected by electrophoretic currents less than -100 nA) resulted in the enhancement of the amplitude of evoked potentials (up to three times pre-drug levels) with no change in the waveform (phase I); the enhanced potentials were very similar in appearance to normal evoked potentials. Phase I usually occurred within five to ten minutes after the start of penicillin ejection and occurred before the onset of spontaneous epileptiform spiking. Larger concentrations of penicillin (ejected by electrophoretic currents greater than -150 nA) resulted in an enhancement of duration (up to three times the pre-drug levels) as well as further increases in the amplitude (up to nine times pre-drug levels) of evoked potentials (phase II). Phase II was associated with the onset of spontaneous epileptiform spikes, which were very similar in appearance to the enhanced evoked potentials in phase II.

The concept of two phases in penicillin epileptogenesis was further substantiated by the findings that the refractory period of the potentials enhanced by small doses of penicillin (phase I) was the same as the refractory period of normal potentials (less than 100 ms for SEPs and 40 ms for DEPs). However the refractory period of enhanced evoked potentials in phase II was much greater (in excess of 300 ms) than that of both normal potentials and evoked potentials in phase II.

The stimulus strength response relationship of normal evoked potentials and evoked potentials in phase I were very similar to one another and both followed a sigmoidal relationship. The stimulus strength response relationship of evoked potentials in phase II, which was very nearly a square wave relationship, was very different from that of evoked potentials in phase I and normal evoked potentials.

It was therefore concluded that the underlying activity in phase I of penicillin epileptogenesis was no different from normal activity. However, the activity underlying evoked potentials in phase II was different from that of both phase I and normal activity.

The critical mass of cortex required to generate the different phases of penicillin epileptogenesis was investigated by the sub-pial isolation of 'blocks' of cortex of different dimensions. It was found that a threshold mass of cortex (about 0.6 x 0.6 mm on the cortical surface) was

necessary for the generation of both phase I and phase II of epileptogenesis. Any mass of cortex smaller than the threshold did not generate any enhancement of evoked potentials, although normal potentials could be evoked. On no occassion was a particular volume of cortex able to support only phase I without phase II. Therefore, the cortical mass requirement of both phase I and phase II was the same and greater than the requirements of normal activity.

The similarity between the physiological characteristics of phase I and normal evoked potentials implied that the excitatory receptor mechanisms mediating the underlying activity might also be the same (kainate/quisqualate receptors). The activity underlying phase II of penicillin epileptogenesis, because of its similarity to epileptic activity and differences form normal and phase I activity, might be mediated by a different group of excitatory receptors (N-Methyl-D-Aspartate receptors; NMDA). Therefore, the pharmacological basis of the phases of hyperexcitability was investigated. Both phase I and phase II enhancement of evoked potentials was blocked by the NMDA receptor antagonists, ketamine and 2,5 amino phosphonovaleric acid (APV). This implied therefore, that all hyperexcitability in the penicillin epileptiform focus was mediated by NMDA receptors; a reduction in normal GABAergic inhibition by penicillin resulted in hyperexcitability mediated by NMDA receptors. The

epileptiform activity resulting from the action of strychnine was also blocked by NMDA antagonists. Therefore, a general reduction of cortical inhibition resulted in hyperexcitability which is, at least in these two procedures, mediated through the activation of NMDA receptors.

INTRODUCTION

LITERATURE REVIEW

WHAT IS EPILEPSY?

Defining epilepsy has never been an easy task. A mechanistic definition, '...a state produced by an abnormal excessive neuronal discharge within the central nervous system' (Penfield and Jasper, 1954), is perhaps the simplest definition, although what is abnormal and what is excessive in the central nervous system is open to de bate. The 'seizure'therefore, is a consequence of where, when and how the excessive neuronal discharge occurs and in a system as complicated as the human central nervous system a multitude of different types of 'seizure' will inevitably occur when different parts of the brain are involved. The complicated and long list of the types of epilepsy (Bancaud et al., 1981) only serves to underline the problems inherent in defining a condition which can present itself in so many different forms.

Conditions originating within the brain have always been subject to fears, misunderstandings, and deep suspicions. Nowhere has this been more evident than in the common attitudes towards the sufferers of epilepsy. Even today, many people regard a person who suffers from epilepsy as somehow repulsive and best kept at a distance. This is not helped by oversimplified and extremely misleading deifinitions of

epilepsy, such as that given in The Concise Oxford Dictonary; 'Nervous disease in which patient falls to ground unconscious ....'. It is worth pausing therefore, to reflect briefly on some of the attitudes towards epilepsy through the ages.

HISTORICAL PERSPECTIVES ON EPILEPSY

The commonly held view of epilepsy, until relatively recently, has been of a disease with origins in the unknown, spiritual world; this 'sacred disease' was sent upon an individual by particular deities as a form of punishment, or was seen as the work of demons, or even a retribution for sins committed against Selene, the moon goddess. Whatever the cause, the underlying belief was of an infliction or possession by some 'power' greater than man. Inevitably, the cures for such a disease could only have been through superstitious or magical remedies, which included the drinking of human blood, the consumption of burnt human bones and other such delights (Temkin, 1971).

The earliest recorded comprehensive text on epilepsy is one of the books of the Hippocratic collection of medical writings, 'On the Sacred Disease' (circa 400 BC), in which it was stated that the underlying cause of the disease was purely a physical phenomenon originating within the brain and that the 'sacred disease' was no more sacred than any other. In the light of the commonly held beliefs of that time, these writings represented a major step forward in moving away from a supernatural towards a medical and scientific view of epilepsy.

Such progress in thought continued into the third and second centuries BC, when some physicians considered one of the causes of epilepsy to be a physical insult to the meninges. Galen insisted that epilepsy was due to an 'affection' of the brain either arising within the brain itself, or was a secondary effect, resulting from disease of another part of the body. A cure for the latter was to ligate that part of the body to prevent the affected humors reaching the rest of the body or the brain. Other remedies included trephining of the skull and bleeding at various points to disperse the bad humors that had collected in the brain.

The enlightened views of the physicians did not permeate into society however, and throughout the ensuing centuries there was if anything, a regression in the plight of sufferers of epilepsy, who were now often regarded as being possessed by satanic influences. Lunatics and epileptics were considered to be one and the same, both being affected by the moon and the stars. Indeed, some physicians believed that the moon heated the atmosphere surrounding the earth, melted the brain and thus provoked a seizure. During these times religious, superstitious and medical thinking came together to provide a hotchpotch of theories and treatments for epilepsy. There were few physicians who would not readily recommend a regimen of prayer, fasting and dietary treatments as well as remedies

borrowed from the sorcerers and magicians, such as the consumption of testicles of wild boar and the stomach and blood of weasel.

Only in the sixteenth century, nearly twenty centuries after first being proposed, was a physical cause of epilepsy once more sought. Attention was now being paid to the correlation between head injuries and the occurrence of any ensuing seizures (Berengarius da Carpi, 1518; Duretus, from Hollerius, 1623, see Temkin, 1971 pp186-187). Such observations were to broaden the clinical knowledge of epilepsy and in the nineteenth century this line of thought was still being vigorously pursued. Post mortem analyses of the brains of some epileptic patients showed various tumors and diseased meninges (Bright, 1831, see Temkin, 1971 pp308) and a causal relationship with seizures was inferred. It was also inferred that other forms of seizures, which could not be directly traced to an obvious underlying cause, were also the result of some form of brain damage, but that this damage could not be so easily pinpointed (Portal, 1827, see Temkin, 1971 pp272).

In the late nineteenth century, the concept that particular parts of the cerebral hemispheres contained centres for certain motor and sensory functions began to take root (Fritsch and Hitzig, 1870, see Brazier, 1973; Ferrier, 1873). This had very important implications for epilepsy, for it was then but a short step to the notion that a seizure which involved a particular part of the body was due to damage to the part of the brain associated with it. John Hughlings Jackson stated that a fit was triggered by an 'excessive discharge' in a localised part of the brain; if the discharge spread then neighbouring 'sane cells' were also made to behave in the same manner; thus as the discharge spread in the brain so the seizure also spread from its origin to include other parts of the body. Furthermore, he concluded that if the discharging cells were located in the temporal lobes then epilepsy of a 'dreamy state' ensued, whilst if the discharging centre was in sensorimotor areas then convulsions and bodily tremors occured (Hughlings Jackson, 1931). Much of Hughlings Jackson's work rested on findings from post-mortem studies which showed that some cases of epilepsy were associated with the presence of organic brain diseases: There was still no explanation for the causes of epilepsy which had no demonstratable associated brain damage.

EARLY EXPERIMENTAL EPILEPSY AND THE ELECTROENCEPHALOGRAM (EEG)

Much of the present knowledge of the cellular and chemical abnormalities of brain function that may lead to epilepsy has arisen through the investigation of various experimental models of seizure activity.

The finding that seizure states could be induced experimentally (eg. Fritsch and Hitzig, 1870; see Brazier, 1973) and the discovery of the electrical activity of the brain (Caton, 1875; see Brazier, 1973), were major steps forward that were eventually to lead to a greater understanding of the nature of the 'excessive discharge' postulated by Hughlings Jackson.

The discovery of the electrical activity of the brain provoked little interest in the West for over thirty years. Meanwhile Kaufman (1912, see Brazier, 1973), in St. Petersburg, recorded the EEG from dogs whilst stimulating the cortex electrically to induce tonic and clonic seizures. He had proposed that the epileptic seizure would be accompanied by an abnormal electrical discharge in the brain. Cybulski and Jelenska-Macieszyna (1913, see Brazier, 1973) provided the first photographic records of an abnormal EEG in experimentally induced epilepsy in dogs and monkeys. It was

only when records of the EEG from human sufferers of epilepsy were published (Berger, 1931, see Brazier, 1971; Gibbs et al., 1935) that the clinical implications of this method, which had been ignored for so long, were finally realised. Use of the experimental induction of epileptiform activity in animals has since flourished in an attempt to understand the basic mechanisms underlying the excessive electrical discharge recorded by Cybulski and Jelenska-Macieszyna (1913, see Brazier, 1971).

RECENT HISTORY OF EXPERIMENTAL EPILEPSY

In the different experimental models that have been used over the years, experimenters have tried to mimic the human forms of both generalised and focal epilepsy. The various methods that have been used are too many and too diverse for the scope of this review, hence only a selection of these will be considered in an attempt to highlight the problems, insights and discrepancies afforded by the experimental investigation of 'The Epilepsies'.

(I) MODELS OF FOCAL EPILEPSY

Focal epilepsy is a state in which the origins of the abnormal electrical discharge are localised in a particular region of the brain. Certain aspects of this situation are relatively easy to mimic experimentally. The chronic models of focal epilepsy come closest to resembling clinical focal seizures in man and all involve the application of various metals, such as cobalt (Ward, 1972), ferric chloride (Reid et al, 1978), tungstic acid gel (Ward, 1972; Elazar and Blum, 1974) and alumina gel (Kopeloff et al., 1942; Wyler et al., 1975) to the cortex.

Much of the electrophysiological knowledge of the cellular mechanisms of epileptiform activity have arisen from the use of acute, in vivo and in vitro, focal models of epilepsy. These have relied mainly on the use of certain drugs to induce, quickly and reliably, a state of neuronal activity in which the hallmark of epileptic behaviour, the epileptic spike, is produced. This is a relatively short duration (about 50msec) potential that arises from the normal electrical activity, and occurs inbetween overt seizure, or ictal, events. Hence it is also known as the interictal spike.

The most commonly used drugs, such as strychnine and penicillin, are those that reduce the state of normal inhibitory influence within the CNS. Penicillin was first discovered to be a convulsive agent when injected into the cerebral ventricles of a human patient in the treatment of a brain infection (Johnson and Walker, 1945). Since this finding, penicillin has become one of the drugs of choice for use in experimental models of epilepsy because of the rapid onset and reversibility of its action and its predictable and repeatable effects.

A review of some of the important findings that have arisen from the use of models of focal epilepsy will now be considered.

(a) THE PAROXYSMAL DEPOLARISATION SHIFT

The paroxysmal depolarisation shift (PDS), first coined as a phrase by Matsumoto and Ajmone-Marsan (1964), is a large (20 to 30mV), long (50 to 150 msec) depolarisation of the neuronal membrane, from which a burst of action potentials arises. This is followed by a long after-hyperpolarisation (AHP). The large depolarisation was found to occur during the surface negative phase of cortical paroxysmal activity (the epileptic spike) (Goldensohn and Purpura, 1963) and the interictal spike is the extracellular manifestation of the synchronised firing of PDSs by neurones within the focus (Matsumoto and Ajmone-Marsan, 1964). The PDS is found to occur in almost all neurones in the acute strychnine (Li, C.-L., 1959), penicillin (Matsumoto and Ajmone-Marsan, 1964) and freeze foci (Goldensohn and Purpura, 1963) models of epilepsy and in the chronic foci of monkeys (Prince and Futamachi, 1968) and humans (Prince and Wong, 1981).

Because the PDS was recognised to be, at the cellular level, a fundamental 'signature' of focal epileptiform activity, an understanding of the basis of this phenomenon was important in elucidating the mechanisms by which single neurones participate in epileptic activity. Kandel and Spencer (1961), who recorded these large depolarisations in hippocampal neurones, and thought that these were the result of a summation of a large number of excitatory post-synaptic potentials (EPSPs), differed in their interpretation from Matsumoto and Ajmone-Marson (1964), who considered the PDS to be due to '..unaltered synaptic influences..' and '... determined by intrinsic alterations within the cell itself'. Thus the stage was set for an argument which still rages in some quarters (see Johnston and Brown, 1986) as to which of the hypotheses, the 'giant EPSP' or the 'endogenous burst', best explains the mechanisms of PDS generation.

The generation of the paroxysmal depolarisation shift

(i) Giant excitatory post-synaptic potential?....

The 'giant EPSP' hypothesis rapidly gained favour after the finding that the PDS amplitude varied with membrane potential (Prince, 1966) and that the PDS had an apparent reversal potential (Prince, 1968). Furthermore, Dichter and Spencer (1969a; 1969b) showed that in the feline hippocampus the PDS could only be elicited by orthodromic stimulation;

intracellular depolarisation of single cells did not result in a PDS. Further support for this hypothesis came from the finding that the PDS was associated with an increase in membrane conductance (Ayala et al, 1970) and that it could be graded in amplitude (Matsumoto et al, 1969). Ayala et al. (1973) stated that the PDS was a '...compound EPSP produced by recurrent excitatory feedback synapses.. '. More recently, Johnston and Brown (1986) have provided further and direct evidence, using single electrode voltage and current clamp techniques, for the notion that the PDS is a 'giant EPSP'.

(ii)....Or intrinsic cellular abnormality?

The advent of the in vitro brain slice technique (Yamamoto and Kawaii, 1967) has allowed a detailed characterisation of the properties of hippocampal neurones. It has been shown that pyramidal cell bodies (Schwartzkroin and Slawsky, 1977) and dendrites (Wong and Prince, 1979) have the ability to produce slow, regenerative calcium potentials. A large depolarisation and burst of action potentials can be induced in CA3 neurones by intracellular depolarisation; therefore, this must be generated by the intrinsic properties of the neuronal membrane (Wong and Prince, 1981). As a result of such findings attention began to shift towards the 'endogenous burst' hypothesis, in which a normal input triggers slow, voltage-dependent, inward calcium currents which could form

the basis of the PDS (Schwartzkroin and Wyler, 1980) when normal inhibition is reduced (Wong and Prince, 1979). The bursting of CA3 neurones was blocked by the injection of hyperpolarising current into the cell; this manoeuvre uncovered a depolarising afterpotential (DAP), which is probably calcium mediated (Johnston et al., 1980; Wong and Prince, 1979; Wong and Prince, 1981) and may be the mechanism by which the PDS is sustained.

(b) PACEMAKER POPULATIONS OF EPILEPTIFORM ACTIVITY

Another important concept that has arisen from the study of acute models of epilepsy has been that of the presence of a population of neurones which act to drive 'normal' cells into an epileptic state. This has arisen from the findings that a normal afferent input to the CA3 region in the penicillin treated hippocampus produces extracellularly recorded epileptiform bursts. CA1 neurones are not so susceptible to such behaviour; however, the spontaneous bursting of CA3 neurones drives bursts in the CA1 cells (Schwartzkroin and Prince, 1978; Wong and Traub, 1983) through the excitatory Schaffer collaterals; cutting these connections prevents the CA1 cells undergoing epileptiform activity (Hablitz, 1984; Schwartzkroin and Prince, 1978). Thus a certain population of neurones which are intrinsically susceptible to generating epileptiform activity may drive normally 'stable' cells into

an 'unstable' mode.

Further support for the concept of a pacemaker population comes from the neocortex, where there are a small number of neurones in the middle layers, which may be interneurones (Connors et al., 1982) or a class of pyramidal neurones (McCormick et al, 1985), which have the intrinsic membrane properties predisposing these cells to generate a large depolarisation and burst of action potentials (Connors et al., 1982). These cells may have the ability to act as a pacemaker population (Gutnick et al., 1982; Connors, 1984) by triggering non-bursting cells into a bursting mode, in a similar way to the triggering of CA1 cells by CA3 cells in the hippocampus.

In the neocortex the middle layers are the most sensitive to penicillin epilepsy (Lockton and Holmes, 1983; Ebersole and Chatt, 1986) and this may be due to the existence of this bursting group of neurones within these layers, which may act to amplify and transfer a particular stimulus when the normal constraints of inhibition are depressed (Prince and Connors, 1986).

The findings discussed above, that certain cell types are more susceptible to epileptiform behaviour than others, has also provided a means of bringing together the two hypotheses of PDS generation.

The Marrying of the two Hypotheses

When first proposed, it appeared that the two theories of PDS generation were mutually exclusive, but over the years it has become clear that both mechanisms can, and must, exist together (see Prince and Connors, 1986). As already described, there are some cells within the hippocampus and the neocortex that have the intrinsic ability to fire in bursts (hippocampal CA3 and some middle layer neocortical neurones) and there are those that do not (hippocampal CA1 and most neocortical pyramidal cells). The relative contributions of intrinsic membrane properties and synaptic currents to the generation of the PDS must vary from structure to structure and cell to cell, depending on the intrinsic properties of a given cell and its synaptic connections.

(c) THE REQUIREMENT OF A POPULATION OF NEURONES FOR EPILEPSY

An understanding of the mechanisms of epilepsy can never be achieved by considering the properties of single cells alone because a seizure is the consequence of the synchronised discharge of many hundreds or thousands of neurones (Jasper, 1969). Miles et al., (1984) have estimated that approximately 1000 hippocampal CA3 neurones are required before these cells can produce an extracellularly recorded manifestation of epileptiform activity. Other workers have shown that the minimum population requirements of the neocortex are a single cortical column (Gabor et al., 1979) or the grey matter underlying a surface area of 0.7 sq mm (Reichenthal and Hochermann, 1977). It is clear then that within the higher centres of the central nervous system, epileptiform activity only comes about when a critical number of neurones is present.

(d) MECHANISMS OF POPULATION SYNCHRONISATION

Because a large population of neurones is essential for the generation of epilepsy in the mammalian brain, this population must have the ability to synchronise its behaviour in order to generate this abnormal activity. The manner in which neurones achieve such synchronisation may be through several factors. The most intensively studied has been the inherent excitatory

synaptic pathways, the importance of which was recognised by Ayala et al (1973). Non-synaptic mechanisms, such as extracellular ionic disturbances (rev. Lux et al., 1986; Somjen, 1984) and electrical interactions between cells (Dudek et al., 1986), may also play an important role.

(i) The importance of synaptic transmission:

If synaptic transmission is blocked in a convulsant-treated hippocampal slice, the CA3 population does not produce epileptiform activity (Oliver et al, 1978). GABA antagonism does not result in epileptiform activity in all neuronal structures (eg cerebellum and dentate gyrus) and this may be because of the lack of excitatory recurrent pathways within these structures (Ayala et al., 1973; Brookhart et al., 1950). Similarly, CA1 cells lack such a mechanism for mutual re-excitation and are less susceptable to producing spontaneous epileptiform activity (Schwartzkroin and Prince, 1978).

CA3 cells are linked to one another via excitatory collaterals, as described by Lorente de No (1938) and recently demonstrated electrophysiologically (MacVicar and Dudek, 1980). A small local stimulus sufficient to excite only a few of these cells results in a synchronised population burst in the disinhibited slice (Wong and Traub, 1983) by the

rapid spread of activity from a few to most other cells within the population. Traub and Wong (1983) have modelled such behaviour and have found that the random connection of each single neurone to only four or five others, through excitatory synapses, is sufficient to produce rapid synchronised firing of the whole population.

The neocortex also has the necessary excitatory synaptic interconnections, in the form of excitatory recurrent collaterals (Szentagothai, 1978; Takahashi et al., 1967; Gilbert and Wiesel, 1979), to enable the rapid synchronisation of neurones throughout the cortical laminae. The importance of the activity of layer IV, as that which initiates epileptiform activity in the neocortex, has already been outlined. In addition, the excitatory connections of the cortex can pass enhanced activity from layer IV to superficial layers via the excitatory collaterals. This layer may then activate the deeper layers through recurrent collaterals of the layer II/III pyramidal cells; the deeper layers may in turn reactivate layer IV through excitatory recurrents of the deep pyramidal cells. Thus a self reactivating circuit allowing the synchronisation of many cells, as postulated by Ebersole and Chatt (1986), may be set up when normal inhibitory control is diminished.

(ii) Importance of non-Synaptic factors

Electrical interactions between cells, although less well understood than the role of synaptic transfer, may also be important in synchronising the neuronal population. The finding that the blockade of synaptic transmission prevented the occurrence of population events in the CA3 region of the hippocampus (Oliver et al., 1978) demonstrated the importance of normal synaptic function in that area. This was not so in the CA1 region however, where population events occured in the absence of synaptic transmission (Jeffreys and Haas, 1982). It was thus postulated that in such a situation, electrical interactions between cells, possibly through gap junctions, may provide the necessary means of inter-cellular communication in order for cell synchrony to occur. What role such mechanisms may play in the presence of normal synaptic function is difficult to assess.

Gap junctions between CA1 (Andrew et al., 1982), CA3 (MacVicar and Dudek, 1980) and neocortical neurones (Gutnick and Prince, 1981) have been demonstrated by the injection of dyes, such as Lucifer yellow, into single cells; cells directly coupled to the injected cell also take up the dye. The most direct evidence for such coupling is provided by intracellular recordings from two individual cells; if these are coupled, then depolarising or hyperpolarising pulses in

one cell result in the same in the coupled cell (MacVicar and Dudek, 1981). Whether electrical coupling has the ability to bring about the rapid synchronisation of a large population of cells by itself remains to be demonstrated, but the potential may exist for the synchronisation of small groups within the population as a whole (Dudek et al., 1986).

(II) MODELS OF GENERALISED EPILEPSY

Generalised epilepsy, in contrast to focal epilepsy, is a state in which abnormal electrical activity arises simultaneously from widespread regions of the brain. The feline penicillin model of generalised epilepsy, described by Prince and Farrell (1969) utilises the parenteral injection of large amounts of penicillin and results in bursts of generalised, synchronous spike and wave (SW) discharges, with a frequency of about 3 Hz, which is similar to that occurring in generalised human absence attacks (Gibbs et al., 1935; Jasper and Drooleever-Fortuyn, 1946), as are the outward motor manifestations (Gloor, 1984). Neurophysiological investigations have shown that during this activity there is an oscillatory discharge occurring between linked cortical and thalamic neurones (Avoli et al., 1983) which drives the SW discharges at 3 Hz. The oscillating circuit exists in the 'normal' brain, but operates to produce 10Hz 'spindles' in the EEG, which are associated with the normal occurrence of certain phases of sleep.

It has been shown that in this model of generalised epilepsy there is no decrease of GABAergic inhibitory function, nor do cells undergo a paroxysmal depolarisation shift (PDS) (Kostopoulus et al 1983, 1986; Giaretta et al., 1987). The penicillin concentration in the brain appears to be

an order of magnitude less than that used in models of focal epilepsy (Quesney and Gloor, 1978; Davenport et al., 1979) and, in some way, causes an excitation of neurones without any decrease in inhibition. This results in powerful EPSPs and an increased firing of action potentials, which in turn, produces a greater than normal degree of recurrent cortical inhibition. This increase in cortical inhibition prevents the thalamic input from triggering the cortex at the normal 10 Hz, thereby gradually converting the spindles to the slower SW discharges (McLachlon et al., 1984). Furthermore, it has been shown that increased GABAergic function, perhaps by increasing recurrent inhibition, serves to increase the likelihood of generalised SW discharges (Fariello et al., 1983).

It is clear that the proposed mechanisms for generalised epilepsy are radically different from those postulated for focal epilepsy.

MECHANISMS OF PENICILLIN ACTION

Because pencillin has been such a widely used convulsant agent in different models of epilepsy it is important to consider the mechanisms of action of this drug in the CNS.

The primary inhibitory transmitter of the higher centres of the CNS is the amino acid, gamma amino butyric acid (GABA) (Krnjevic, 1974), which acts at a membrane-bound receptor molecule to open channels for chloride ions.

Most evidence to date suggests that the primary action of penicillin, in focal epilepsy, is to antagonise GABAergic inhibition. Recent evidence from patch-clamp studies (Chow and Mathers, 1986) indicates that the antagonism is non-competitive, which contradicts the findings of MacDonald and Barker (1978) of competitive antagonism. Penicillin acts as a specific antagonist in the lower millimolar range of concentrations (Macon and King, 1979; MacDonald and Barker, 1978); the concentrations which produce focal epileptiform activity. Macon and King (1979) however, found that the smallest concentrations of penicillin which produced cellular hyperexcitability, did so without there being any depression of GABAergic inhibition. These concentrations did not result in focal epileptiform activity and the concentrations of penicillin required before overt focal epileptiform activity

occurred did result in GABA antagonism. Avoli (1984) found that small concentrations of penicillin (0.17mM) produced an excitation of cortical cells without any GABA antagonism at the soma, and suggested that the hyperexcitability was due to inhibitory antagonism at a dendritic level. Therefore, the action of penicillin appears to be dependant on its concentration and as already indicated smaller concentrations of penicillin than result in focal epilepsy may produce generalised epilepsy by a means other than GABAergic antagonism.

THE ROLE OF A REDUCTION OF INHIBITION IN EPILEPSY

Since much of the knowledge of epileptiform activity arises from the use of experimental models that rely on the reduction of GABAergic inhibition, an obvious question to ask, as recognised by Iwama and Jasper (1957), was: 'Is epilepsy actually caused by a disruption of normal inhibition ?'. Unfortunately, there has been no simple answer to this seemingly simple problem.

Many models have been proposed to show that a reduction of inhibition will lead to seizures (see Roberts, 1986) but any experimental evidence demonstrating that epilepsy is caused by a decrease in inhibition has been largely circumstantial, inconclusive and contradictory. Some of the evidence in favour of this theory is that levels of the inhibitory transmitter GABA have been found to be lower than normal in the CSF of some patients with some types of epilepsy (Wood et al., 1979). A decrease in the GABA-synthesising enzyme, GAD, and a decrease in postulated inhibitory synapses has been found in the alumina cream epileptiform focus (Ribak et al., 1981) and the action of some of the current anticonvulsants, such as sodium valproate, may be through an enhancement of GABAergic transmission (Chapman et al., 1982). The impairment of GABA synthesis also leads to generalised seizures (Horton and Meldrum, 1973; Beart and Bilal, 1979; Ashton and Wauquier,

1979). This begs the question whether epilepsy itself occurs as a result of an impariment of GABAergic inhibition. The removal of tissue from the foci of patients undergoing surgical treatment for epilepsy showed that 60 to 70% had a lower level of GAD in epileptic than in non-epileptic tissue (Lloyd et al., 1986). However, this study could only be carried out on patients with focal seizures so nothing is known of the state of the inhibitory system in generalised epileptic activity. Moreover, most of the patients in the study of Lloyd (1986) had intractable epilepsy and so may constitute a somewhat biased group.

Other investigators have found little evidence in favour of the above hypothesis. For example, agents elevating GABA levels by up to five times had little effect on seizure susceptibility (Elliot, 1965). A strain of mice genetically prone to seizures was not found to have lower than normal GABA levels correlated with the timing of seizure susceptibility, which is only from 21 to 28 days of age (Ticku, 1979). Furthermore, the current hypothesis of generalised epilepsy (Gloor and Fariello, 1988) would suggest that the generation of this type of seizure occurs without any decrease in GABA inhibition. Increasing GABA levels by the inhibition of the enzyme GABA aminotransferase (GABA-T) has been shown to have only a small anticonvulsant effect (Kuriyama et al., 1966). Therefore, the function of GABA inhibition in the onset of

epilepsy is still far from clear.

THE ROLE OF EXCITATORY MECHANISMS IN EPILEPSY

A powerful mechanism by which a state of hyperexcitability can be achieved is through an enhancement of the excitatory mechanisms of the CNS.

The work of Curtis and his colleagues was vital in elucidating the excitatory transmitter role of glutamate and aspartate in the spinal cord (Curtis et al., 1960), while Krnjevic and Phillis (1963) subsequently demonstrated that glutamate produced excitation of neurones in the cerebral cortex. That glutamate is the major excitatory transmitter of the CNS is now fairly well established and the evidence has been reviewed recently (Fagg and Foster, 1983; Fonnum, 1984)

Increased levels of glutamate in the brain can bring about the generation of seizures (Hayashi, 1959; Delgado et al., 1971), but whether epilepsy is brought about by an over-vigorous excitatory system remains a matter of contention. It is quite possible that in the generation of different forms of epilepsy the excitatory and inhibitory systems of the central nervous system play different roles (van Gelder, 1987).

EXCITATORY TRANSMITTER RECEPTORS

One of the explosion areas in neuroscience research in the last few years has been the question of the role of N-methyl-D-aspartate (NMDA) receptors in the CNS. NMDA was originally synthesised as a glutamate/aspartate receptor agonist (Watkins, 1962) and the importance of this molecule came to light when it was found that an increase in extracellular Mg ions reduced the excitation by NMDA but had little effect on the excitation by glutamate (Evans et al., 1977). Hence, there was the possibility that NMDA and glutamate acted at different types of glutamate receptor, which were in some way differently modified by magnesium. Since that finding, the use of various antagonists and agonists has led to glutamate/aspartate receptors being divided into three groups, named after the most potent agonists for each type; these are NMDA, kainate and quisqualate (Watkins Evans, 1981).

It has since been found that the different excitatory receptor types subserve different forms of activity. The kainate and quisqualate types are often classified as 'non-NMDA' receptors and these appear to subserve 'normal', 'fast' excitatory synaptic transmission in the hippocampus whereas NMDA receptors are not important in such transmission (Collingridge et al., 1983b), despite their abundance in the

hippocampus (Monaghan et al., 1983; Greenamyre et al., 1985). The reason for this became clear with the finding that in normal circumstances NMDA receptor operated channels are subject to a voltage dependant magnesium block (Nowak et al., 1984; Mayer et al., 1984). When the magnesium block of NMDA operated channels was alleviated in the cerebral cortex (Thomson and West, 1986), or when NMDA was applied into the neocortex (Flatman et al., 1983), the neurones underwent a long, slow depolarisation from which arose a burst of action potentials. The resemblance of this behaviour to the PDS and burst firing in epileptiform activity was striking and it was not long before the implications of NMDA receptor involvement in epileptiform activity were realised.

NMDA receptor antagonists attenuate epileptiform activity induced by antagonism of GABAergic inhibition in hippocampal slices (King and Dingledine, 1986), aswell as in mice (Croucher et al., 1982;) susceptible to sound induced seizures, in kindling induced epileptic activity (Peterson et al., 1983a; 1984) and in the photosensitive baboon (Meldrum et al., 1983).

METHODS

METHODS

All the experiments described in this thesis were performed on Wistar male rats, weighing between 190 and 210g.

ANAESTHESIA

Rats were anaesthetised with 25% urethane, given intraperitoneally, at a dosage of 1.9g/kg. This was usually sufficient to produce a depth of anaesthesia which abolished the hindlimb withdrawl reflex. If the level of anaesthesia was not sufficient, supplementary doses of 0.2ml of urethane were given so as to abolish the withdrawl reflex, before any surgery was carried out.

SURGICAL PROCEDURES

(i) Tracheostomy

The anaesthetised rat was laid supine on an operating board. A midline incision was made in the skin from the hyoid arches towards the sternum to expose the sternohyoid muscles. These were cut and separated in order to gain access to the trachea. The trachea was separated from the underlying tissues and a thread passed underneath. A cut was made in the trachea between adjacent cartilagenous rings, a canula inserted and securely tied in place. The skin incision was tied closed.

(ii) Craniotomy

The rat was laid prone, and in order to expose the skull, a midline incision was made from between the ears to between the eyes. The rat was then placed on a heated table so as to maintain its body temperature. The head was placed in a head holder consisting of two ear bars and a mouth piece which could firmly clamp the head and thus prevent any significant movement of the skull.

The following procedures were then carried out under x6-x40 magnification, provided by a Zeiss binocular dissecting microscope.

The skin covering the head was separated from the skull and surrounding muscles by cutting the thin connective tissues. The connective tissues overlying the skull were removed, primarily from the area where the bone was to be excised. The surrounding temporal muscle was also cut away in order to expose the side of the skull. A craniotomy was then performed over the coronal suture and about 5mm lateral to the sagital suture using Kaltenbach and Voight dental drilling apparatus. The skull was cut through on four sides of a 'square' (approximately 3x3mm) until the bone could be pulled away from the underlying tissues and the surrounding skull using a pair of fine forceps. Care was taken to ensure that the bone was

lifted straight up in order to prevent any undue pressure on the underlying cortex. With experience it was a matter of routine to get to this stage without injuring the cortex in any obvious way.

To remove the dura a surgeons triangular needle, which had been bent back so as to make a little hook, was pulled lightly over the dural surface until the membrane was lifted up. The dura could then be split and reflected from the cortex. The cortical surface was immediately covered with a drop of warm liquid paraffin. The skin flaps were tied up to a metal ring above the head and this 'pool' was filled with liquid paraffin.

(iii) Sub-pial isolation of blocks of cortex

In some experiments it was required to isolate small blocks of cortex from the sorrounding cortical tissue. A fine tungsten wire was bent to an L shape and the short part of the L (about 1mm long) was pushed vertically into the cortex through the pia mater. The longer length was used to manipulate the short piece within the cortex through 90 degrees so this became visible immediately below the pial surface; thus one face of the cuboid was formed. The wire was rotated to its original position and withdrawn. A second cut was made from the same point of insertion to produce another face of the cuboid at right angles to the first. The procedure was repeated from a point diagonally opposite the first point of insertion; in this way a piece of cortex was isolated on four sides from the surrounding cortex. The pial integrity was maintained except for the two points of insertion of the wire and the block was not undercut in order to maintain the integrity of the afferent input. The method of sub-pial isolation is illustrated in figure 2.1.

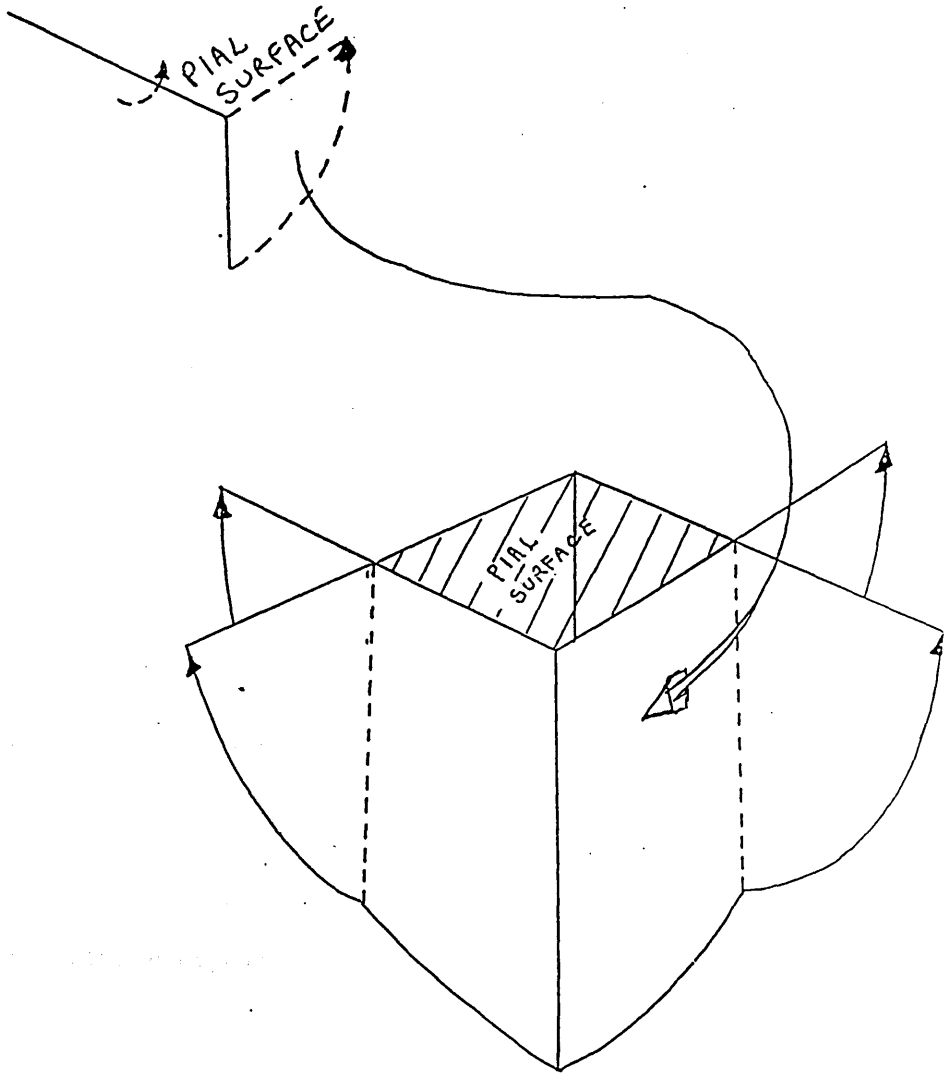


Figure 2.1: Diagrammatic representation of the method used to sub-pially isolate a cuboid of cortex. An 'L'-shaped needle is pushed into the cortex and rotated through 90 degrees to form one face of the cuboid. This procedure is repeated to complete the isolation, which is not undercut. See text for fuller details.

ELECTROPHYSIOLOGY

Recording: Apparatus and general outline

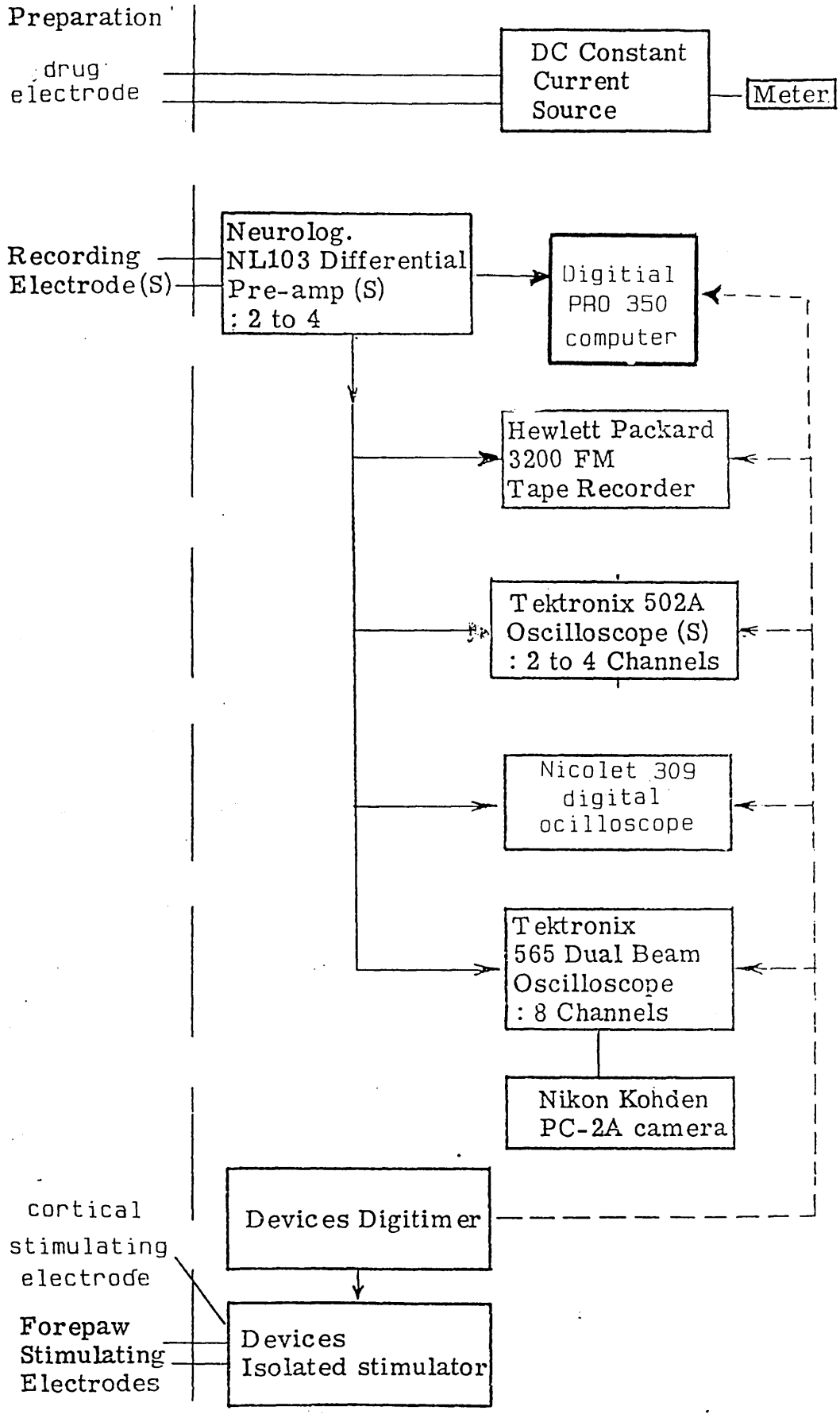
The electrocorticogram (ECoG) was recorded by a chlorided silver ball electrode on the pial surface. Intracortical field potential recordings were made with saline-filled (4M NaCl) glass microelectrodes. All recordings were made with reference to an indifferant chlorided silver ball electrode placed on the skull surface. The signals were led from the glass electrodes by chlorided silver wires.

Conventional signal amplification techniques were used: all signals were fed into Digitimer Neurolog a.c. preamplifiers (NL 103; X100, 0.1hz-3kHz) and thence into Digitimer Neurolog a.c. amplifiers (NL 105; X1 to X50). The amplified output was fed into a 'cherry board' which allowed the signals to be led into any number of the following devices see (fig. 2.2).

1) A four beam Tektronix storage oscilloscope for constant monitoring of the ongoing cortical activity.

2) A Nicolet 309 digital and Tektronix 502A, d.c. coupled, dual beam oscilloscopes, for monitoring the evoked potentials.

Figure 2.2: Diagrammatic representation of the electronic apparatus used.


Preparation
drug
electrode
DC Constant Current Source
Meter
Recording Electrode(S)
Neurolog. NL103 Differential Pre-amp (S) : 2 to 4
Digitial PRO 350 computer
Hewlett Packard 3200 FM Tape Recorder
Tektronix 502A Oscilloscope (S) : 2 to 4 Channels
Nicolet 309 digital ocilloscope
Tektronix 565 Dual Beam Oscilloscope : 8 Channels
Nikon Kohden PC-2A camera
Devices Digitimer
cortical stimulating electrode
Forepaw Stimulating Electrodes
Devices Isolated stimulator

3) A Tektronix RM565 4 beam oscilloscope with an attatched Nihon Kohden PC-2A camera.

4) A 6 channel Bell and Howell VR 3200 FM magnetic tape recorder (0-10kHz) for storing the data for later analysis.

5) The amplified evoked potential signals recorded from the depth electrodes were also digitised and stored on computer (Digital PRO 350) for off-line analysis.

All the recording and stimulating apparatus was triggered by a Devices Digitimer.

ELECTRODES

The electorcorticogram (ECoG) was recorded by a chlorided silver ball electrode (0.5mm dia.). This was lightly touched on the pial surface under visual inspection. The voltage deflection as seen on the oscilloscope recording trace also indicated that contact with the pial surface had been made.

Recording from within the cortex was achieved using glass microelectrodes made from borosilicate glass tubing ('kwik-fil', outside diameter 2.0mm, Clarke Electromedical) These were pulled in a lab-made puller and filled with 4M NaCl. Electrodes with resistances of 10-20 MOhms were regularly pulled in this manner. The electrode tips were

broken back to achieve a resistance of 2-10 MOhms.

It was found necessary to break back the electrode tips because high resistance electrodes suffered the problem of being very sensitive to cortical oscillations. This may be a function of the very fine tip being highly flexible and moving with the cortical oscillations. In such cases very large deflections, corresponding to each up and down movement of the brain, masked any cortical activity on the recording trace. Lower resistance electrodes usually overcame this problem, perhaps because of the less flexible electrode tip and shank.

This procedure could not be taken too far however, because if the electrode tips were too large, simply piercing the cortex could be a problem. This could also presumably, lead to excessive injury of the grey matter.

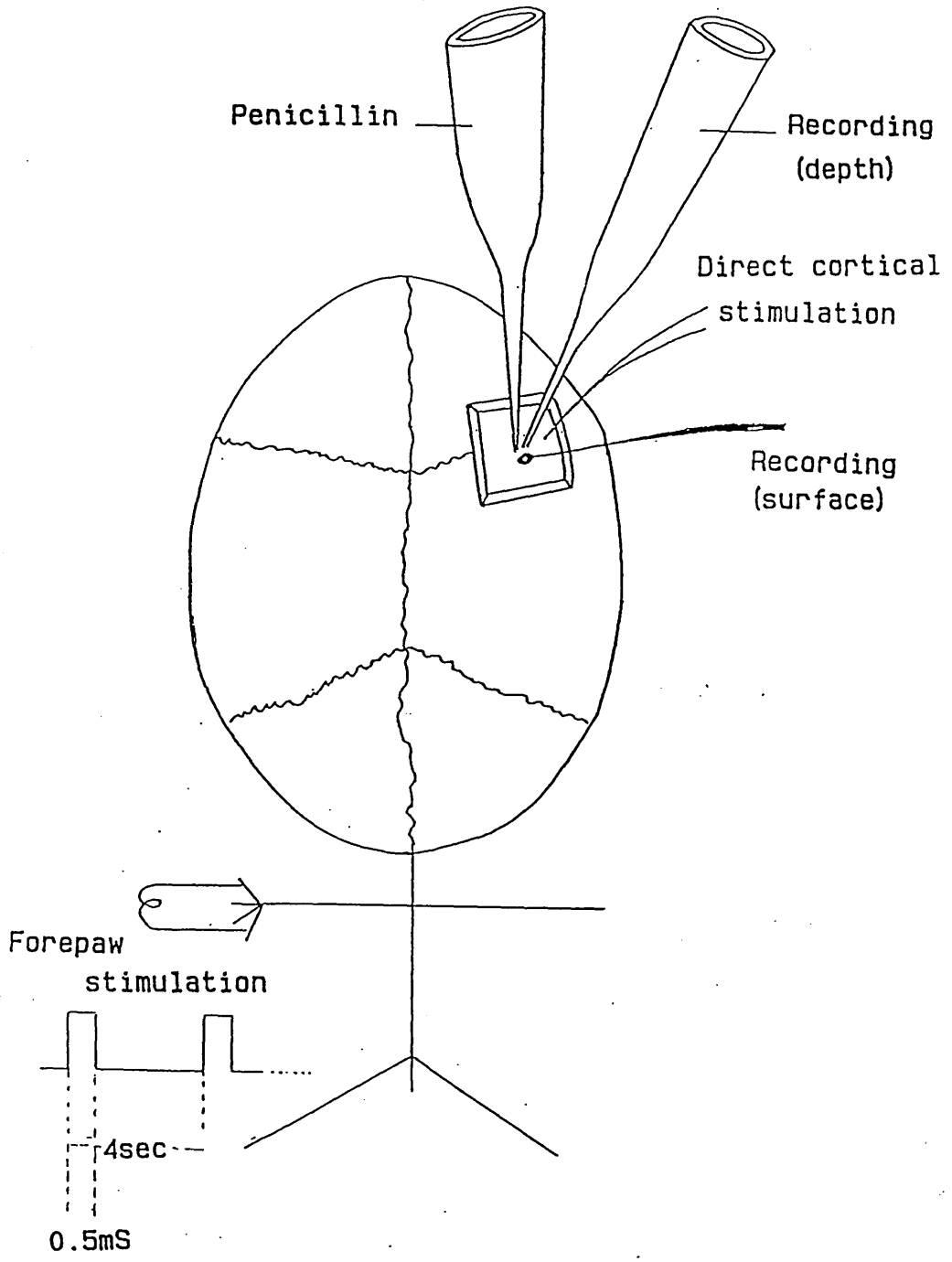


Figure 2.3: Diagrammatic representation of the experimental set up using separate recording and drug electrodes.

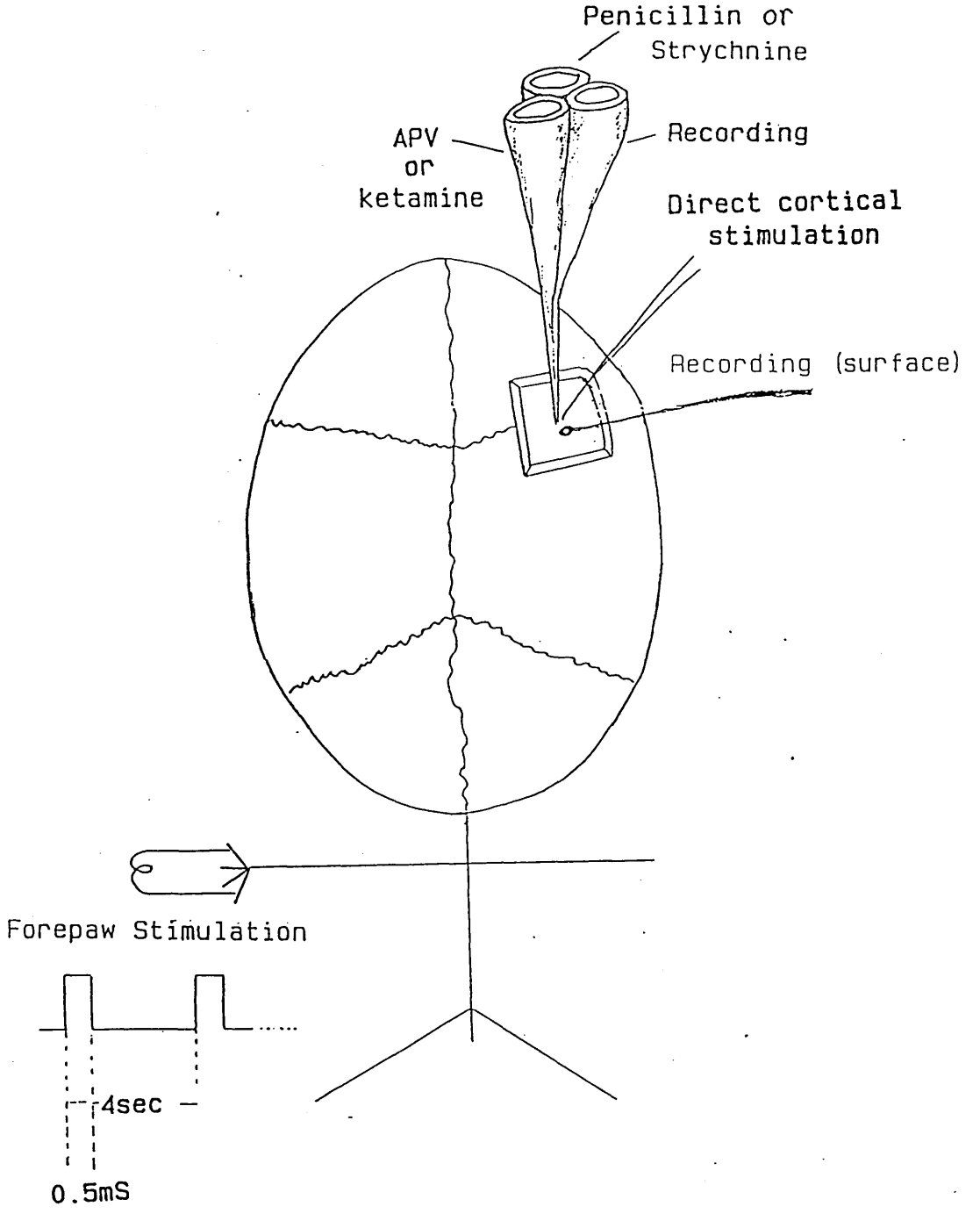


Figure 2.4: Diagrammatic representation of the experimental set up using multi-barrelled electrodes.

ELECTROPHORESIS

Electrophoresis was the method used to eject a localised, small concentration of drug into the cortex. Glass microelectrodes were pulled in the same way as already described and filled with the required drug. It was normally the case that a penicillin filled electrode had a greater resistance than a saline-filled equivalent. Penicillin electrodes very often had resistances of over 50 MOhms.

The high resistance of the penicillin filled electrode was often a problem because with such a high resistance the driving voltages required to pass the required currents would be far larger than the output of the iontophoretic device. Therefore the drug electrode tips had to be broken back so that their resistances were such that the electrodes could be used with ease for iontophoresis. It was often the case that the insertion of an electrode with a relatively large tip caused the evoked potentials to be reduced in size, perhaps due to some damage of the cortex. The usual electrode resistance that was used, and proved to be a useful compromise, was 2-5 MOhms for penicillin-filled electrodes. Figure 2.3 illustrates the experimental set up.

In many experiments a procedure of using multi-barrelled pipettes was used ('kwik-fil', fused, multi barrelled glass, Clarke Electromedical). These were pulled in the same way as for single barrelled electrodes. Generally, 3 barrelled electrodes were used. If only one drug was being used, a second barrel would be employed for recording and the third was filled with normal saline. When using two drugs the normal saline was substituted for the other drug. Figure 2.4 illustrates the experimental set up.

The use of multi-barrelled electrodes has two main advantages over two or three single electrodes.

i) That the drug electrode and the recording electrode are at the same point in the cortex. When using separate drug and recording electrodes any variation in distance between the two electrode tips, in both horizontal and vertical planes, were of concern. This problem would be exaggerated the more individual electrodes are used.

ii) The second advantage was purely a practical one in that there was a limit to the number of electrode holders that could be placed around the preparation. There was also a limit to the number of electrodes that could be placed close to one another without touching and bumping whilst still trying to ensure that the separate electrode tips were all within a very

confined space.

Therefore the multi-barrel micropipette technique was generally employed when two drugs were being used together. In time, this technique also came to be used as a matter of routine even for the use of a single drug and single recording electrode, to try to overcome any errors which might have occurred because of differances in tip separation in differant experiments.

However, the use of multibarelled electrodes was not without its problems. One of these was that invariably the recording and pencillin-containing electrodes ideally needed to be of differant tip sizes; the penicillin electrode tip needed to be larger so as to have a small enough resistance to be able to eject penicillin without overloading the current source device. This had the result of the recording electrode being of a very low resistance. Therefore one had to be satisfied with a compromise situation. One other problem was of cross-talk between the two barrels. The current passing through the penicillin electrode could be picked up by the recording barrel. This was often in the form of very large oscillations, which were perhaps a function of the continuously changing resistance of the penicillin barrel and the constant current source's alterations to this.

Both of these problems were minimised by the introduction of an updated DC powered constant current source. This was an advantage because it reduced any a.c. interference. Secondly, this device had a 90 volt driving voltage and allowed the use of electrodes of larger resisitance than had previously been feasible.

FOREPAW AND DIRECT CORTICAL STIMULATION

Forepaw stimuli were delivered from a Digitimer isolated stimulator (0-90V, 0.2mS) through two wires attatched to the outer digits. Direct cortical stimulation was by a Neurolog isolated current source (10-1000 microA, 0.2mS) which was in turn driven by a Digitimer stimulator.

The cortex was stimulated directly via an electrode placed into the middle layers of the grey matter. Two types of electrodes were used:

i) via a tungsten stimulating electrode (Clarke Electromedical, tip less than 1 micron, shank dia. 0.25mm), insulated except for the very tip.

ii) via a 4M NaCl filled glass microelectrode.

In both cases the stimulating electrodes were placed close to the recording electrodes with their tips within layer IV. Stimulation by both means was able to produce evoked potentials which were negative going within the cortex and a complex of positive waves recorded on the surface. Stimulus strengths were set so that both forms of evoked potentials were of similar amplitude and waveform.

The stimulus cycle was controlled by a Digitimer and various cycling modes were used:

i) Forepaw stimulation every 4 sec.

ii) DCS every 4 sec.

iii) FP and DCS stimuli alternating every 4 sec; i.e. one FP every 8sec alternating with one DCS every 8 sec.

iv) Pairs of FP stimuli every 4 sec or pairs of DCS every 4 sec.

v) Pairs of FP stimuli alternating with pairs of DCS, as in (iii).

vi) Pairs of FP and DCS, DCS and FP, FP and DCS, etc., every 4 sec.

DRUG PREPARATION

i) Sodium benzyl penicillin (Glaxo) was dissolved in 1 ml of distilled water; final concentration of 1.7 M.

ii) Strychnine hydrochloride (Sigma) was dissolved in 120 mM NaCl; final concentration 10mM.

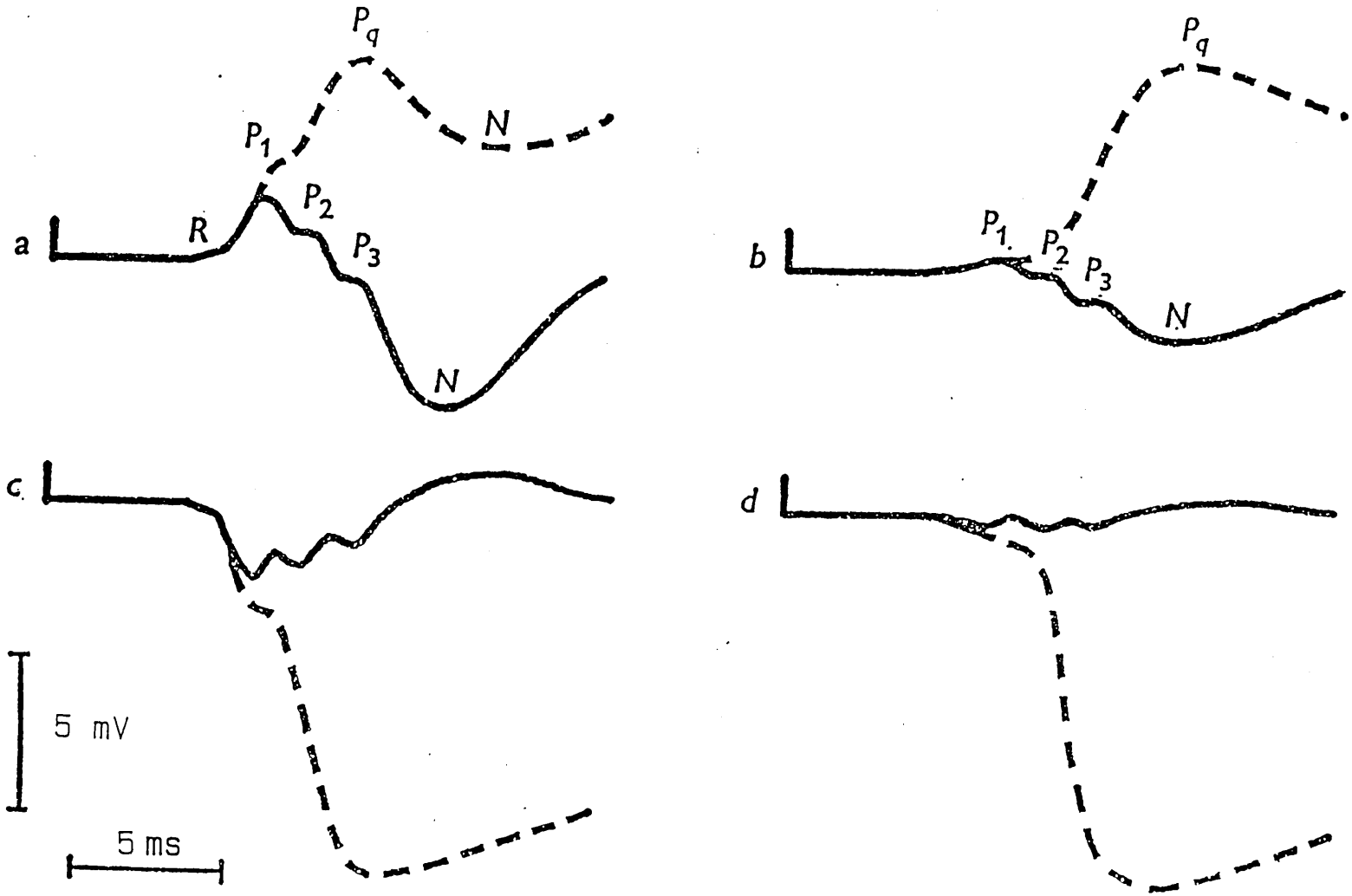
iii) D,L-APV (Sigma) was prepared in 120 mM NaCl; final concentration 50 mM. The pH was adjusted to 8 by the addition of molar NaOH.

iV) Ketamine hydrochloride (Vetalar; 100 mg/ml) was dissolved in 120 mM NaCl; final concentration 20 mM. The pH was adjusted to 4 by addition of HCl.

LOCALISATION OF PRIMARY RECEIVING AREA

It was important to ensure that the area of the cortex used in the study was always the same. This region was the primary somatosensory receiving area of the forepaw. This was localised at the start of the experiment by stimulating the contralateral forepaw and recording from the surface of the exposed cortex (see fig. 2.5). The surface recorded potential is an initially positive-going potential with at least two positive-going components. The first of these is small and a reflection of the afferent volley arriving into the cortex. This can only be recorded in the area of cortex overlying the afferent input and has a latency to onset of between 4.5 and 5.0 msec. Following this initial component is a second positive-going deflection, the amplitude and duration of which is variable and dependent on the ongoing cortical activity. Thus by mapping out the surface of the exposed cortex one could locate the primary receiving area. The depth recorded evoked potentials were also composed of several negative-going components which may reflect the same activity as the components in the surface recorded potentials. The form of the components of the evoked potentials was highly dependant on the state of the ongoing spontaneous cortical activity (fig. 2.5).

Figure 2.5: Surface (a and b) and depth (c and d) recorded somatosensory evoked potentials. The influence of spontaneous cortical activity (solid responses) and quiescent cortex (broken lines) on the evoked responses recorded close to the centre of the primary receiving area (a and c) and at a distance of 1 mm anterior to this (b and d). Components in the surface responses are associated with 'wavelets' in the depth responses. From Carter et al., 1968).


a
R
P1
Pq
P2
P3
N
b
P1
Pq
P2
P3
N
c
d
5 mV
5 ms

HISTOLOGY

At the end of some experiments it was necessary to remove the brain for histological verification of electrode marking and sub-pial cutting.

The rat was decapitated and the brain swiftly removed and placed in formalin solution. Subsequent histological sectioning and staining were carried out by the histology department.

DATA ANALYSIS

As already mentioned, signals from the depth electrode were digitised (A-D convertor constructed by Dept of Physiology electronics workshop) and stored on the hard disk of Digital PRO 350 computer.

Programs for the sampling of the signals by the computer were written by Dr. Ian Logan (Trans1, Appendix A). The computer and A-D were triggered by the same digitimer output which triggered all other apparatus. This signal started the A-D conversion and the sampling by the PRO 350. When the PRO 350 had sampled for as long a period as required it sent a voltage pulse to the A-D convertor to stop the conversion.

Several programs were composed to analyse the recorded data. After some initial experiments it became clear which parameters of the evoked potentials were of greatest interest and needed to be measured. These were: i) the maximum negative amplitude of the potential; ii) the delay to the maximum amplitude; iii) the delay before the amplitude decayed to 25% of its maximum. Fig 2.6 illustrates these parameters.

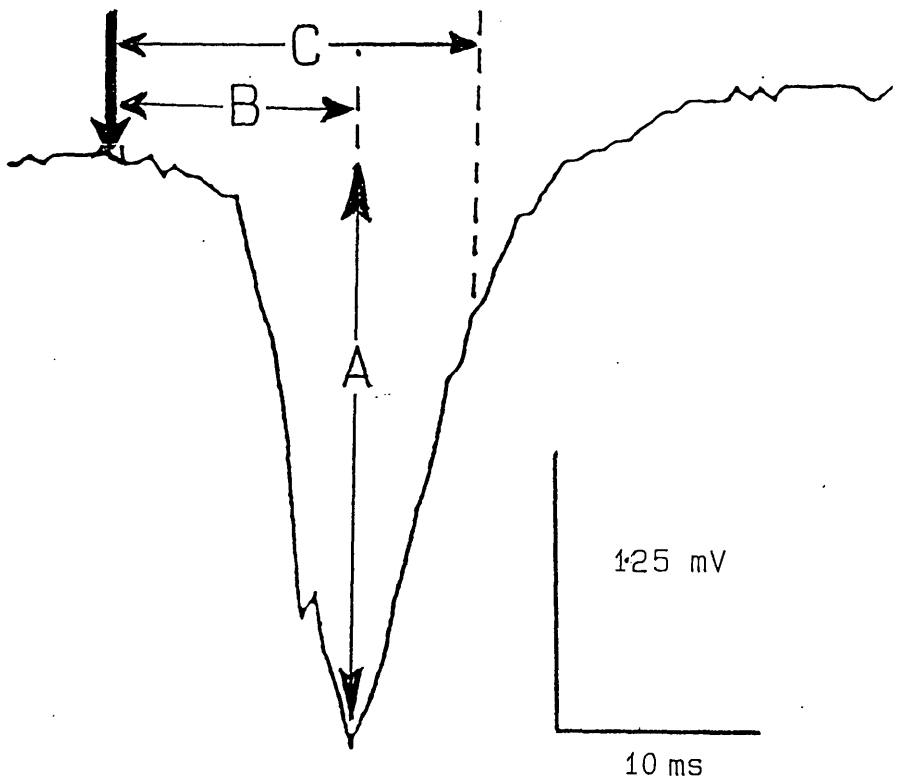


Figure 2.6: Depth recorded somatosensory evoked potential. Thick arrow marks the stimulus. Arrow A is the peak amplitude, B is the time to the peak and C is the duration of the response (time for peak amplitude to recover to 25% of the peak).

The initial program to calculate these parameters (Calc, Appendix A) was written by O.Holmes and continually developed and updated by myself and O.H. The basic function of this program was to take the stored data and work on each transient sequentially until the last record requested. The program worked through the signal and looked for the stimulus artefact. This was taken as time zero and all delays were measured from this point. The DC offset was removed from the signal so that any amplitude measures were taken from a common zero. The program then ran along the signal to find the point of maximum negativity and then the point at which this maximum decayed to 25%. Thus the delay to the peak, the peak amplitude, and the duration were calculated. From this, the area under the wave was calculated by integration. These four values were then stored in a 4Xn array, n being the number of records.

All of the evoked potentials could be displayed on the VDU for visual inspection. Various commands were included in the program to take account of amplification changes, to look for any number of evoked potentials along the whole record, to remove stimulus artefacts, etc.

The calculated data obtained from the evoked potentials was read into another file. Programs were written, initially by O.H., and then modified by O.H. and myself as required, to read the stored calculations and present these graphically (Program Plot., Appendix A). In this way, any of the calculated parameters could be displayed as a function of time with respect to drug application. Such data could be displayed by (i) Calculating the mean of the values prior to drug ejection and setting this mean to 100%. Any changes in the values were then expressed relative to this pre-drug level. (ii) Alternatively, the absolute amplitudes of the potentials could be displayed.

The above programs were modified by myself in order that the screen display from any of the above programs could be output to a Hewlett Packard 7475a plotter to provide a hard copy of these results. Most of the graphical results presented in this thesis were obtained in this way. The raw evoked potential traces could also be output in this same way to provide hard copies. This became the favoured method of presenting such data, succeeding the previous method of photographing transients from oscilloscope screens.

Unfortunately, a drawback of the PRO 350 was that it was only able to sample from a single channel at any one time. Therefore, although most of the work presented is concerned with evoked potentials recorded at depth it was required to investigate the surface evoked potentials also. When this was the case this data was re-read from the tape recorder into the computer at a later time. This was costly in terms of time and computer storage but was the only short-term practical solution to the problem. It was then a simple enough case to pair these two pieces of data together.

SIMPLE OUTLINE OF EXPERIMENT

The animal and the recording set up were prepared as described above. In the urethane anaesthetised rat the ECoG is characterised by periods of electrical activity which are interspersed by periods of quiesence; the deeper the anaesthesia the longer the periods of quiesence. The level of anaesthesia was set such that the ECoG was characterised by approximately equal active and quiescent periods. This level of anaesthesia was maintained throughout the experiment by administration of supplementary doses of urethane when periods of activity began to dominate the ECoG.

In most experiments, the recording electrode was placed at a depth corresponding to the middle layers of the cortex, because these have been shown to be the most sensitive to the epileptogenic effects of penicillin (Lockton and Holmes, 1983).

In the simplest of experiments a single recording electrode and a penicillin-containing electrode were placed at a depth of 0.7mm. Forepaw somatosensory evoked potentials were recorded from depth and from the surface and stored on tape and computer. Penicillin was ejected from the electrode by a known anionic current for a given period of time and then to a cationic 'holding' current. The evoked potentials were

continually monitored throughout the period prior to, during, and for a time after penicillin ejection. The data was recorded and analysed as described.

Placing the penicillin containing electrode into the cortex sometimes resulted in a temporary decline in the amplitudes of evoked potentials recorded by a nearby depth electrode. If this effect, when it occured, was only small the potentials normally recovered within a few minutes. If there was little or no recovery the experiment was abandoned. However this was only a problem when using the 1st constant current device; this had a driving voltage of only 10 volts and so it was necessary to use larger tipped (smaller resistance) electrodes so that penicillin could be ejected without the constant surrent source overloading. The newer constant current device had a driving voltage of 90 volts. Therefore overloading was no longer a great problem and smaller tipped electrodes could be used. The electrode could be placed in the cortex without any detrimental effect on the evoked potentials. Furthermore with smaller tipped electrodes there was less spontaneous leakage from the electrode. A holding current (+10 to +50 nA) was automatically applied to the electrode once it touched the cortical surface and, in most cases, the electrode could be left for long periods of time without any effects on SEPs.

On some occassions, after the insertion of the penicillin electrode into the cortex, spontaneous epileptiform spikes began to occur, despite there being no ejecting current applied to the electrode. If such a situation arose, the electrode was removed and its resistance tested; if the resistance was 'large' (> 2 MOhms) the electrode was thoroughly washed and used again; in this case the effect of unwanted spontaneous spiking may have been attributable to the adherance of penicillin to the outside of the electrode. If the electrode resistance was small however, the effect was more likely due to leakage of penicillin from the electrode, which was then replaced by one with a higher resistance.

RESULTS

RESULTS: PART 1

I. The Characteristics of Normal Somatosensory Evoked Potentials

Somatosensory evoked potentials (SEPs) and potentials evoked by direct cortical stimulation (DCS) were recorded from within layer IV and from the surface of the somatosensory cortex of the urethane anaesthetised rat. Penicillin was ejected from a neighbouring glass microelectrode or from one barrel of a multi-barrelled electrode (of which another barrel was used for recording) by electrophoretic expulsion into layer IV.

Both SEPs and potentials evoked by DCS, when recorded in layer IV, had negative going waveforms. SEPs in the centre of the primary receiving area were composed of a series of 'wavelets' which may correspond to the different components of the surface recorded potential (described in the methods) and reflect the various stages in the processing of the afferent information. Away from the centre of the primary receiving area depth recorded SEPs consisted of a simple negative going deflection (see methods). The amplitude of the potentials was calculated from the point of the stimulus artifact to the maximum negativity of the evoked potential.

In the absence of penicillin ejection (with a retaining current applied to the penicillin containing electrode) there was a random variation in amplitude between successive SEPs, aswell as between potentials evoked by DCS, but there was no overall change in the mean amplitudes of the potentials (eg. fig. 3.1.2, prior to penicillin ejection) with time. The random variability between successive potentials may be a function of the state of ongoing spontaneous cortical activity (Carter et al., 1969), the effect of which on surface recorded potentials has already been discussed in the methods.

As there was no overall change of evoked potential amplitude with time, the effects of penicillin on cortical excitability could be investigated by monitoring the evoked potentials and the ECoG with the knowledge that any changes would not be due to spontaneous variability with time.

II. The Enhancement of Somatosensory Evoked Potentials by Penicillin

(a) Penicillin Ejected by Small Electrophoretic Currents: -50 to -100nA

After the start of penicillin ejection (-50 to -100nA) into layer IV of the cortex the depth recorded evoked potentials started to increase in amplitude so that after several minutes the amplitude of these enhanced potentials was 2 to 3 times larger than that of the pre-drug potentials (fig. 3.1.1). Furthermore, it can be seen from figure 3.1.2 that when the depth recorded SEP was composed of several components (ie it was recorded from the centre of the receiving area) the first of these was virtually unaltered whilst the second negative going wave was enhanced by the effects of penicillin. A similar feature was also true for the surface recorded response in that the initial positive wave (P1) was unchanged but the second positive wave (P2) was enhanced in amplitude by penicillin (fig. 3.1.1c). Since the same afferent input into the cortex resulted in a larger evoked potential than in the absence of penicillin, this implied that the ejection of penicillin resulted in an enhancement of cortical activity. Furthermore, the enhancement of the P2 component of the surface recorded response and the enhanced

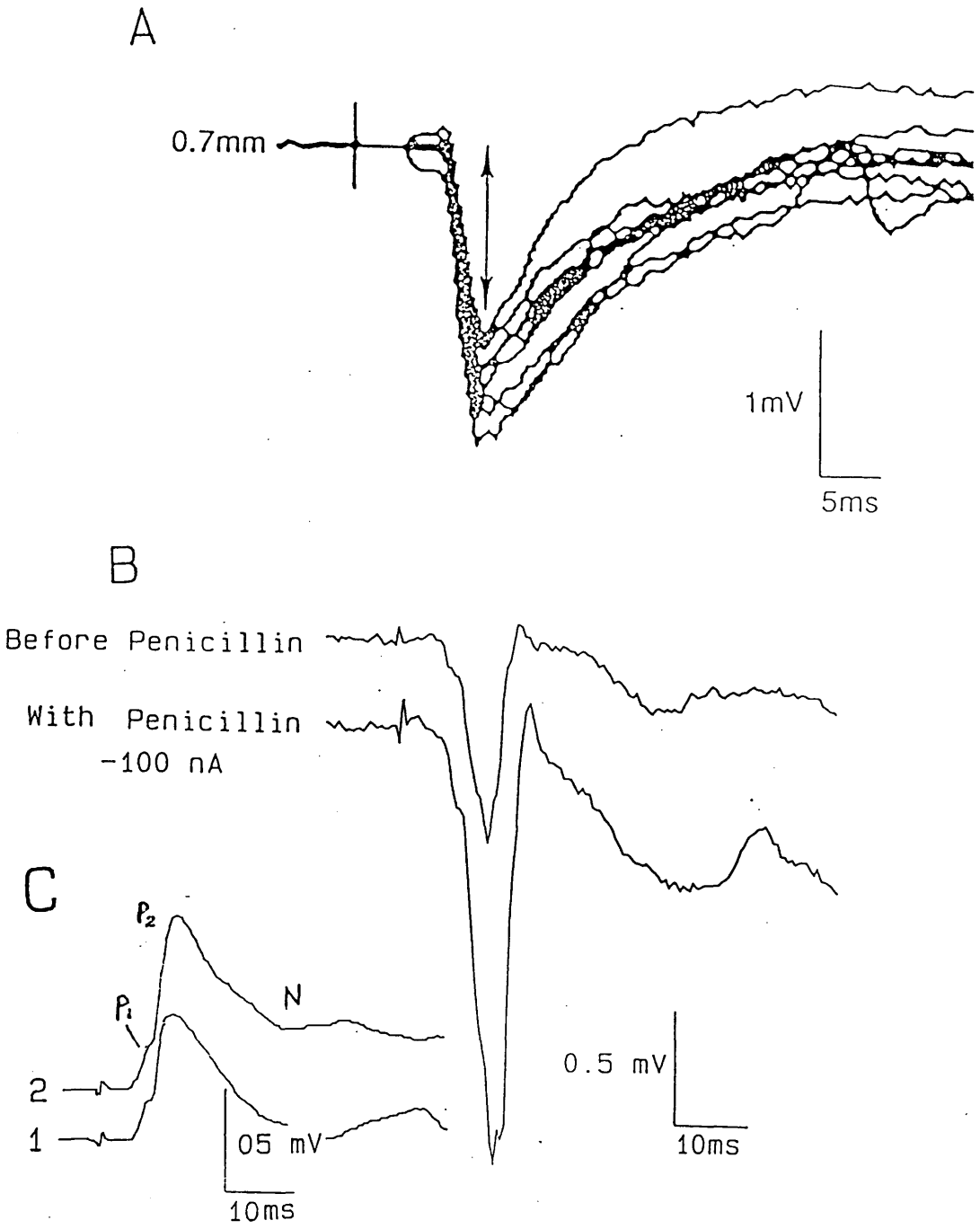


Figure 3.1.1: The enhancement by penicillin, ejected by small (-100 nA) electrophoretic currents, of somatosensory evoked potentials recorded from layer IV. (A) The smallest evoked potential (its amplitude indicated by the length of the arrow) was evoked before penicillin and the successive potentials were evoked every four seconds after the start of penicillin ejection. (B) Individual depth recorded potentials prior to and after several minutes of continued penicillin ejection (-100nA). The stimulus artifacts are just visible in the recording traces.

(C) The surface recorded potential was also enhanced by penicillin but this enhancement was confined to an increase in the amplitude of the P2 component.

amplitude of the depth response may reflect the same underlying enhancement of cortical activity.

Penicillin, when ejected by electrophoretic currents of up to -100nA, although resulting in an enhancement of evoked activity, did not produce any spontaneous epileptiform spikes. This implied therefore, that although the cortex was in a state of hyperexcitability this was insufficient to result in the occurrence of spontaneous epileptiform spikes.

The time course of the enhancement of the depth recorded evoked potentials was also monitored and the result of a typical enhancement by a small flux of penicillin is illustrated in figure 3.1.2. After the start of penicillin ejection there was a delay (usually 1 to 3 min) during which there was no detectable change in evoked potential characteristics. The evoked potentials then began to increase in amplitude and over the next few minutes followed a sigmoidal curve to reach a plateau level at about 300% of the pre-drug levels (fig. 3.1.2b). The plateau level was maintained for as long as penicillin ejection was continued. When penicillin ejection was terminated the amplitude of the evoked potentials, after a short delay, began to decline back towards normal levels. The time course of the recovery of evoked potential amplitude was similar to the time course of the enhancement.

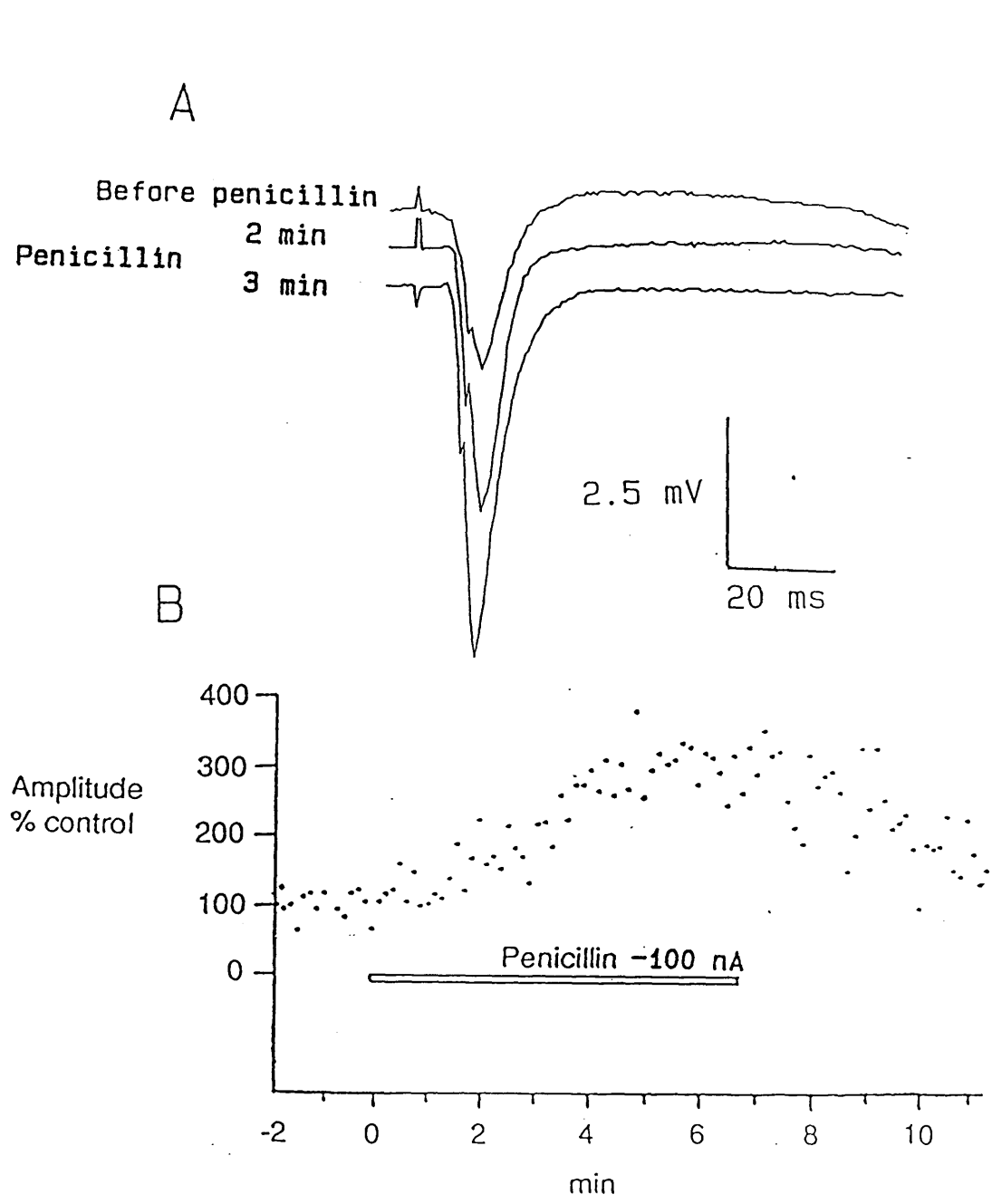


Figure 3.1.2: The enhancement of somatosensory evoked potentials by penicillin ejected by small (-100 nA) electrophoretic currents. (A) individual SEPs before and at various times during the onset of enhancement. Note that the second negative going component is enhanced to a greater extent (>200%) than the first component (50%). The stimulus artifacts are visible in the individual traces. (B) Time course of the penicillin induced enhancement of amplitude. Each point is the amplitude of an individual potential evoked every four seconds. The mean amplitude of potentials before penicillin ejection is taken as 100%. The amplitude of potentials is expressed as a percentage of this mean. Penicillin ejection is started at time 0.

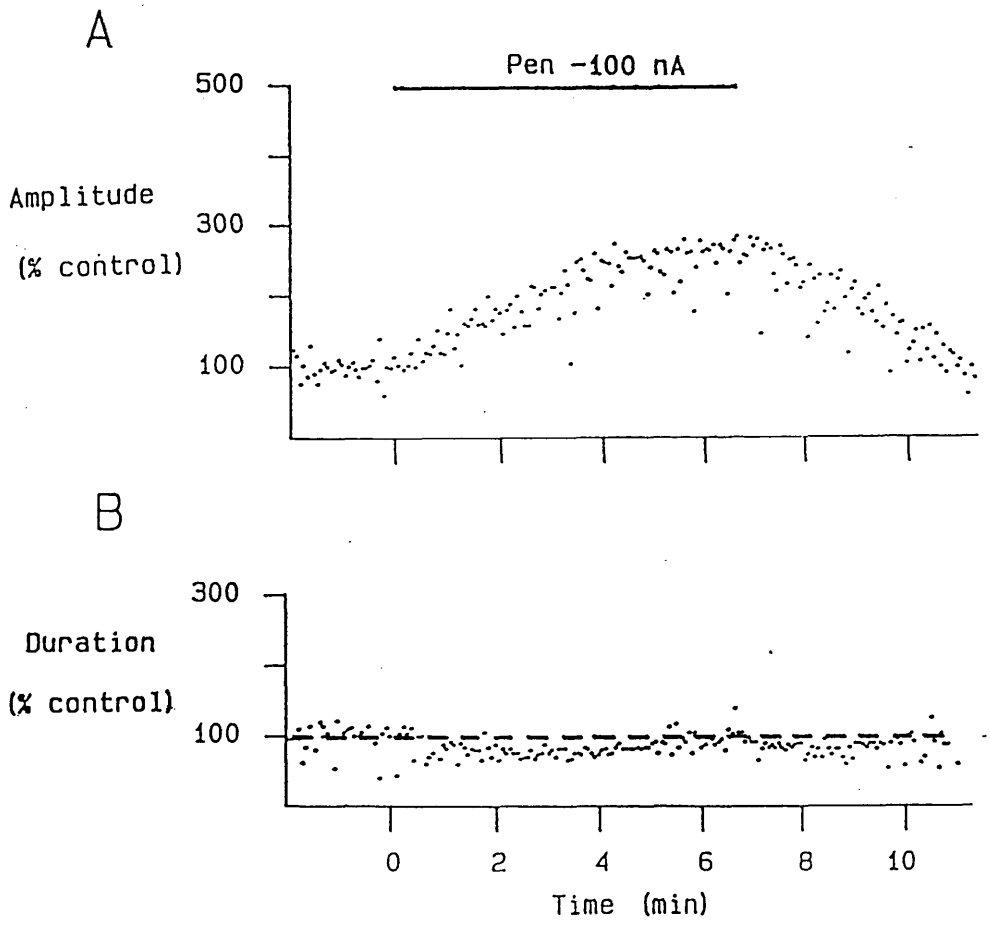


Figure 3.1.3: The effect of penicillin, ejected by small electrophoretic currents (-100 nA), on the amplitude (B) and duration (C) of somatosensory evoked potentials.

The waveform of the enhanced potentials, illustrated in figure 3.1.2a, suggested a lack of any effect of penicillin on the duration or the time to peak of the evoked potentials. Figure 3.1.3, in which the effect of the ejection of penicillin on the duration (time to recovery to 25% of maximum amplitude) of the potentials has been plotted, illustrated that despite the enhancement of amplitude there was indeed no enhancement of the duration of the evoked potentials. If there was any effect this was a decrease in the normal variability of the duration.

The results described above were typical for the effects of penicillin ejected by small electrophoretic currents. It will be clear from table 1, which summarises all the experimental values, that there was a certain degree of variability between the various parameters in different experiments. Any reasons for such differences will be considered further in the discussion.

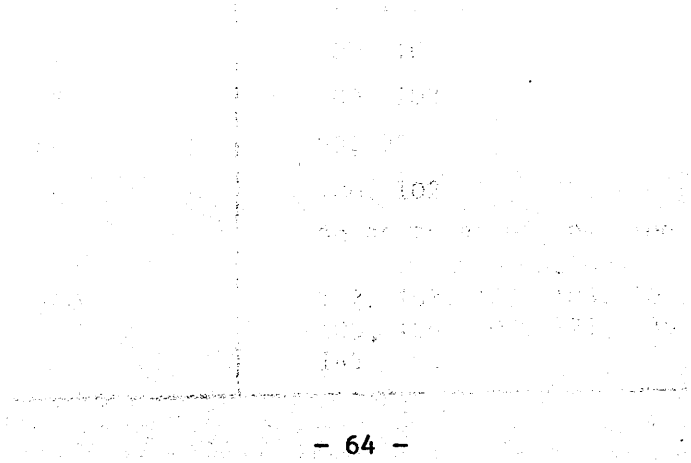


Table 1: Summary statistics of the Enhancement of Evoked Potentials by small Doses of Penicillin.

	Penicillin Dose	
	-50 to -90 nA n=12	-100 nA n=28
Delay to Onset of Enhancement (min)	2 - 6	1 - 4
Delay to Plateau Level (min)	7 - 14	5 - 12
Plateau Level (% control)	150 - 200	150 - 350

Penicillin (nA)	Enhancement of duration (% control)
-50	97, 100, 100, 104
-55	100, 102
-65	100, 103
-80	93, 95
-90	100, 102
-100	90,92,95,95,97, 97, 100 (9 observations) 102, 103, 103, 103, 105,105, 105, 106, 107, 125, 135,140, 145

The preceding results showed that small doses of penicillin resulted in an enhancement of evoked potentials and that this enhancement was confined to an increase in amplitude, without any change in the waveform of the potentials. The enhancement of evoked potentials by small doses of penicillin illustrated that a state of hyperexcitability existed within the cortex but that this was insufficient to result in the generation of spontaneous spiking. It was important therefore, to investigate how evoked activity in the epileptiform focus was enhanced when sufficient penicillin was ejected to result in spontaneous epileptiform spiking.

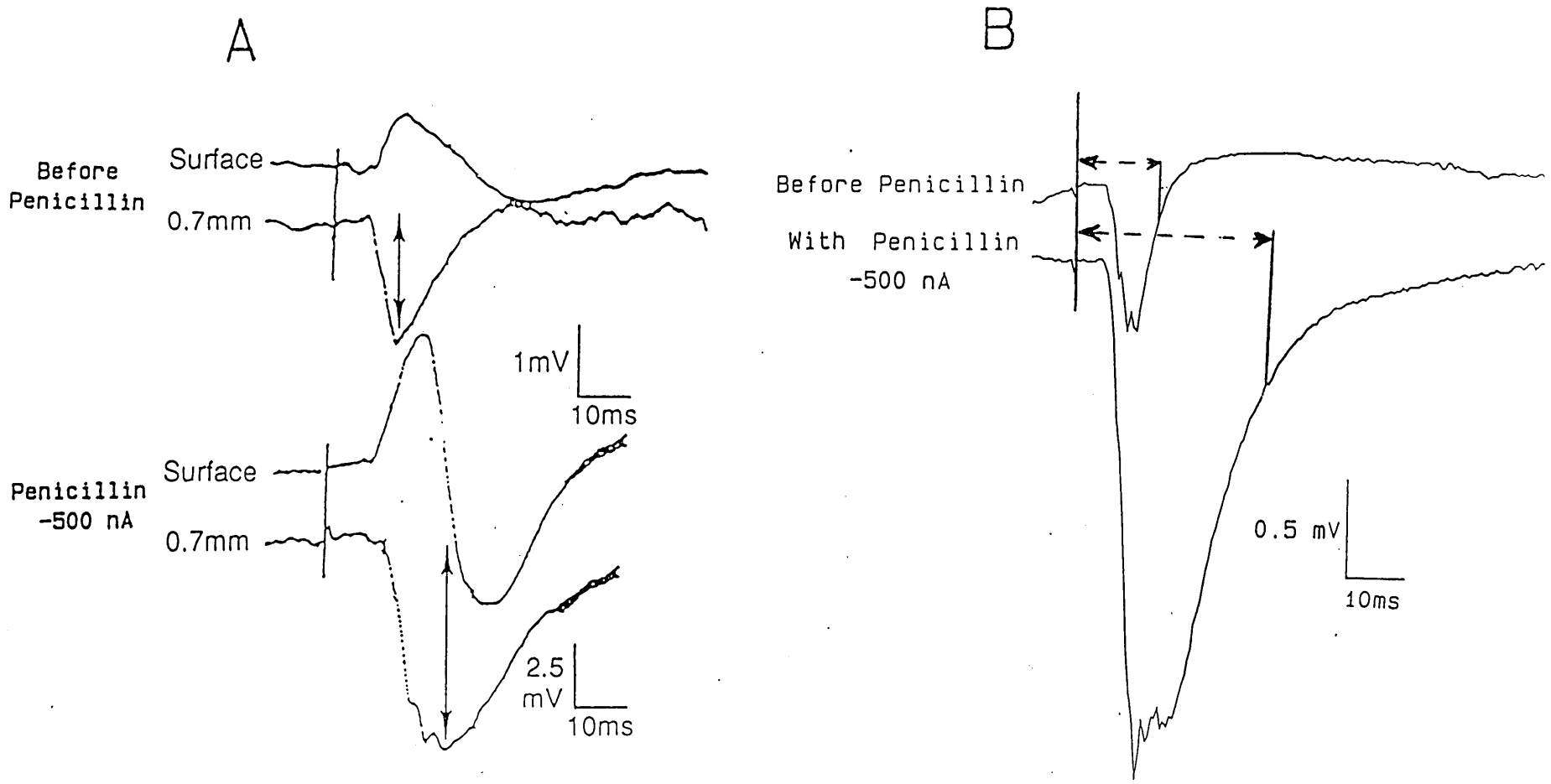
(b)Penicillin Ejected by Large Electrophoretic Currents: -500 nA

After the start of penicillin ejection (-500 nA) the depth recorded evoked potentials increased in amplitude so that after several minutes these were very much larger (in excess of 5mV) compared to normal (fig. 3.1.4) and also larger than evoked potentials enhanced by small doses of penicillin (see fig. 3.1.5). As well as the large increase in amplitude, there were also dramatic changes in the waveforms of the evoked potentials; there was an increase in the delay to the peak and an increase in the overall duration of the depth recorded potentials. By the time the enhancement had reached a steady level the duration of the depth recorded potentials was much

larger (50 to 100 msec) than that of normal evoked potentials (10 to 15msec). Spontaneous epileptiform spikes also began to occur during the enhancement of the evoked potentials.

The surface recorded positive going wave (P2) was also enhanced in amplitude. In addition, the later negative-going wave (N2) of the surface recorded response became very large in amplitude and duration and dominated the surface recorded evoked potential (fig. 3.1.4a). During this stage the depth recorded enhanced evoked potentials, except for the initial component, were very similar in appearance to spontaneous epileptiform spikes (fig. 3.1.6) except that the wavefront of the enhanced evoked potential was very much steeper than that of the spontaneous spike. Furthermore, the surface recorded enhanced SEP had a very large P2 wave which, since it reflects the evoked activity, was absent from the surface record of spontaneous spikes. Clearly then, the ejection of larger doses of penicillin, sufficient to induce spontaneous epileptiform spiking, resulted in a further enhancement of evoked activity in addition to that brought about by small doses of penicillin.

Figure 3.1.4: The effect of penicillin, ejected by large electrophoretic currents (-500nA), on surface recorded (A) and depth recorded somatosensory evoked potentials (A and B). The arrows marked indicate the maximum amplitude (A) and the duration (B) of the responses.


A
Before Penicillin
Surface
0.7mm
1mV
10ms
Penicillin -500 nA
Surface
0.7mm
2.5 mV
10ms
B
Before Penicillin
With Penicillin -500 nA
0.5 mV
10ms

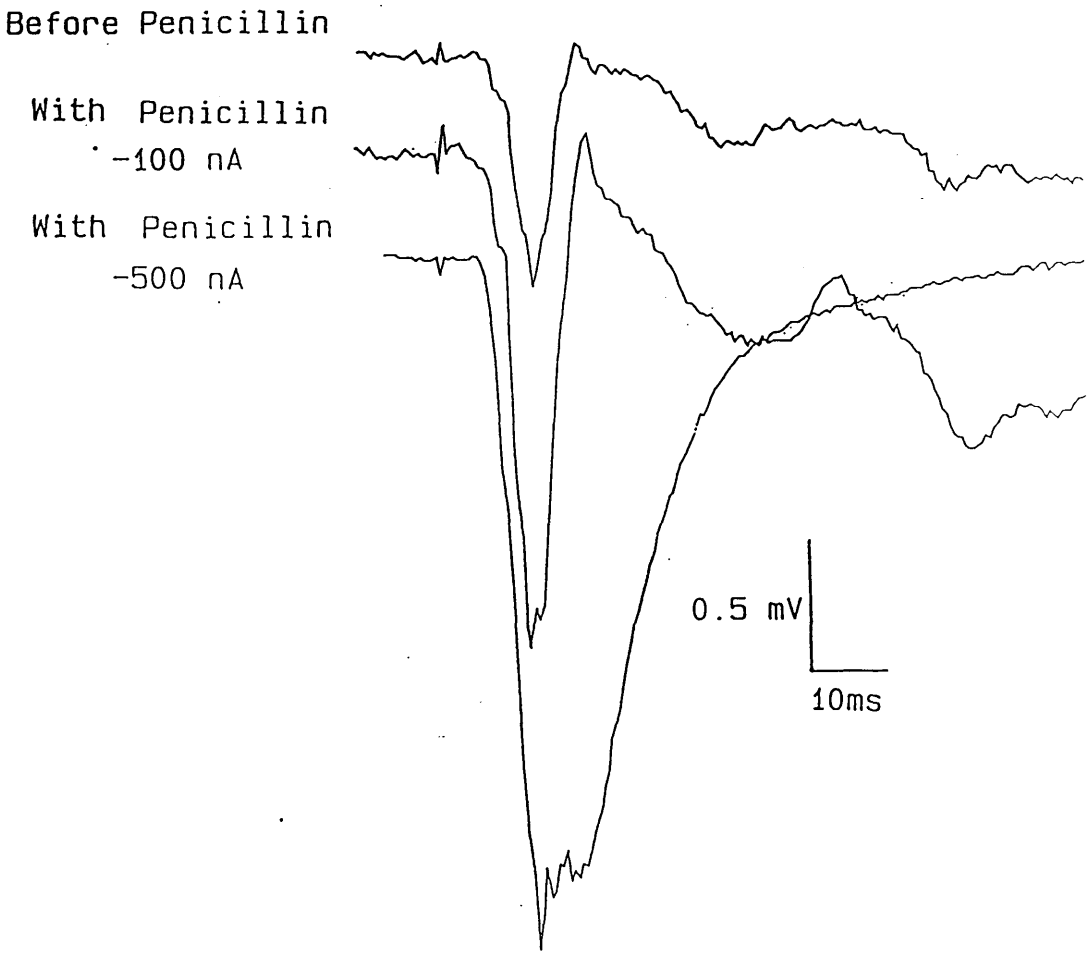
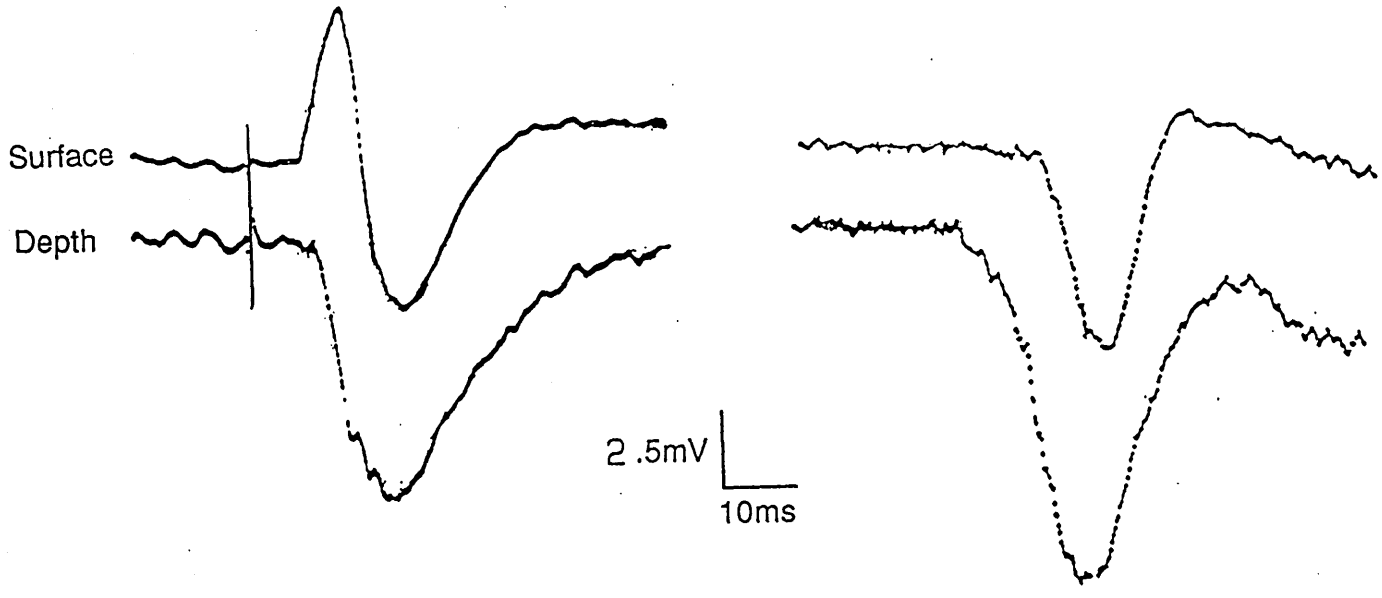


Figure 3.1.5: A comparison of the effects of small (-100nA) and large (-500nA) doses of penicillin on depth recorded somaatosensory evoked potentials. Note the dramatic increase in the duration due to larger doses of penicillin compared to that in the absence of penicillin or in the enhancement by smaller doses of penicillin.

Figure 3.1.6: Comparison of somatosensory evoked potential, enhanced by large doses of penicillin, and a spontaneous epileptiform spike. The waveform of the spontaneous spike is very similar to that of the enhanced evoked potential. The only difference is in the initial component; the surface P2 wave and the very steep rising phase of of the depth recorded EP are absent from the spontaneous spike waveform. This suggests that the activity underlying the later component of the enhanced EP may be similar to that which underlies the spontaneous spike.



Somatosensory evoked potential

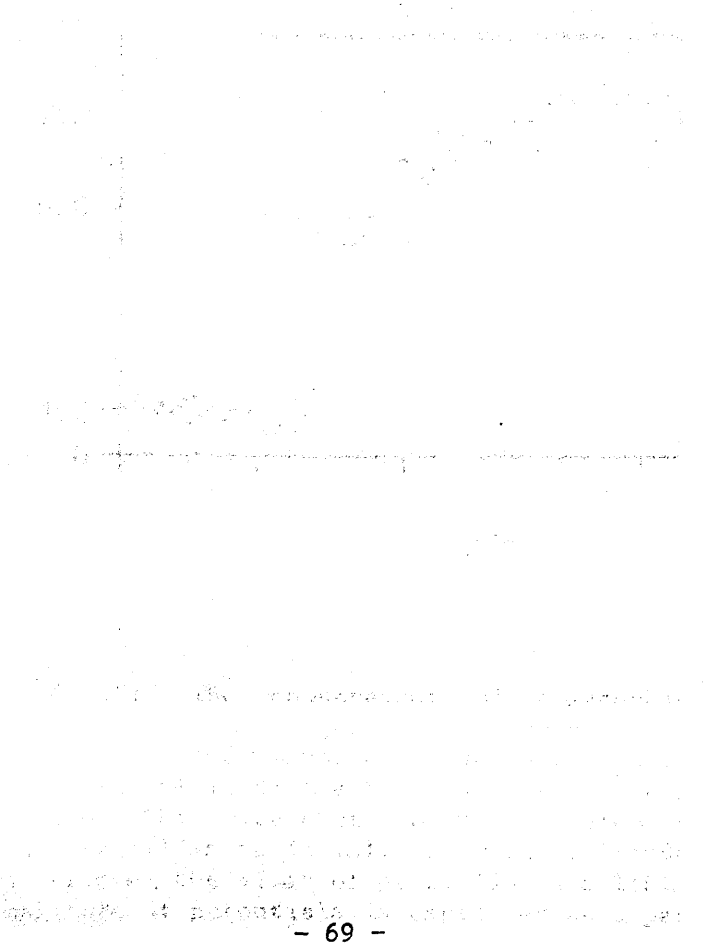
Spontaneous epileptiform spikes

The Time Course of Enhancement

After the start of penicillin ejection (-500nA) there was a delay, generally shorter than that with smaller penicillin ejecting currents, before any change in the evoked potentials occurred. After this delay there was an increase in the duration (fig. 3.1.8), and an enhancement of amplitude of the evoked potentials (fig. 3.1.7 and 3.1.8). As illustrated in figure 3.1.8, there was no difference between the time course of enhancement of amplitude and the enhancement of duration; at the onset of the enhancement (point A in fig. A7) both the amplitude and duration increased together. The rate of enhancement was much more rapid than had been evident with smaller penicillin fluxes. The increase in amplitude and duration continued until both reached plateau levels of enhancement within a few minutes. The plateau level of enhancement was maintained for as long as penicillin ejection was continued.

On termination of penicillin ejection there was a short delay before the recovery of the evoked potentials started. In contrast with the sequence of events during the onset of enhancement there was now a considerable difference in the time courses of the recovery of the amplitude and the recovery of duration. The amplitude of the evoked potentials recovered more slowly than the duration so that there was a phase (after

point B in fig. A7) during which the duration of the potentials had returned to normal levels although the amplitude was still greater than pre-drug levels. This situation was analagous to the enhancement brought about by smaller penicillin ejecting currents: ie. an enhancement of amplitude with no change in duration of the evoked potentials.



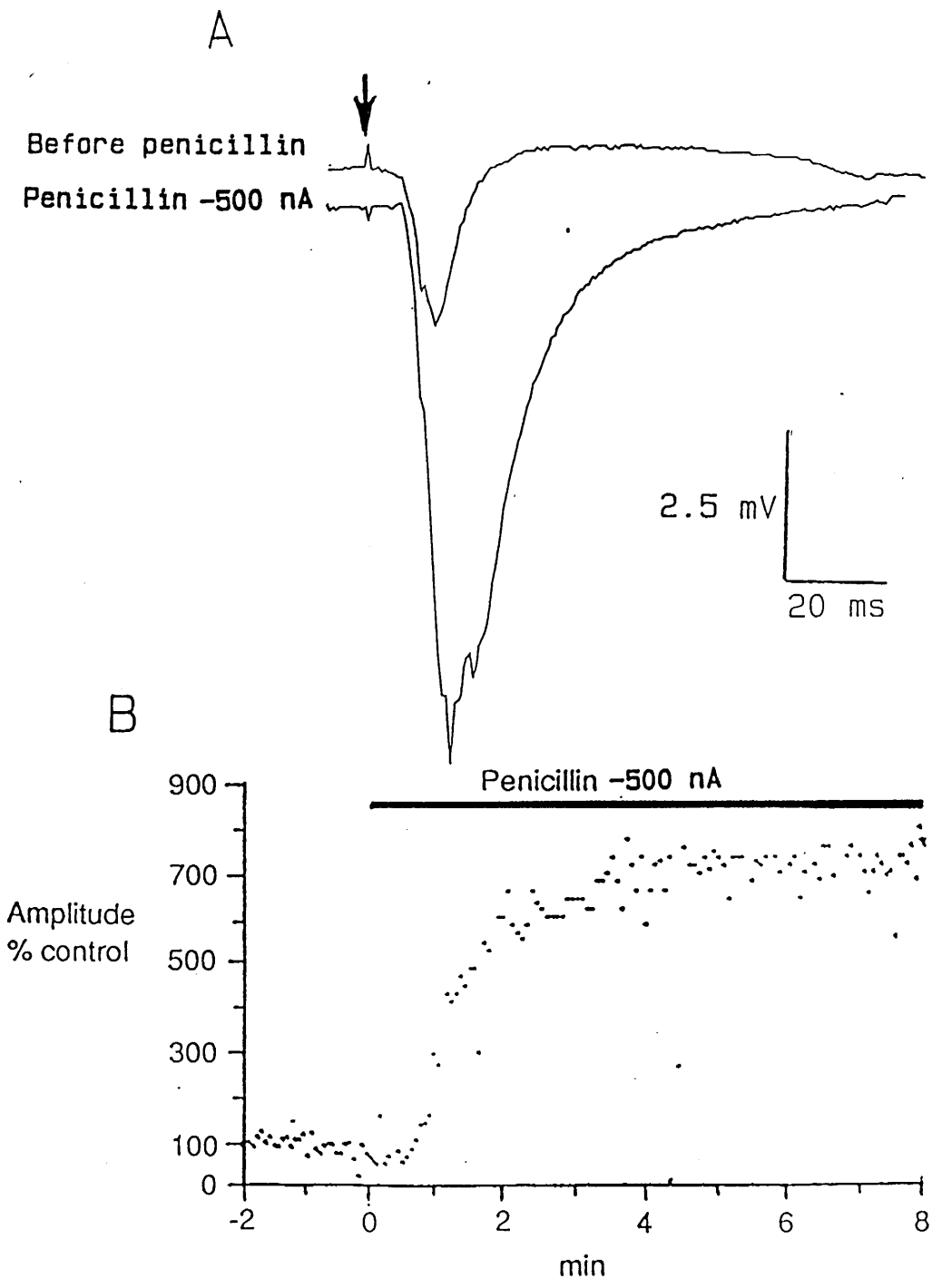


Figure 3.1.7: The enhancement of somatosensory evoked potentials by penicillin ejected by large electrophoretic currents (-500 nA). Individual responses before and after about 1.5 min after the start of ejection (A). Arrow indicates time of stimulation. The time course of the enhancement (B) by the same dose of penicillin as in (A). The mean amplitude of evoked potentials before the onset of pencillin is calculated as 100% and the amplitude of potentials is expressed as a percentage of the mean.

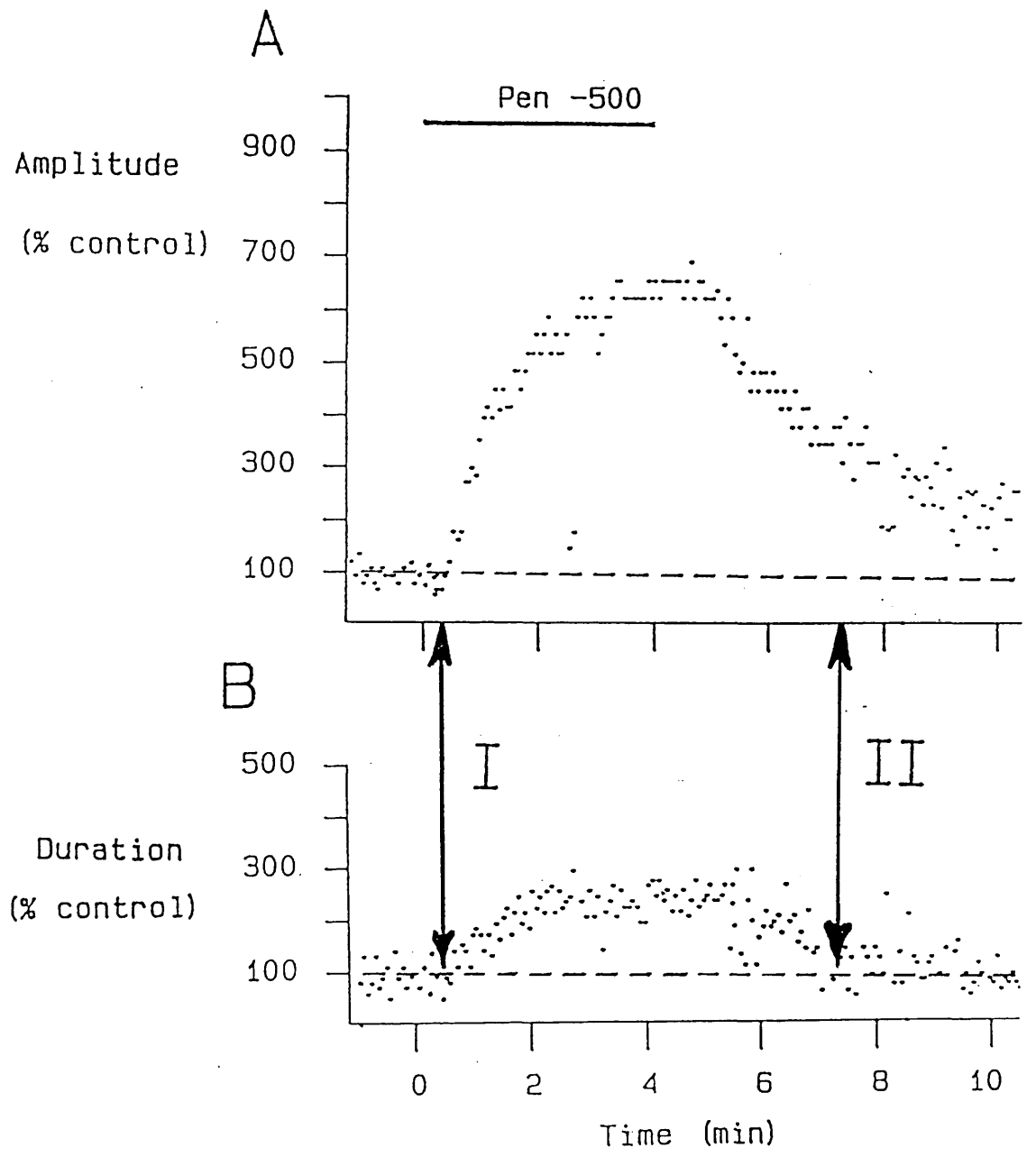


Figure 3.1.8: The enhancement of amplitude (A) and duration (B) of somatosensory evoked potentials by penicillin ejected by large electrophoretic currents (-500 nA). Arrow I indicates the onset of enhancement; enhancement of both amplitude and duration starts at the same time. Arrow II indicates the time at which the duration of the SEPs returns to pre-drug levels. Note that at this time the amplitude of the potentials is still considerably greater than pre-drug levels.

Figure 3.1.7 and 3.1.8 describe the time course of a typical enhancement of depth potentials resulting from large penicillin fluxes; a summary of all the experimental values is given in table 2.

Table 2: The Enhancement of Evoked Potentials by Large Doses of Penicillin.

	Penicillin Dose		
	-250 nA n=15	-500 nA n=25	> -500 nA n=13
Delay to Onset of Enhancement (min)	1 - 4	0.5-2.5	0.5-2
Delay to Plateau Level (min)	5- 11	3.5-8	3.5-7
Plateau Level (% control)	200-400	350-1200	350-1100
Enhancement of Duration (% control)	150-200	150-300	200-350

These results showed that using larger doses of penicillin the enhancement of evoked potentials was very different to the enhancement brought about by smaller doses of penicillin. There was a dramatic change in the waveform of the evoked potentials, and the occurrence of spontaneous spiking, which indicated that additional changes in cortical hyperexcitability to those produced by smaller doses of penicillin were occurring.

The first phase (phase I) of cortical hyperexcitability, reflected by a simple enhancement of the amplitude of evoked potentials without any change in the waveform, was only apparent when penicillin was ejected by small electrophoretic currents (eg. fig. 3.1.2) or during the recovery from the enhancement by a large dose of penicillin (eg. fig. 3.1.8). There were no spontaneous epileptiform spikes during phase I, which implied that the hyperexcitability was insufficient to induce spontaneous paroxysmal events.

The second phase of hyperexcitability only occurred when penicillin was ejected by larger electrophoretic currents and was associated with an increase in the duration and time to peak of the evoked potentials. This change in waveform suggested the onset of a different form of neuronal activity to that underlying phase I and normal evoked potentials and will be referred to as phase II. This second phase was

associated with the appearence of spontaneous epileptiform spikes.

Because of the requirement of different magnitudes of penicillin ejecting currents for the production of the different phases of epileptogenesis, one possible interpretation of the above results was that each phase of evoked potential enhancement required a critical threshold concentration of penicillin (smaller for phase I than phase II) at the edges of a given volume of cortex, in the same way as a critical concentration is required for the generation of spontaneous epileptiform spikes (Lockton and Holmes, 1983). A small ejecting current achieved the concentration of penicillin at the edges of the volume which was necessary to result in phase I. A larger ejecting current achieved a higher concentration at the edges of the volume and resulted in phase II. However, the rate of increase of the concentration when using an ejecting current of -500 nA was so rapid that phase II occurred almost immediately and phase I was not apparent. Only after the termination of penicillin ejection, when the penicillin concentration field declined slowly, did phase I occur by itself (see fig. 3.1.8).

(III) The Enhancement of Evoked Potentials by Penicillin Ejected by Intermediate Electrophoretic Currents

If the above assumption was correct, and two distinct phases of cortical excitability did occur in the development of the epileptic focus, then to disentangle the two phases during the development of the focus required that the threshold penicillin concentration for phase II be achieved over a longer time course than that during the ejection of penicillin by the large ejecting current in the previous section (-500nA; part IIb), which immediately resulted in phase II. Penicillin ejected by electrophoretic currents smaller than -500nA was sufficient to produce spontaneous epileptiform spikes and therefore, presumably sufficient to result in phase II of evoked potential enhancement. The ejection of penicillin by a smaller electrophoretic current should however, result in a slower rate of increase of penicillin concentration at the edges of the volume and therefore, may allow the temporal seperation of phase I and phase II.

After the start of ejection of penicillin with an 'intermediate' electrophoretic current (-250 nA) there was indeed an initial enhancement of the amplitude of the evoked potentials without any change in the duration, indicating the presence of phase I in the development of the epileptic focus.

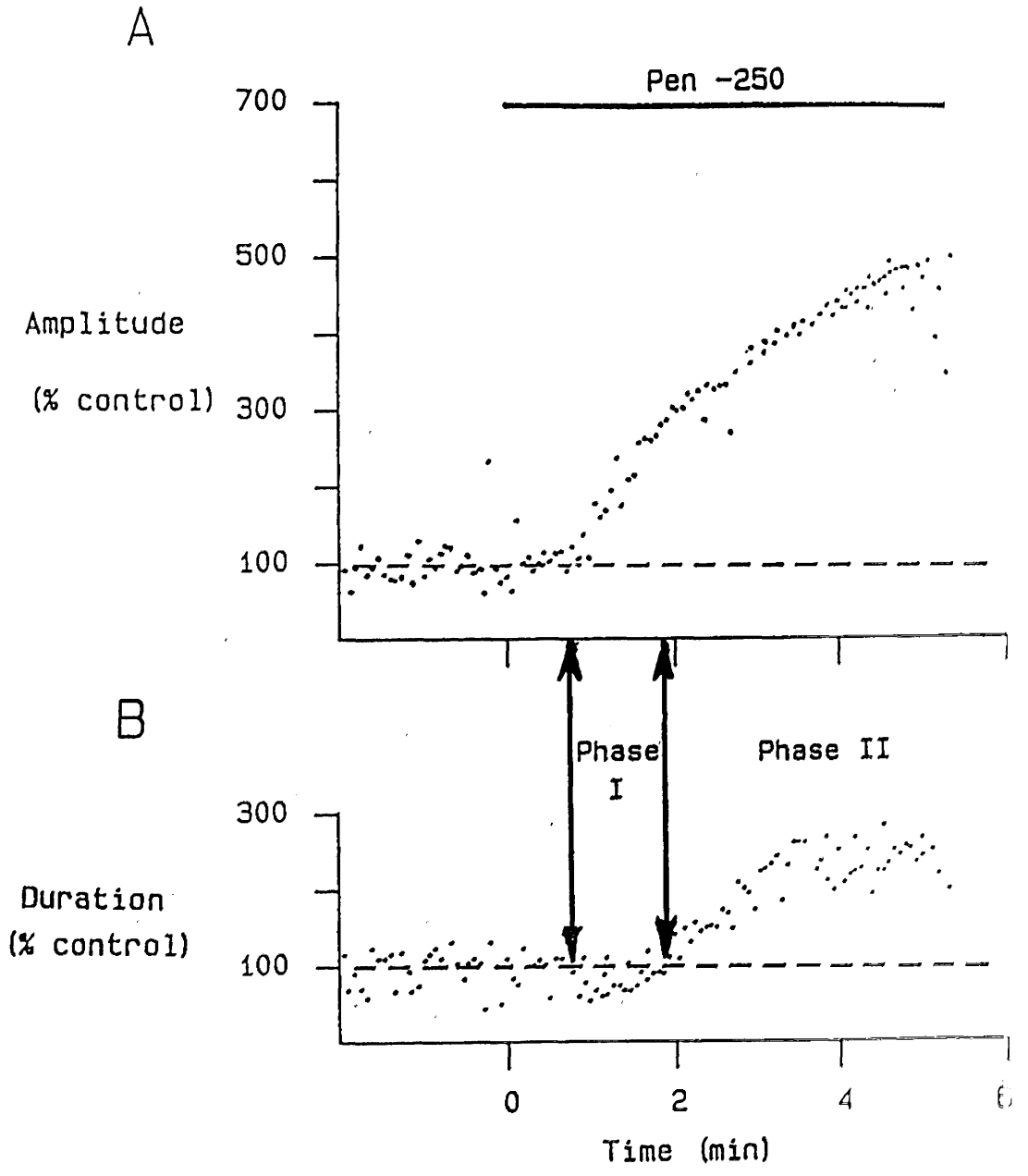


Figure 3.1.9: The effect of penicillin ejected by 'intermediate' electrophoretic currents (-250 nA) on the amplitude (A) and the duration (B) of somatosensory evoked potentials. The first arrow indicates the onset of phase I, in which there is an enhancement of amplitude without any increase in duration. Second arrow indicates the onset of phase II when the enhancement of duration begins.

This is illustrated in figure 3.1.9 in which the duration of the potentials were not enhanced during the first two minutes after the start of penicillin ejection. Meanwhile, the amplitude of the potentials were enhanced to about 300% of pre-drug values. As the enhancement of amplitude continued, the duration of evoked potentials then also started to increase, which indicated the onset of phase II. The enhancement of amplitude and duration continued until plateau levels for both parameters were reached.

(IV)Penicillin Ejected by Stepped Functions of Ejecting Current

Another possible means of obtaining a separation of the two phases during the development of the epileptiform focus was to step the penicillin ejecting current from a small to a large value once phase I had already been established. This should then result in the onset of phase II.

Penicillin ejection was initially started with small ejecting currents (-100 nA to -150nA). After several minutes, the enhancement of amplitude reached a plateau (between 200% and 300% of pre-drug levels) without any enhancement of the duration of the potentials (fig. 3.1.10 and 3.1.11). This indicated that phase I enhancement of the evoked potentials had been achieved. The penicillin ejecting current was then

increased (-250 nA to -500nA) and there was almost immediately a further increase in amplitude which, this time, was accompanied by an enhancement of the duration (phase II). Both parameters then reached their respective plateau levels (between 400 and 500%; amplitude and 200 and 300%; duration) (fig. 3.1.10 and 3.1.11).

Thus, the two phases of enhancement of the evoked potentials could be separated during the development of the epileptic focus.

On termination of penicillin ejection the duration and the amplitude of the evoked potentials both started to decline from their respective plateau levels. After about two minutes the duration of the enhanced potentials returned to pre-drug levels, whilst the amplitude was still greater than its pre-drug level (phase I). Over the next few minutes the amplitude of the potentials also returned to normal levels (fig. 3.1.10 and 3.1.11).

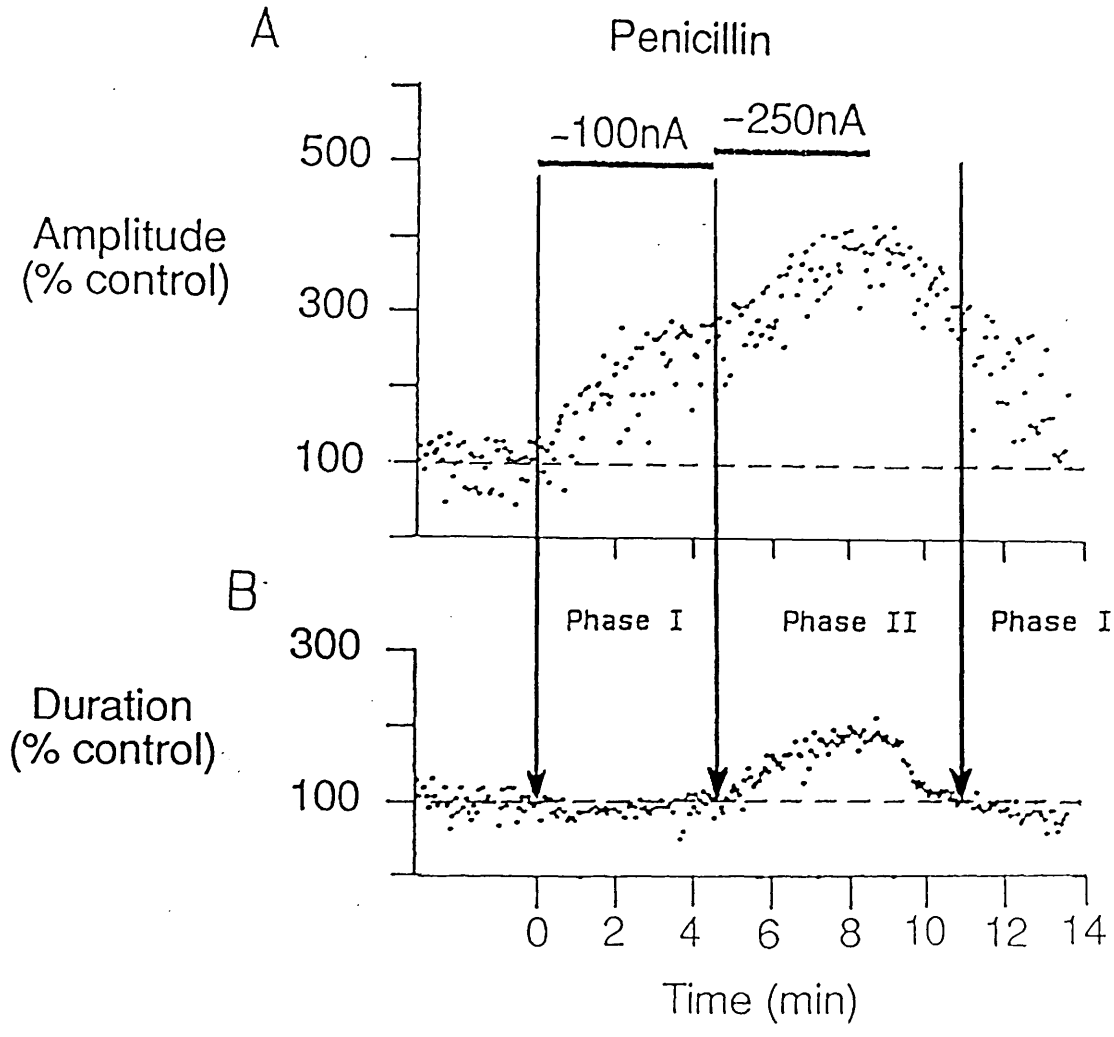


Figure 3.1.10: The effect of penicillin ejected by small (-100 nA) and larger (-250 nA) electrophoretic currents on the amplitude (A) and duration (B) of somatosensory evoked potentials. The arrows indicate the demarcation between phase I, an enhancement of amplitude with no increase in duration, brought about by small doses of penicillin and phase II, an increase in amplitude and duration, resulting from larger doses of penicillin. Note that phase I occurs once again after the termination of the ejection of the larger dose.

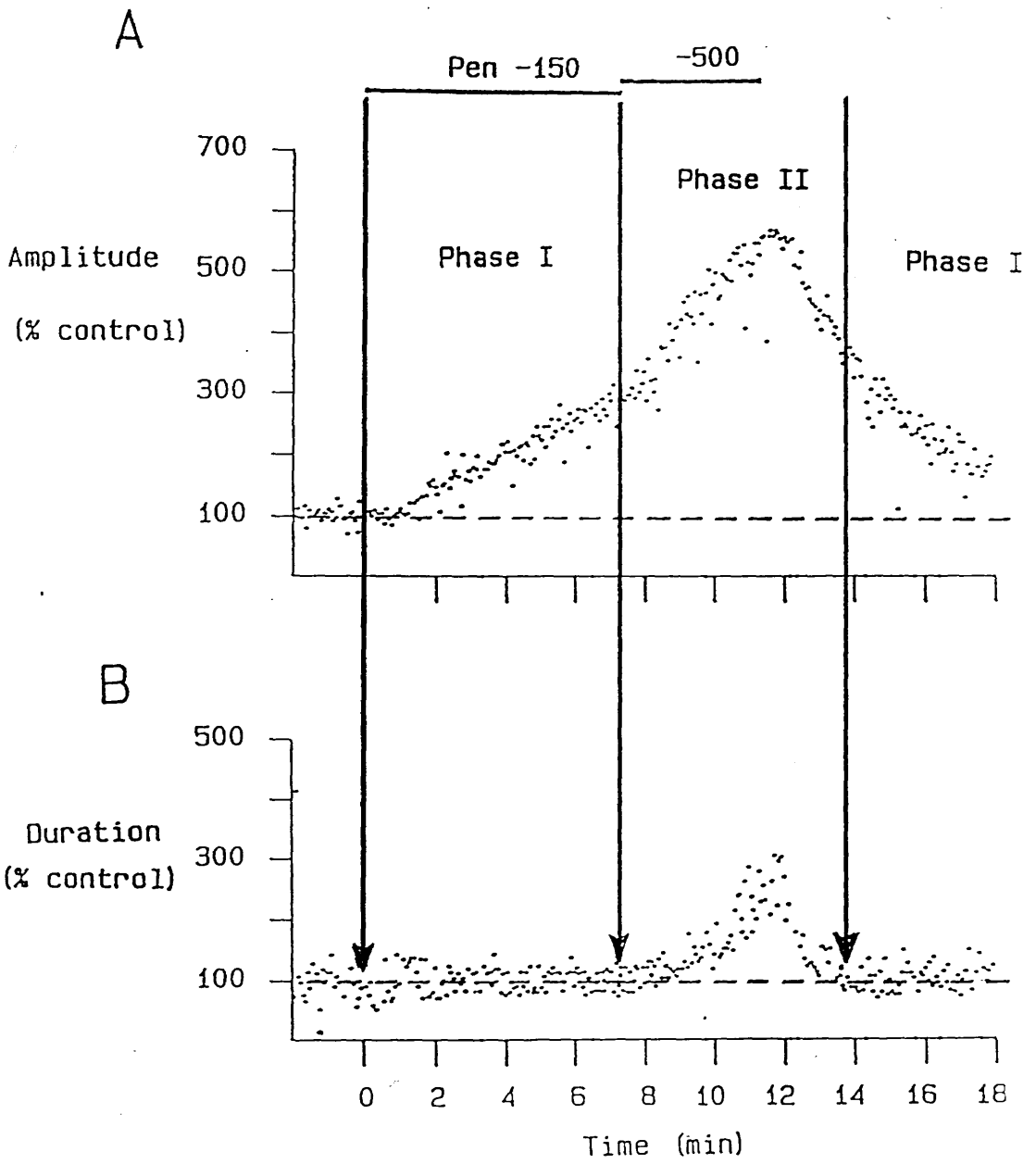


Figure 3.1.11: The effect of penicillin ejected by small (-150 nA) and larger (-500 nA) electrophoretic currents on the amplitude (A) and duration (B) of potentials evoked by direct cortical stimulation (DCS). The arrows indicate the demarcation between phase I, an enhancement of amplitude with no increase in duration, and phase II, an increase in both amplitude and duration. Note that phase I occurs by itself again during the recovery from phase II.

Conclusion The above results suggested that when sufficient penicillin to result in spontaneous epileptiform spiking was ejected there were two phases in the enhancement of evoked activity. The first of these was reflected by an enhancement of the amplitude of EPs, which resulted from the ejection of small doses of penicillin. Phase II, which resulted from larger penicillin doses, was reflected by a change in waveform of evoked potentials which only occurred together with, or after, an enhancement of the amplitude and never by itself. Furthermore, the second stage of enhacement (the increase in duration) was associated with the onset of spontaneous epileptic spiking. Evoked potentials in phase I resembled normal potentials whereas evoked potentials in phase II resembled evoked epileptiform spikes. The two different phases suggested the presence of two different forms of activity during the development of the epileptiform focus. It was important therefore to investigate whether there were any differences in the physiological characteristics of the evoked potentials in the two phases of penicillin epileptogenesis because of the possibility that the activity underlying phase I was the same as normal, whereas that underlying phase II might be 'epileptic' in nature. The next section of the results describes the investigation into some of the physiological properties of the evoked potentials during the two phases of epileptogenesis.

RESULTS: PART 2

The Physiological Characteristics of Activity Underlying the Different Phases of Epileptogenesis

One possible means of differentiating between 'epileptic' and 'normal' activity was on the basis of the refractory periods of the evoked potentials during the different stages of their enhancement. In a well established penicillin focus, however vigorous the spiking, there is always an apparent minimum separation between two epileptiform spikes and it has been shown that epileptiform spikes, whether spontaneous or evoked, have a much longer refractory period than normal potentials (Matsumoto and Ajmone-Marsan, 1964).

A conditioning/test paradigm was adopted to test the refractory periods of the potentials in the different stages of pencillin induced enhancement. Before assessing the properties of the enhanced potentials the refractory periods of normal evoked potentials were tested.

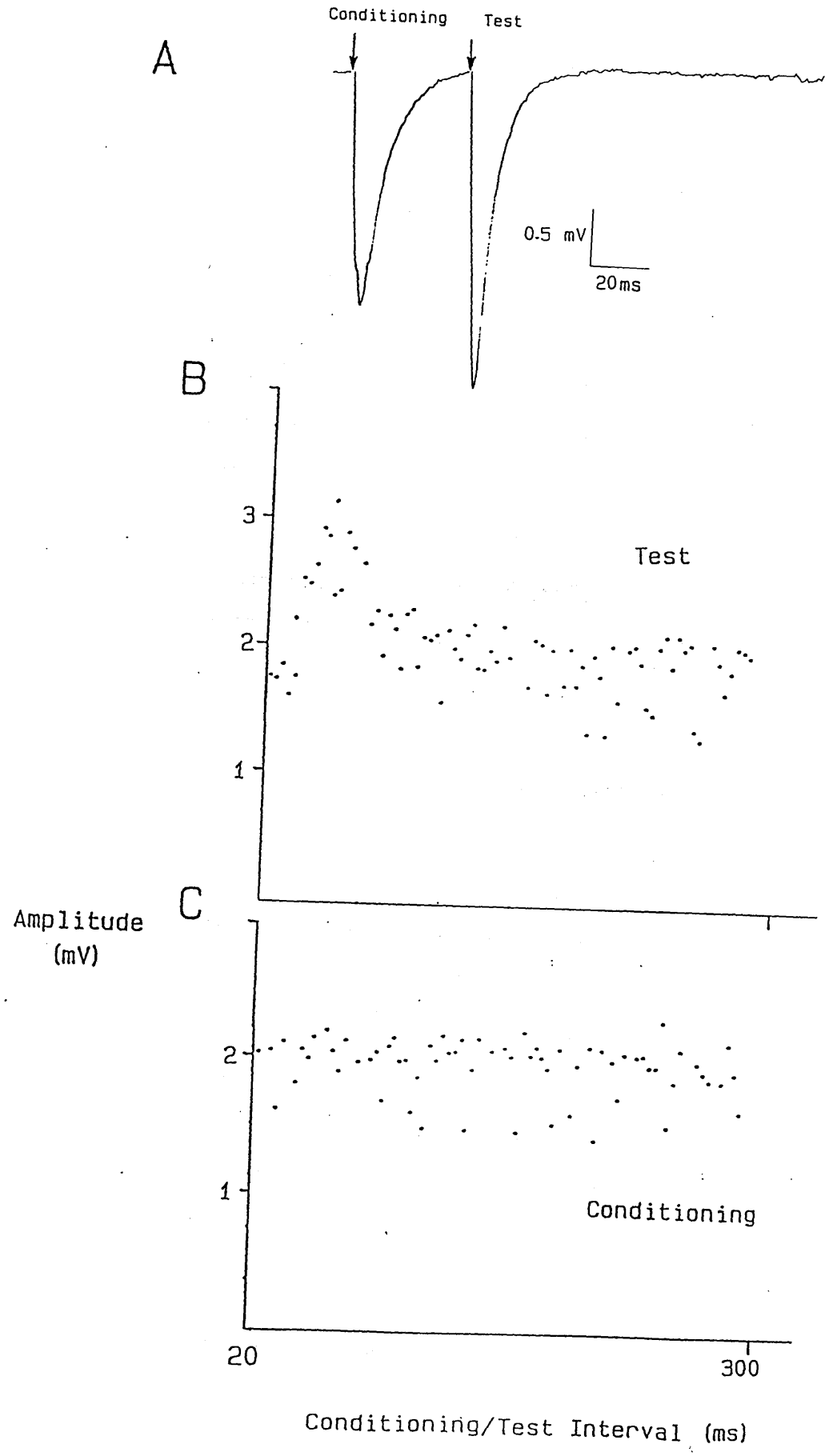
In order to ascertain the refractory periods of normal potentials, pairs of stimuli were delivered every 4 seconds and the delay between the pair was increased, in 20 msec steps, from 20 to 300 msec. The amplitudes of the conditioning and test potentials were monitored. The amplitudes of both the individual responses are shown in some cases (eg. fig. 3.2.1) and in other cases the mean amplitude and standard error of five responses at each delay are illustrated (eg. fig. 3.2.2).

I(a) The Refractory Period of Normal Potentials Evoked by Direct Cortical Stimulation

For potentials evoked by direct cortical stimulation (DCS) there was no discernable absolute refractory period; with small delays the test potential arose out of the waveform of the conditioning potential and the true amplitude of the test and conditioning potentials could not easily be ascertained. Therefore, data was only taken with delays greater than 20 msec, by which time the test stimulus occured after the end of the conditioning potential. With delays greater than 20ms the amplitude of the test potential was similar to that of the conditioning potential. However, a further increase in the delay, to between 40 and 60 msec, resulted in a supra-normal phase in which the test potential amplitude was larger than that of the conditioning potential (fig. 3.2.1). With a further increase in the delay the amplitude of the test

potentials was once more comparable to that of the conditioning potentials.

Figure 3.2.1: The refractory period of potentials evoked by direct cortical stimulation (DCS) tested by the amplitude of responses to test stimuli (B) compared to responses to conditioning stimuli (C) as a function of increasing conditioning/test interval from 20 to 300 msec in 20 msec steps. Five responses were evoked and are shown at each delay. Apart from a supra-normal phase for the test response amplitude, between 40 and 60 msec (A and B), both conditioning and test responses were of a similar amplitude.


A
Conditioning
Test
0.5 mV
20ms
B
3
2
1
Test
C
Amplitude
(mV)
2
1
Conditioning
20
300
Conditioning/Test Interval (ms)

I(b)The Refractory Periods of Normal Somatosensory Evoked Potentials

The refractory period of normal Somatosensory evoked potentials (SEP) was greater than that of normal potentials evoked by DCS, being about 100ms. There was no supra-normal phase at smaller delays as there was with potentials evoked by DCS. Furthermore, absolute and relative refractory periods, which had not been evident for potentials evoked by DCS, were about 25 and 80ms respectively.

II The Effect of C/T Interval on the Test Potentials During the Different Phases of Epileptogenesis

a. Phase I

For potentials evoked by DCS, when phase I was established, by the ejection of penicillin by a small electrophoretic current, the effect on the test potentials of changing the C/T interval was similar to that already described in the absence of penicillin; with intervals greater than 20msec, apart from the supra-normal phase, the test potentials were of a similar amplitude to the conditioning potentials at all delays; the response to the test stimulus was enhanced to the same extent as the response to the conditioning stimulus. For SEPs in phase I, the effect on the test potential of changing the C/T

interval was the same as for SEPs in the absence of penicillin (see fig. 3.2.2).

b. Phase II

During phase II the effect of changing the C/T delay on the test potentials was markedly different to that during phase I or in the absence of penicillin (fig. 3.2.3). For potentials evoked by DCS, with the smallest delays the test potentials were slightly depressed compared to the conditioning potentials. As the delay was increased the test potentials were further depressed so that with a delay of between 80 to 140 msec the amplitude of the test potentials was at its smallest level and only with larger delays did these begin to recover; by 300 msec the amplitude of the test potentials was comparable to that of the conditioning potentials. For SEPs, apart from the absence of the supra-normal phase, the situation was similar to that for potentials evoked by DCS. Instead of the refractory period of normal or phase I potentials there was a very long delay before the test potentials were comparable to the conditioning potentials.

These results indicated that during the enhancement of EPs by penicillin, because there was no depression of test responses compared to the conditioning responses, the activity underlying phase I had a refractory period which was no different to normal. However, because test responses during

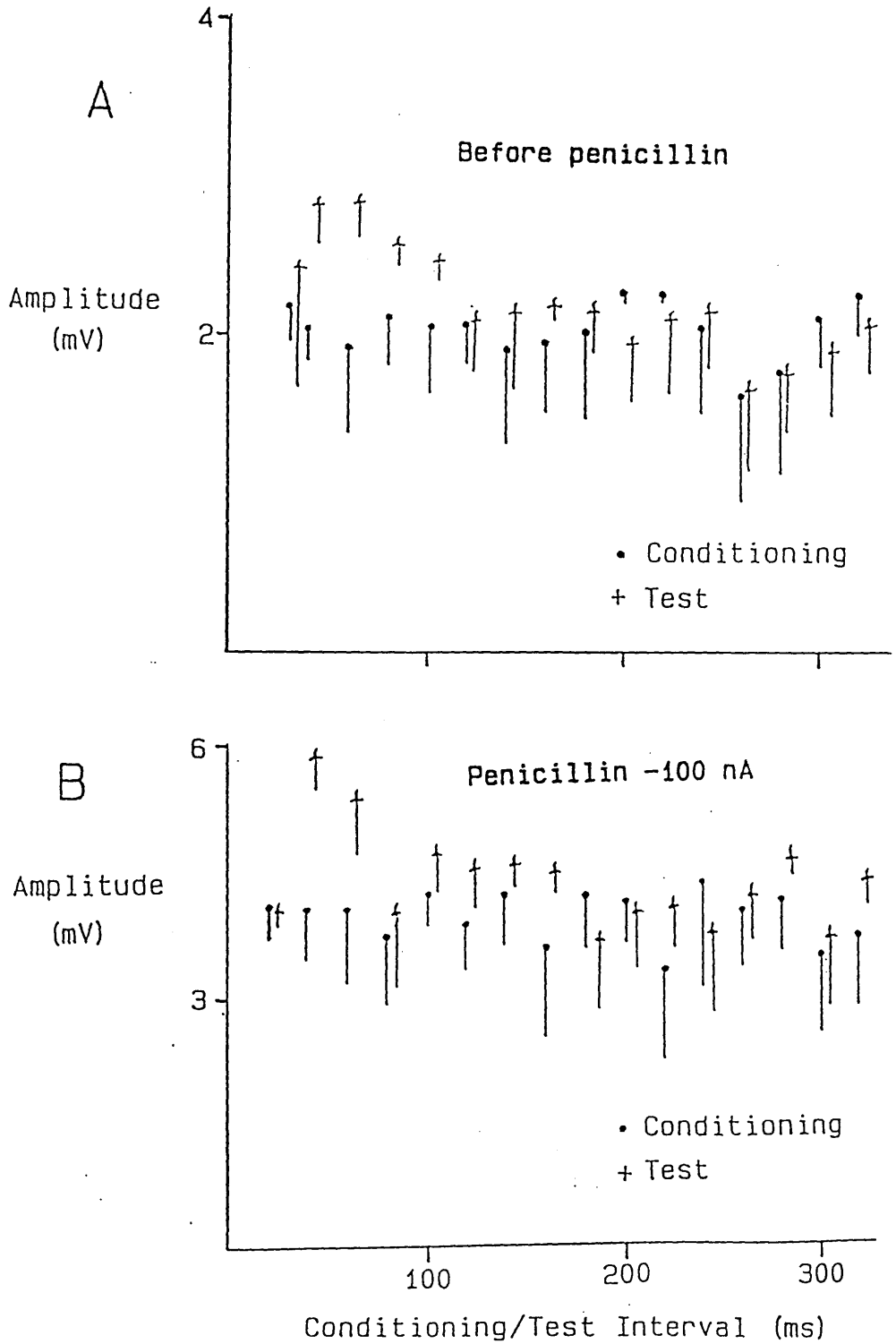


Figure 3.2.2: The refractory period of potentials evoked by DCS tested by increasing the C/T interval before (A) and during phase I (B) of penicillin epileptogenesis. Means and SEM of five responses at each delay (at 20 msec intervals) between 20 and 300 msec. Responses to test stimuli are shown offset from the conditioning responses for clarity.

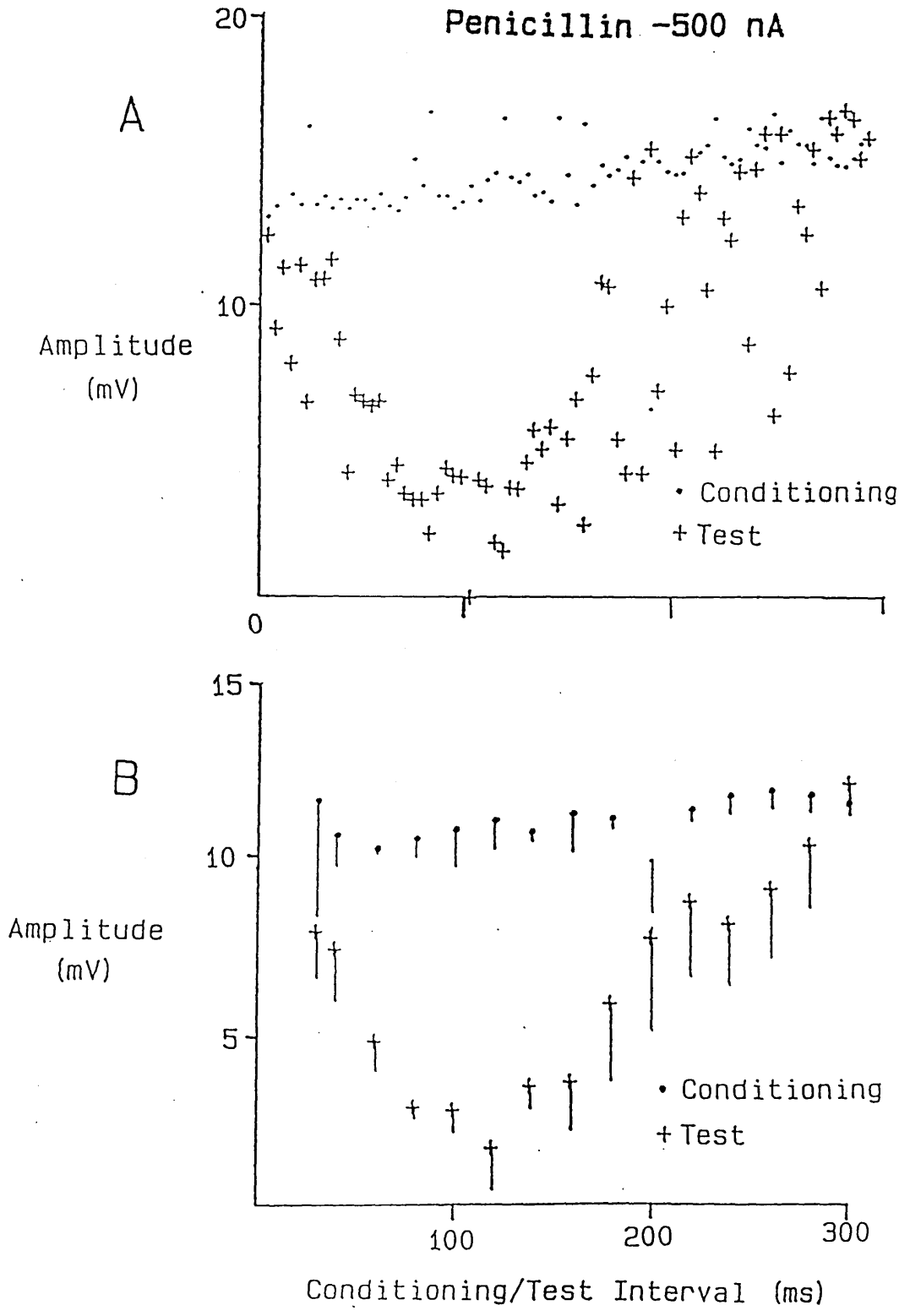


Figure 3.2.3: The refractory period of potentials evoked by DCS, tested by increasing C/T interval, during phase II of penicillin epileptogenesis. Individual responses (A) and means and SEM of five responses at each delay (20 msec intervals) between 20 and 300 msec (B).

phase II were depressed compared to the conditioning responses, the activity underlying phase II had a refractory period which was much greater than that of both normal and phase I evoked activity. However, the above experiments were conducted during the plateau of phase I and phase II enhancement and revealed no information as to when the refractory period changed from normal to be greater than normal during the development of the epileptiform focus. A delay of 150 msec was therefore set between conditioning and test potentials; this delay was greater than the refractory period of 'normal' EPs, yet smaller than that of 'epileptic' potentials. If during epileptogenesis the refractory period became greater than 150 ms this would be indicated by the test potential being depressed compared to the conditioning potential.

III. The Refractory Periods of Evoked potentials During Enhancement by

(a) Penicillin Ejected by Small Electrophoretic Currents

When penicillin was ejected by small electrophoretic currents (-100nA; phase I) both conditioning and test potentials were enhanced in amplitude, with little or no change in waveform compared to normal. This was the case for both SEPs (fig. 3.2.4) and potentials evoked by DCS (fig 3.2.5). Furthermore, the degree of enhancement of the test potentials was the same as that of the conditioning potentials.

The time course of enhancement of the test potentials was also the same as that of the enhancement of conditioning potentials: the delay to onset, the rate of enhancement and the plateau level of enhancement was the same for both conditioning and test potentials (fig. B4b, B5b). As the test responses were able to match the enhancement of the conditioning responses without being depressed in any way, the refractory period of activity underlying these enhanced potentials was less than 150ms and therefore, no different from that of normal evoked potentials. Hence, when the evoked potentials were enhanced such that there was only an enhancement of amplitude (phase I) the activity underlying the

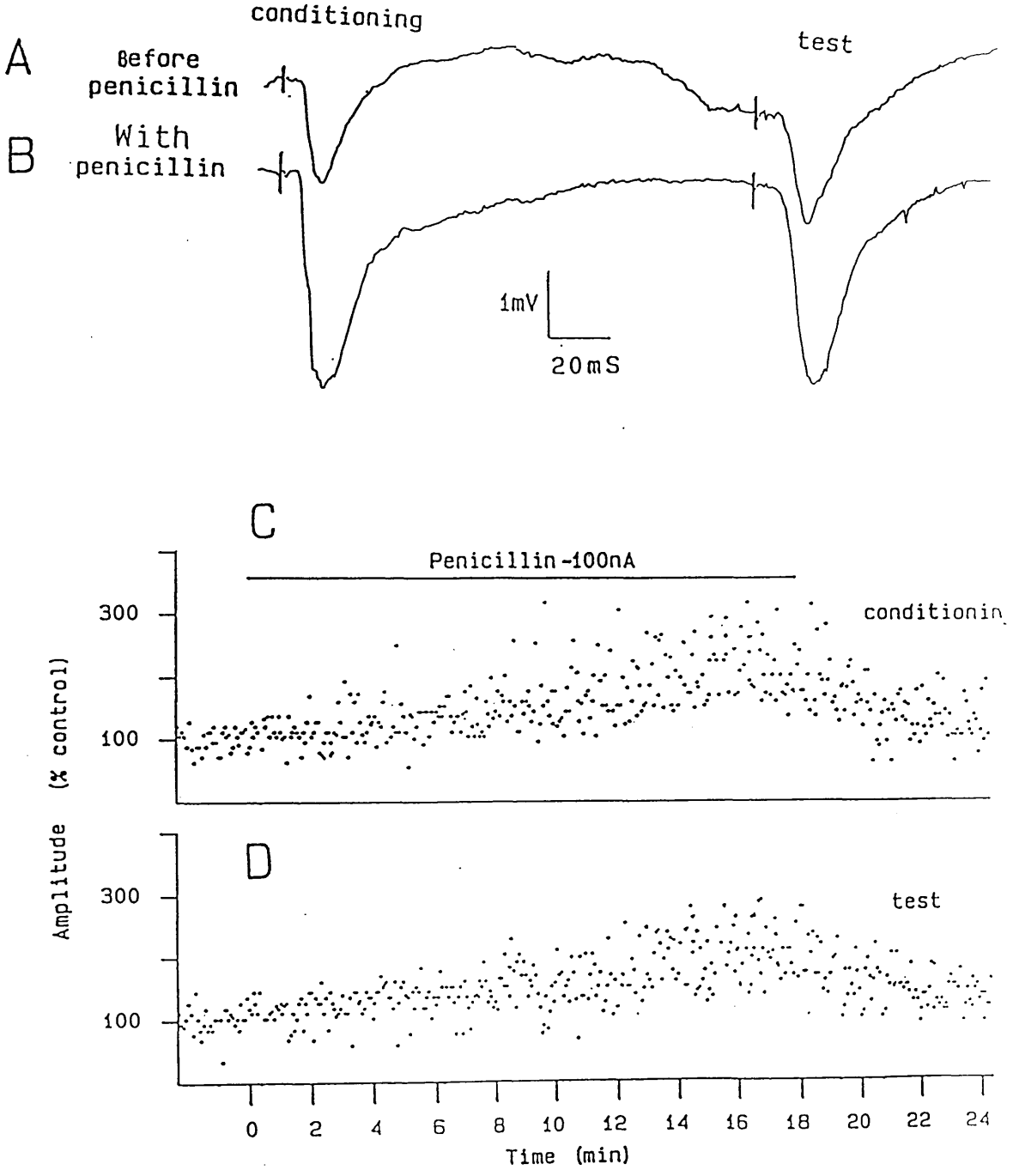


Figure 3.2.4: The effect of the development of phase I of penicillin epileptogenesis on the refractory period of somatosensory evoked potentials tested by a C/T interval of 150 msec. Responses to conditioning stimuli and test stimuli are similar in amplitude and waveform both before (A) and during phase I; penicillin -100 nA (B). The time course of enhancement during the development of phase I shows no difference between responses to conditioning (C) or test (D) stimuli.

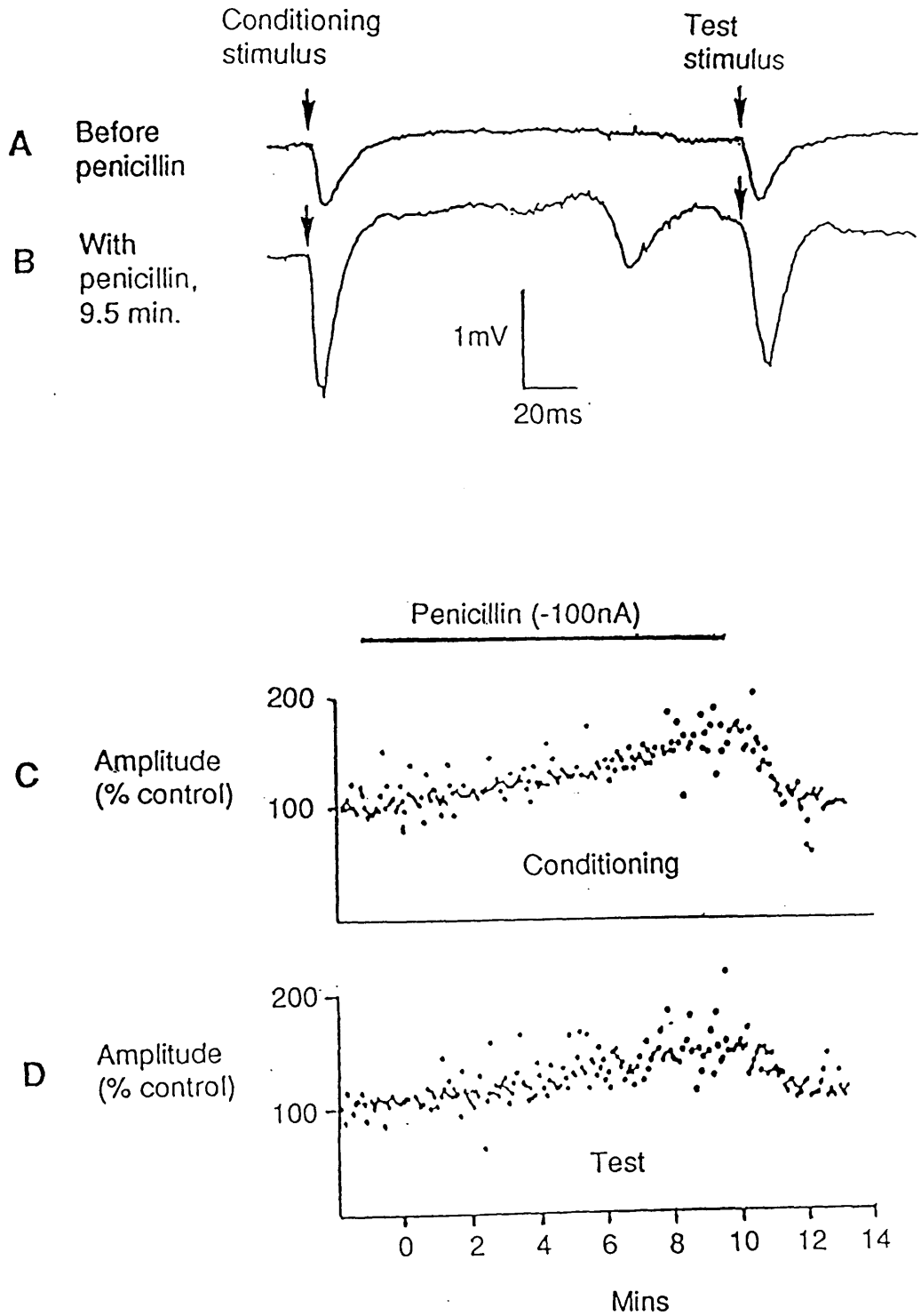


Figure 3.2.5: The effect of phase I on the refractory periods of potentials evoked by DCS. Responses to conditioning and test stimuli are similar in amplitude and waveform both before (A) and during phase I; penicillin -100 nA (B) with a C/T interval of 150 msec. The time course of and the maximum enhancement of both conditioning (C) and test (D) responses are the same during the development of phase I.

enhanced potentials had a refractory period which was the same as that of normal evoked potentials.

(b) Penicillin Ejected by Large Electrophoretic Currents > -500 nA

When penicillin was ejected by large electrophoretic currents (-800nA) the enhancement of conditioning potentials was as already described for the enhancement of evoked potentials by a large flux of penicillin (results part A): there was a large increase in amplitude, duration and the time to peak. The test potentials however, evoked with a delay of 150msec, did not exhibit any enhancement and in most cases were depressed below the normal pre-drug levels (fig. 3.2.6c).

Because of the depression of the responses to the test stimulus during the more drastic alteration of evoked potentials by a larger penicillin concentration (phase II), the refractory period of the underlying activity was greater than 150msec and thus very different to that of normal activity and activity underlying the simple enhancement of amplitude of evoked potentials (phase I).

(c) Penicillin ejected by large electrophoretic currents: about -500 nA

After the start of penicillin ejection (-500nA), there was an initial period during which the test potentials kept pace with the enhancement of the conditioning potentials (fig. 3.2.6b); this was in contrast to the lack of any enhancement of test potentials with the larger penicillin doses (see part IIb above and fig. 3.2.6c). Therefore, during this initial phase of enhancement, the refractory period of the underlying activity was similar to the refractory period of normal activity. As the enhancement of the conditioning potentials continued however, the test responses were unable to follow suit and were depressed towards pre-drug levels. During the plateau phase of enhancement of the conditioning potentials the mean of the test potentials was at about pre-drug levels (fig. 3.2.6b) although individual responses were very variable, some being enhanced and others depressed compared to normal. The refractory period of the underlying activity was now greater than normal and indicated the presence of phase II. After the termination of penicillin ejection, the amplitude of the conditioning potentials began to decline; as it did, the test responses increased in amplitude. This concurrent decline and enhancement of conditioning and test responses respectively continued until both potentials reached

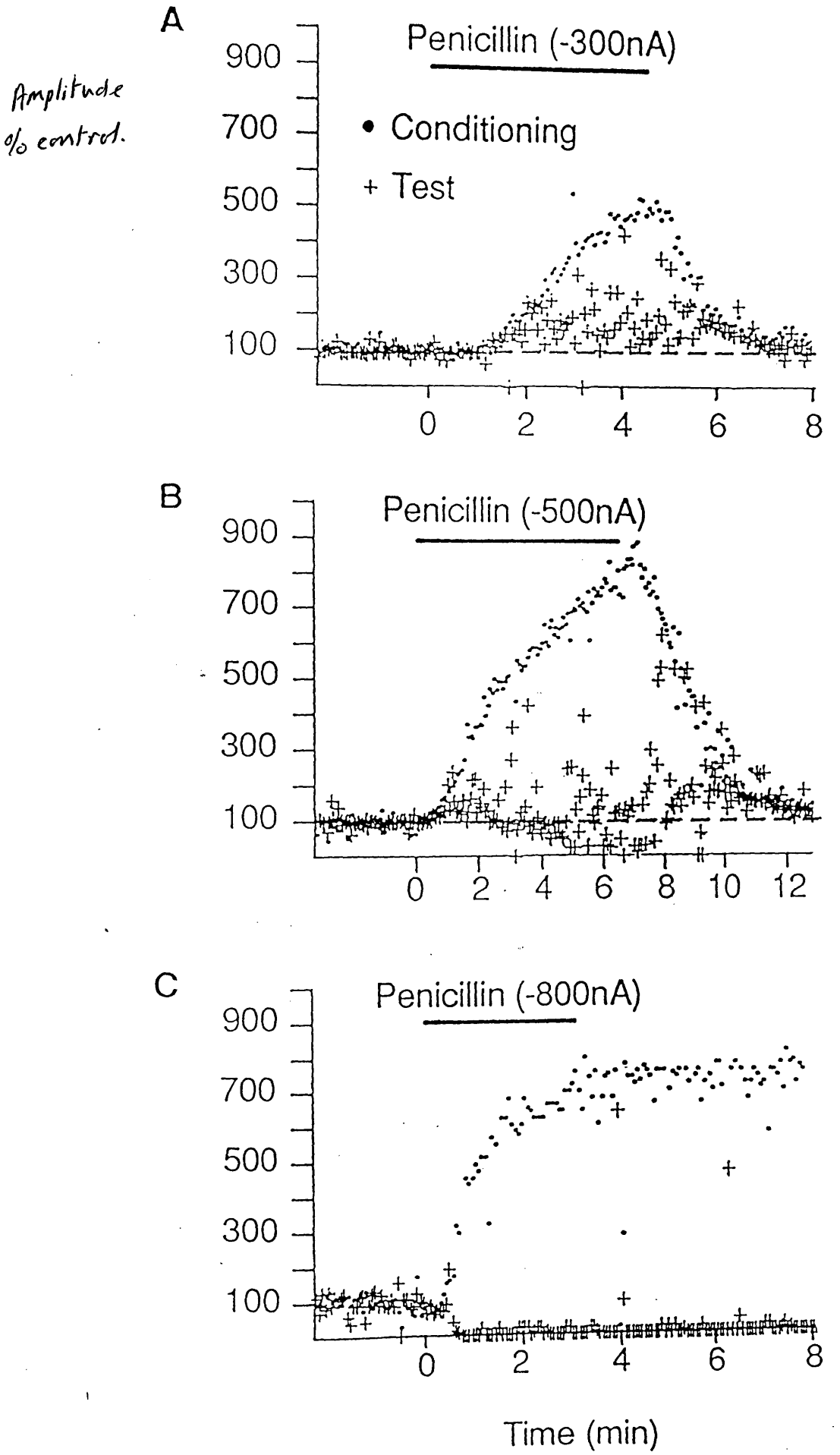
the same amplitude at a level greater than the normal pre-drug level (fig. 3.2.6b). Hence, the refractory period of the activity was once again less than 150msec and therefore, similar to the normal activity and that underlying phase I. Thereafter, both responses declined over the same time course back to pre-dug levels.

(d) Penicillin ejected by Intermediate ejecting currents: -200 to-300 nA

Penicillin ejected by this range of ejecting currents resulted in an enhancement of the amplitude and duration of the conditioning potentials. The test potentials were also enhanced but this enhancement was less than that of the conditioning potentials and was confined to an increase in amplitude.

After the start of pencillin ejection, the conditioning and test responses initially increased in amplitude together. After a certain level of enhancement, the test responses did not follow the continuing enhancement of the conditioning responses and levelled off to a plateau (200% of pre-drug; fig. 3.2.6a) whilst the conditioning potentials reached a plateau level which was greater than that of the test responses. After the termination of penicillin ejection, the conditioning potentials rapidly declined to reach the same amplitude as the enhanced test responses (at the plateau level of the test enhancement). Thereafter both conditioning and test responses declined together back to pre-drug levels. Therefore, in this, as in the enhancement by -500nA described above, there was initially an enhancement in which the refractory period of enhanced activity was similar to normal. Only once a greater degree of enhancement had taken place did

Figure 3.2.6: The effects of different doses of penicillin on the refractory periods of potentials evoked by DCS, with a C/T interval of 150 msec; penicillin ejected by 'intermediate' (A), and large (B and C) electrophoretic currents. If the refractory period is greater than 150 ms the test responses are depressed relative to the conditioning responses.


A
Penicillin (-300nA)
Amplitude
% control
900
700
500
300
100
• Conditioning
+ Test
0
2
4
6
8
B
Penicillin (-500nA)
900
700
500
300
100
0
2
4
6
8
10
12
C
Penicillin (-800nA)
900
700
500
300
100
0
2
4
6
8
Time (min)

the refractory period of the evoked activity exceed 150ms.

These results illustrated that the penicillin induced enhancement of evoked potentials could be divided into an enhancement with the same properties as normal potentials and a further enhancement with different properties to normal. When the enhancement resulted from a small dose of penicillin then the properties of the activity underlying this enhancement were the same as the activity underlying normal evoked potentials (phase I). However, when the enhancement of evoked potentials resulted from a large dose of penicillin the properties of the enhanced potentials were fundamentally different to both the normal evoked potentials and potentials enhanced by small doses of penicillin.

Large ejecting currents resulted in an enhancement of evoked potentials which almost immediately entered into phase II (see figs. 3.26b and c). Phase I was rapidly overtaken by phase II, as indicated by the rapid depression of the test potentials. During the recovery phase however, both conditioning and test potentials were enhanced, and of the same amplitude, indicating the occurrence of phase I. As shown in the preliminary results (part 1.III) a gradual transition from normal through phase I and then phase II could be attained by using smaller penicillin ejecting currents (fig. 3.2.6a) or stepped functions of ejecting current. This is

illustrated in figure 3.2.7 which shows that penicillin ejected by smaller electrophoretic currents resulted in the enhancement of both conditioning and test potentials. An increase in the penicillin flux resulted in further enhancement of the conditioning responses but a depression of the test responses. On termination of penicillin ejection the conditioning responses declined, and as the refractory periods decreased so the test responses increased in amplitude to match the conditioning responses. Thereafter, both potentials declined in amplitude together.

In part 1 of the results the possibility of two phases of epileptogenesis was postulated: phase I was identified as being that in which the enhancement of evoked potentials occured with no change in the duration; the enhanced potentials were of a similar waveform to normal potentials. Phase II was that in which evoked potentials had a greater duration than the normal. Two phases have been further identified in the results just described; phase I was that in which the refractory periods of the evoked potentials were the same as for normal evoked potentials and phase II was indicated by the evoked potentials having refractory periods greater than normal or phase I evoked potentials.

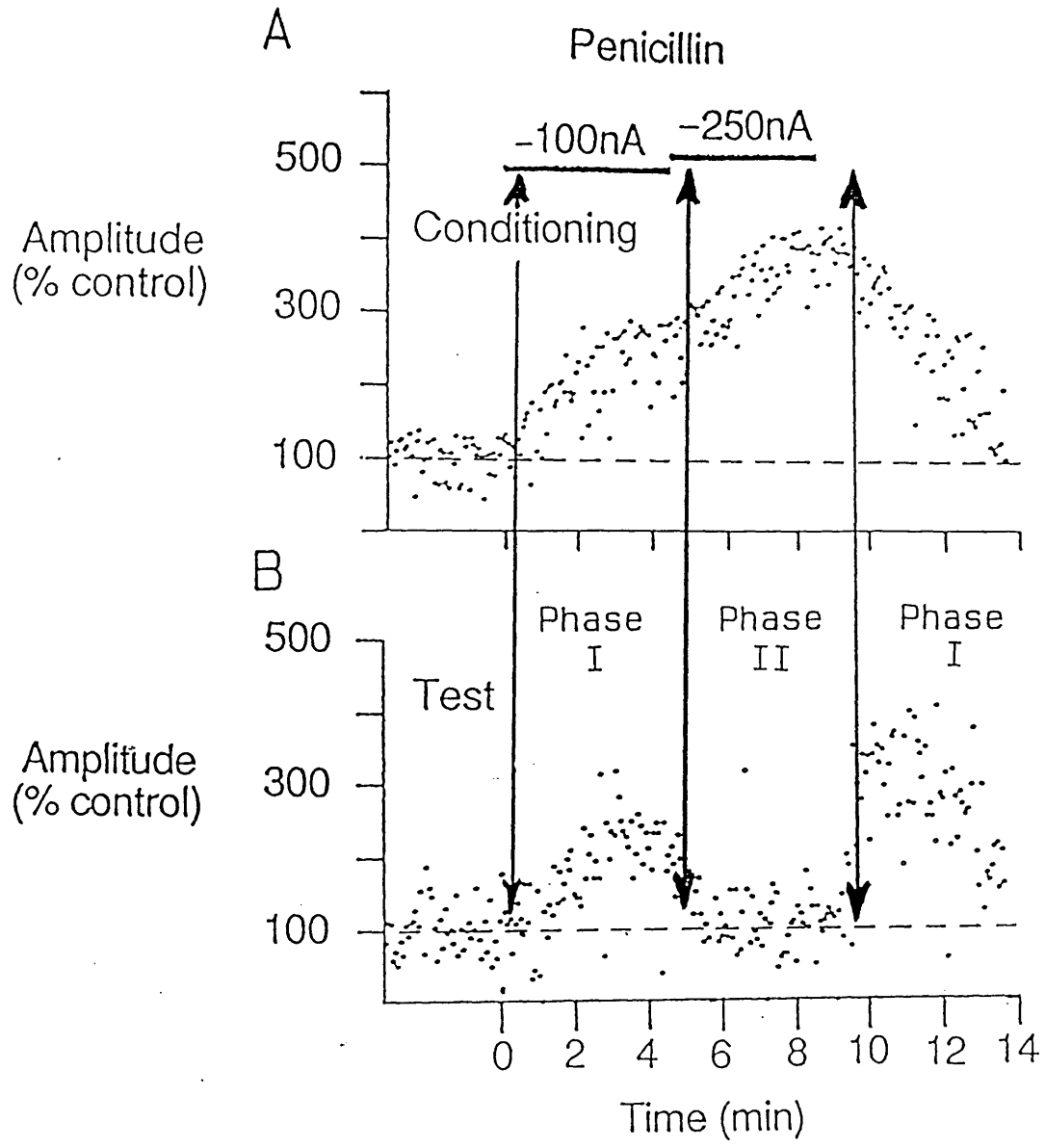
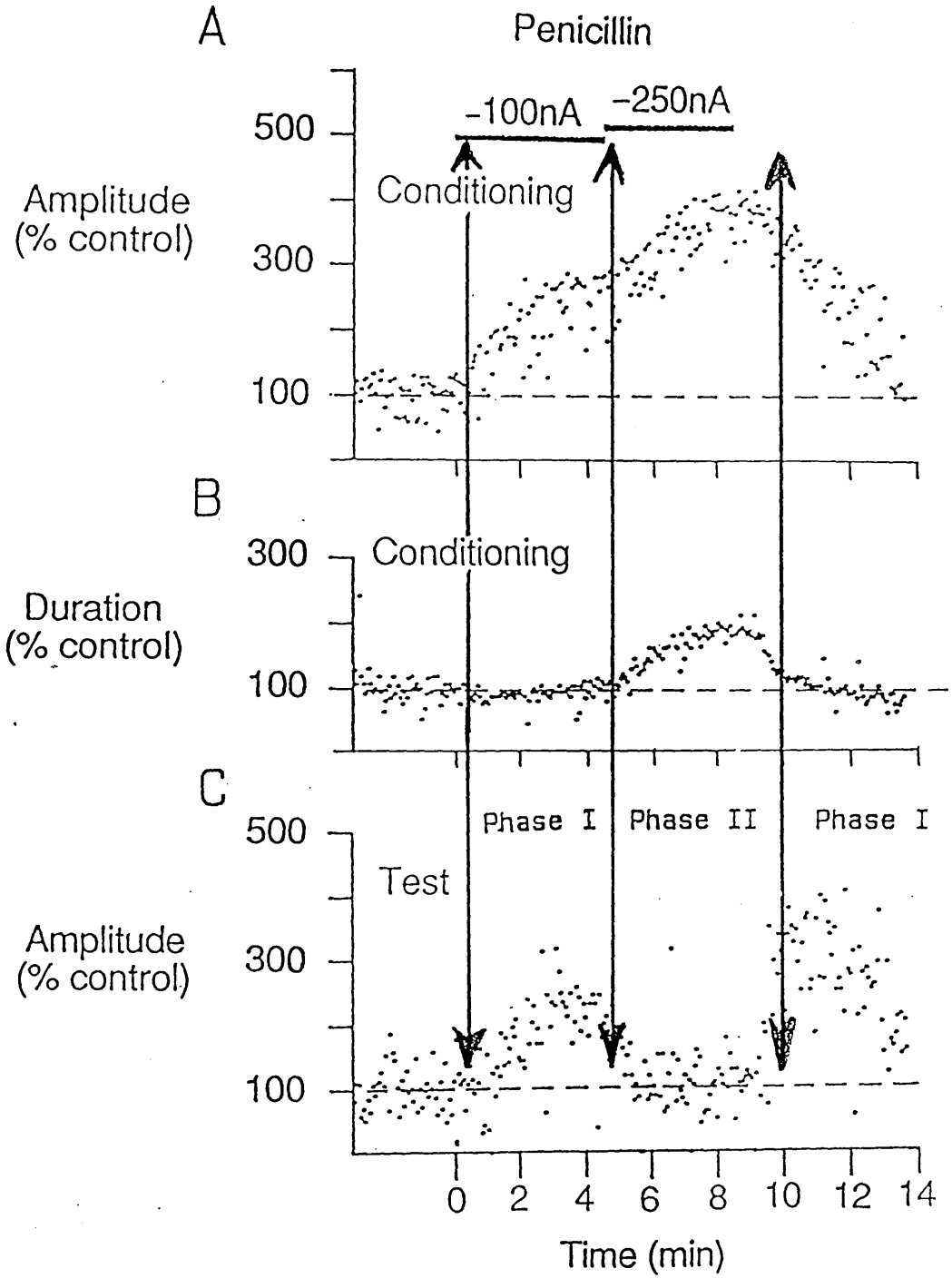


Figure 3.2.7: The effect of penicillin ejected by small (-100 nA) and large (-250 nA) electrophoretic currents on the amplitude of both conditioning (A) and test (B) responses indicates that phase I of epileptogenesis is reflected by an enhancement of both conditioning and test responses but that the onset of phase II is signalled by a depression of the amplitude of the test response. Phase I reappears during the recovery from phase II.

The two phases identified by these two different means occurred at the same time, as illustrated in fig. 3.2.8. This shows that while there is an enhancement of EPs with no change in the waveform (ie. no increase in duration), the refractory period was the same as normal (test and conditioning responses enhanced together). When Phase II started there was an increase in the duration of the EPs which was also associated with an increase in the refractory period of the activity underlying this phase (a depression of the test responses). This implied that the increase in refractory period and the increase in duration reflected the same underlying changes in cortical excitability at the onset of phase II.

Figure 3.2.8: Graphs to illustrate the changes in evoked potential characteristics that reflect the onset of phase I and phase II. Phase I occurs as a result of the ejection of penicillin by small (-100 nA) electrophoretic currents. There is an enhancement of the amplitude (A) and no change in the duration (B) of the evoked potential. Test responses (C) are not depressed; the refractory period during this enhancement is no different to that of normal potentials. Phase II occurs as a result of the ejection of pencillin by large (-250 nA) electrophoretic currents. There is an enhancement of the duration (B) aswell as of the amplitude (A) of evoked potentials. This is associated with a decrease in the amplitude of responses to test stimuli; the refractory period during phase II is greater than during normal or phase I. Phase I reappears during the recovery from phase II.


A
Penicillin
-100nA
-250nA
500
300
100
Amplitude
(% control)
Conditioning
B
300
100
Duration
(% control)
Conditioning
C
500
300
100
Amplitude
(% control)
Test
Phase I
Phase II
Phase I
0
2
4
6
8
10
12
14
Time (min)

IV. Stimulus strength characteristics of the EPs in the different phases of epileptogenesis

Another method of testing the physiological characteristics of the different phases of hyperexcitability was by the response of the evoked potentials to different stimulus strengths.

(a) normal potentials

The amplitude of the normal potentials, as a function of stimulus strength, followed a sigmoidal curve (fig. 3.2.9a).

(b) Phase I

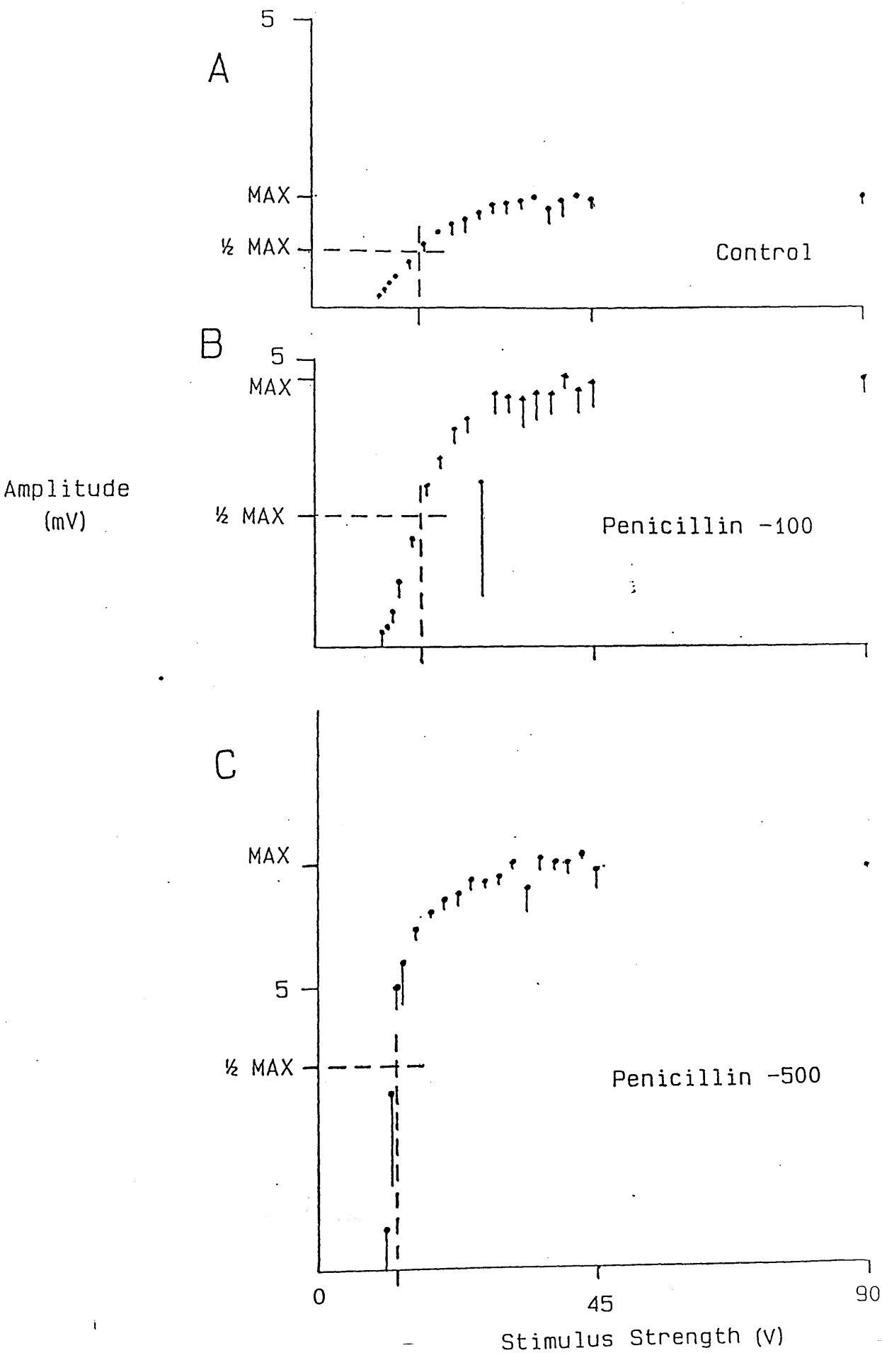
During phase I enhancement, the shape of the curve of amplitude as a function of stimulus strength was similar to that of normal, unenhanced potentials. The only difference was a quantitative one, in that the curve was shifted up the Y axis (compare figs. 3.2.9a and b), indicating that a larger potential compared to normal was evoked for each stimulus. The stimulus strength required to evoke the half maximal EP in both normal and phase I situations was very similar.

(c) Phase II

During this phase of enhancement the shape of the curve of amplitude as a function of stimulus strength was very different from the sigmoidal curve of phase I and normal potentials. On increasing the stimulus strength there was a very steep increase to a plateau, indicating an almost all or none nature underlying the generation of these potentials (fig. 3.2.9c). The stimulus required to evoke a half maximal EP was smaller than that of normal and phase I potentials. A stimulus which was half maximal for normal and phase I potentials evoked a potential which was about 80% to 90% of the maximum amplitude of the potentials during phase II.

In fig 3.2.10 which shows the individual responses in a stimulus strength test during phase II, there was a stepwise increase from small evoked potentials evoked with the smallest stimuli, to the maximum amplitude evoked potentials. In the circumstances of this experiment there were on several occassions small evoked potentials which resulted from large stimuli. In these instances the potential was evoked closely following a spontaneous spike and so fell within the refractory period of the epileptiform spike and was therefore depressed. It is interesting to note that the EPs which were depressed in this way fell to a level which was similar to the EPs which were evoked with stimuli just too weak to elict the

Figure 3.2.9: The stimulus strength response relationship of normal somatosensory evoked potentials (A) and during phase I (B) and phase II (C) of penicillin epileptogenesis. Means and SEM of five responses at each strength. Note the change in the shape of the curve and the smaller stimulus required to evoke a half maximum response during phase II.


A
5
MAX
½ MAX
Control
B
5
MAX
½ MAX
Penicillin -100
Amplitude
(mV)
C
MAX
5
½ MAX
Penicillin -500
0
45
90
Stimulus Strength (V)

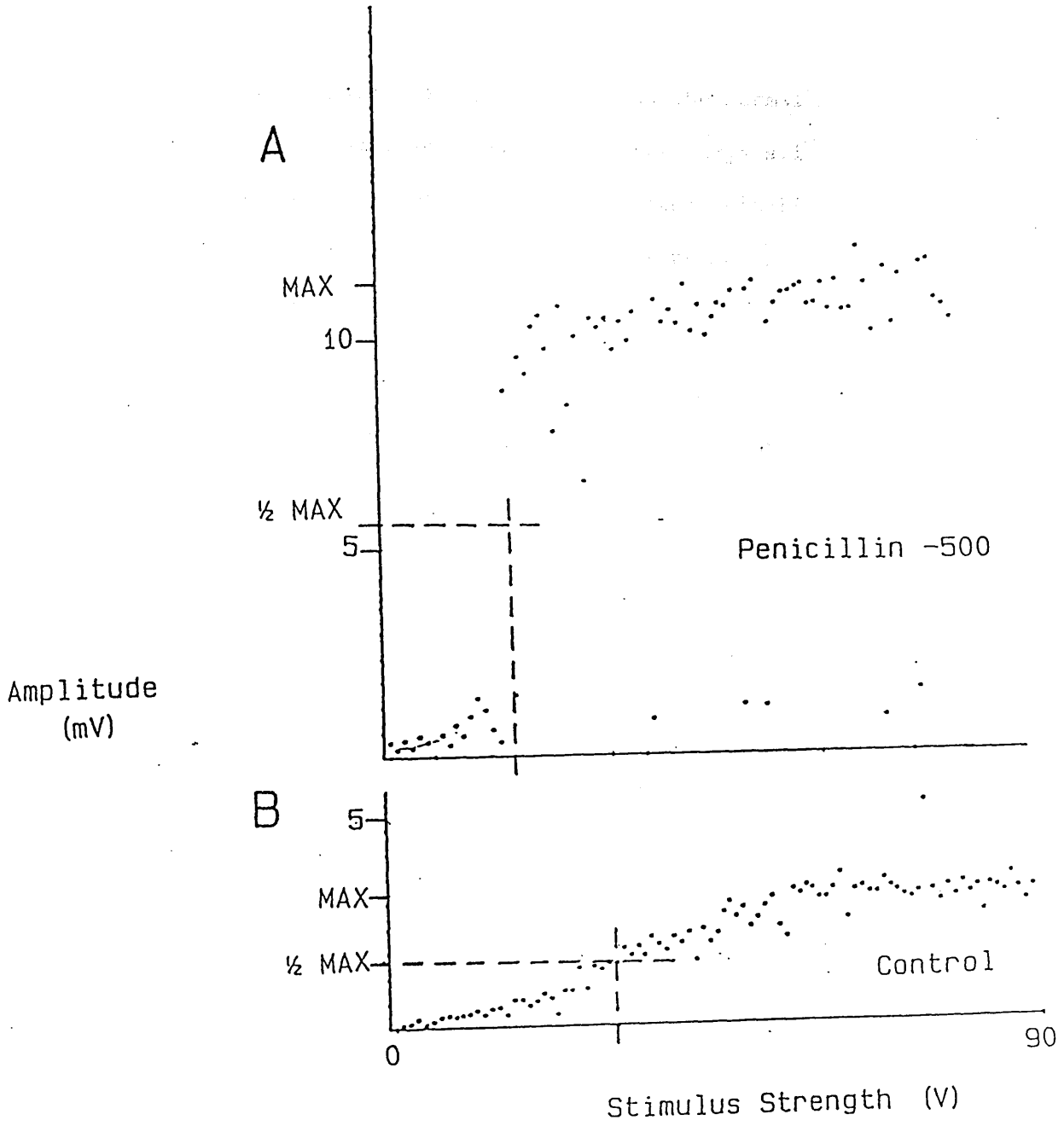


Figure 3.2.10: Stimulus strength response relationship of normal SEPs (A) and potentials during phase II (B). Individual responses, five at each stimulus strength. Note the change in the shape of the curve and the smaller stimulus required to evoke a half maximum stimulus during phase II. The presence of smaller responses during the plateau of the curve during phase II are discussed in the text.

near maximum enhanced potentials. This would indicate that these depressed potentials are similar to the normal EP (which results from the smallest stimuli) and the large all or none enhancement which is added on by the larger stimuli (and that which is depressed by a preceding spontaneous spike) is epileptiform in nature.

These results provide further evidence that phase I activity was essentially no different to normal activity, whereas the activity underlying phase II epileptogenesis was of a very different form.

The preceding results indicated that the different phases of enhancement of the evoked potentials, which were implied by the findings in part 1, do indeed have differant physiological properties. Phase I enhanced potentials were found to be very similar in nature to normal potentials, whereas phase II potentials were different to both normal and phase I potentials, but had characteristics similar to those of epileptiform acitivity. Therefore, the activity underlying phase I was fundamentally different from that underlying phase II.

IV. The Critical Cortical Mass Required to Generate the Phases of Penicillin Epileptogenesis

After finding that there were two different phases during penicillin epileptogenesis, which could be differentiated on account of their physiological properties, it was decided to investigate whether there were any differences regarding the critical cortical mass required to generate these different phases of epileptogenesis.

It has been shown in previous studies that a critical cortical mass is a prerequisite for the generation of spontaneous epileptiform spikes and this has been found to be roughly the size of a cortical column (Reichenthal and Hocherman, 1977), and has indeed been suggested to be the physiologically defined cortical column (Gabor et al., 1979).

To test if there were any differences in the structural requirements of the different phases of epileptogenesis, the sub-pial isolation of cuboids of cortex of varying magnitudes was carried out (see methods). The isolated cuboids were not undercut so that the integrity of the afferent input was maintained. After isolation of the cortex the recording and penicillin containing electrodes were placed within the cuboid. Initially spontaneous and evoked activity was usually depressed compared to that before the isolation but normally

recovered within an hour, so that both forms of activity appeared to be no different to that before the sub-pial isolation. This indicated that the isolated cortex was not suffering any undue damage. If, as on some occassions, there was no recovery of activity, presumably because of excessive neuronal damage, then the experiment was terminated. Only when cortical activity had returned to be similar to normal was the experiment continued. Histological verification of the magnitude of the isolated cuboid was performed at the end of the experiment.

In blocks of cortex in which the cuts were approximately 0.6X0.6 mm apart, or greater (see fig. 3.2.12), the ejection of penicillin resulted in both phases of enhancement of evoked potentials (fig 3.2.11; table 3). Furthermore, spontaneous epileptiform spiking also occurred when sufficient penicillin was ejected. Therefore, blocks of cortex of these dimensions generated all the normal enhancement of evoked potentials.

In blocks of cortex in which the cuts were closer together than approximately 0.6mm (see fig. 3.2.14) no enhancement of evoked potentials occurred when penicillin was ejected by small or by large electrophoretic currents (-500nA; fig. 3.2.13) even though normal potentials could be evoked in cuboids of such sizes. Furthermore, on no occassion did a block of cortex generate phase I but not phase II. Therefore,

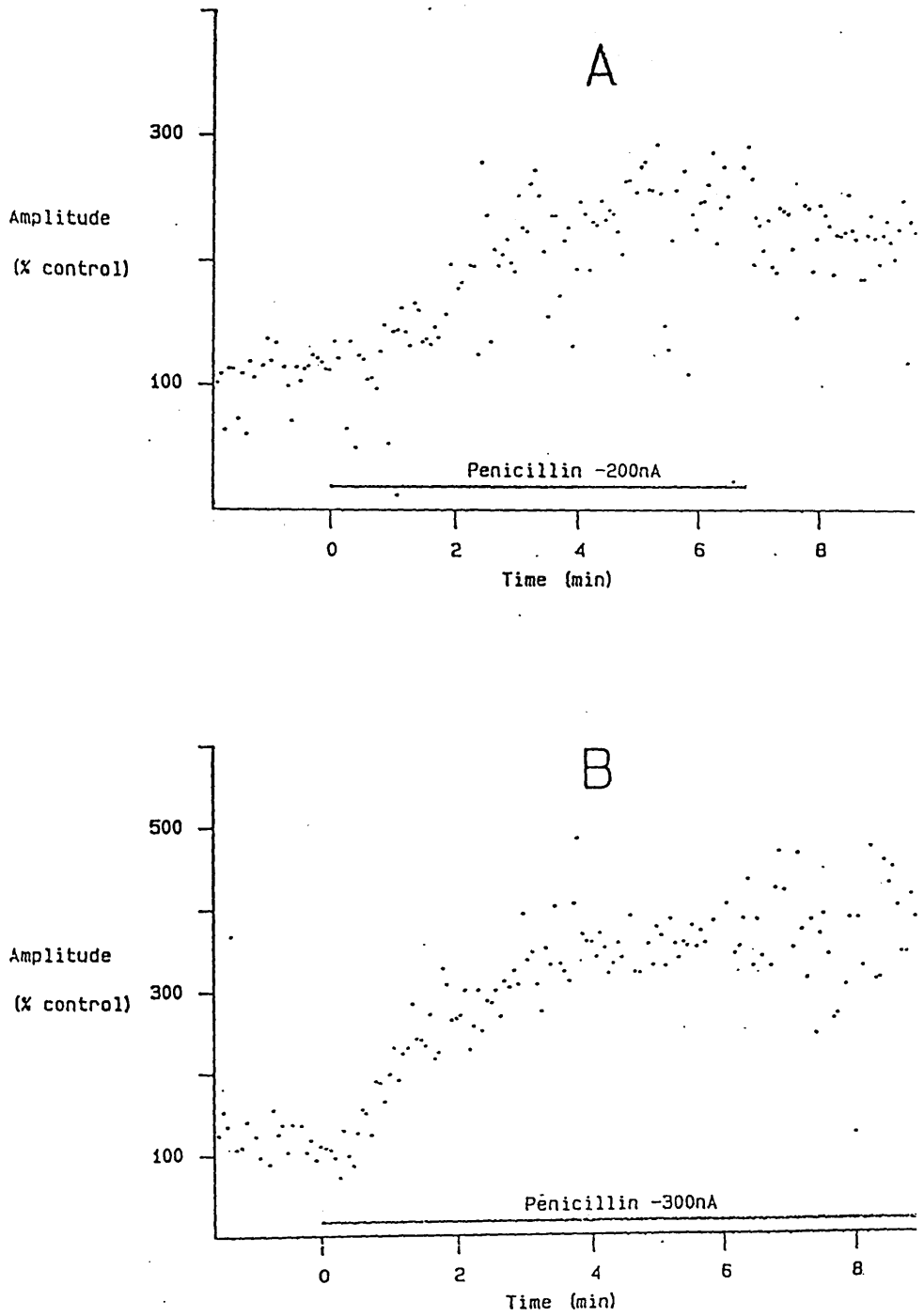


Figure 3.2.11: The enhancement of SEPs in normal cortex (A) and in sub-pially isolated cortex (B) in which the separation between the cuts at the cortical surface was greater than the threshold for generation of epileptogenesis (about 0.6mm).

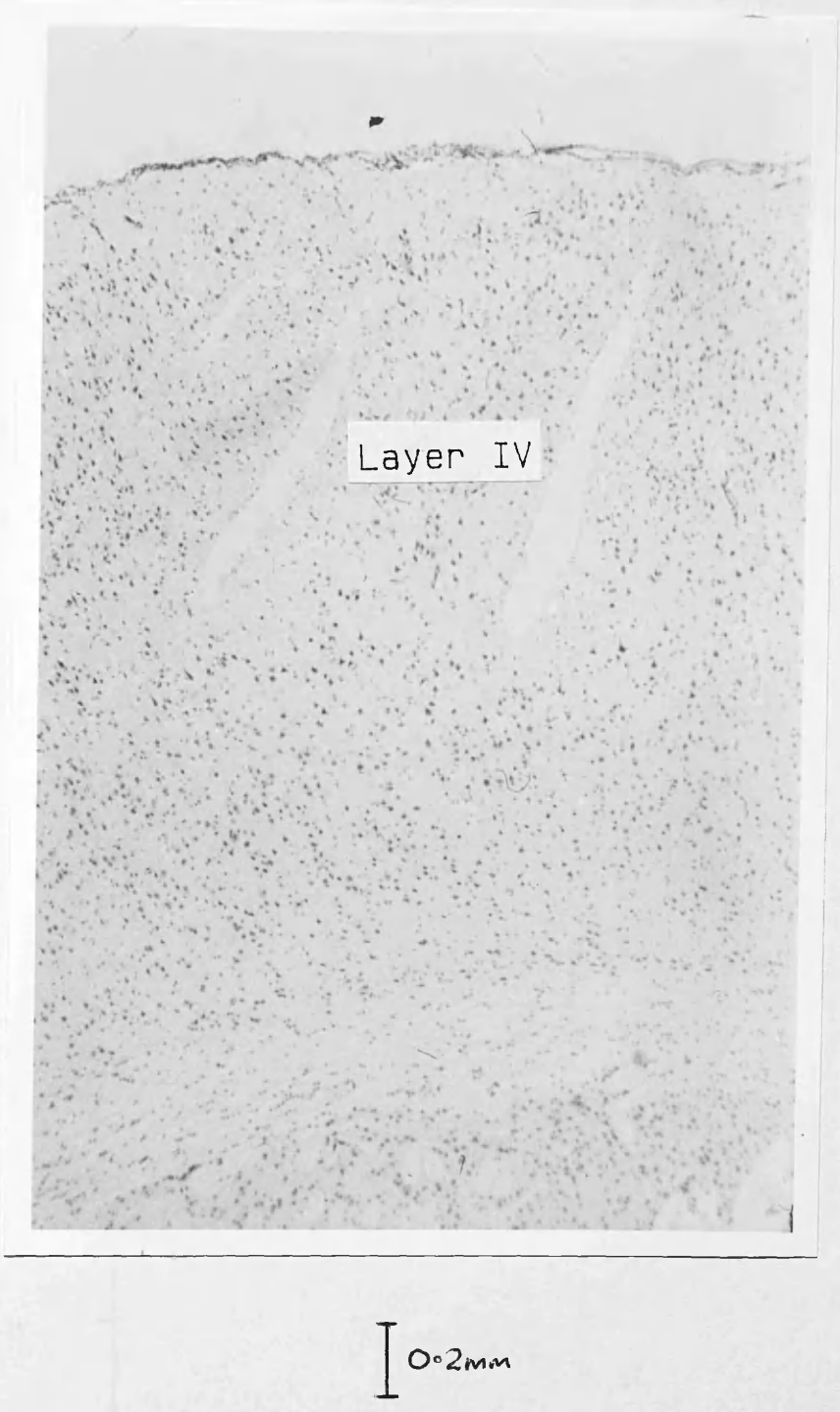


Figure 3.2.12: Section through the somatosensory cortex showing the surgical cuts resulting in the sub-pial isolation of a cuboid of cortex. The approximate location of layer IV is indicated. Note that the cuts do not extend the full depth of the grey matter. Separation between the cuts is about 0.7 mm.

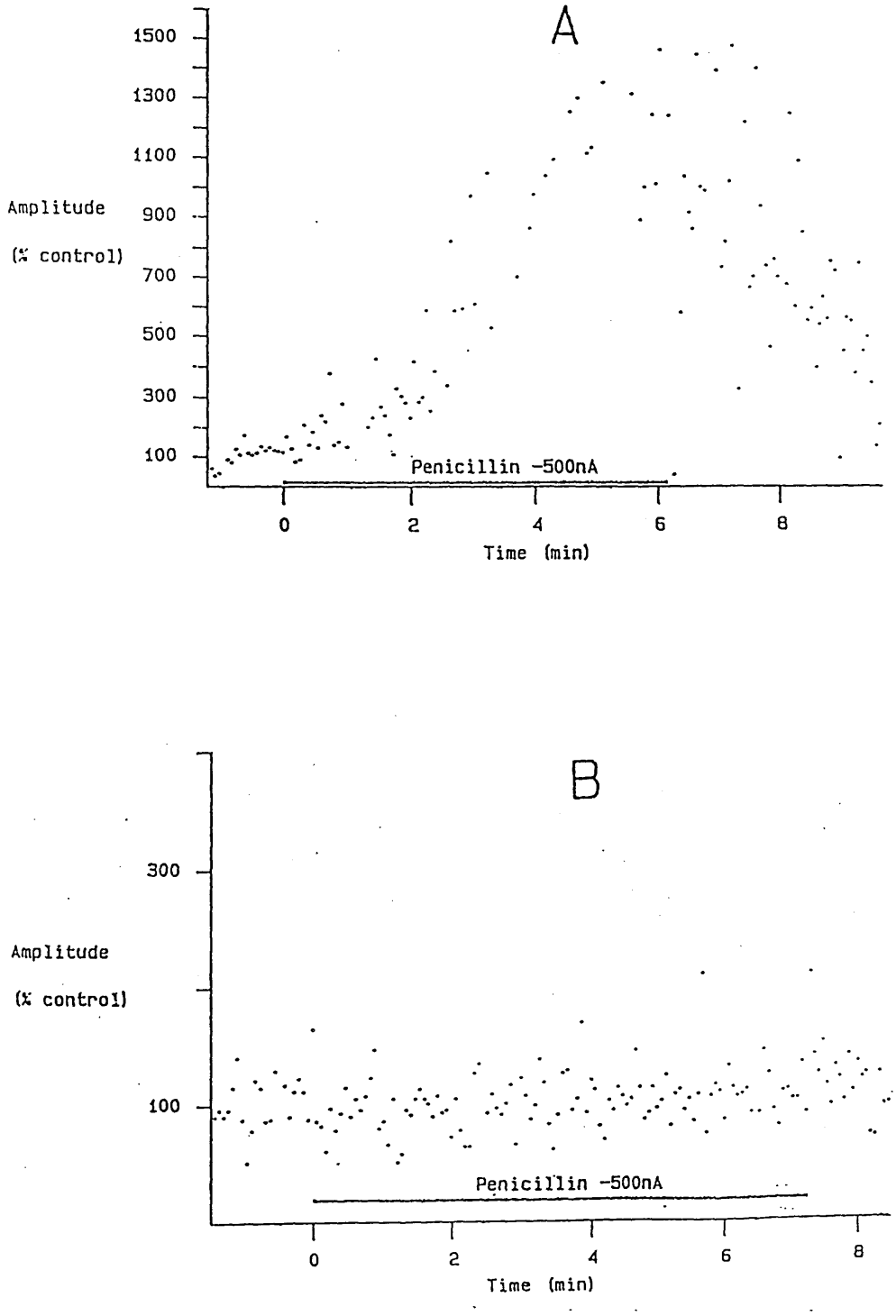


Figure 3.2.13: The enhancement of SEPs in normal cortex (A) by large fluxes of penicillin. In sub-pially isolated cortex with a separation between the cuts less than 0.6 mm no enhancement of the SEPs occurred; the isolated cortex was smaller than the threshold for generation of epileptogenesis.

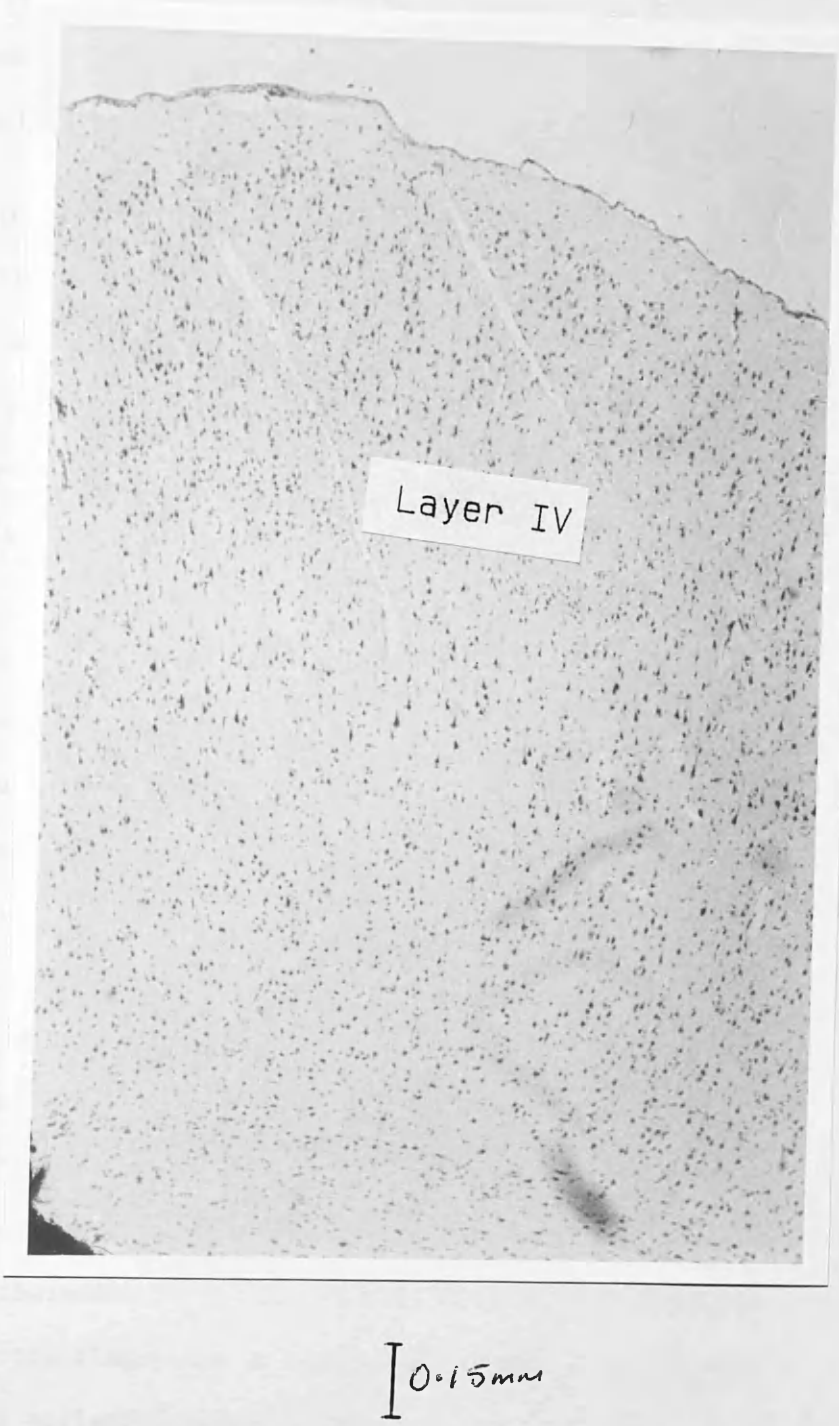


Figure 3.2.14: Section through the somatosensory cortex illustrating the sub-pial isolation of a small cuboid of cortex. The approximate location of layer IV is indicated. Seperation between the cuts is approximately 0.5 mm.

both phases of epileptogenesis required a cortical mass which had threshold size of approx 0.6 X 0.6mm.

It should be noted from the figures of the isolated cuboids of cortex (figs. 3.2.12 and 14) that the cuts were not usually perpendicular to the surface of the cortex. In such a situation less of a vertically organised piece of cortex would be included in the isolation than if the cuts had been perpendicular to the surface and of the same separation. Therefore, the dimensions of the isolated blocks (or the separation between the cuts) should only be considered to be very approximate if using such sizes in relation to physiological cortical columns. Because of the gradient of the cuts in figure 3.2.14 the isolated piece of cortex does not include an entire vertical extent of the cortex and furthermore this piece of cortex did not generate any of the phases of epileptogenesis. The isolation in figure 3.2.12, which did generate evoked potential enhancement, does include an entire vertical extent of cortex. The actual width of the piece of cortex including an entire vertical extent is very small and therefore it may be that isolated peices of cortex smaller than the dimensions of a cortical column are capable of generating epileptiform activity.

		Enhancement (% pre-drug)	
		Penicillin Dose	
		100 nA	500 nA
Distance between cuts (mm) < 0.6 mm	0.4	100	100
	0.35	100	100
	0.45	100	100
	0.5	103	108
Distance between cuts (mm) >0.6 mm	0.65	200	430
	0.7	180	400
	0.7	260	520
	0.9	225	500
	1.0	240	550

Table 3. The effect of the sub-pial isolation of cuboids of cortex of different dimensions on the ability to generate epileptogenesis by the ejection of small and large doses of penicillin.

RESULTS: PART 3

The Involvement of NMDA Receptor Mediated Activity in the Phases of Penicillin Epileptogenesis

The dicarboxylic amino acids, glutamate and aspartate, are excitatory neurotransmitters in the mamma lian cerebral cortex. The receptors for these amino acids have been divided into three types (kainate, quisqualate and N-methyl-D-aspartate (NMDA)) on account of the affinity of these different pharmacological agonists. Because of the lack of sufficiently specific antagonists to differentiate between the activity of kainate and quisqualate receptors the classification of the excitatory receptors is often considered to be of NMDA and non-NMDA receptors, with the non-NMDA receptors responsible for the conduction of normal, fast synaptic transmission. As discussed in the introduction, the importance of a role for NMDA receptors in epilepsy and other neurological disorders has recently come to light (Meldrum, 1985). It was important therefore, to carry out an investigation into the role of these receptors in the penicillin model of focal epilepsy.

Some reports have suggested that that the initial part of the cellular paroxysmal depolarisation shift (PDS) in epileptiform activity is non-NMDA receptor mediated and the later part of the PDS is mediated by NMDA receptors (King and Dingledine, 1985). This was particularly exciting in relation to the results described concerning the phases of epileptogenesis because it raised the possibility that phase I, which has already been shown to be similar to normal activity, might be mediated by non-NMDA receptors and that phase II, which was shown to be more 'epileptic' in nature, might be mediated by NMDA receptors. Hence, an investigation into the excitatory receptors responsible for the activity underlying the two phases of penicillin epileptogenesis was undertaken.

2-amino-5-phosphonovaleric acid (APV) is one of a class of highly potent, competitive NMDA antagonists (Davies et al., 1981) and it was this that was initially used to investigate the role of NMDA receptors in penicillin epileptogenesis. APV (50 mM) was prepared in distilled water and the pH (4.4) was adjusted to 8.0 by the addition of molar NaOH.

When employing electrophoretic ejection of APV and penicillin, multibarrelled electrodes were used to try to get around the problems (discussed in the methods) of using several separate electrodes. One barrel of a three barrelled microelectrode contained APV, the second contained penicillin and the third was used to record extracellular field potentials from layer IV of the somatosensory cortex.

It was important to investigate the specificity of APV so as to ensure that any effects of APV were due to specific NMDA receptor antagonism. NMDA receptors are widely considered to be unimportant in the generation of 'normal', fast cortical activity (Collingridge et al., 1983a, b; Salt, 1986) and this knowledge provided a convenient method for testing for non-specific effects of the NMDA antagonist; a depression of normal potentials must result from non-specific effects of APV.

I The Specificity of action of APV

APV was ejected by electrophoretic currents of varying magnitudes (-25 to -500 nA) from one barrel of a three barrelled electrode, whilst one of the other barrels was used to record evoked activity. The ejection of APV by currents up to and including -200nA had no discernible effect on evoked potential characteristics, even with prolonged ejection (fig. 3.3.1). APV ejected by electrophoretic currents of -250 nA and larger resulted in a very small depression (fig. 3.3.1), or a reduction of the normal random variability, of the amplitude of evoked potentials (fig. 3.2.2). Even very large ejecting currents of APV (-500nA) resulted in only a very small depression of EPs (fig. 3.3.2).

The above results showed that doses of APV produced by ejecting currents up to -200nA had no effect on normal evoked potentials. This ruled out the possibility of APV having non-specific depressant effects in the circumstances of these experiments. The depression of normal potentials by larger doses of APV (produced by ejecting currents greater than -250nA) may have therefore been due to non-specific depressant effects of the drug on normal cortical excitability.

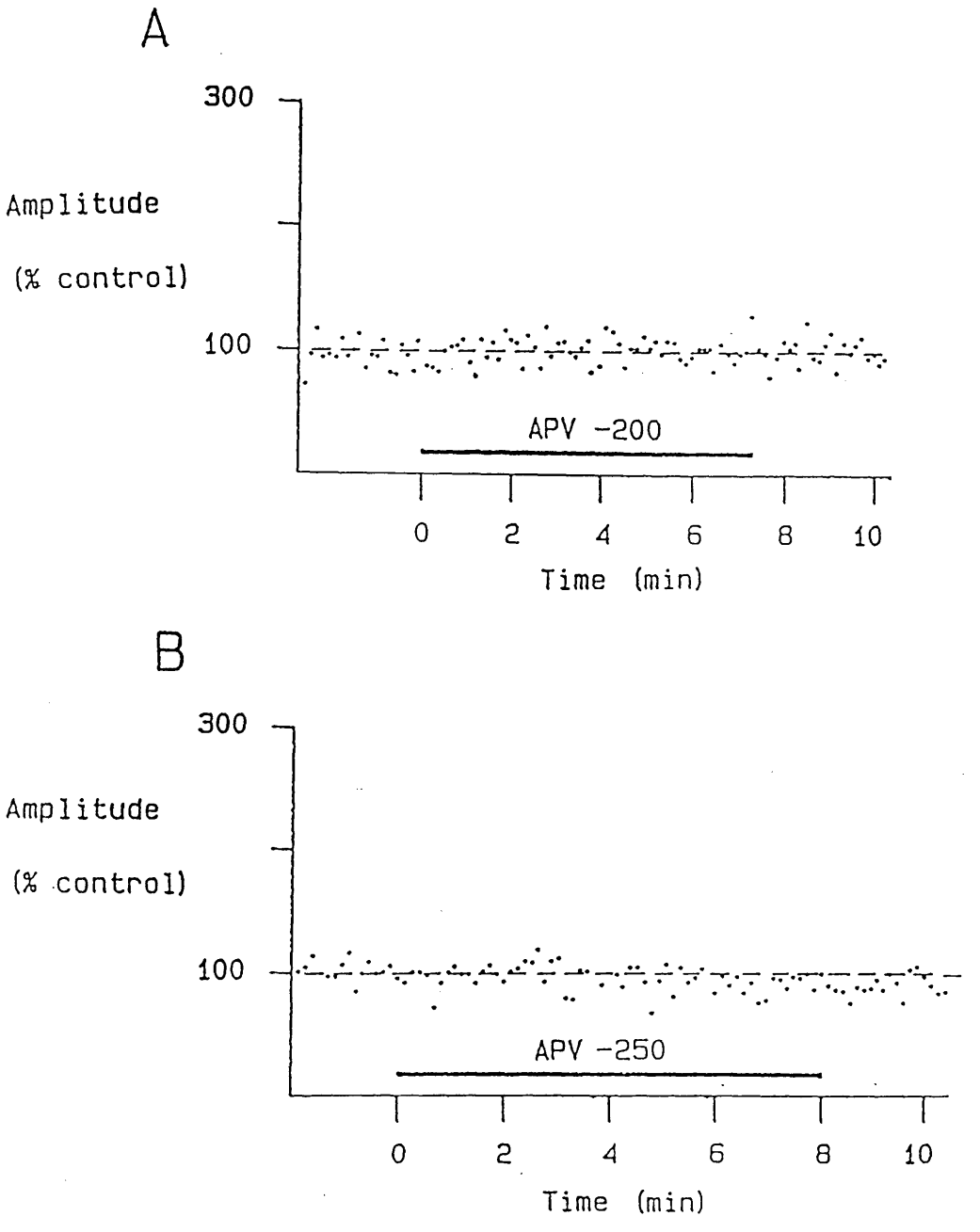


Figure 3.3.1: To test the specificity of APV the drug was ejected by itself into layer IV of the somatosensory cortex. APV ejected by large electrophoretic currents; -200 nA (A) had no discernable effect on SEP amplitude. APV ejected by larger currents; -250 nA (B) had a small depressant effect on the amplitude of SEPs.

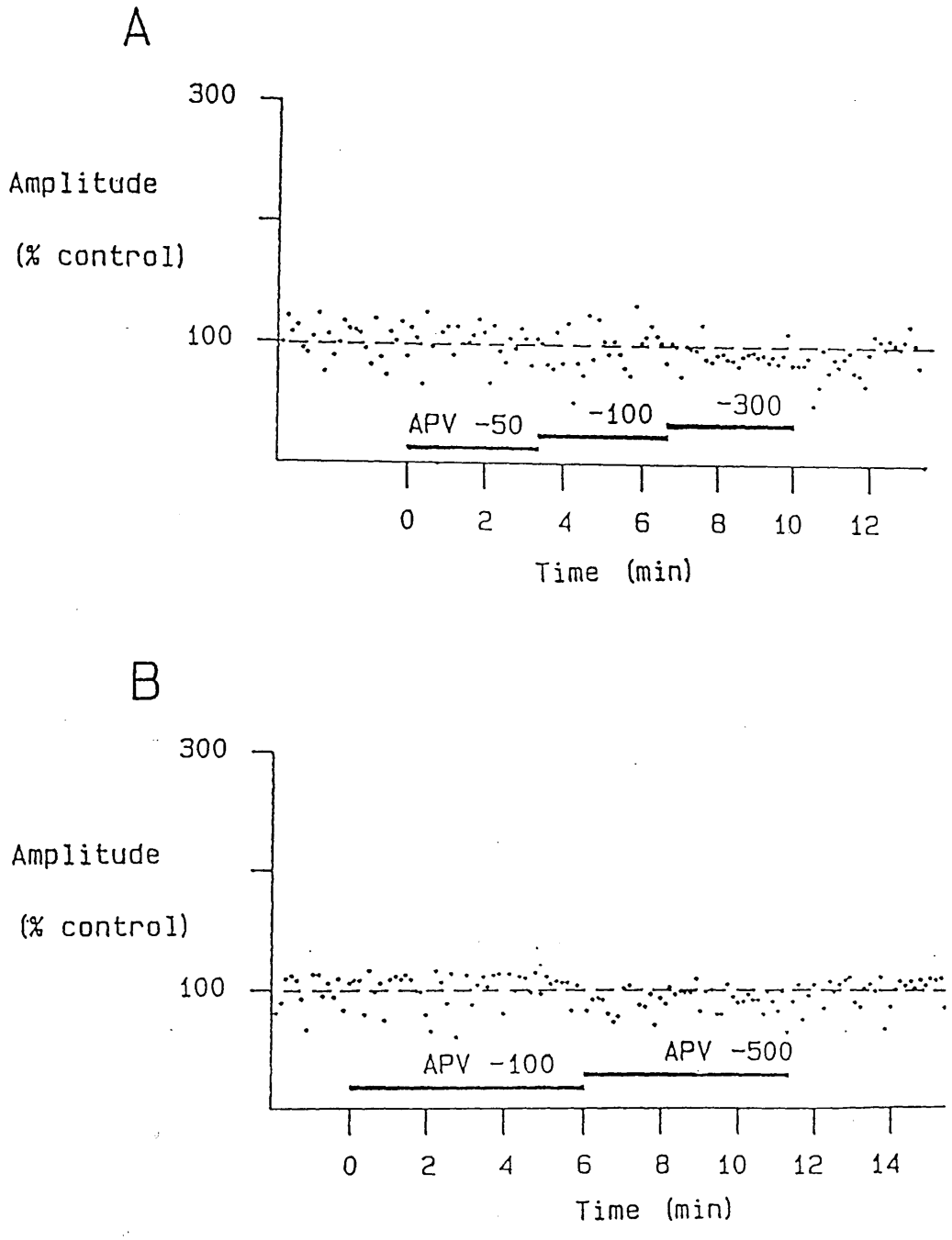


Figure 3.3.2: The effect of a range of doses of APV, ejected by itself, on the amplitude of somatosensory evoked potentials. APV ejected by -300 nA (A) and -500 nA (B) resulted in a decrease in the normal variability and/or a small depression of the peak amplitude of evoked potentials. The ejection of APV by small currents had no effect on SEP amplitude.

II (a) The Effect of NMDA Receptor Antagonism on Phase I of Penicillin Epileptogenesis

The ejection of penicillin by itself (-100nA), resulted in the enhancement of EPs (fig. 3.3.3) and within a few minutes after the start of ejection the enhancement reached a plateau level (phase I). This effect of penicillin was predictable and repeatable (see results part 1) and provided a control against which any effects of APV could be tested.

To test the effects of NMDA antagonism on phase I of penicillin epileptogenesis, the ejection of penicillin (-100 nA) and APV (-75 nA) was started simultaneously; the concurrent ejection of the two drugs resulted in no enhancement of the evoked potentials whatsoever, even after many minutes of continued ejection (fig. 3.3.3).

The ejection of normal saline by the same ejecting currents as used for APV (-75 nA) had no effect on the penicillin induced enhancement of evoked potentials. Hence, the blockade of enhancement during the concurrent ejection of APV was not some artefact due to the passage of current in a neighbouring barrel to that from which penicillin was being ejected. As the ejection of penicillin by itself had already been shown to enhance the evoked potentials (fig. 3.3.3a), the lack of enhancement in the presence of APV must have been due to the action of the NMDA antagonist. However, these results did not

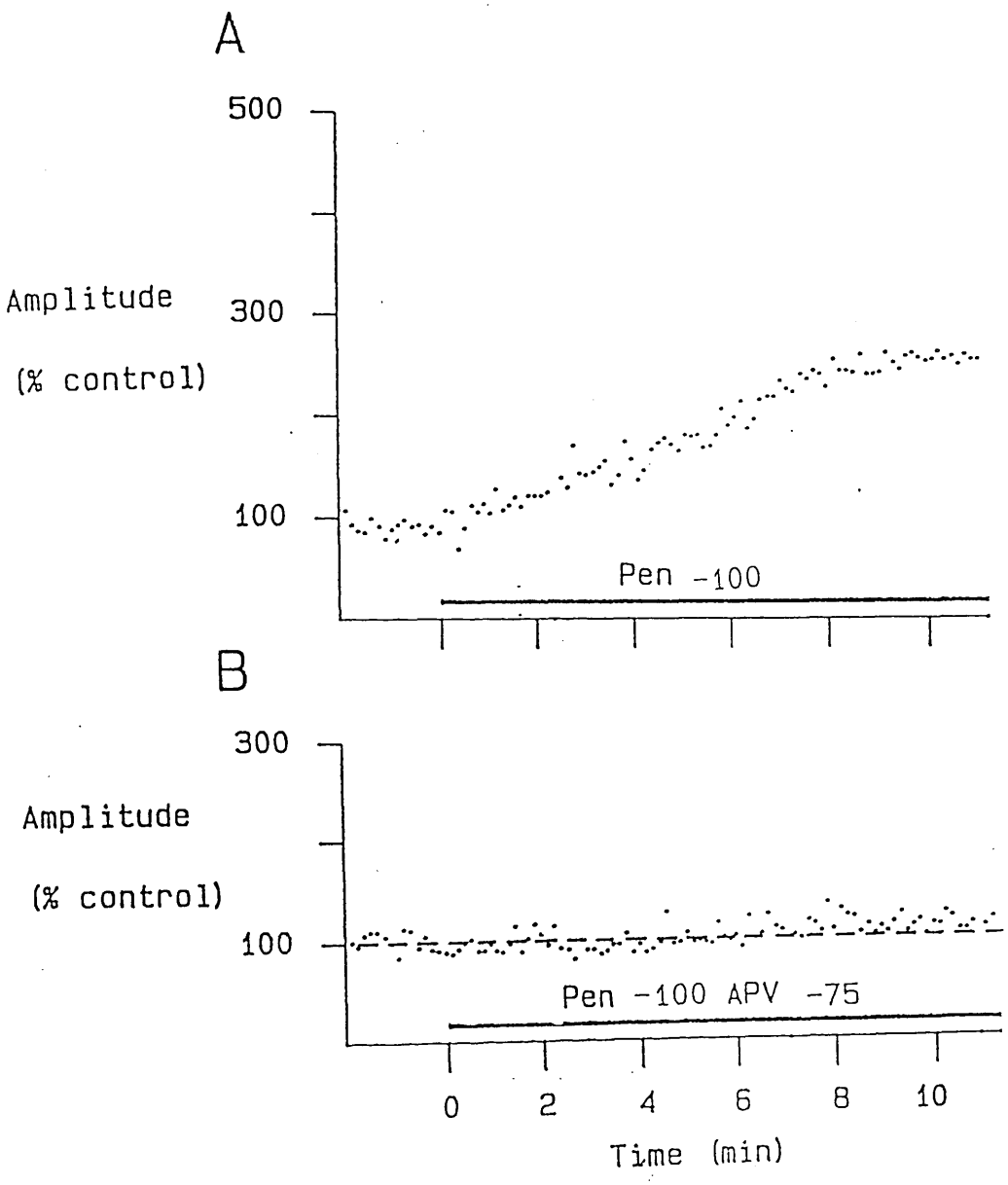


Figure 3.3.3: The enhancement of SEPs by small doses of penicillin (-100 nA) ejected by itself (A). The concurrent ejection of APV (-75 nA) prevents the enhancement that would normally result from the ejection of penicillin (B) although there is a very small increase in the mean amplitude after many minutes of the ejection of both drugs.

support the prediction that phase I was mediated by non-NMDA receptors, but suggested the very opposite: that the activity underlying phase I was in fact totally dependant on NMDA receptor mechanisms.

II (b). The Effect of NMDA Receptor antagonism on Phase II of Penicillin Epileptogenesis

The ejection of a flux of penicillin sufficiently large to result in phase II (-250nA) and the ejection of APV (-75nA) was started simultaneously. The ejection of penicillin alone would have resulted in a maximal enhancement of the amplitude and duration of the EPs (phase II) within five or six minutes. However, in the presence of APV, only after about four or five minutes did some of the potentials begin to be enhanced so that these were a little larger than normal (fig. 3.3.4a). This small enhancement, when it occured, was to only 150% of pre-drug levels. When this enhancement occurred it did so only after a relatively long time after the start of penicillin ejection, by which time penicillin ejected by itself would have produced a near maximal and much greater enhancement (compare figs. 3.3.3a and 3.3.4a). These results showed therefore, that the ejection of APV did not totally block, but did substantially reduce, the enhancement of evoked potentials which normally resulted from large fluxes of penicillin.

The effects of NMDA receptor antagonism by APV (-100nA) on the enhancement by a still larger flux of penicillin (-500 nA) was also tested. Once again in the presence of APV, a small, slow enhancement occurred. The enhancement was far less than would have occurred if penicillin had been ejected by itself and in the experimental circumstance of fig. 3.3.4b the enhancement was to just over 200% of pre-drug levels. This should be compared to fig. 3.3.3a in which a small penicillin flux by itself (-100nA) produced a more rapid and larger enhancement than resulted from the ejection of this large flux of penicillin (-500nA) in the presence of APV. In both of the above situations the ejection of large fluxes of penicillin did result in an enhancement of evoked potentials despite the ejection of APV. However, this enhancement was much less drastic than penicillin would have normally produced when ejected by itself.

As already described, NMDA receptor antagonism by APV resulted in the total blockade of any enhancement of evoked potentials which would normally have occurred in the presence of penicillin ejected by small electrophoretic currents (-100nA) but did not totally block the enhancement by larger fluxes of penicillin (-250nA and -500nA). This was compatible with the suggestion that the hyperexcitability underlying phase I was mediated by NMDA receptors, whereas the further

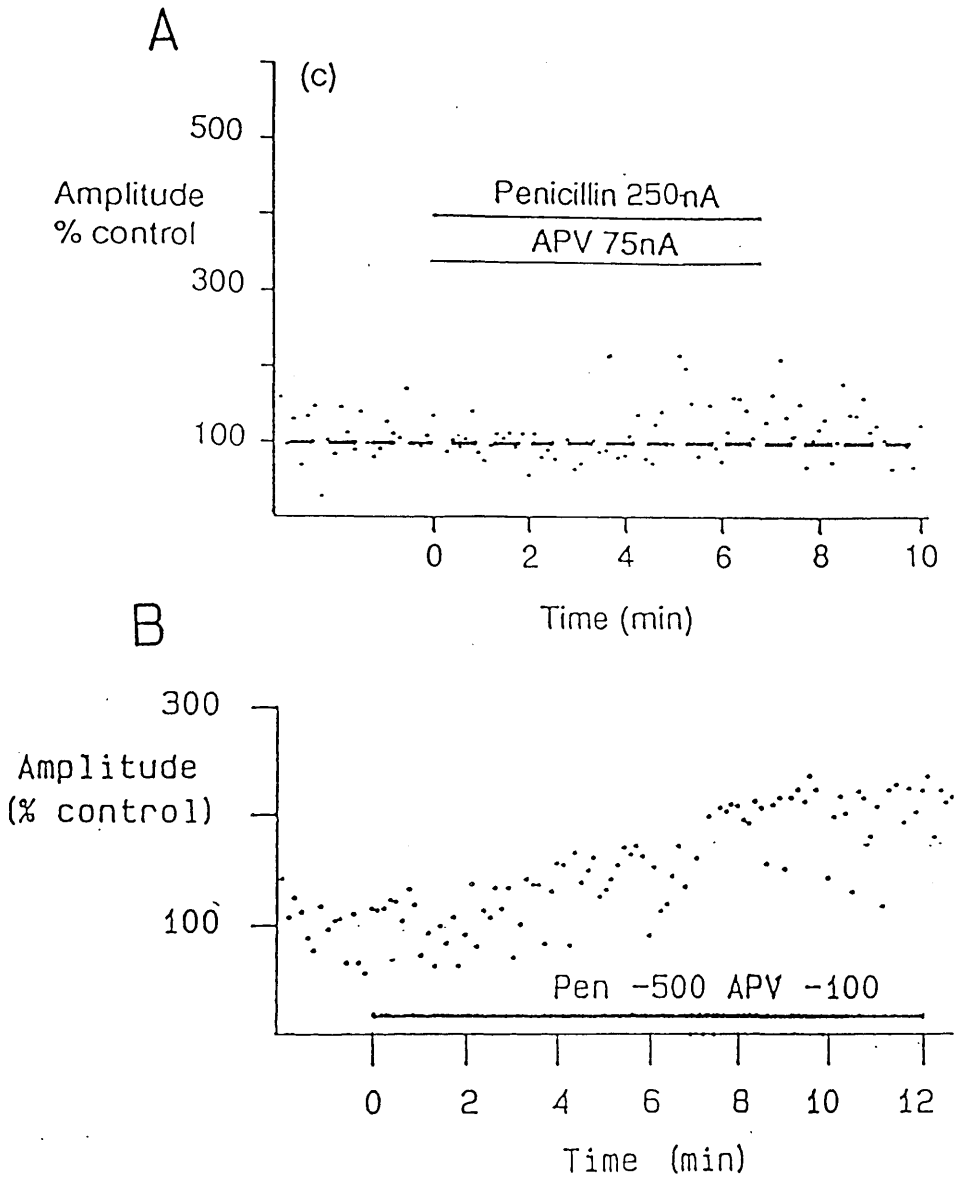


Figure 3.3.4: The enhancement that would normally result from the ejection of larger doses of penicillin; -250 nA (A), -500 nA (B) is substantially curtailed by the ejection of APV; -75 nA (A), -100 nA (B). These small enhancements that occurred should be compared to the enhancement by penicillin ejected by itself (-250 nA; see fig. 3.1.9, -500 nA; see fig. 3.1.7).

hyperexcitbility, which constituted phase II, might be mediated by non-NMDA receptors. However, this seemed unlikely in the light of the findings by other workers that the initial stages of the epileptic paroxysmal depolarisation shift are non-NMDA receptor mediated and the later, more 'epileptic' stages were NMDA mediated. Furthermore, it is generally regarded that 'normal' activity is non-NMDA receptor and 'epileptic' activity is NMDA receptor mediated.

Another possible explanation for the above findings was that the small enhancement that had resulted from the ejection of large doses of penicillin and APV was in fact mediated by NMDA receptors but that these were at some distance from the site of ejection of APV (into layer IV). It was therefore possible that in the above experimental conditions the concentration field of APV was too limited to provide a sufficient antagonsim of distant NMDA receptors which may be activated by larger fluxes of penicillin.

III The Effect of NMDA Receptor Antagonism by APV Ejected by Larger Ejecting Currents

In order to test this possibility the ejection of penicillin (-250nA) and a larger flux of APV (-200nA) was started simultaneously; the concurrent ejection of the large flux of APV and penicillin usually resulted in no enhancement of evoked potentials even after many minutes of continued ejection. Occasionally there was a very small enhancement (eg. 25% in the circumstances of fig. 3.3.5a) which occurred after a long time. This should be compared to fig. 3.3.4a in which a smaller flux of APV (-75 nA) was less effective in blocking the enhancement by the same penicillin flux, the resulting enhancement being to 150% of pre-drug levels.

When a still larger flux of penicillin (-500 nA) and APV (-200 nA) were ejected simultaneously, the enhancement that had occurred (200%) with smaller APV fluxes (-75nA) and this same flux of penicillin (-500nA) was drastically reduced (compare figs. 3.3.5b and 3.3.4b). When any enhancement did occur this was only about 25% as compared to at least 400% in the absence of APV (compare figs. 3.3.6a and 3.3.6b).

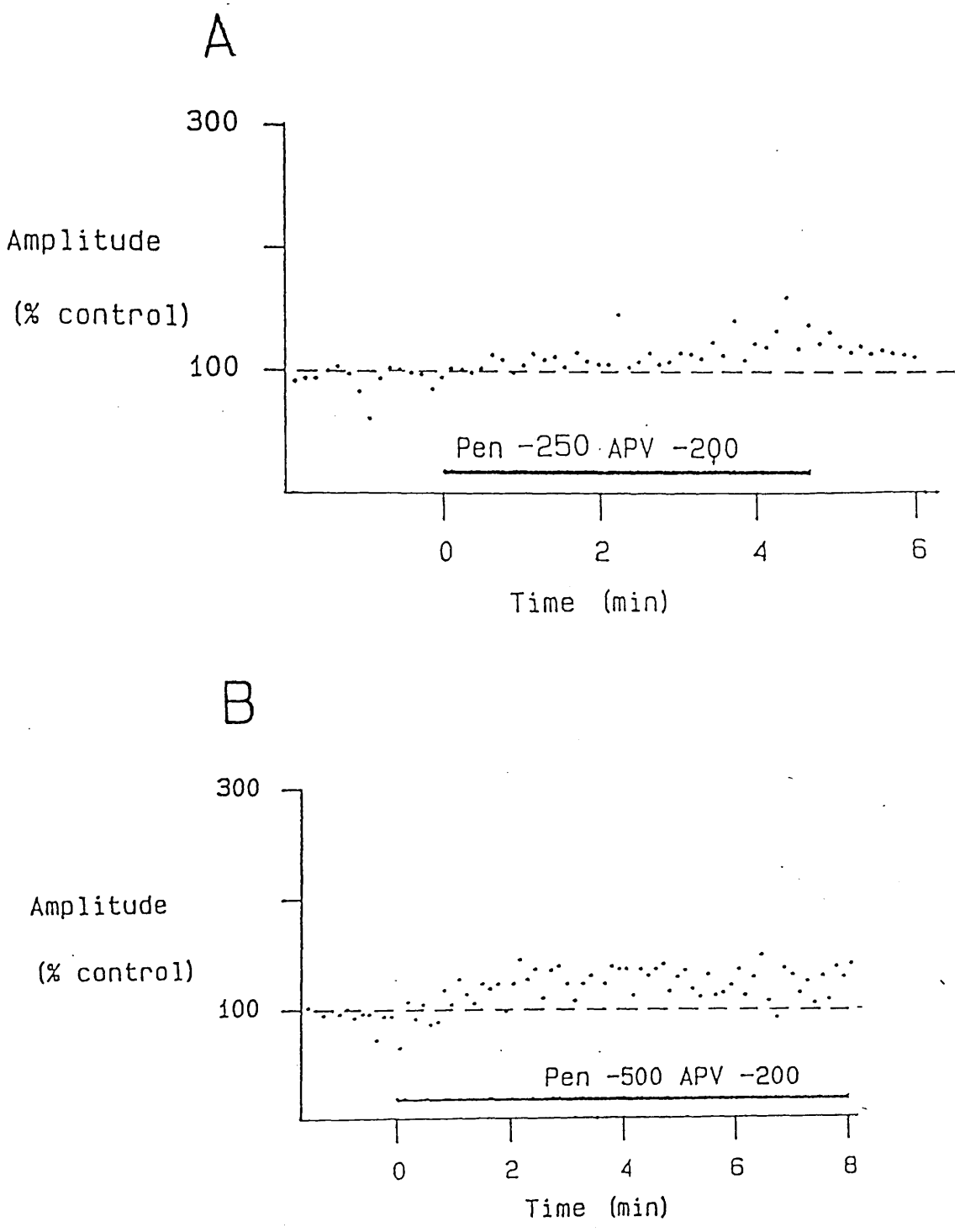


Figure 3.3.5: The effect of larger doses of APV (-200 nA) on the enhancement by large doses of penicillin; -250 nA (A), -500 nA (B). APV prevents any substantial enhancement by penicillin and reduces the enhancement that occurred in the presence of smaller doses of APV (-75 nA; see fig. 3.3.4)

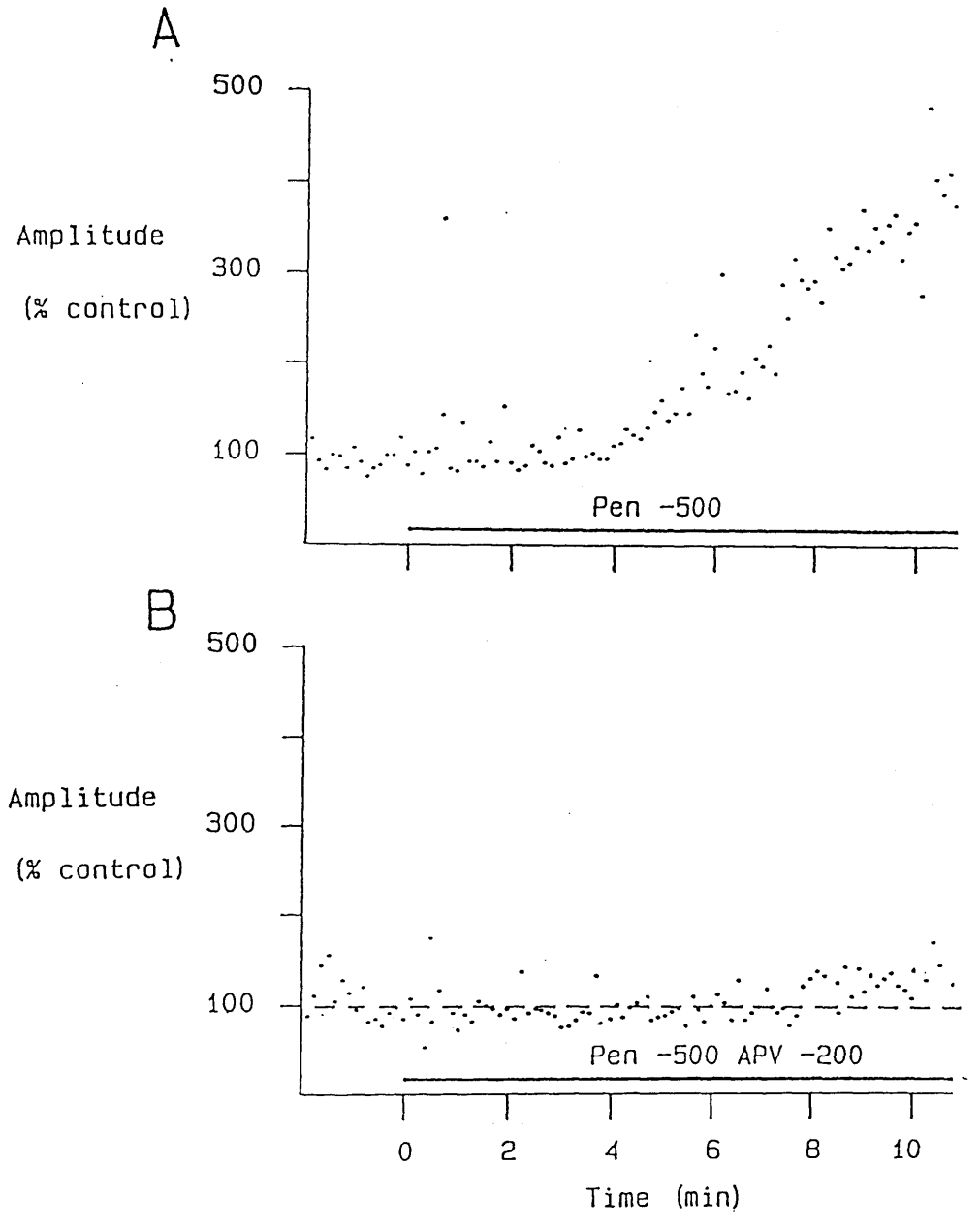


Figure 3.3.6: The enhancement of the amplitude of evoked potentials by a large dose of penicillin (-500 nA; A). The concurrent ejection of APV (-200 nA) prevents the occurrence of any such enhancement (B) although there is a very small increase in the mean amplitude of the EPs after many minutes.

Since the larger flux of APV, which was still specific (see part I) for NMDA antagonism, was effective in blocking the enhancement by larger penicillin fluxes, the previous enhancement that had occurred with large penicillin fluxes and smaller APV fluxes (part IIb) was not a non-NMDA receptor mediated event but presumably resulted from an inadequacy of NMDA receptor antagonism, thereby allowing activation of some NMDA receptors by the larger doses of penicillin.

It is not unreasonable to assume, since the sink in the evoked potential voltage field during phase I enhancement was no different from the initial sink in the field of the normal evoked potential (see results part 1 and discussion), that phase I enhancement was a reflection of activity mediated by excitatory receptors close to pyramidal cell bodies close to layer IV and that, since the sink in the voltage field of phase II enhanced potentials was centered in the superficial layers of the cortex (see results part 1 and discussion), that phase II was the result of activity mediated by receptors either on the superficial parts of the pyramidal cell apical dendrites or on pyramidal cells distant from layer IV. Furthermore, since both phases were blocked by the ejection of APV, this implied that both phases of penicillin epileptogenesis were NMDA receptor mediated. The hyperexcitability underlying phase I may therefore be mediated by receptors confined to a small distance from layer IV. A

small APV flux nevertheless resulted in a sufficient concentration field to produce complete NMDA receptor antagonism of these receptors and therefore blocked phase I. However, if large penicillin fluxes produced activity which was mediated by superficial NMDA receptors these receptors may not be blocked because of the limited extent of the APV concentration field produced by small APV fluxes. A more extended concentration field of APV resulted in a block of these superficial receptors and prevented the enhancement that had occurred previously.

IV Blockade of Phase II of Penicillin Epileptogenesis by APV Ejected by Small Electrophoretic Currents

Another way to test the above hypothesis, that the enhancement that had occurred was due to a lack of NMDA receptor antagonism, was to eject a small flux of APV over a longer period. This would also result in an extensive APV concentration field and should also block the enhancement by larger penicillin fluxes.

APV ejection (-75nA) was started 2 to 5 mins before the start of pencillin ejection. During this period there was no effect on the SEPs. The ejection of penicillin (-500nA) was then started. After many minutes of the concurrent ejection of APV and penicillin there was no enhancement of the evoked

potentials (fig. 3.3. 7); by this time penicillin ejection by itself would have produced a maximal enhancement.

These results showed that APV was effective in blocking both phase I and phase II of penicillin epileptogenesis when ejected concurrently with penicillin.

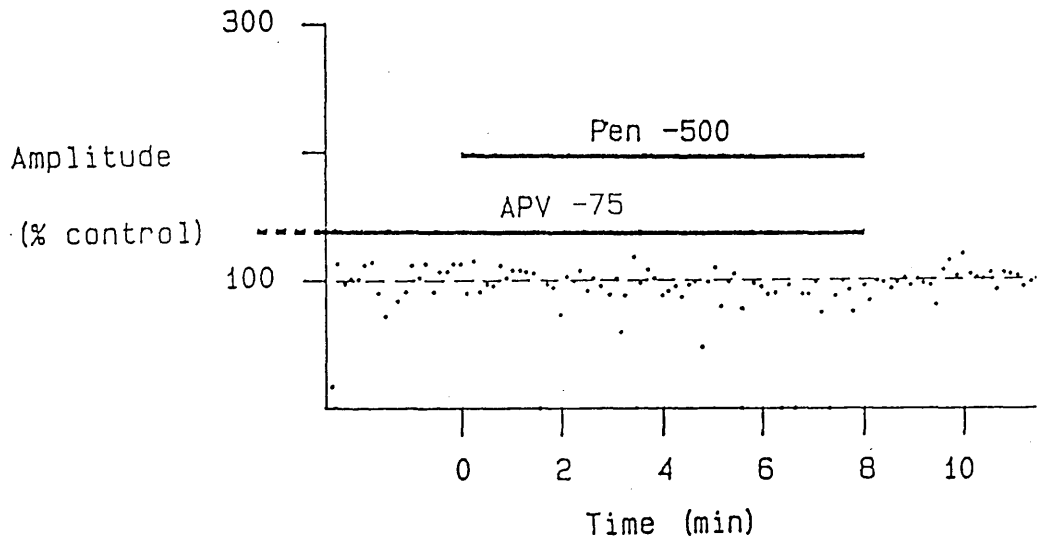
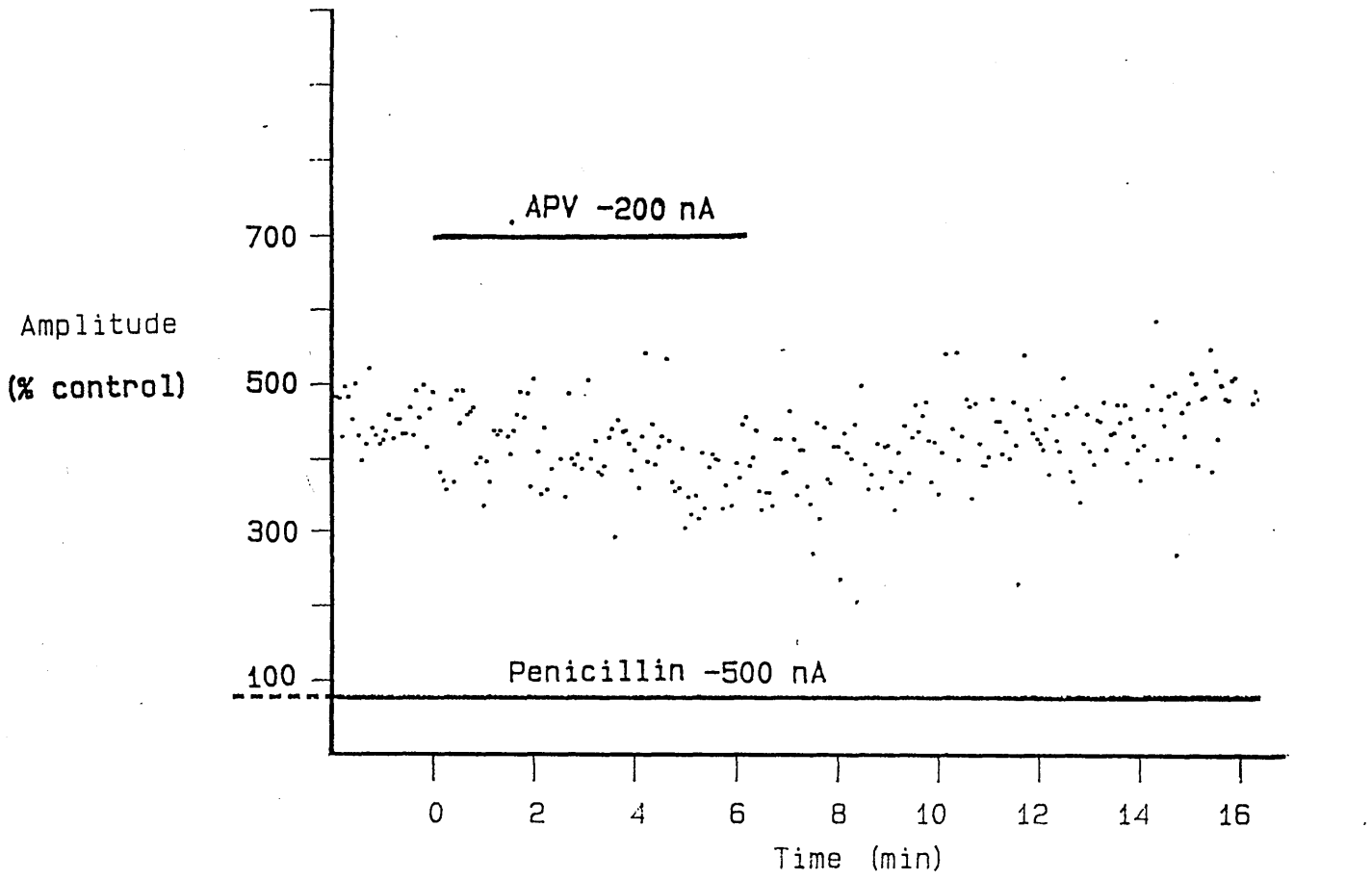


Figure 3.3.7: The effect of a small dose of APV (-75 nA) ejected prior to the start of penicillin ejection (-500 nA). In this way a small dose of APV prevents the enhancement that would normally result from a large dose of penicillin.

V. Circumstances in Which APV Ejection was Ineffective in preventing Penicillin Enhancement of Evoked Potentials

The ejection of APV (small or large fluxes) did not abolish the enhancement of evoked potentials which resulted from large penicillin fluxes if APV ejection was started once the plateau level of enhancement had been attained; there was only a small reduction of the plateau level of the enhanced potentials to a lower level (fig. 3.3.8). Therefore, APV was only effective in blocking penicillin induced enhancement of evoked potentials if ejected prior to or concurrently with penicillin, but was virtually ineffective in reducing the enhancement of evoked potentials once established. Possible reasons for this will be considered in the discussion.

Figure 3.3.8: The effect of a large flux of APV (-200 nA) on the plateau level of enhancement of somatosensory evoked potentials resulting from the ejection of large doses of penicillin (-500 nA).


APV -200 nA
700
500
300
100
Amplitude
(% control)
Penicillin -500 nA
0
2
4
6
8
10
12
14
16
Time (min)

RESULTS: PART 4.

The non-competitive NMDA antagonist, ketamine, was used in order to try and provide further evidence for the role of NMDA receptors in penicillin epileptogenesis. Ketamine hydrochloride was prepared (20mM) in 120mM NaCl. The pH of this solution (6.0) was adjusted to 4.0, by the addition of molar HCl.

I. The Specificity of ketamine

As with APV, it was important to test the effects of ketamine on normal evoked cortical activity to find out the range of doses of ketamine free of non-specific depressant effects.

Ketamine was ejected into layer IV (+50 to +400nA) to test the effects on normal evoked activity. Ketamine ejected by electrophoretic currents up to +400nA had no effect on the amplitude (fig. 3.4.1a) or waveforms of the evoked potentials, even after many minutes of ejection. When ketamine was ejected by electrophoretic currents of +450nA there was sometimes a very small reduction in the amplitude of evoked potentials (fig. 3.4.1b) with no detectable effects on the waveforms of these potentials. A current of the opposite polarity but of the same magnitude (-450nA) resulted in no effect on the EPs (fig. 3.4.1b).

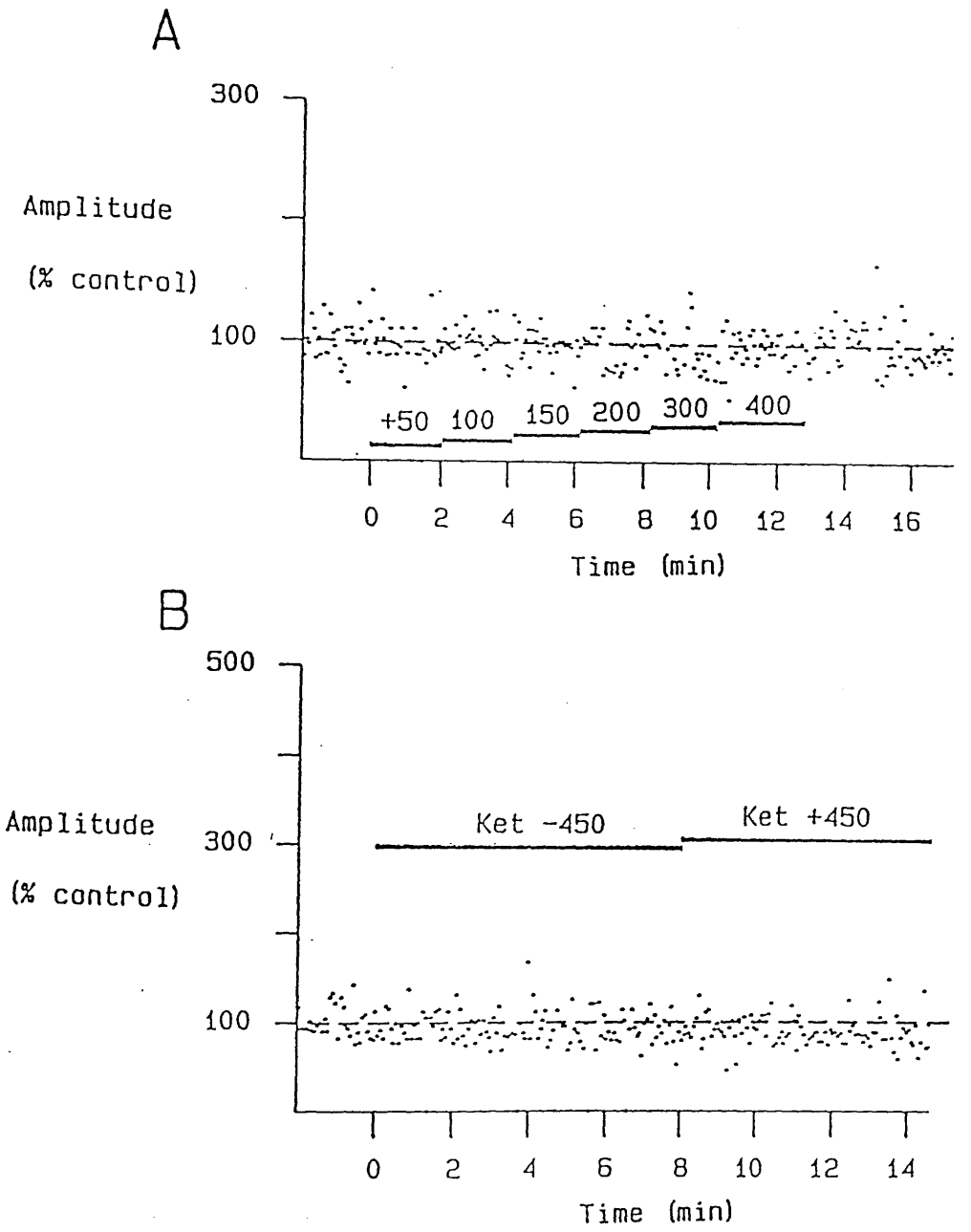


Figure 3.4.1: To test for the specificity of ketamine the drug was ejected by consecutively larger electrophoretic currents (A) and the effect on the amplitude of the evoked potentials was monitored. (B) Large doses of ketamine (+450 nA) resulted in a small depression of the the mean amplitude of EPs. An application of the same magnitude but opposite polarity of current had no effect on the evoked potentials.

As already described for APV, doses of the antagonist that had no effect on normal evoked activity were regarded as being free of non-specific depressant effects, whereas those resulting in a depression of evoked potentials were regarded as having non-specific effects.

II. The Effects of Ketamine on an Established Penicillin Enhancement

Because of the possibility that the very small depression of evoked potentials by larger ketamine doses (+450nA) may have been due to some non-specific depressant effect, smaller doses of the drug, which had no effects whatsoever on normal evoked activity, were used to test for the involvement of NMDA receptors in the penicillin induced enhancement of evoked potentials.

Penicillin was ejected by itself (-200nA) into layer IV so as to produce an enhancement of evoked potentials, as already described in the earlier results (part 1). When the enhancement of the amplitude of evoked potentials had reached a plateau level (300% of pre-drug levels), the ejection of ketamine (+250nA) was also started. After about two minutes the amplitude of evoked potentials began to decline from the plateau level (fig. 3.4.2); this decline continued for about 2 to 3 minutes until the amplitude of the potentials reached a

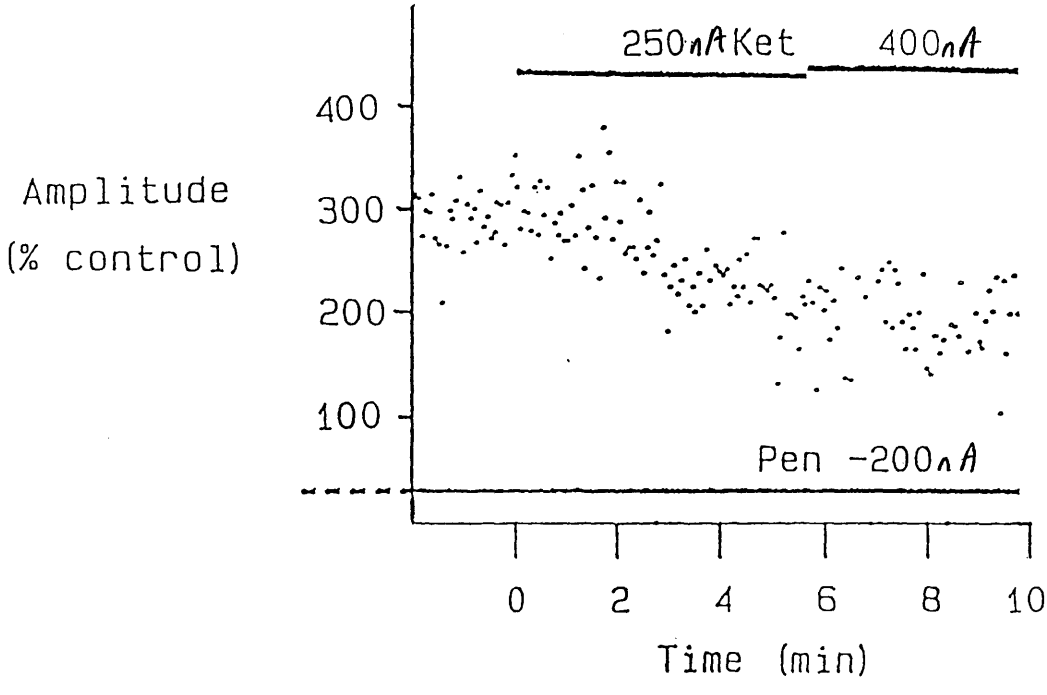


Figure 3.4.2: The enhancement of evoked potential amplitude by penicillin (-200 nA) was reduced from its plateau level by the ejection of ketamine (+250 nA). An increase in the ejecting current of ketamine (+450 nA) resulted in no further depression of evoked potentials.

steady level (200% of pre-drug levels). The ketamine dose was then increased (ejecting current: +400nA) but this resulted in no further depression of the evoked potential amplitude. When ketamine ejection was terminated the amplitude of the evoked potentials began to increase and reached the plateau level (300%) which had originally been present proir to ketamine ejection.

The effect of ketamine was then tested on the enhancement of potentials resulting from larger doses of penicillin (-700nA). When a large plateau level (about 600% of pre-drug levels) had been established (fig. 3.4.3) ketamine ejection (+250nA) was started; once again there was a delay of about two minutes after which the amplitude of the potentials began to decline. The depression of the enhanced evoked potentials continued until a steady level, at about 400% of pre-drug levels (fig. 3.4.3) was attained after about 5mins.

In the above preliminary experiments, the ejection of ketamine reduced the penicillin induced enhancement of evoked potentials when tested against an already established enhancement. However, ketamine did not totally abolish the enhancement. This was not due to an insufficient antagonist concentration since increasing the ketamine flux resulted in no further reduction of the amplitude of the enhanced EPs. As described earlier the effect of APV in reducing penicillin

epileptogenesis was very limited when APV was administered after the establishment of a plateau level of evoked potential enhancement (results 3.V), but was far more effective when ejected prior to or concurrently with penicillin. Hence the simultaneous ejection of penicillin and ketamine was also tested.

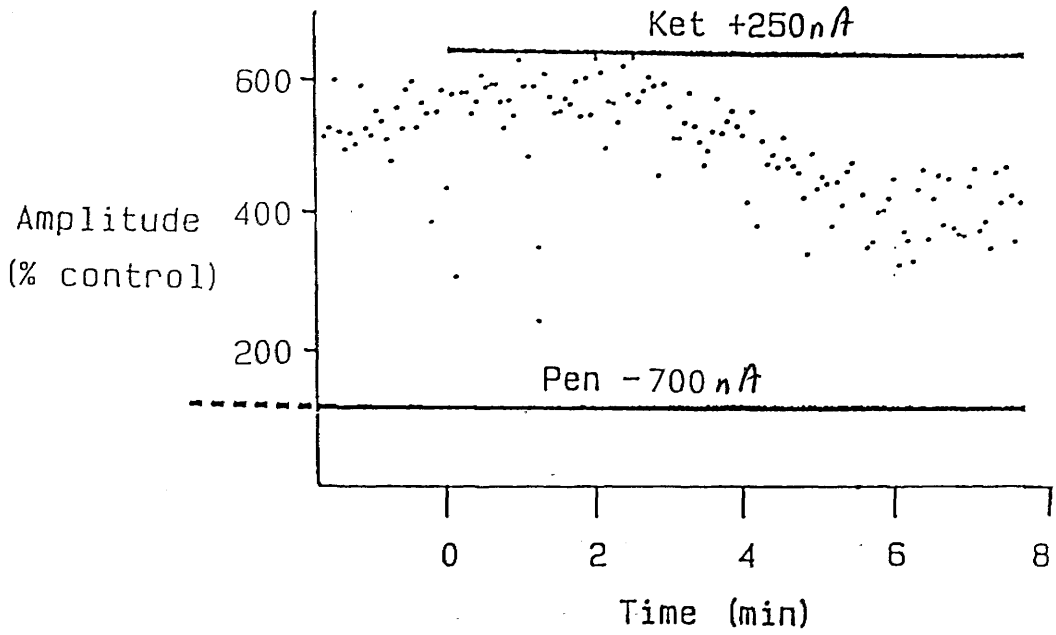


Figure 3.4.3: The plateau level of enhancement of evoked potential amplitude by a large dose of penicillin (-700 nA) was reduced by the ejection of ketamine (+250 nA).

III. The Effect of the Concurrent Ejection of Ketamine and Penicillin

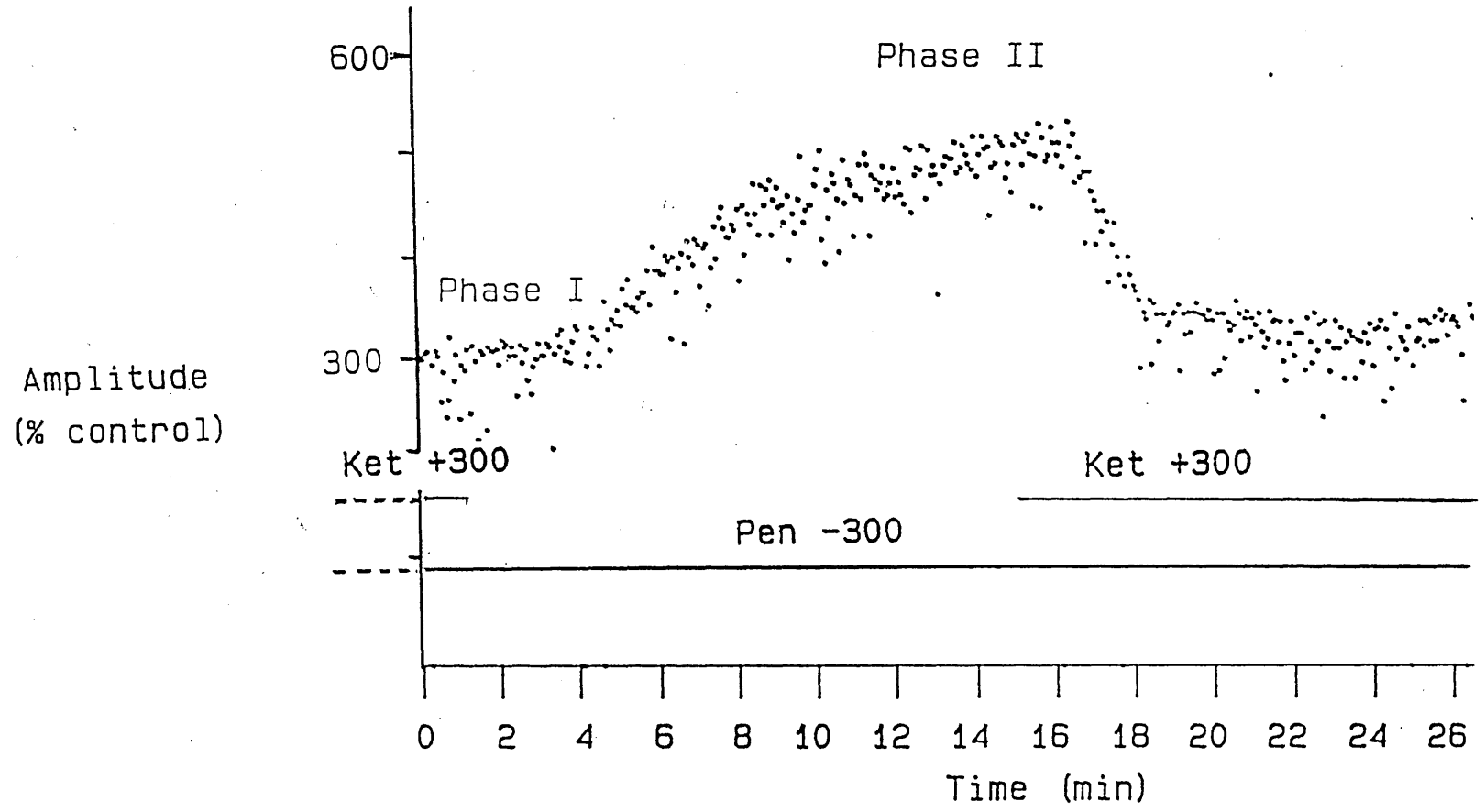
With the concurrent ejection of ketamine and penicillin there was still invariably an enhancement of the amplitude of the evoked potentials. This is illustrated in fig. 3.4.4 in which the ejection of penicillin (-300nA) and ketamine (+300nA) resulted in a plateau level of enhancement at about 300% of pre-drug levels. During this plateau phase, the ejection of ketamine was terminated; after a delay of about 2-3 mins the evoked potentials began to increase in amplitude so that a new plateau level was attained at about 500% of pre-drug levels. Ketamine ejection was once again started (+300nA) and the evoked potentials began to decline in amplitude from the 500% plateau level to the 300% plateau which had originally been present with the concurrent ejection of both penicillin and ketamine.

Thus, ketamine ejection, even when started at the same time as penicillin ejection, only depressed and did not prevent the enhancement of evoked potentials by penicillin. In the presence of ketamine, even with large penicillin doses, the evoked potentials were only enhanced in amplitude and there was no change in the waveform. This was similar to the earlier identified phase I of epileptogenesis which resulted from small penicillin doses. Despite the use of a large penicillin

ejecting current (fig. 3.4.4), in the presence of ketamine, the enhancement of evoked potentials had the form that was normally associated with phase I epileptogenesis. On termination of ketamine ejection the evoked potentials were enhanced in the fashion characteristic of phase II epileptogenesis (see fig. 3.4.4).

The refractory period properties of evoked potentials in the presence of penicillin and ketamine were investigated to find out if ketamine was preferentially abolishing phase II enhancement whilst allowing the expression of phase I activity.

Figure 3.4.4: The concurrent ejection of ketamine (+300 nA) and pencillin (-300 nA) resulted in a plateau level of enhancement of evoked potentials. During this plateau level ketamine ejection was terminated. This resulted in a further enhancement of the evoked potentials to a new plateau level which was depressed back to the original plateau level of enhancement when ketamine ejection (+300 nA) was once more started.


600
300
Amplitude
(% control)
Phase I
Phase II
Ket +300
Ket +300
Pen -300
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Time (min)

IV. The effects of Ketamine on the refractory period properties of penicillin enhanced potentials

To test whether ketamine was producing a selective depression of phase II enhancement, a conditioning/test paradigm was used, with a delay of 150msec between the two potentials. The refractory period of normal potentials is less than 150msec, as is that of phase I enhanced potentials; phase II activity has a refractory period in excess of 150msec (see Results part 2).

Penicillin (-300nA) was ejected by itself and the amplitude of the conditioning potentials reached about 600% of pre-drug levels. The amplitude of the test potentials however was not enhanced and remained at, or slightly below, pre-drug levels (fig. 3.4.5a). This situation corresponded to phase II of penicillin enhancement (as described in part B); the enhanced potentials having a refractory period in excess of the refractory period of normal potentials. The effect of ketamine ejection on the refractory periods of penicillin enhanced potentials was then tested.

(a) The ejection of ketamine (+250nA) and penicillin (-300nA) was started simultaneously. After about two minutes both conditioning and test potentials began to increase in amplitude until they both reached a plateau level at about 500% of pre-drug levels (fig. 3.4.5). With penicillin alone such large enhancements of the conditioning response were accompanied by a depression of the test potentials to, or below, pre-drug levels (fig. 3.4.5a). Hence, this dose of penicillin by itself resulted in phase II of epileptogenesis, but in the presence of ketamine, only phase I occurred. Ketamine had prevented the generation of phase II but not phase I enhancement of evoked potentials when ejected concurrently with penicillin.

(b) The effects of ketamine on the refractory periods of evoked potentials in an already established penicillin enhancement were also tested. Penicillin (-250nA) was ejected so that a plateau level of enhancement of the conditioning potentials was achieved (350% of pre-drug levels; fig. 3.4.6). The test potentials meanwhile, were depressed a little below their pre-drug levels. Therefore, the refractory period of the enhanced potentials was greater than 150 msec and phase II of penicillin epileptogenesis prevailed. The ejection of ketamine (+250nA) was then started and after about two minutes the conditioning responses began to decrease in amplitude to about 200% of pre-drug levels. Meanwhile, the test responses

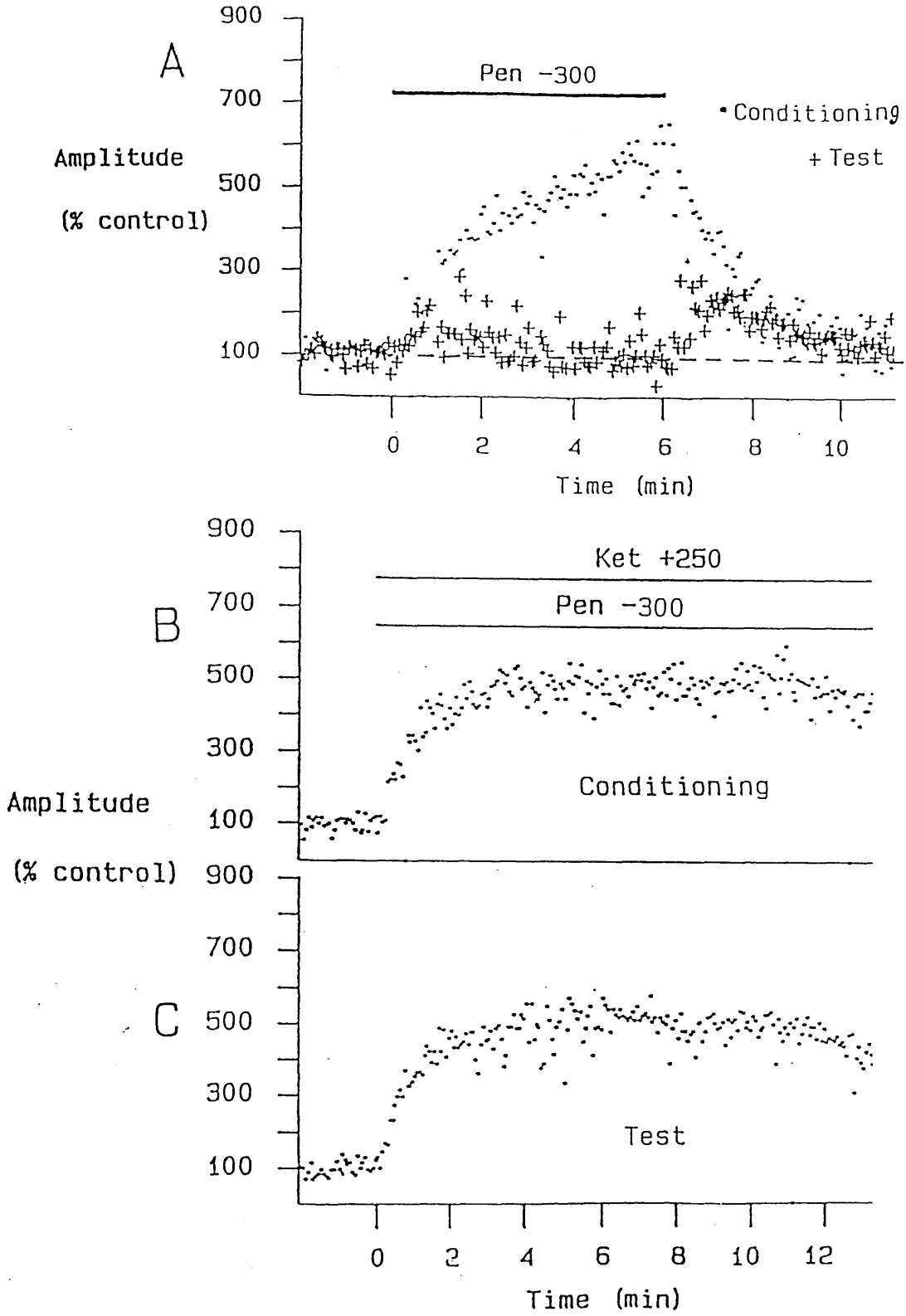
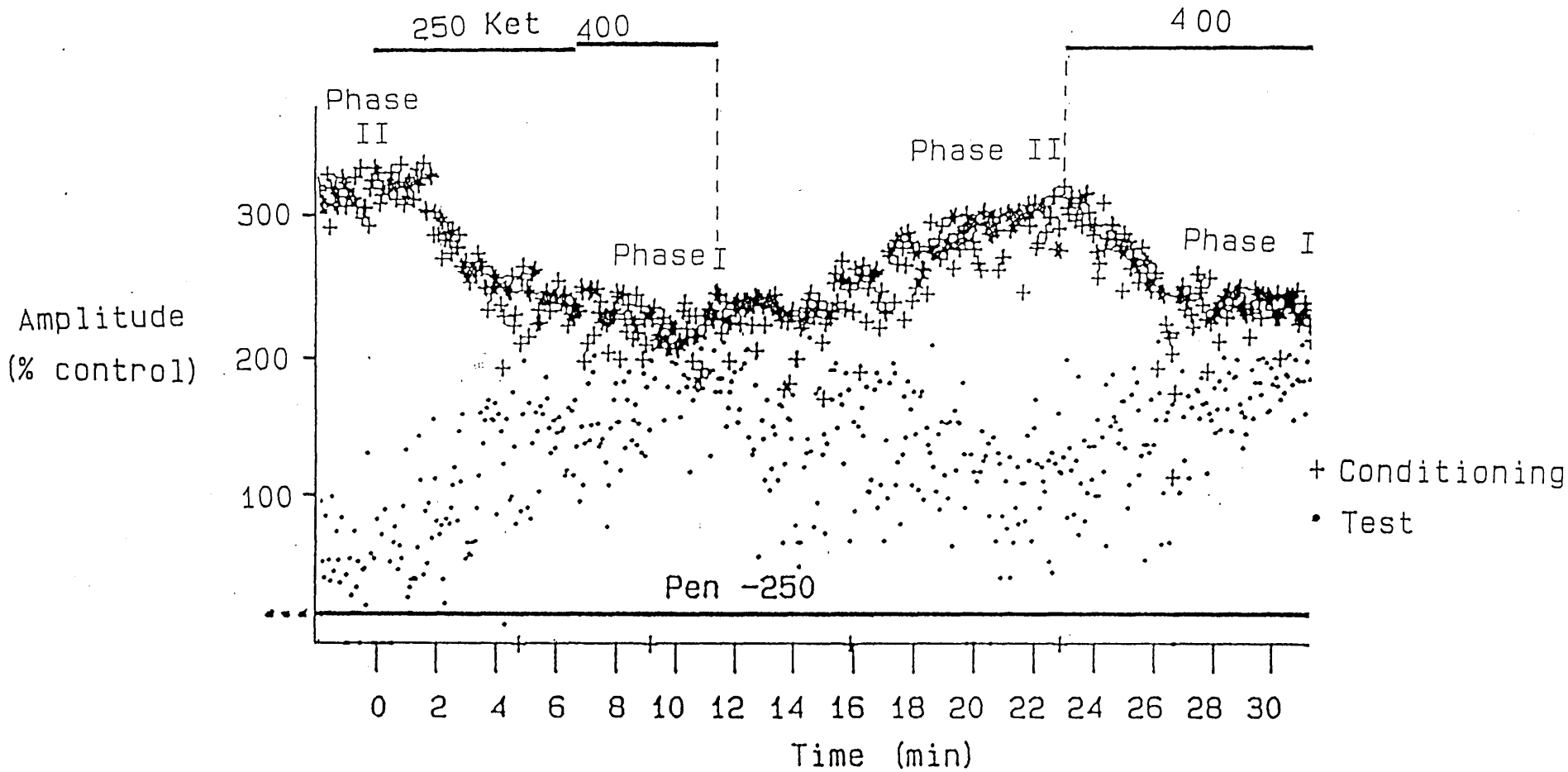


Figure 3.4.5: The ejection of a large dose of penicillin (-300 nA) by itself resulted in phase II of epileptogenesis; the refractory period of enhanced potentials being greater than that of normal potenials (A). (B) The ejection of the same dose of penicillin in the presence of ketamine (+300 nA) resulted in the enhancement of both conditioning and test responses; the enhanced potentials had a refractory period which was the same as that of normal potentials (phase I).

Figure 3.4.6: The ejection of penicillin (-250 nA) results in phase II of epileptogenesis. The subsequent ejection of ketamine reduces the enhancement to phase I. An increase in the dose of ketamine results in no further depression. This effect of ketamine is reversible and repeatable.


250 Ket 400
400
Phase II
Phase I
Phase II
Phase I
Amplitude
(% control)
300
200
100
Pen −250
+ Conditioning
• Test
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (min)

increased in amplitude to nearly 200% of pre-drug levels, being only a little smaller than the conditioning responses (fig. 3.4.6). That the conditioning and test responses were now of a similar amplitude indicated the presence of phase I. Ketamine ejection was then increased (+400nA); there was no further decrease in the amplitudes of the evoked potentials. Ketamine ejection was then terminated, and after a small delay both potentials began to return to their original, pre-ketamine levels so that the conditioning potentials were large and the test potentials depressed, indicating a return to phase II. The effect of ketamine was repeatable and reversible (see fig. 3.4.6).

These results raised the possibility that in these experimental circumstances phase I was mediated by non-NMDA receptors and phase II by NMDA receptors. Furthermore, the dose of ketamine was not a limiting factor in the inability to reduce phase II back to normal (See IIa and IIIbii) because, as described above, a further increase in the dose of ketamine did not reduce the amplitude of the evoked potentials any further. This conclusion was in agreement with the earlier prediction of phase I being non-NMDA receptor and phase II being NMDA receptor-mediated. However this conclusion contradicts the conclusions reached from the APV findings that both phase I and phase II were NMDA receptor mediated. In the results so far ketamine had not been tested against phase I

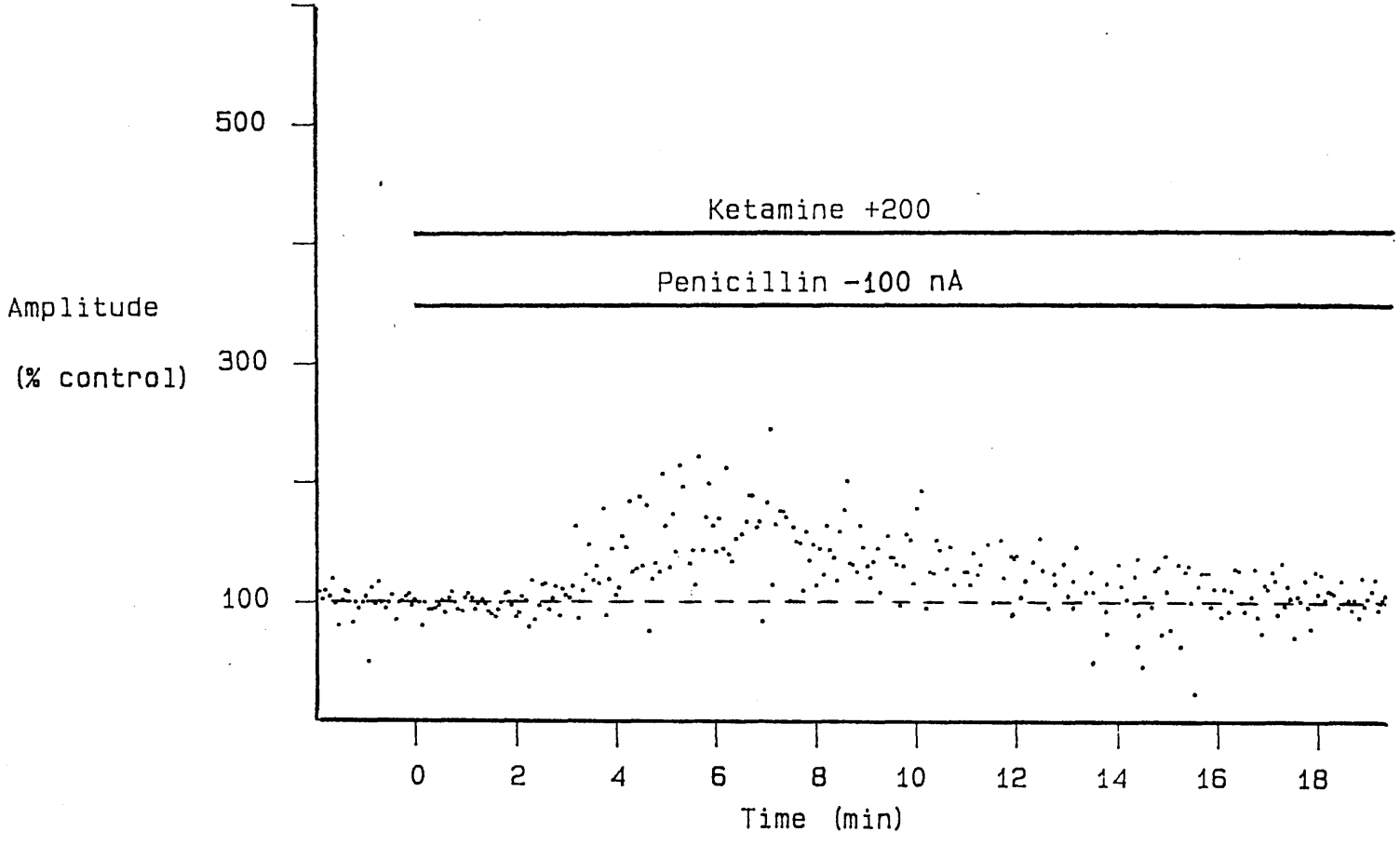
itself.

To test whether phase I was itself mediated by NMDA receptors, this was established by the ejection of penicillin by small ejecting currents (-100nA) and ketamine ejection (+250nA). (fig 3.4.7) The concurrent ejection of the two drugs resulted in the amplitude of the evoked potentials slowly increasing from normal levels. However, the enhancement was unlike that by penicillin ejected alone; the potentials began to increase in amplitude and then began to slowly decline, with the concurrent ejection of ketamine and penicillin, back towards normal levels. This form of blockade was suggestive of a use dependant antagonism; the NMDA receptor/channels had to be used before the antagonism occurred.

Thus, phase I must have been mediated by NMDA receptors and this result confirmed the findings obtained using APV that the activity underlying phase I enhancement was NMDA receptor mediated.

However, an anomaly still existed in these results in that ketamine was able to block the activity underlying both phase I and Phase II but was unable to block phase I after phase II had been established. This will be considered further in the discussion.

Figure 3.4.7: The effect on somatosensory evoked potential amplitude of the concurrent ejection of ketamine (-200 nA) and a small flux of penicillin (-100 nA). The ejection of penicillin would normally result in the sustained enhancement of evoked potential amplitude.


Ketamine +200
Penicillin −100 nA
Amplitude
(% control)
500
300
100
0
2
4
6
8
10
12
14
16
18
Time (min)

V. NMDA Receptor Involvement in Strychnine Epileptogenesis

To test whether the epileptogenic effects of penicillin were in some way peculiar in their necessary requirements for NMDA receptor activation, another epileptogenic agent, strychnine, was used. Strychnine is a glycine receptor antagonist and the topical application or ejection into the cortex results in epileptiform activity in the cerebral cortex.

Strychnine had similar epileptogenic properties to penicillin in that small doses of strychnine failed to result in spontaneous epileptic spiking but produced an enhancement of evoked potential amplitude (fig. 3.4.8). However, the delay to onset of enhancement was longer and the rate of enhancement was much slower than that resulting from the ejection of penicillin.

The ejection of APV (-70nA) prevented the enhancement of evoked potentials which strychnine ejected by itself would have produced (fig. 3.4.8). Even after many minutes of of continued ejection, by which time strychnine enhancement would have been maximal, there was no enhancement.

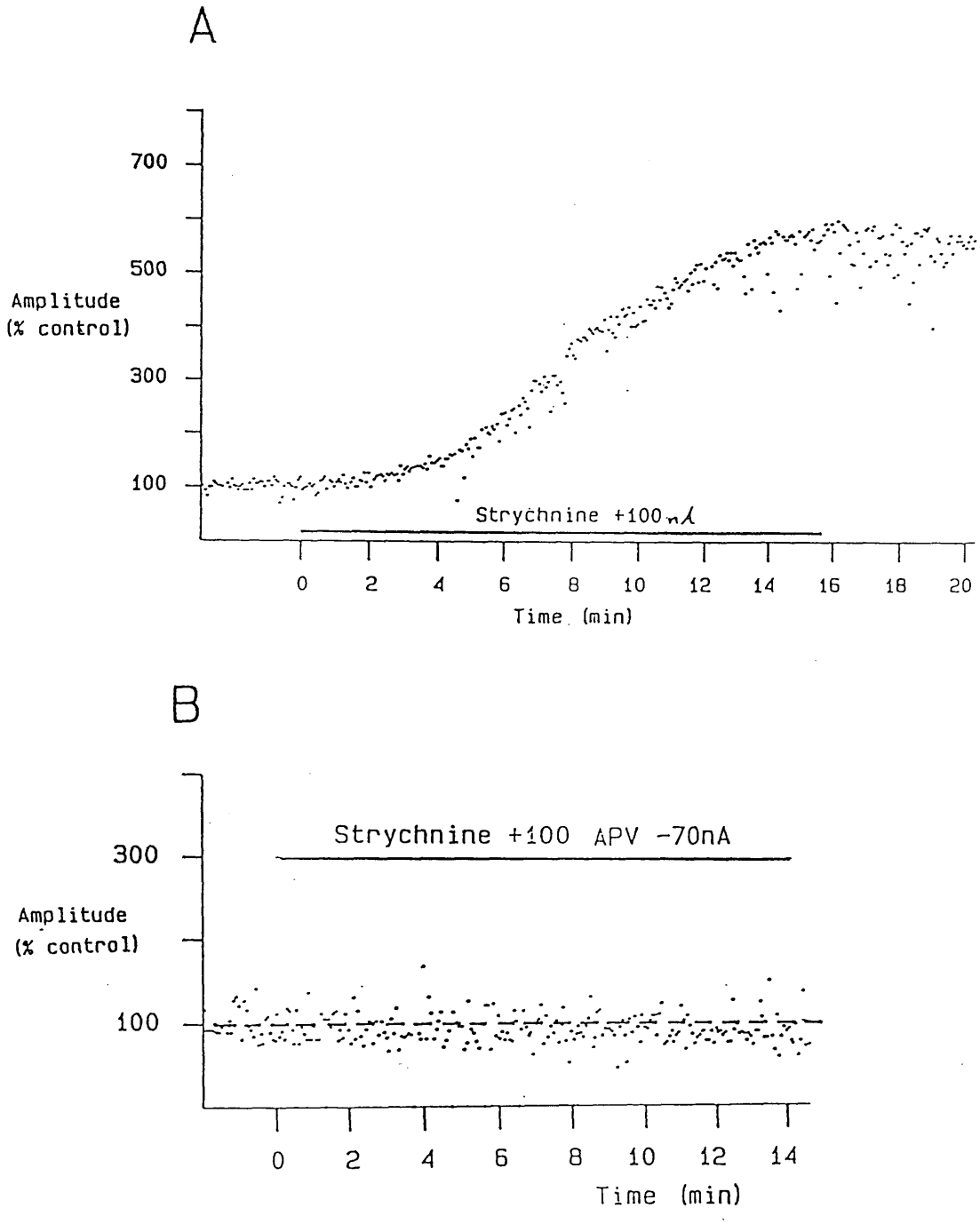


Figure 3.4.8: The ejection of stychnine (-100 nA), like penicillin, resulted in the enhancement of evoked potential amplitude (A). The concurrent ejection of APV (-75 nA) prevented the enhancement that would normally result from the ejection of stychnine (B).

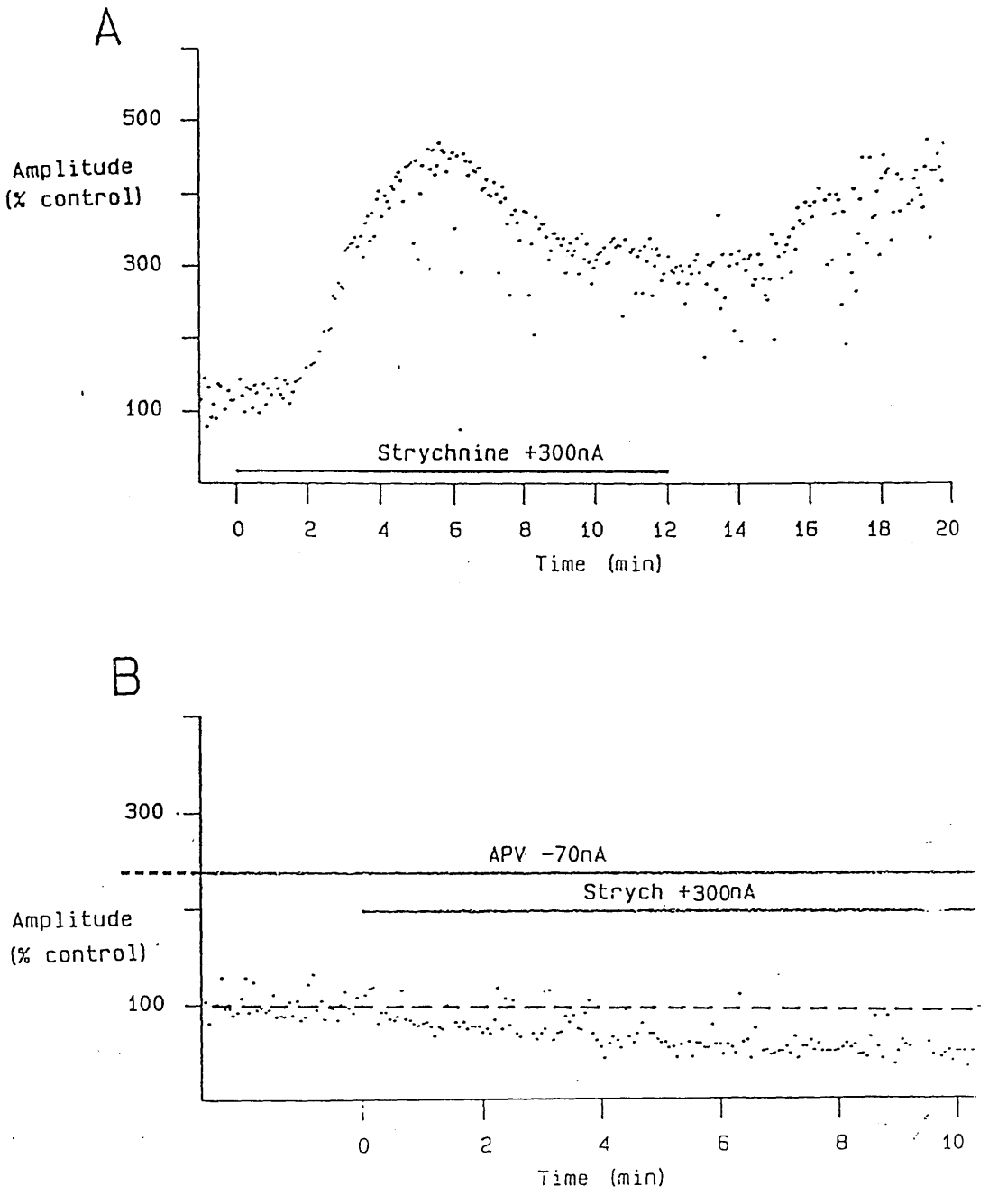


Figure 3.4.9: The ejection of larger doses of strychnine (+300 nA) resulted in the enhancement of evoked potentials, although the continued ejection resulted in a later depression phase which was then alleviated by the termination of strychnine ejection (A).

(B) The ejection of APV by a small electrophoretic current (-75 nA) prior to and during the ejection of strychnine prevented the enhancement that would normally result from the ejection of strychnine. A depression, which is probably the same as that occurring in the presence of strychnine by itself, did occur.

Strychnine ejected by larger electrophoretic currents (-300nA) resulted in a different pattern of enhancement (fig. 3.4.9a) to that resulting from smaller doses of the drug. An initial enhancement of the EPs was followed by a reduction of the amplitude to a level which was however, still greater than the pre-drug levels. On termination of ejection the amplitude of the SEPs recovered to the initial maximal enhanced voltage. The depression phase may have been a non-specific effect due to a larger concentration field of strychnine (there being no such effect with smaller doses).

APV (-70nA) ejection was started a few minutes before the start of strychnine ejection. This prevented any enhancement of SEPs which would normally have resulted from the ejection of strychnine alone (fig. 3.4.9). There was a small slow depression of SEPs and this may have reflected the non-specific effect of strychnine mentioned earlier, occurring in isolation.

Therefore, epileptiform activity induced by both glycine receptor antagonism, by strychnine, and GABA receptor antagonism, by penicillin, was blocked by NMDA receptor antagonism, which implies that all hyperexcitability resulting from such manoeuvers is mediated by the activation of NMDA receptors.

DISCUSSION

DISCUSSION

Summary of the Evidence for two Phases in Penicillin Epileptogenesis

These experiments have provided several lines of evidence to suggest that there are two physiologically distinct stages in the development of the acute penicillin epileptic focus in the rat neocortex.

Penicillin ejected by small electrophoretic currents did not result in spontaneous epileptiform spiking but did produce a simple increase in the amplitude of somatosensory evoked potentials and potentials evoked by direct cortical stimulation (DCS) (phase I). This enhancement was present when penicillin was ejected by small electrophoretic currents, or as the last remaining vestige of hyperexcitability during the recovery from a fully developed epileptiform focus.

Penicillin ejected by large electrophoretic currents resulted in additional changes in evoked potential characteristics; an increase in duration and time to peak (phase II). This change in waveform implied the presence of an additional and different form of cortical activity as compared to normal and was associated with the appearance of spontaneous epileptiform spikes. The waveform of enhanced evoked potentials in phase II and spontaneous epileptiform

spikes was very similar, which was suggestive of the fact that the enhanced evoked potentials were evoked epileptiform spikes.

Further evidence for two distinct phases in epileptogenesis arose from the finding that the refractory period of evoked potentials which showed a simple enhancement of amplitude was no different from the refractory period of normal evoked potentials. However, the refractory period of evoked potentials which showed a prolongation in waveform was considerably greater than the refractory period of both normal evoked potentials and evoked potentials enhanced only in amplitude. This change in refractory period also indicated the presence of a different form of activity. These results implied that the activity underlying phase I was no different from that of normal potentials, whereas the activity underlying phase II of penicillin epileptogenesis was different from that of both phase I and of normal activity.

Furthermore, the increase in the refractory period and the increase in duration of the evoked potentials, which had seperately been used to identify the onset of phase II, both occurred at the same time during the development of the focus and presumably both were associated with the onset of the same changes in cortical activity.

The stimulus strength response relationship of evoked potentials in the different phases was also tested: for phase I evoked potentials, the relationship followed a scaled up version of the normal sigmoidal curve. This was further evidence that phase I activity was a simple enhancement of normal activity. In contrast, the strength response relationship of phase II evoked potentials was very nearly a step function, indicating an all or none character of the underlying activity. This implied that the activity underlying phase I evoked potentials was similar to that underlying normal evoked potentials but different from that underlying phase II.

The above results have demonstrated that phase II evoked potentials have all the classical characteristics of epileptiform activity. However, before such activity occurs there is a simpler form of enhancement of cortical excitability (phase I) and the evidence suggests that this is an enhancement of activity underlying normal evoked potentials.

Variabilty of the Experimental Results

The degree of enhancement, delay to onset of enhancement, rate of enhancement etc. sometimes showed considerable variability even when resulting from a given ejecting current. When penicillin was ejected by large electrophoretic currents the absolute amplitude of the enhanced evoked potentials was remarkably similar, irrespective of the amplitude of the potentials prior to penicillin. This was demonstrated in the stimulus strength relationship of potentials before and after penicillin. As the level of enhancement was expressed relative to the amplitude of potentials prior to drug ejection it was possible to have a large variability in the percentage enhancement brought about by larger penicillin doses if the amplitude of the pre-drug potentials was very different. Such variability was not so great in the enhancement by smaller doses of penicillin since the amplitude of the enhanced potentials bore a relationship to the amplitude of the pre-drug potentials, as was demonstrated in the stimulus strength response relationship.

The delay to the onset of and the rate of enhancement of evoked potentials after the start of penicillin ejection could be very variable, even when using the same electrodes in the same experiment. The delay to the onset of changes in the evoked potential may reflect the delay before a critical

population of neurones is affected by a threshold level of penicillin and the rate of enhancement was presumably a function of the rate of increase of penicillin concentration after the threshold level had been achieved. The variabilty of these parameters may have been due to the use of different durations and magnitudes of the retaining current applied to the electrode before a period of ejection (Bradshaw and Szabadi, 1974). The retaining current could not be dispensed with however as it was required to prevent penicillin 'leakage' from the electrode which would normally occur by diffusion. In some cases, if no retaining current was applied to the electrode the enhancement of evoked potentials and the appearence of spontaneous epileptiform spikes occurred, without the application of an ejecting current. This suggests that the spontaneous leakage, since it resulted in spontaneous epileptiform spiking, might be equivalent to an ejecting current of greater than -100nA. However, it was not necessary to use retaining currents greater than +50nA (unless the electrode tip had broken in which case no amount of retaining current could reverse the leakage), probably because the efficacy of a retaining current is dependant on its duration as well as its magnitude (Bradshaw et al., 1973); a retaining current initially insufficient to counteract diffusional leakage will nevertheless slow down the diffusional leak until this becomes zero some time after the application of the

retaining current. If the retaining current is maintained for longer than this length of time the terminal part of the electrode tip will become devoid of drug ions. Once an ejecting current is then applied to the electrode the depleted region of the tip will firstly have to be refilled before there is any release of drug ions from the electrode (Bradshaw and Szabadi, 1974). The delay before this occurs and the delay before the rate of ejection attains a constant level is dependant on the duration and magnitude of the retaining current. However, this should only affect the inital delay before any ejection starts and the initial rate of ejection (ie delay to onset and initial rate of enhancement) and not affect the final equilibrium ejection rate or the final concentration field (ie the final degree of enhancement), which should theoretically be dependant only on the ejecting current, for a given electrode (Bradshaw and Szabadi, 1974).

The variablity in results between different experiments for a given ejecting current may be a function of the differences in retaining currents discussed above, and additionally due to the fact that a given ejecting current is unlikely to result in the same rate of ejection when different electrodes are used because of the different geometery of individual electrodes (Purves, 1979; 1981). Indeed even electrodes of similar tip diameter and resistance cannot be relied upon to

produce similar rates of ejection when the same electrophoretic current is applied, as has been demonstrated by Bradshaw et al. (1973) studying the ejection of C(14) labelled noradrenaline. Therefore, the variability in the rate of drug ejection which exists between electrodes was probably one of the major factors in the variability of the rate of and delay to onset of enhancement of evoked potentials between experiments.

The Generation of Cortical Field Potentials

In the cerebral cortex extracellular current arising from normal potential changes across the membranes of pyramidal cells are probably responsible for the generation of the 'fast' evoked extracellular potentials. The apical dendrite, cell body and axon of pyramidal cells lie in a straight line perpendicular to the cortical surface and extend the full extent of the grey matter from the cortical surface to the white matter (Cajal, 1955). A change in the membrane potential in one region of the cell (the sink) will result in spatially extensive extracellular current (Holmes and Houchin, 1967) as current flows out (sources) from the rest of the pyramidal cell towards the sink. All pyramidal cells in the cerebral cortex of the rat are arranged in a parallel fashion, hence the activiation in the same region of a number of such cells will result in the summation of the extracellular currents arising from individual cells.

Stellate cells are unlikely to contribute to any great extent to the field potential, firstly because the symmetrical arrangement of the dendritic processes will tend to result in very little net extracellular current and secondly, because of the random arrangement of these cells in the cortex any extracellular current generated will tend to cancel. It is perhaps too much of a generalisation to state that all

stellate cells are small and will only generate small spatial currents since there are stellate cells within the cortex which have very extensive dendritic process (see Szentagothai, 1978).

It is by no means true that pyramidal cells are the generators of evoked potentials in all regions of the CNS. For example, glial cells in the retina (the Muller cells) are thought to be responsible for the extracellular potentials which make up the electroretinogram (Newman, 1980; Newman and Odette, 1984). These glial cells are elongated and oriented parallel to one other, perpendicular to the neuronal and plexiform layers. As discussed with regard to pyramidal cells this may allow for the generation of spatially extensive extracellular currents.

It is likely that the membrane potential changes due to postsynaptic potentials (PSPs), rather than due to action potentials, will contribute more to the extracellular field potential. The extracellular field potential probably has a wider spatial distribution when the membrane potential change is of a long duration (Humphrey, 1968a; 1968b) and the current flow resulting from PSPs can be recorded extracellularly at a greater distance from the cell membrane than the current flow resulting from APs, as has been demonstrated by Humphrey (1968a; 1968b). Ammassian et al. (1964) were able to depress

evoked action potential firing in the cat cortex by nitrogen breathing. Despite the depression of action potentials, while there was no depression of the evoked excitatory post synaptic potentials (EPSPs) there was not any depression of the evoked potential, which also suggests that the field potential was due to EPSPs rather than APs. Only once EPSPs were depressed were field potentials also depressed.

A theoretical estimate carried out by Mitzdorf (1985) of the contribution of different membrane potential changes to the generation of field potentials suggests that EPSPs are at least 2 to 3 times more effective than APs. Normal (somatic or axonal) action potentials are considered to be relatively ineffectual in the generation of the evoked potential but dendritic action potentials may be more effective. Many studies have shown a corellation between action potentials and evoked potentials (eg. Fromm and Bond, 1967; Rebert, 1973) but such a corellation would be expected, given that action potentials result from EPSPs. Furthermore, in the cat visual cortex the antidromic activation of pyramidal cells, just sufficient to induce action potentials, resulted in no evoked extracellular potential (Mitzdorf and Singer, 1978).

However, it seems that not all PSPs are equally effective in producing extracellular potentials. Mitzdorf (1985) has estimated that EPSPs are an order of magnitude more effective in the generation of extracellular potentials than inhibitory post synaptic potentials (IPSPs). In a model construction of field potential generation by Towe (1966) it was calculated that IPSPs were at least 5 times less effective than EPSPs in the generation of the extracellular evoked potential in the cat neocortex. Furthermore, the enhancement of IPSPs in the feline visual cortex by the application of pentobarbital, resulted in no enhancement of the extracellular field potential whereas the reduction of IPSPs by picrotoxin, and the consequent enhancement of EPSPs did produce an enhancement of the field potential (Mitzdorf, 1985). However IPSPs which generate field potentials have been identified in some pathological cases. For example the wave in the spike and wave complex of epilepsy (Pollen, 1969; Pollen et al., 1964) has been shown to be due to large recurrent IPSPs.

The Generation of the Somatosensory Evoked Potential

In view of the above discussion I shall assume that the generation of the 'fast' evoked potential is due to the current flow resulting from EPSPs on pyramidal cells. The effect of action potentials and IPSPs will be considered to be minimal.

The somatosensory evoked potential (SEP) recorded at depth in the urethane anaesthetised rat is a negative going wave, which reflects a sink of activity due to EPSPs located around the middle layers. Since the depth evoked potential becomes less negative in the deeper layers there cannot be any great extent of synchronised EPSP activity centered on pyramidal cells in the deeper layers. The surface recorded SEP is a complex of a small positive wave (P1) followed by a larger but variable positive wave (P2) and an even more variable negative going wave (N). The surface P2 wave is considered to be the reflection of the synaptic activation of the pyramidal cells at depth, probably on the basal dendrites. The resulting sink at depth will have sources located in the superficial apical dendrites and it is these sources which produce the positive going P2 wave. P2 is associated with the negative wave at depth. If these do indeed reflect the same activity they might be expected to vary together in size and duration. This has indeed been shown to be the case (once the surface N wave, which interferes with the size and duration of the preceding P2 wave, has been abolished)(Bindmann and Lippold, 1981) which further indicates that the surface P2 wave is a reflection of the activity which has its sinks centered about the middle layers (although the negative wave remains large over quite a large distance indicates that EPSPs are occuring on pyramidal cells located over a large vertical extent).

The surface negative (N) wave of the evoked potential may be due to IPSP activity at the somatic level of the pyramidal cells or due to a new sink of excitatory activity located in the superficial layers. Since IPSP involvement in the generation of the evoked potential is considered minimal it is probable that this part of the evoked potential is due to EPSP activity in the superficial parts of the apical dendrites. This would set up a superficial sink which the surface electrode records as a negative wave. Because this is a later event in the EP it may be mediated either through a multisynaptic pathway or through a slower afferent pathway. There is an inverse relationship between the amplitude of the depth response and the surface negativity (Bindmann and Lippold, 1981), which would be expected if the sink of activity moves into the superficial layers and the region of the initial sink at depth now acts as a source.

The use of more elaborate means, eg current source density (CSD) methods, to elucidate the underlying sequence of events has also proposed a similar sequence of excitatory activation (Mitzdorf and Singer, 1978; Mitzdorf, 1985) during the evoked potential in the cat neocortex.

The Enhancement of Evoked Potentials During Epileptogenesis: Phase I

In the neocortex, EPSPs in pyramidal cells are the most likely generators of the 'fast' evoked potential (Ammassian et al., 1964). Andersen et al. (1971) have shown in the hippocampal slice that the enhancement of the evoked potential amplitude (with increasing stimulus strengths) was correlated with an increase in the number of single units which fired an action potential. As action potential generation is dependant on suprathreshold EPSPs, the larger amplitude evoked potential was probably a function of the enhancement of previously subthreshold EPSPs.

Phase I of penicillin epileptogenesis was characterised by a simple increase in amplitude of the evoked potentials which suggested that this might also be generated by larger EPSPs. Furthermore, there was no change in the time to peak of the evoked potentials which suggests that the enhanced EPSPs must have been occurring in cells which were synaptically no further from the termination of the afferent input than those cells generating the normal evoked potential.

The enhancement of amplitude of the depth recorded evoked potentials which resulted from small doses of penicillin was associated with an increase in the amplitude of the second positive going wave (P2) without any detectable changes in the first positive (P1) or the later negative going wave (N2) of the surface recorded evoked potential.

The activity underlying the P2 component of the evoked potential has its sink centered around the middle or deeper layers (see earlier discussion) of the cortex and is probably a reflection of the activity of cells at least one synapse removed from the afferent input and underlies the initial stages of the normal evoked potential. As discussed earlier, P2 and the depth negative wave are intimately associated and since P2 was the one component of the surface response that was enhanced by the action of penicillin in phase I it may reflect the same enhancement of activity as the enhancement of the evoked potential recorded at depth. Corticothalamic projections, which have been suggested to depress the activity of the thalamus (Gutnick and Prince, 1974) do not appear to interfere to any great extent with the afferent volley. The P1 component of the surface response, which reflects the magnitude of the afferent volley, was unaltered. This will be considered further later in the discussion.

It has been shown in the hippocampal slice preparation that penicillin causes a reduction in the IPSP of the EPSP/IPSP complex which results from orthdromic stimulation (Dingledine and Gjerstad, 1979). The depression of the IPSPs unmasks a much larger EPSP. It is probable that in the neocortex the reduction of GABAergic inhibition by penicillin similarily results in the unmasking of a larger EPSPs, despite the same afferent volley. Penicillin produces a larger field potential without any change in the extracellular EPSP gradient (Wheal, 1984) in the hippocampal slice preparation. This suggests that there is no enhancement of the excitatory synaptic drive in the prescence of penicillin but that the enhanced EPSPs simply resulted from the removal of the inhibitory drive.

It can be postulated therefore, that phase I is due mainly to the enhancement of sub-threshold EPSPs in pyramidal cells which are responsible for the normal P2 wave of the surface response. GABAergic antagonism by penicillin allows the same excitatory drive to transform the previously subthreshold EPSPs into larger amplitude EPSPs, thus resulting in the larger amplitude evoked potential. Because this is associated with an increase in the amplitude of only the P2 component of the surface response then the enhanced activity does not apply to cells any further in the chain of cortical processing. Furthermore, the enhanced activity is an enhancement of normal

activity, as was shown by evoked potentials in phase I having a normal stimulus strength relationship and having refractory periods that were no different to the refractory periods of normal activity.

The enhancement of activity underlying the normal evoked potential as the first effect in the development of the epileptiform focus, resulting from the topical application of strychnine to the cat neocortex, has also been suggested by Towe et al. (1981). They conducted a current source density investigation of somatosensory evoked potentials before and during the development of the strychnine focus and subtracted the current flow of normal evoked potentials from that of the strychnine enhanced evoked potentials and found that the initial part of the evoked potential, represented by P2 in the surface recorded response, was enhanced in amplitude by the action of strychnine. Furthermore, the refractory period of the enhanced component was similar to that of the normal evoked potentials. These authors also suggested that enhanced evoked potentials in the epileptiform focus had a component which represented an enhanced version of normal activity.

Ebersole and Chatt, (1981, 1986) have also postulated two stages in the development of penicillin epileptogenesis in the cat visual cortex. They showed that the first effect of penicillin, when ejected into layer IV of the visual cortex, was a simple increase in the amplitude of normal depth recorded visual evoked potentials, which they called the enhanced physiological response (EPR). This was associated with an increased firing frequency of single cells in layer IV without any alteration in the surface recorded response, and may be considered to be equivalent to the phase I described in the present work.

However, in that study Ebersole and Chatt suggest that the EPR is the direct result of the increased firing frequency of single cells. The single unit recordings, on which these assumptions are based, are from layer IV and, as suggested by the authors, are likely to be stellate cells (Ebersole and Chatt, 1986). The effect of stellate cell activity on the EP is considered to be minimal and it is debateable whether such activity would contribute directly to enhancing the evoked potential to any great degree (see the discussion of field potential generation). 'Small' fluxes of penicillin do indeed transform neurones which normally fire single action potentials into a mode of bursting activity but it has been demonstrated by Walsh (1971), in the cat somatosensory cortex,

that this effect occurs without any changes in the evoked response. It may be that an additional effect, resulting from the increased firing frequency of single cells, such as enhanced activity in pyramidal cells, is responsible for the enhancement of the evoked potential.

PHASE II

Assuming that the evoked potential is a reflection of summed EPSPs then EPSPs in phase II are necessarily prolonged and/or not so synchronised and larger in amplitude compared to normal. It is well known that the post synaptic potential in epileptiform activity (the PDS) is of a much longer duration (up to 100msec) than the normal EPSP (eg. Matsumoto and Ajmone-Marsan, 1964). Therefore, activity in phase II might be considered to be the equivalent to the 'fully formed' epileptic state, in which cells are generating PDSs.

The onset of phase II of penicillin epileptogenesis was indicated by the appearance of the increased duration of the evoked potentials. This was associated with a large negative going component in the surface recorded response and both changes may reflect the same changes in cortical activity. The surface negative component (N2) is present in normal evoked potentials (Carter et al., 1969) but is very variable and is a later part of the EP than the positive going P2 and probably

results from activation of pyramidal cells by synapses at least two places removed from the termination of the afferent input (Mitzdorf, 1985). The generation of the current sink responsible for N2 may be due to excitatory synaptic activation of the more superficial parts of pyramidal cell apical dendrites and may be conducted by upwardly directed axon collaterals. This may be followed by excitatory activation of pyramidal cells in the deeper layers which explains the surface record going positive after the large negative wave.

The topical application of the GABA antagonist, picrotoxin, results in an enhancement of all the sinks and sources of the normal evoked potential, with the greatest enhancement being of the N2 component (Mitzdorf, 1985). Since the activity underlying N2 is present in normal potentials this may suggest that the enhancement of N2 (or the presence of phase II), like phase I, may simply be an enhancement of normal activity. However, the results of the present work showed that this second component in the development of the focus had a much longer refractory period and a different stimulus strength response curve compared to both normal and phase I enhanced potentials.

There is evidence that cortical spontaneous spikes cause a depression of afferent activity at the thalamic level (Gutnick and Prince, 1974; Schwartzkroin et al., 1974a; 1974b) and this may result in the depression of the response to the second of a pair of stimuli and hence cause the long refractory period that occurred in phase II. In order to test this possibility pairs of potentials were evoked by DCS and these were also found to have longer refractory periods compared to normal. Hence the long refractory period was not an effect of depression of the afferent input at the thalamus but must have a cortical component.

Towe et al., (1981) have also shown, by the topical application of strychnine to the cat somatosensory cortex, that although the current generating this later phase of enhancement of the evoked potential was very much an enhanced version of the current flow in normal potentials, it had radically different characteristics from normal activity in that the refractory periods of this activity were far greater than normal and the current underlying the later negativity travelled through the cortex at a much slower rate than normal, indicating that this was polysynaptically mediated.

It is possible that the application of a large dose of strychnine (Towe et al., 1981) or picrotoxin (Mitzdorf, 1985) to the cortical surface might produce the greatest enhancement of activity in the superficial layers because the greatest concentration of the drug would necessarily be in these layers. This has been demonstrated by the application of C(14)-labelled penicillin to the cortical surface (Nobels and Pedley, 19). However, it has recently been demonstrated (Ebersole and Chatt, 1985; 1988), by the discrete intracortical application to various cortical layers that the superficial layers are more sensitive to the effects of strychnine than the deeper layers. The GABA antagonists, penicillin and bicuculline, are more effective in the middle layers of the cortex (Lockton and Holmes, 1983; Cambell and Holmes, 1984) and, by analogy, one would expect the same to be true for picrotoxin. Despite the different sites of action of strychnine and the GABA antagonists they result in remarkably similar forms of epileptiform activity which, in its fully developed form, is dominated by a later component with its sink of activity in the superficial layers even when both drugs are ejected into their most sensitive layers. This large enhancement of activity in the superficial layers is not due to the topical application of the convulsant drugs but suggests the importance of the superficial layers in the generation of PDS like activity in both strychnine and

penicillin epileptogenesis.

It has been suggested by Mitzdorf (1985) that the upwardly directed axon colaterallls may be the mediators of the intense synaptic activity which is reflected by both the large surface recorded negative going wave and the increased duration of the depth recorded potential during epileptiform activity. The importance of recurrent axon collaterals has long been implied in the generation of neocortical epileptiform activity (Ayala et al., 1973, Dichter and Spencer, 1969b) and the importance of the superficial layers in the generation of epileptiform activity has been demonstrated by the surgical transection of the upper layers of the cortex, which resulted in the abolition of epileptic activity (Morrell, 1973; Reichenthal and Hocherman, 1977).

Ebersole and Chatt (1981,), studying visually evoked potentials (VEPs) in the cat, also suggest that there is a second phase in the development of the penicillin focus which occurs after a simple enhancement of the amplitude of the VEP. A distinct and new component (the late response; LR) occurred after the appearence of the first alteration of the VEP (the EPR) and at a longer latency than the normal visually evoked response or the EPR. Ebersole and Chatt have suggested that the activity underlying the LR (or by analogy phase II in the present study) is due to the recruitment of cells in the more

superficial and deeper layers activated by enhanced activity of cells during the EPR. The LR was considered to be mediated by normal synaptic pathways and it occurred in layers which were directly connected to layer IV, from which the EPR originates. Furthermore, this component had a longer recovery period than the normal evoked potential and was only produced by larger doses of penicillin and was suggested by the authors to be a 'larval' PDS. The LR can be considered to be equivalent to the increased duration of the evoked potentials during phase II in the present study.

A model of the Stages of Epileptogenesis

Taking into account the results and the above discussion one can propose a simple model to explain the findings of the present study by assuming a simplified cortical circuit comprised of pyramidal cells A,B and C (fig. 4.1). For simplicity the afferent input is shown reaching the pyramidal cells directly, but may also act via excitatory stellate interneurones (Lund et al., 1979; Szentagothai, 1978; Gilbert and Wiesel, 1979,). The only non-pyramidal cells illustrated are the inhibitory cells. Feed forward inhibition has been demonstrated in the CA1 area of the hippocampus (Alger and Nicoll, 1980) and in the pyriform cortex (Haberly and Bower, 1984). There is no concrete evidence for such inhibition in the neocortex but the requisite circuitry and connections are present (Globus and Schiebel, 1967; DeFelipe and Fairen, 1982; Peters and Poskauer, 1980). The presence of this inhibition is shown in figure 4.1 by cells I(f).

(a) The Normal Evoked Potential

(see fig. 4.1) The feed forward inhibition, mediated by cells I(f), is activ ated by the afferent input and acts on pyramidal cells A and C to keep the EPSP, evoked by afferent input, small in these cells. Cell B is not inhibited in this way and produces a large supra-threshold EPSP. The EPSPs (row 1) and the resulting extracellular potentials for each cell

(row 2) and the combined evoked potential (row 3) are shown. In this situation the bulk of the normal evoked potential is due to cell B (see row 3).

(b) Phase I

(see fig. 4.2) Assuming that small doses of penicillin renders the feed forward inhibition inoperable (inhibitory interneurones therefore not shown) this would result in larger EPSPs in cells A and C (see row 1), which results in the larger field potential (rows 2 and 3). The enhancement of the evoked potential is due to additional activity in cells synaptically no further removed from the afferent input than those producing the normal evoked potential. The additional activity is in cells which normally only produce small, subthreshold EPSPs, and do not contribute greatly to the normal evoked potential. The possibility now exists for mutual reexcitation between pyramidal cells via recurrent colaterals but this is prevented by the feedback inhibition mediated by cells Ib (see fig 4.3). The presence of feed back inhibition in the neocortex is fairly well established and has been demonstrated by, amongst others, Phillips (1959) and Stefanis and Jasper (1964). Evidence for the axon colaterall pathways is well established; Lorente de No (1938) illustrated the presence of these pathways histologically and these were later shown to exist by physiological studies (Takahasi et al.,

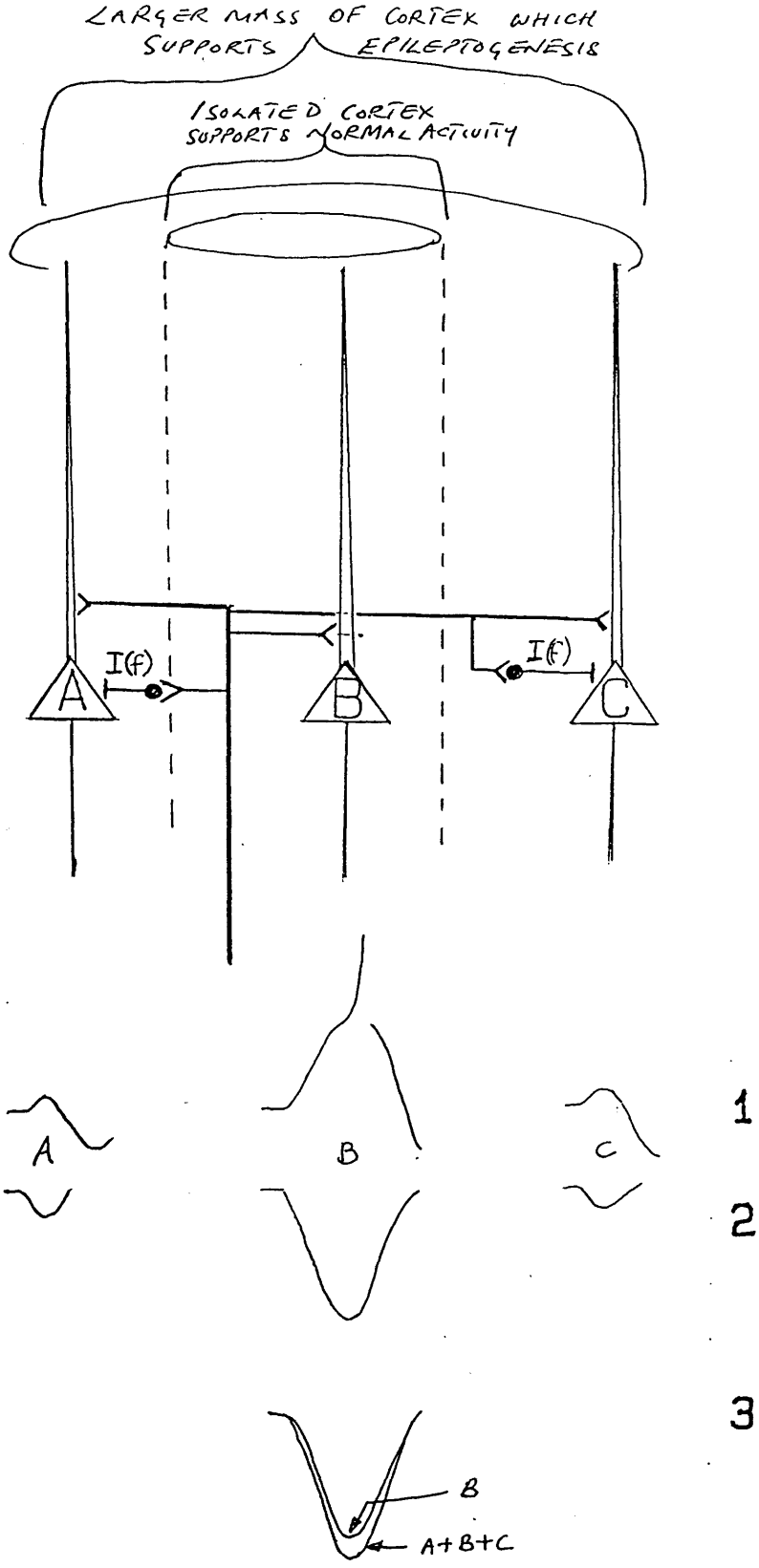


Figure 4.1 see text for details

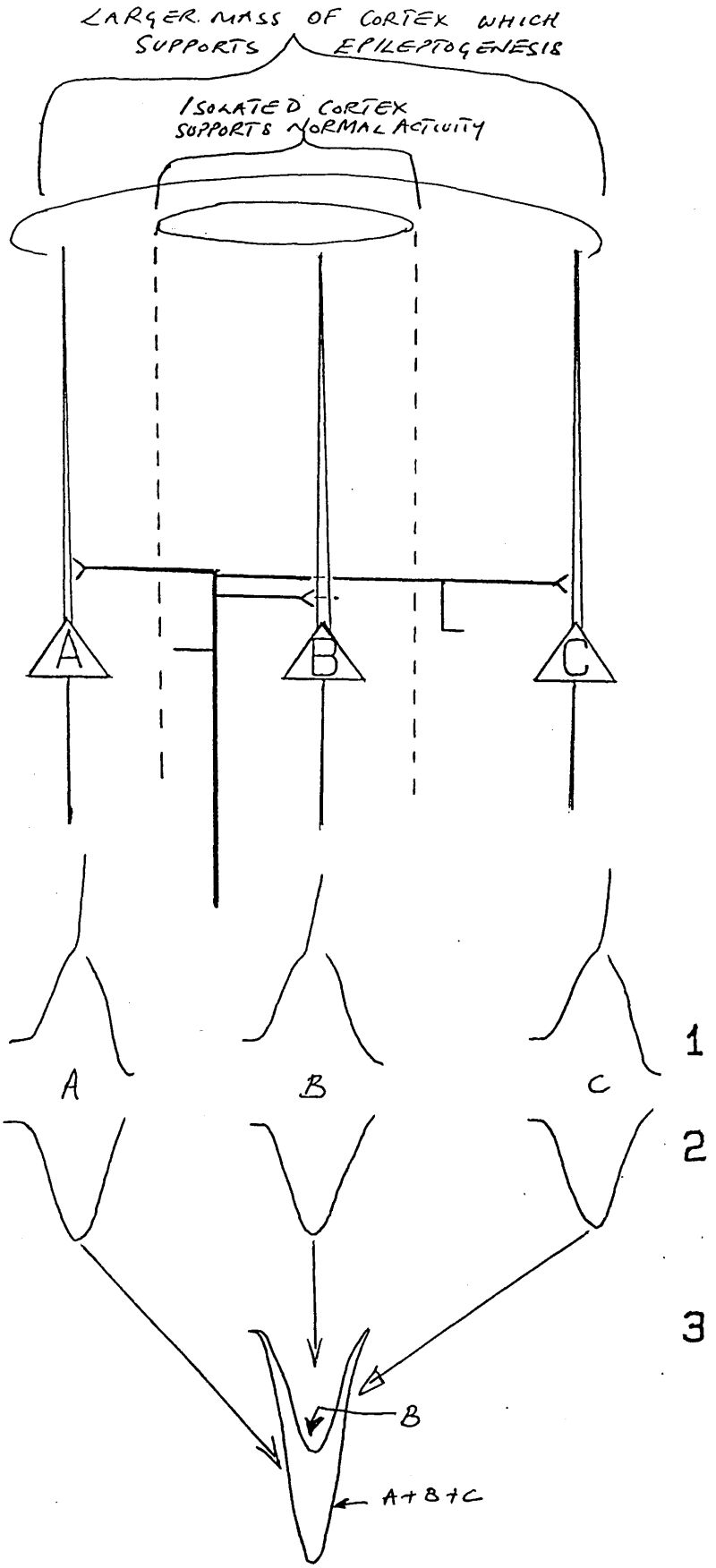


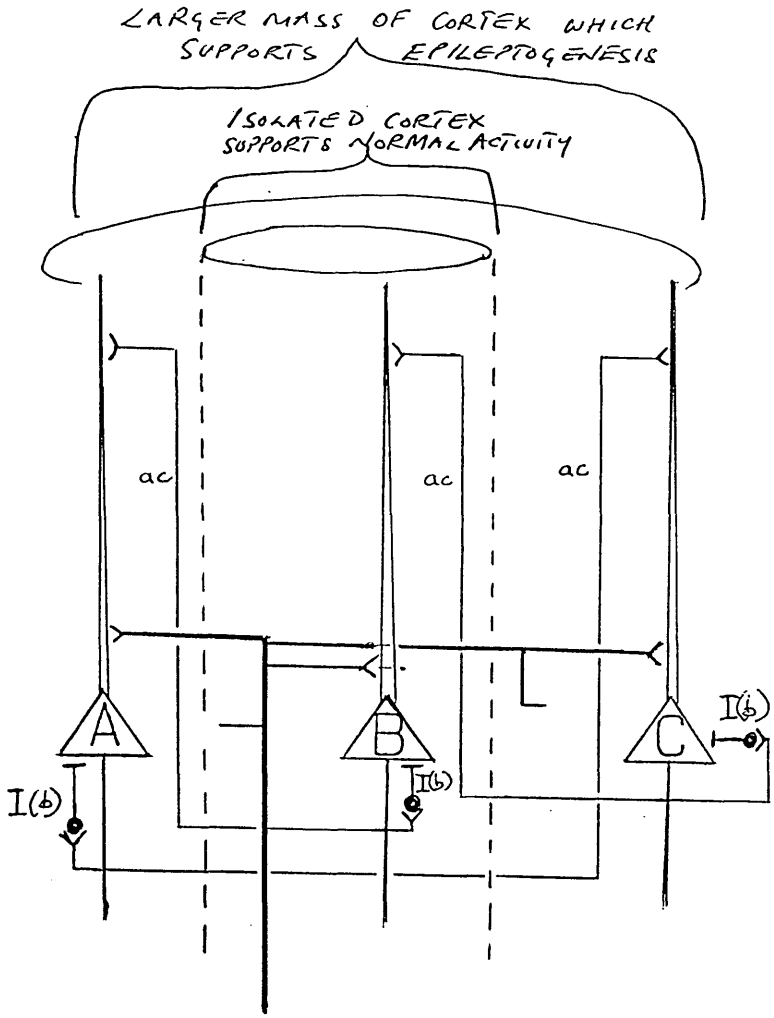
Figure 4.2 see text for details

1967). Another mechanism which may be responsible for reciprocal excitation is electrical synapses between pyramidal cells, which have been demonstrated by Gutnick and Prince (1981) but are not illustrated in this circuit.

(c) Phase II

(See fig. 4.4) Phase II occurs in the same cortical mass as phase I and does not result from activity in any cells outside the population that generated phase I. A further increase in penicillin concentration results in a decrease in GABAergic inhibition of the feedback circuit (inhibitory interneurones Ib therefore not illustrated) which allows the recurrent excitatory activation via the upwardly directed axon collaterals resulting in the intense synaptic activation of the superficial regions of apical dendrites of pyramidal cells. This results in the larger longer EPSPs (PDSs) in single cells, seen as the enhanced evoked potentials of phase II.

In order for this model to be more accurate we need to explain why the effects of penicillin are most effective in layer IV (Lockton and Holmes, 1983; Ebersole and Chatt, 1986), and not in the pyramidal cell layers, as implied by this model. This may be a function of the oversimplification of the model: the afferent input enters layer IV and terminates on excitatory stellate cells which may themselves be regulated



1

2

3

Figure 4.3 see text for details

ac - axon collaterals.

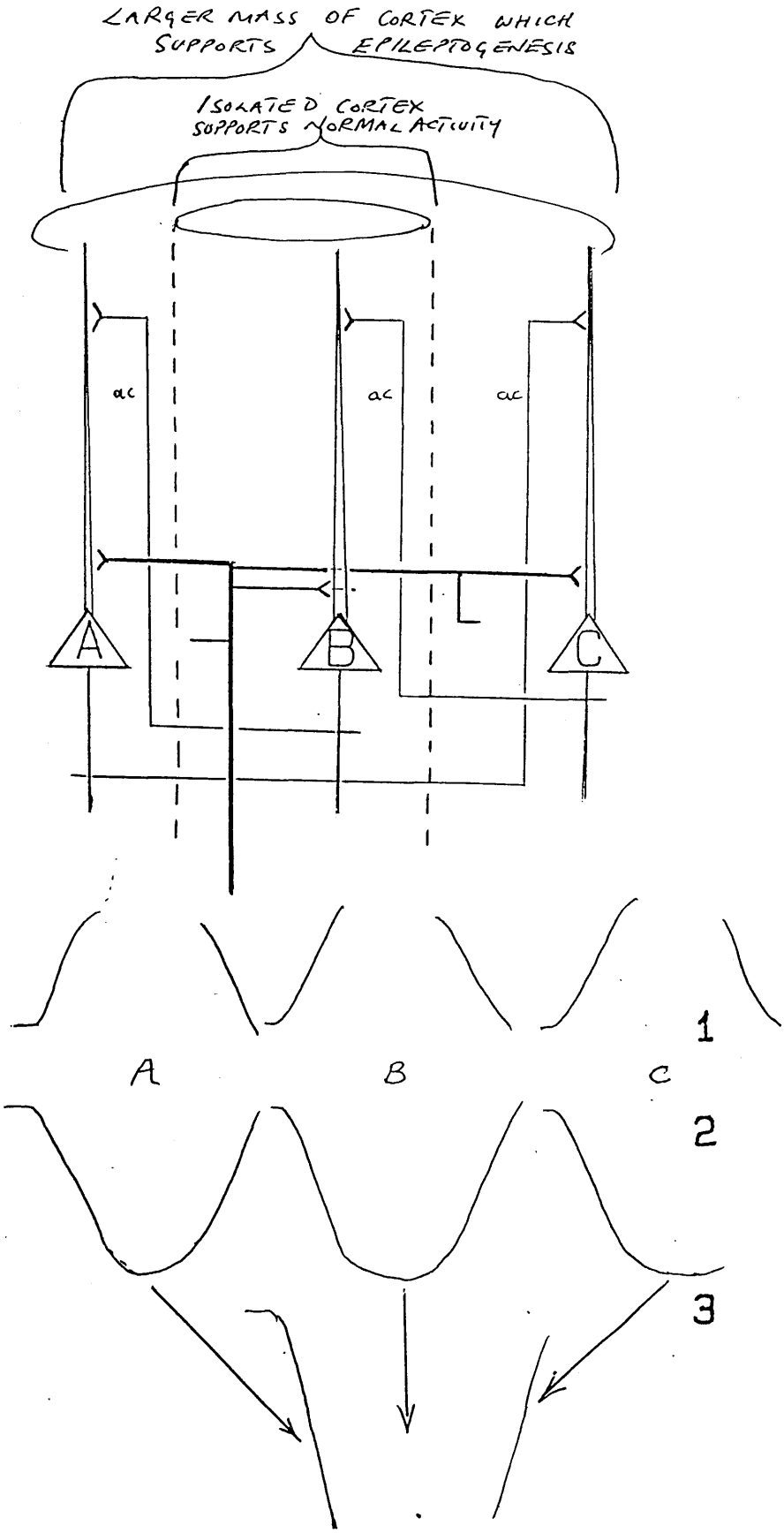


Figure 4.4 see text for details

by feed forward inhibition activated by the afferent input, then penicillin in layer IV relieves the inhibition on these excitatory interneurones and hence the afferent input causes a greater activity of these cells (Ebersole and Chatt, 1986) which in turn results in a greater activation of the pyramidal cells and hence phase I. Because greater concentrations of penicillin are required for phase II, we can assume that the feedback inhibition, as already proposed, may be outside layer IV and is active on the pyramidal cells themselves.

Critical Cortical Mass for Penicillin Epileptogenesis

There was no difference in the critical cortical mass that was required to generate phase I or phase II but both phases of epileptogenesis required a greater cortical mass than did normal evoked potentials. In this respect, phase I is unlike normal activity but is similar to phase II activity. This is contrary to the earlier hypothesis that phase I was similar to normal activity but that both were different from phase II activity.

However, these results, in finding that epileptiform activity has a critical cortical mass, agree with previous work (Reichenthal and Hocherman, 1977) in which epileptiform foci, produced by the application of penicillin-soaked pledgets to the cortical surface, were surgically divided by sub-pial cuts. The authors deduced that the minimum cortical

surface area required for epileptiform activity was between 0.5 and 0.6 sq mm. There was always the possibility however, that the lack of any epileptiform activity in these smaller isolated pieces of cortex may have been due to excessive cortical damage; the authors did not specifiy any effects of the sub-pial isolation on normal spontaneous activity. Eidelberg et al., (1959), who demonstrated that the sub-pial isolation of an area of approximately 1 sq cm in the cortex of macaca mulatta rendered this island of cortex refractory to the development of afterdischarges by electrical stimulation, suggested that this may have been due to cortical insult. However, in the present work spontaneous and evoked activity was monitored before and after sub-pial isolation and was found to be little affected by the surgical procedures. On this evidence the partially isolated cortex was in a healthy condition.

Small concentrations of penicillin (less than -25nA) did not result in any detectable changes in evoked potential characteristics but do produce changes in single cell activity as shown by Walsh (1971) in the feline neocortex. This provides further evidence that, as for spontaneous spikes (Lockton and Holmes, 1983; Reichenthal and Hocherman, 1977), a critical neuronal population must be affected by the threshold penicillin concentration before an evoked potential event undergoes any alterations; affecting only a few cells in a

population is not sufficient to produce any changes in the evoked potential.

Phase I was the first observable change in the extracellularly recorded population response during the onset of penicillin epileptogenesis. This implied that the generation of phase I required the smallest concentration of penicillin and/or the smallest number of cells affected by the convulsant. This is in agreement with the suggestion also proposed by Ebersole and Chatt (1986). In the present work it has been shown that the requirement for the critical mass for phase I was not any different from that for phase II it seems likely that the concentration of convulsant is the more important factor, provided that the minimum population is available.

Gabor et al., (1979) suggested that the minimum cortical mass for epileptiform activity is the cortical column. They showed a close correlation between the borders of a penicillin epileptiform focus and the functionally defined cortical column in the cat visual cortex. In the present study a cortical mass similar to the size of a cortical column, and similar in size to the minimum cortical mass for epileptiform activity as described by others (Reichenthal and Hochermann, 1977), generated epileptiform activity; any mass of cortex smaller than this did not produce any phase of epileptogenesis

but did produce normal activity. It was possible that in the situation of the present work a cortical column was being reduced in size and that this 'mini'-column was still sufficiently organised to be able to generate an evoked potential but insufficiently organised to generate epileptiform activity. Whether activity in this 'mini'-column is normal, simply because it looks no different from that before the sub-pial isolation is debateable. However, in the surgical treatment of epilepsy, using similar methods (Morrell and Hanberry, 1969), the procedure has been shown to prevent focal epileptic activity without any obvious signs of impairment of normal cortical function. Furthermore, Duysens and McLean (1974) found that the sub-pial isolation of a 2X2 mm area, prevented epileptiform activity in the feline cobalt-gelatin epileptiform focus without affecting the normal paw placing reflex of the cat.

The model already described for the generation of phase I and phase II activity also illustrates how phase I and phase II can occur in the same cortical mass, which is larger than that for normal activity. Normal activity occurs in cells A,B and C and these constitute a single cortical column. Cells A and C only contribute a little to the normal evoked potential and so removing these from cell B will not result in any great effect on the normal evoked potential (fig. 4.1). However, cells A and C are essential for the generation of phase I and

phase II, since only an enhancement of EPSPs in these cells can result in phase I and therefore phase II. Removing these cells from cell B prevents the occurence of any epileptiform activity (see fig. 4.2).

NMDA Receptor Involvement in the Phases of Penicillin Epileptogenesis

Because of the similarity of phase I evoked potentials to normal evoked potentials it was considered possible that activity underlying phase I may be mediated via the same excitatory receptors as mediate 'normal' activity and that the different and more 'epileptic' activity underlying phase II may be mediated by NMDA receptors.

The results in the present study showed that the concurrent ejection of penicillin and small doses of APV did not result in any enhancement of evoked potentials. Therefore, phase I enhancement of evoked potentials, which normally resulted from small doses of penicillin, must be dependant on the activation of NMDA receptors.

However, with small APV doses and penicillin doses which would normally result in phase II, there was some enhancement of evoked potentials although this was less than would be expected from the ejection of penicillin alone. This small enhancement was prevented by the ejection of larger doses of APV and therefore, may have occurred because of insufficient NMDA receptor antagonism. This could occur if some of the NMDA receptors involved in the hyperexcitability resulting from large penicillin doses, were at some distance from the site of APV ejection and the APV concentration field was too small to

reach these receptors. The effect of APV on an already established enhancement of evoked potentials was minimal.

As discussed earlier, phase I enhancement may be due to the activation of receptors close to pyramidal cell bodies, close to the middle layers. Phase II, with a superficially located sink, may be due to the activation of excitatory receptors located superficially on the pyramidal cell apical dendrites. Hence, the activated NMDA receptors mediating phase I were presumably close to the site of APV ejection (into layer IV). Small doses of APV resulted in a concentration field of sufficient extent to block these receptors and prevent phase I. However, the activation of distant NMDA receptors which may be responsible for phase II might not blocked by the smaller APV doses (as discussed above) because of the limited extent of the APV concentration field. Thus, there is the possibility of further hyperecitability being mediated through these unantagonised receptors. The extent of the APV concentration field may also be limited by the binding of APV to NMDA receptors near to the site of ejection. This would limit the spread of the drug ejected by small electrophoretic currents but could be overcome by the ejection of larger doses of APV. The implication of these results is that all hyperexcitability resulting from a reduction of GABAergic antagonism, whether phase I or phase II, requires NMDA receptor activation.

As already discussed, the waveform of enhanced evoked potentials resulting from the application of strychnine and penicillin were very similar and this implied that the underlying activity may also be very similar and may be conducted via the same excitatory transmitter receptors in both cases. APV blocked all the enhancement of evoked potentials which strychnine would normally produce when ejected by itself, which suggested that indeed both epileptogenic agents resulted in the activation of NMDA receptors.

APV Specificity

As indicated in the results (part C.II) it was important to ascertain the specificity of action of APV. It has been reported (Collingridge et al., 1983a) that in the hippocampal slice small doses of L-APV resulted in a depression of activity which was non-NMDA receptor mediated. Thomson (1986b) has reported that, in slices from rat cerebral cortex, twice the effective dose of APV for specific NMDA receptor antagonism depressed responses due to kainate and quisqualate. However, Davies and Watkins (1982) found that large doses of L-APV (150nA) had little effect on the excitation by kainate and/or quisqualate, where as similar doses of D,L-APV resulted in some depression of kainate/quisqualate induced excitation in the cat spinal cord. In the frog spinal cord Davies et al.,

(1981) found that 50micromolar d l-APV completely abolished all NMDA induced excitation with very little effect on kainate and quisqualate responses. A ten-fold increase in APV concentration did not result in any further depression of the excitation due to kainate or quisqualate. It appears therefore, that in different situations the doses of APV required to induce non-specific effects vary markedly over and above the doses required for specific NMDA receptor antagonism.

The Involvement of NMDA Receptors in Normal Synaptic Transmission

The method of determining APV and ketamine specificity in this study relied on there being no significant NMDA receptor involvement in the normal evoked response. This has been concluded from the results of studies in the hippocampal slice (Collingridge et al., 1983b, 1988; King and Dingledine, 1986; Koerner and Cotman, 1982; Slater et al., 1985; Cherubini et al., 1988). However, Thomson (1986a) and Thomson et al. (1986) have shown in the neocortical slice, and Dale and Roberts (1985) have shown in frog motoneurones that in particular situations EPSPs can be evoked which are NMDA receptor mediated. Hablitz and Langmoen (1985) have reported a partly NMDA receptor mediated EPSP in the hippocampal slice, although this may have been due to a large concentration of the racemic

mixture of APV (Collingridge et al., 1988). Forsythe and Westbrook (1988) have demonstrated an NMDA receptor component in monosynaptic EPSPs between pairs of cultured hippocampal neurones, but only in the presence of picrotoxin, to abolish inhibitory activity, and zero magnesium to prevent the normal magnesium block of NMDA receptor channels.

Armstrong-James et al. (1985) have shown that spontaneously bursting cells in layer IV of the somatosensory cortex of the urethane anaesthetised rat are APV sensitive. However, the bursting was of a relatively slow nature (a time course of seconds) and furthermore, these cells fired action potentials in response to peripheral stimulation in the presence of APV; faster evoked events were not NMDA receptor mediated but the slower and later firing was.

It may be that the means of stimulation in the present study resulted in there being no NMDA receptor mediated component in the normal evoked potential. Salt (1986, 1987) has shown that in the ventrobasal nucleus of the thalamus NMDA receptors are not activated by brief electrical stimulation of the afferent pathway or brief physiological stimuli but are activated by prolonged high frequency electrical stimuli or a prolonged physiological stimulus.

The NMDA receptor mediated EPSP described by Thomson (1986a) in the neocortical slice was only evoked by itself by stimuli too small to evoke any other EPSPs or IPSPs. With larger stimuli EPSPs with non-NMDA and a small NMDA receptor mediated component were evoked. It may be therefore, that with larger stimuli, as in the present study, the NMDA receptor component constituted only a small portion of the overall normal EPSP and hence blockade of this component had little effect on the overall evoked potential.

It may also be possible that any effect of NMDA receptor blockade may not be seen if the peak of the evoked potential is used to test for this, because of the slow time component of the NMDA receptor EPSP. It has been shown that the NMDA receptor mediated current occurs during the later part of the EPSP in cultured hippocampal neurones (Forsythe and Westbrook, 1988). Artola and Singer (1988) have demonstrated, in slices of rat visual cortex, that EPSPs in presumed pyramidal cells were virtually unaffected by bath applied APV; the only effect was a small reduction of the declining phase of the EPSP. It is possible that if there was such a component in the normal evoked potential in the present study, that this too may contribute more to the later part of the potential and have little effect on the peak amplitude. Furthermore, it may be possible that with larger stimuli sufficient to induce IPSPs, that these would limit the possibility of occurence of both

the 'early' NMDA receptor mediated component described by Thomson (1986a) and the later NMDA receptor component of the EPSP described by Artola and Singer (1988) and Forsythe and Westbrook (1988).

Furthermore under a state of anaesthesia it may be possible that there is an enhancement of the activity of the inhibitory system. This has been demonstrated to be true for barbiturate anaesthesia (Curtis and Lodge, 1977; Barker and McBurney, 1979) and is presumably also the case for urethane anaesthesia (Lodge and Curtis, 1978). If this is so, NMDA receptor activation would be damped and it could be another possible reason why there is no NMDA receptor mediated activity in the normal evoked potentials in this study.

If the normal EPs in the present sudy had no NMDA receptor component, the small depression of evoked potentials by larger APV fluxes must have been due to a non-specific effect of APV. The doses of APV used in this study to determine the involvement of NMDA receptors in epileptogenesis were less than those producing a depression of normal EPs and so cannot be considered to have non-specific depressant effects. Furthermore, the higher ejecting currents of APV (-200nA) used in this study were by some standards quite large and one would expect that the doses of APV resulting from such ejecting currents could not have failed to produce some depression of

the evoked potentials if there had been an NMDA receptor component present in the normal potentials. In addition the doses of APV having no effect on normal potentials blocked all the enhancement which would normally result from penicillin ejection and therefore, must have been capable of NMDA receptor antagonism.

An inherent problem of the iontophoretic technique of drug application lies in the concentration profile which results from the ejection of drugs from a point source. It is possible in single unit studies to test the specificity of an antagonist, despite the non-linear drug concentration, simply by ejecting the agonist from one of the adjacent barrels and altering the antagonist concentration field to be specific for that particular cell. The use of that particular ejecting current can produce a specific antagonist effect for that one particular cell in that particular situation. However, such a method cannot be used when studying population responses because one has to try to affect the whole active cell population with a concentration of drug that will be specific in its effects. Clearly this is an impossible task; all receptors close to the point of ejection will be affected by a high concentration, which might result in non-specific effects, whilst those further away will be affected by smaller concentrations which might be within the range for specific antagonism. Cells or receptors still further away from the

site of ejection might not be affected by the antagonist (Curtis et al., 1971). Furthermore, the classical agonist/antagonist dose response cannot be undertaken because of the probability of different diffusion characteristics and binding properties of different drugs. This can result in an agonist or antagonsit producing its results simply by acting at more distant receptors which may not reached by the antagonist (Curtis et al., 1971).

Ketamine Antagonism of NMDA Receptor Activity in Penicillin Epileptogenesis

In keeping with previous studies (eg. Thomson et al., 1986; Thomson, 1986a) electrophoretic currents several times larger than those resulting in specific NMDA antagonism by APV, resulted in specific NMDA antagonism by ketamine. Furthermore, several times the effective dose of ketamine on NMDA receptor antagonism had no effect on quis/kain responses in cerebral cortical slices (Thomson, 1986a).

The experiments in this study which used ketamine as an NMDA receptor antagonist (Anis et al., 1983) rather than APV, resulted in an attenuation rather than a total blockade of penicillin induced epileptogenesis when sufficient penicillin to normally induce phase II of epileptogenesis was ejected. The enhancement that occurred in the presence of ketamine was limited to a phase I enhancement, where the enhanced evoked potentials had refractory periods the same as that of normal potentials.

A reason for this limited effect of ketamine may have been that the dose of ketamine was insufficient to prevent all the enhancement induced by penicillin. However, this was clearly not the case since increasing the dose of ketamine, after phase II had been reduced to phase I, resulted in no further

depression of the evoked potentials.

This implied that phase 1 enhancement was mediated, in these conditions, by non-NMDA receptors. This possibility was however excluded by the finding that APV blocked all penicillin induced enhancement and that ketamine blocked phase I when this occurred by itself due to small penicillin fluxes. This apparent anomaly may be due to the mechanism of action of ketamine, which is a non-competitive antagonist (Harrison and Simmonds, 1985) and is thought to bind within the NMDA receptor operated channel. Before ketamine can be effective however, the natural agonist must activate the receptors to open the channels and currrent will presumably flow before ketamine can block the channel. Hence, ketamine can only be effective after NMDA channels have been opened. This could explain why in the presence of ketamine and small doses of penicillin there was an initial enhancement which was then slowly blocked and the enhancement returned to normal levels; this was not the case with the concurrent ejection of APV and small doses of penicillin because of the different nature of antagonism by APV. However, if sufficient penicillin to produce phase II was ejected, then some enhancement did occur in the presence of ketamine. The NMDA receptor/channel activation which resulted from large penicillin doses may have been sufficient, before being blocked by ketamine, to induce some intracellular mechanism which could then maintain a

degree of hyperexcitability through non-NMDA receptors which was reminiscent of phase I. However, any further enhancement (phase 2) required further NMDA receptor involvement because the presence of ketamine prevented phase II enhancement. If sufficient penicillin to result in only phase I was ejected the enhancement mediated by NMDA receptors was blocked by both ketamine and APV. Therefore, when only a small degree of reduction of inhibition occurred this did not result in sufficient NMDA receptor activation to trigger the mechanisms to maintain some degree of hyperexcitability, as did a large degree of reduction of inhibiton.

Such a mechanism, by which NMDA receptor activation triggers some intracellular change which then maintains the hyperexcitability, is reminiscent of long term potentiation (LTP), in which high frequency stimulation causes a pronounced long lasting hyperexcitability of synaptic activity (Bliss and Lomo, 1973). It has since been demonstrated that the activation of NMDA receptors is responsible for the initiation of LTP, but is not required for the maintainence of this phenomenon. The application of APV prior to or during high frequency stimulation prevents the induction of LTP (Collingridge et al., 1983; Wigstrom and Gustafsson, 1984) whereas APV is ineffective if applied after the establishment of LTP (Collingridge et al., 1983). A similar effect has been

described by Anderson et al., (1987) with regard to epileptiform activity. They demonstrated that, in the hippocampal slice, stimulus induced epileptiform bursting in CA1 cells was prevented by the application of APV prior to and during the stimulation but was much less effective when applied after epileptiform activity had been established. This suggests that in this situation epileptiform activity was initiated by NMDA receptors but that these were not necessary or responsible for the maintenance of this activity.

A similar result was seen in the present study in that APV was only totally effective when ejected prior to or during the enhancement by penicillin. Ejection of APV after the establishment of enhancement only reduced the hyperexcitability but did not abolish it.

One mechanism by which the maintainence of hyperexcitability could be triggered is by the increase in intracellular calcium which results from the entry of calcium into the cell through the NMDA receptor operated channel (MacDermott et al., 1986; Ascher and Nowak, 1986). Calcium is recognised as being a second messenger in many physiological systems and it has been suggested that in LTP (Malinow et al., 1988) a surge in intracellular calcium might trigger a protein kinase system which could result in long term membrane alterations to maintain the hyperexcitiability.

These results, which have shown that NMDA receptor activation is essential for penicillin induced epileptogenesis, are suppported by other studies demonstrating that NMDA receptors play a key role in kindled hippocampal seizures (Peterson et al., 1984; Buterbaugh and Michelson, 1986) and kindled amygdaloid seizures (Peterson et al., 1983), sound induced seizures in rats (Bourn et al., 1983), penicillin induced epileptiform activity in rabbits (Sagratella et al., 1985), epileptiform activity induced by GABA antagonists in hippocampal slices (Hablitz and Langmoen, 1986; Brady and Swann, 1986; King and Dingledine, 1986; Horne et al., 1986) and picrotoxin and strychnine induced epileptiform activity in freely moving rats (Czuczwar and Meldrum, 1982; Frenk et al.,1986). However, the degree of effectiveness of NMDA receptor antagonism in the above quoted studies ranges from the total abolition of epileptic activity (eg. Brady and Swan, 1986) to a reduction in seizure activity (eg. Hablitz and langmoen). Hence, it must be that in different types of seizure activity different excitatory receptors play a leading role, or it may be that, as suggested by Anderson et al. (1987) and Bourn et al. (1983), NMDA receptors underlie the initiation rather than the maintainence of epileptiform activity.

However, one study of note has reported that epileptiform activity in the neocortical slice resulting from GABA antagonism has no NMDA receptor involvement whatsoever (Thomson and West, 1986).

Our results as to the involvement of excitatory receptors in penicillin induced epileptogenesis have shown that all the enhancement of evoked potentials s can be blocked by NMDA receptor antagonists. This suggests that all the hyperexcitability resulting from the reduction of inhibition is, initially at least, mediated through NMDA type receptors. This implies that there is a critical balance between the excitatory and inhibitory mechanisms of the cortex and that a decrease in inhibitory regulation results in the activation of NMDA receptors which can lead to epileptiform activity. The voltage dependant block of NMDA receptor channels (Nowak et al., 1984; Mayer et al., 1984) is ideally suited for such activation. At hyperpolarised potentials the channel is subject to a block by magnesium ions which is alleviated at more depolarised potentials. The channel has a current/voltage relationship unlike normal channels in that more current flows at more depolarised potentials. In the presence of inhibition the membrane potential can be kept from being excessively depolarised and hence the NMDA receptor channel block by magnesium remains. With the reduction of inibition a larger EPSP can result and depolarise the membrane sufficiently to

allow the magnesium block to be alleviated; the modulation of GABAergic inhibition is one mechanism by which NMDA receptor function can be regulated.

Artola and Singer (1987) have shown that in the neocortical slice LTP only occurs after normal GABAergic inhibition is reduced by GABA antagonism. This is a very significant finding because the initiation of LTP relies on NMDA receptor activation. In this situation NMDA receptors only come into play when normal inhibition is reduced. The findings of Artola and Singer are analagous to the findings in the present study in that we only see an involvement of NMDA receptors when there is a reduction of normal inhibition. Therefore, GABA inhibition keeps the cell membrane in the range where the Magnesium ion block of NMDA receptor operated channels is not alleviated. In hippocampal slices GABA inhibition does not need to be depressed by the application of antagonists in order to induce LTP but does facilitate its induction (Wigstrom and Gustafsson, 1985). It is possible therefore, that in the neocortex GABA inhibition has a far tighter reign on excitability and the control of activation of NMDA receptors than in the hippocampus.

APPENDIX

PROGRAM TRANS1: DIGITISES AND STORES EVOKED POTENTIAL DATA

```
C  ***  TRANS1.FTN  -  DIGITISE TRANSIENTS
C
       PARAMETER (NOREC =1000)
       DIMENSION ISTAT(2),IFLAG(2)
       BYTE FILNAM(14),BELL
       DATA FILNAM(13),FILNAM(14) /0,0/
       DATA BELL /"7/
C
C  ***  SET UP DATA COMMON TO ALL ROUTINES
       ISTAT(1) = 0
       ISTAT(2) = 0
       IEFN = 31
       MODE = 1
C
C  ***  ANNOUNCE THE PROGRAM
       WRITE (5,1000)
C
C  ***  OPEN A FILE
       WRITE (5,1010)
       READ  (5,2000) (FILNAM(I), I = 1,12)
       OPEN  (NAME=FILNAM, UNIT=1, ACCESS='DIRECT',STATUS='NEW',
       1      RECL=128)
C
C  ***  SET UP CHANNEL NUMBER
       CALL DSETUP(ISTAT,'S','1000',IEFN,MODE)
       ICHAN = 0
       CALL DOUT(ISTAT,8,ICHAN,,'B',IEFN,MODE)
       IF (ISTAT(1) .LT. 0)   WRITE(5,*) ISTAT(1),ISTAT(2)
C
C  ***  GET A VALUES FROM SELECTED A/D
       DO 20 I = 1,NOREC
C
         CALL DSETUP(ISTAT,'S','IIII',IEFN,MODE)
10       CALL DINP(ISTAT,24,IFLAG(1),,,IEFN,MODE)
         IF ((IFLAG(2).AND.1) .NE. 1) GO TO 10
         IF ((IFLAG(2).AND.8) .EQ. 8) GO TO 30
C
         CALL SAMPLE(I)
         WRITE (5,1020) I,BELL
20     CONTINUE
C
C  ***  RETURN TO POS
30     CLOSE (UNIT=1)
       CALL EXIT
C
C  ***  FORMAT STATMENTS
1000   FORMAT(//' TRANS1'/' ------')
1010   FORMAT(/'$Filename?  ')
```

PROGRAM CALC: CALULATES AND STORES PARAMETERS OF THE EPs

```
C  ***  CALC.FTN - PLOT TRANSIENTS
C  ***  LAST UPDATE-28-6-87
C  ***  TYPE -1 TO EXIT
      INCLUDE 'LB:[1,5]CGL.FTN'                    ! DEFINE CGL PARAMETERS
      PARAMETER (NOSAM = 1024)
      PARAMETER (NPLOT=128)
      DIMENSION ISTAT(2),FLAB(3),Y(NPLOT),Z(NPLOT),OUT(4,500)
      BYTE BDATA(NOSAM),PDATA(NPLOT),FILNAM(14),BLAB(12)
      EQUIVALENCE (FLAB(1),BLAB(1))
      DATA FLAB /'Reco','rd =','    '/
      DATA FILNAM(13),FILNAM(14) /0,0/
C
C  ***  ANNOUNCE THE PROGRAM
      WRITE (5,1000)
C
C  ***  GET THE FILE AND OPEN IT
      WRITE (5,1010)
      READ  (5,2000) (FILNAM(I),I=1,12)
      OPEN(NAME=FILNAM, UNIT=1, TYPE='OLD', ACCESS='DIRECT')
C
C  ***  SET UP DEFAULT VALUES
      IREC = 1
      SF=1.0
      ISPACE=13
      NTRANS=1
      IART=0
      IPEAK=40
      IDELAY=10
      LSHIFT=0
      IALT=1
C
C  ***  TYPE 1 TO FIND NUMBER OF TRANSIENTS
10    WRITE (5,1120)
      READ (5,2010) ITEMP
      IF (ITEMP) 6,6,3
3     I=1
4     READ (1'I,ERR=5)
      I=I+1
      GOTO 4
5     I=I-1
      WRITE (5,1110)
      WRITE (5,2010) I
C
C  ***  READ TRANSIENT NUMBER
6     WRITE (5,1020)
      READ  (5,2010) ITMP
      IF (ITMP) 50,920,910
910   IREC=ITMP
C
C  ***  TYPE 1 FOR PLOTTING PEAK
920   WRITE (5,1025)
      READ (5,2010) IFLAG
      IF (IFLAG) 925,925,100
```

```
C
C  ***  TYPE 1 FOR DUMPING DATA
925      WRITE (5,1095)
         READ (5,2010) IFLAGB
         IF (IFLAGB) 926,926,710
C
C  ***  TYPE 1 FOR SKIPPING TRANS PLOT
926      WRITE (5,1027)
         READ (5,2010) IFLAGA
C
C  ***  TYPE 1 FOR NO ALIGNMENT
         IFLAGC=0
         WRITE (5,1028)
         READ (5,2010) IFLAGC
C
C  ***  SET DELAY OF ARTEFACT
         WRITE (5,1100)
         READ (5,2010) ITEMP
         IF (ITEMP) 928,928,927
927      IDELAY=ITEMP
C
C  ***  SCALING FACTOR
928      WRITE (5,1080)
         READ (5,2020) STEMP
         IF (STEMP) 930,932,930
930      SF=STEMP
C
C  ***  SIZE OF ARTEFACT
932      WRITE (5,1130)
         READ (5,2020) XTEMP
         IF (XTEMP) 935,932,935
935      SA=XTEMP
C
C  ***  SPACING
940      WRITE (5,1050)
         READ (5,2010) ISTMP
         IF (ISTMP) 960,960,950
950      ISPACE=ISTMP
C
C  ***  NUMBER OF ARTEFACT POINTS
960      WRITE (5,1070)
         READ (5,2010) IATMP
         IF (IATMP) 970,975,970
970      IART=IATMP
C
C  ***  SHIFT TO GET SECOND TRANSIENT
975      WRITE(5,1160)
         READ(5,2010)L
         IF(L) 978,978,977
977      LSHIFT=L
```

```
C
C  ***  DECIDE WHETHER ALTERNATE OR EVERY RECORD LOCKED AT
C
978     WRITE(5,1170)
        READ(5,2010)IAL
        IF(IAL)980,980,979
979     IALT=IAL
C
C  ***  NUMBER OF TRANSIENTS TO BE PLOTTED
980     WRITE (5,1040)
        READ (5,2010) NTMP
        IF (NTMP) 995,995,990
990     NTRANS=NTMP
C
C  ***  CGL PRAPHICS PACKAGE PARAMETERS
995     VMAX = FLOAT(NPLOT)
        VMIN = 0.0
        TMAX = 5.0
        TMIN = -5.0
C
        XMAX = TMAX + 10.0
        XMIN = TMIN - 10.0
        YMAX = VMAX + 0.5
        YMIN = VMIN - 0.5
C
C  ***  INITIALISE CGL
        CALL CGL(GIC)                              ! INITIALISE CGL
        CALL CGL(GNF)                              ! NEW FRAME
C
C  ***  DEFINE THE GRAPHICS WINDOW
        CALL CGL(GSO,1)
        CALL CGL(GSW,XMIN,XMAX,YMIN,YMAX)          ! SET WINDOW
C
C  ***  DRAW THE BORDER
        CALL CGL(GMA2,XMIN,YMIN)                   ! DRAW BORDER
        CALL CGL(GRA2,XMAX,YMAX)
C
C  ***  LABEL WITH RECORD NUMBER
        CALL CGL(GMA2,TMAX*0.9/2.0,VMAX*0.7)
        ENCODE(3,3000,BLAB(10)) IREC
3000    FORMAT(I3)
        CALL CGL(GT,BLAB,12)
C  ***  GET TRANSIENT NUMBER FOR STARTING- IREC
C
C  ***  MATHS-RETURN POINT AFTER A TRANSIENT
C  ***  SALVAGE THE TRANSIENT
C
        DO 500 J=0,NTRANS-1
        READ (1'IREC+IALT*J,ERR=40) BDATA
C  ***  TRANSFER FOR MATHS
        DO 20 I=1,NPLOT
20      PDATA(I)=BDATA(I+LSHIFT)
        DO 30  I = 1,NPLOT
          ITEMP = PDATA(I)
          ITEMP = (ITEMP .AND. "377) - 128
          Y(I) = FLOAT(ITEMP) * 5.0  / 128.0
30      Z(I)=0.0
```

```
C
C  ***  SUPPRESS ALIGNMENT IF FLAGC=1
      I=IDELAY
      IF (IFLAGC) 199,199,210
C
C  ***  FIND STIMULUS ARTEFACT
199   I=0
200   I=I+1
      IF (I-100 ) 205,490,490
205   X=ABS(Y(I+1)-Y(I))
      IF (X-SA) 200,200,206
C
C  ***  JUMP IF ARTEFACT IS TOO SOON
206   IF (I-IDELAY) 490,490,210
C
C  ***  ALIGN TRANSIENTS
210   DO 220 K=1, NPLOT-I+IDELAY
220   Z(K)=Y(K+I-IDELAY)

C  ***  GET RID OF DC LEVEL
      X=0
      DO 340 I=1,IDELAY
340   X=X+Z(I)
      XP=X/(FLOAT(IDELAY))
      DO 350 I=1,NPLOT
350   Z(I)=Z(I)-XP
C
C  ***  GET RID OF ARTEFACT
      DO 360 I=1,IART
360   Z(I+IDELAY)=0.0
C
C  ***  FIND TROUGH
      XPEAK=0.0
      IPEAKF=IDELAY
      DO 620 I=1,IPEAK
      IF (-Z(IDELAY+IART+I)+XPEAK) 620,620,610
610   IPEAKF=IDELAY+IART+I
      XPEAK=Z(IPEAKF)
620   CONTINUE
C
C  ***  FIND ENDPOINT FOR SUMMATION
      IEND=IPEAKF
      XEND=XPEAK*0.25
      I=IPEAKF
625   IF (Z(I)-XEND) 630,630,640
630   I=I+1
      GOTO 625
640   IEND=I
C
C  ***  SUMMATE FROM  ADDRESS OF ARTEFACT TO IEND
      XSUM=0.0
      DO 650 I=IDELAY,IEND
650   XSUM=XSUM+Z(I)
C
```

```
C  ***  STORE CALCULATIONS
       IRECA=INT(FLOAT((IREC/IALT)))
       OUT(1,J+IRECA)=FLOAT(IPEAKF-11)
       OUT(2,J+IRECA)=XPEAK*SF
       OUT(3,J+IRECA)=FLOAT(IEND-11)
       OUT(4,J+IRECA)=XSUM*SF
C
C  ***  ?SUPPRESS PLOTTING
       IF (IFLAGA) 651,651,480
C
C  ***  ADD RASTOR, SCALE  AND PLOT THE DATA
651    XRAST=FLOAT(J)*FLOAT(ISPACE)*0.1-14.0
       YRAST=FLOAT(ISPACE)
       CALL CGL(GMA2,0.0,0.0)
       DO 160 I=1,NPLOT
       V=Z(I)*SF-XRAST

         T = FLOAT(I)
160      CALL CGL(GLA2,V,T)
C
C  ***  PLOT TROUGH, END OF SUMMATION AND SUM
       CALL CGL(GSMKS,4)
       V=XPEAK-XRAST
       T=FLOAT(IPEAKF)
       CALL CGL(GMKA2,V,T)
       V=XEND-XRAST
       T=FLOAT(IEND)
       CALL CGL(GMKA2,V,T)
       X=FLOAT(IDELAY)
       V=XSUM/10.0-XRAST
       CALL CGL(GMA2,-XRAST,X)
       CALL CGL(GLA2,V,X)
C
C  ***  RETURN FOR NEXT TRANSIENT
C
480    CONTINUE
490    CONTINUE
500    CONTINUE
C
C  ***  SKIP BACK FOR NEXT RECORD
       GO TO 41
C
C  ***  PLOT ANALYSED DATA
100    CALL CGL(GNF)
       WRITE (5,1026)
       CALL CGL(GSO,0)
       CALL CGL(GSW,-1.0,51.0,-0.1,4.1)
       CALL CGL(GMA2,0.0,0.0)
       CALL CGL(GRA2,50.0,4.0)
       DO 111 K=1,4
       CALL CGL(GSMKS,K,K)
       CALL CGL(GMA2,0.0,0.0)
       DO 110 J=1,50
       X=FLOAT(J)
       YP=ABS((OUT(K,J))/(OUT(K,1)))
       CALL CGL(GLA2,X,YP)
       CALL CGL(GMKA2,X,YP)
110    CONTINUE
111    CONTINUE
```

```
C
C  ***  DUMMY READ
41      READ (5,2010) ITEMP
        GO TO 10
C
C  ***  END OF DATA FOUND
40      WRITE (5,1030)
        GOTO 41
C
C  ***  DUMP THE CALCULATIONS.
710     CLOSE (UNIT=1)
        WRITE (5,1090)
        READ (5,2000) (FILNAM(I),I=1,12)
        OPEN(NAME=FILNAM,UNIT=1,TYPE='NEW',ACCESS='SEQUENTIAL')
        WRITE (1,2030) OUT
C
C  ***  CLEAR UP AND EXIT TO POS
50      CLOSE (UNIT=1)
        CALL EXIT
C
C  ***  FORMAT STATEMENTS
1000    FORMAT(//' PLTRN1'/' ------')
1010    FORMAT(/'$Filename?  ')
1020    FORMAT(/'$Record?    ')
1025    FORMAT(/'$Type 1 for plotting peak.   ')
1026    FORMAT(/'$Plot peaks etc.   ' )
1027    FORMAT(/'$Type 1 to suppress transient plotting.    ')
1028    FORMAT(/'$Type 1 to suppress alignment.   ')
1030    FORMAT(/' End of File')
1040    FORMAT(/'$Number of transients?  ')
1050    FORMAT(/'$Number of spaces between transients?   ')
1060    FORMAT(/'$Number of points per transient?    ')
1070    FORMAT(/'$Number of artefact points?   ')
1080    FORMAT(/'$Scaling factor-decimal?   ')
1090    FORMAT(/'$Filename for writing?    ')
1095    FORMAT(/'$Type 1 for dumping.     ')
1100    FORMAT(/'$Delay of artefact,2 to 10?   ')
1110    FORMAT(/'$Number of transients= ')
1120    FORMAT(/'$Type 1 to count transients.  ')
1130    FORMAT(/'$Size of artefact to be seen; 0.1 to 2.5?  ')
1160    FORMAT(/'$For second transient add 320     ')
1170    FORMAT(/'$Type 1 for every transient or 2 for alternate     '
C
2000    FORMAT(12A1)
2010    FORMAT(I4)
2020    FORMAT(F4.1)
2030    FORMAT(F8.2,F8.2,F8.2,2X,F8.2)
        END
```

```
C  ***  GRAPH.FTN - GRAPHS CALCULATIONS.
C
C  ***  TYPE -1 TO EXIT
C  ***  . IS DELAY OF PEAK
C  ***  + IS HEIGHT OF PEAK
C  ***  * IS DELAY OF DECLINE TO 25%
C  ***  O IS INTEGRAL FROM ARTEFACT TO *
C
C  ***  SET UP FILES
       INCLUDE 'LB:[1,5]CGL.FTN'                ! DEFINE CGL PARAMETERS
C
C *** MAXIMUM NUMBER OF DATA POINTS
C
       PARAMETER (MAXPTS = 500)    ! CHANGE ALSO IN "FETCH" & "SHOW".
C
       DIMENSION ISTAT(2),FLAB(3),ARA(4,MAXPTS),ARB(4,MAXPTS)
       DIMENSION ARC(4,MAXPTS)
C
       CHARACTER*4 NUMSTR
       CHARACTER*2 UMSTR
       CHARACTER*3 BUMSTR
       CHARACTER*20 LUMSTR,TUMSTR,CUMSTR
C
C  ***  ANNOUNCE THE PROGRAM
       WRITE (5,1000)
C
C  ***  CALL THE DATA
       NGRAPH=1
       CALL FETCH (ARA)
       CALL MORE (IM)
       IF (IM) 90,90,20
20     NGRAPH=2
       CALL FETCH (ARB)
       CALL MORE(IM)
       IF (IM) 90,90,30
30     NGRAPH=3
       CALL FETCH (ARC)
       CALL MORE (IM)
       IF (IM) 90,90,90
40     NGRAPH=4
       CALL FETCH (ARD)
       CALL MORE (IM)
       IF (IM) 90,90,60
60     NGRAPH=5
       CALL FETCH (ARE)
C

C  ***  SET DEFAULT VALUES
90     XMAX=50.0
       YMAX=10.0
       ICH=1
       IFLAGB=0
       FF=1.0
       TT=0
       YAVE=0.0
       YAV=0.0
       NA=0
       VA=0.0
       MA=0
       IFLAGD=0
```

```
C
C  ***  RETURN POINT
      WRITE(5,1100)
10    READ (5,2010) ITP
      IF (ITP) 50,114,114
C
C  ***  FOR DOUBLE PLOTTING OR SINGLE
114   WRITE(5,99)
      READ(5,2010)IDBPLO
C  ***  SET XMAX
      WRITE (5,1010)
      READ (5,2020) XTMP
      IF (XTMP) 120,120,115
115   XMAX=XTMP
C
C  ***  SET YMAX
120   WRITE (5,1020)
      READ (5,2020) YTMP
      IF (YTMP) 130,130,125
125   YMAX=YTMP
C
C  ***  WHICH PLOTS ?
130   WRITE (5,1040)
      READ(5,2040) ITEMP
      IF (ITEMP) 132,132,131
131   ICH=ITEMP
C
C  ***  INDENTING LINES
132   WRITE(5,1150)
      READ(5,2010)MAD
      IF (MAD.LE.0)THEN
       GOTO 133
       ELSE
      MA=MAD
      WRITE(5,1160)
      READ(5,2020)VA
      ENDIF
C
C  ***  CHANGING PLOTTING TO DOUBLE SPACING FOR ALTERNATE STIMULI ETC.
133   WRITE(5,1190)
      READ(5,2010)IALT1
      IF(IALT1.LE.0)THEN
        GOTO 134
        ELSE
      IAL1=IALT1
      ENDIF
      WRITE(5,1200)
      READ(5,2010)IALT2
      IF(IALT2.LE.0)THEN
        GOTO134
        ELSE
      IAL2=IALT2
      ENDIF
```

```
C
C  ***  CHANGE PLOTTING ORDE OF LINES
134     WRITE(5,1230)
        READ(5,2010)IZ
        IF(IZ)137,137,135
135     IIZ=IZ
C  ***  PENICILLIN LINE
137     IFLAGB=0
        IFLAGC=0
        WRITE (5,100)
        READ (5,2020) F
        IF (F) 144,145,138
138     IF(F.GE.1.0) FF=F
139     WRITE (5,105)
        READ (5,2020) T
        IF (T) 145,145,140
140     TT = T
        WRITE(5,110)
        READ(5,2080)CUMSTR
2080    FORMAT(A20)
        WRITE(5,113)
        READ(5,2010)INASHT
C  ***  SETTING Y PLACING OF LINE
        WRITE(5,1500)
        READ(5,2020)YSET
        IF(YSET.GT.1)THEN
          YSET1=YSET
        ELSE
          YSET1=20.0
        ENDIF
C  ***  AVERIGING
144     WRITE(5,1120)
        READ(5,2010)IFLAGB
        IF(IFLAGB)145,145,146
C
C  ***  NO AVERAGING
145     WRITE(5,1130)
        READ(5,2010)IFLAGC
146     CONTINUE
        IRPTS=0
C
C  ***  SETTING ANY OTHER LINE
148     CONTINUE
        WRITE(5,180)
        READ(5,2020)B
        WRITE(5,190)
        READ(5,2020)E
        IF(B)147,155,147
147     BZ=B
        EZ=E
C  ***  SET Y CORDINATE OF THIS LINE
        WRITE(5,1600)
        READ(5,2020)YSETB
        IF(YSET.GT.1.0) THEN
          YSET2=YSETB
        ELSE
          YSET2=30.0
        ENDIF
```

```
C  ***  WRITING CURRENT SIZE FOR DRUG AND PLACING OF THIS LABEL
       WRITE(5,109)
       READ(5,2060)LUMSTR
2060   FORMAT(A20)
       WRITE(5,113)
       READ(5,2010)JNASHT
C
C  ***  ANY OTHER LINE
1491   WRITE(5,200)
       READ(5,2010)IFLAGF
       IF(IFLAGF)155,155,149
149    WRITE(5,180)
       READ(5,2020)B2
       IF(B2)155,155,150
150    B2Z=B2
       WRITE(5,190)
       READ(5,2020)E2
       IF(E2)155,155,151
151    E2Z=E2
C
C  ***  SET Y PLACING OF THIS LINE
       WRITE(5,1600)
       READ(5,2020)YSETC
       IF(YSETC.GT.1)THEN
         YSET3=YSETC
       ELSE
         YSET3=30.0
       ENDIF
C  ***  SET CURRENT LABELS ETC
       WRITE(5,109)
       READ(5,2070)TUMSTR
2070   FORMAT(A20)
       WRITE(5,113)
       READ(5,2010)KNASHT
```

```
C  ***  SET UP VIEW AND WINDOW
155     CALL CGL(GIC)
        IF(IPLOT)157,157,156
156     IPLOT=0
        CALL CGL(GDVS,'TI:',3)
        CALL CGL(GIVS,'GH:',3)
        CALL CGL(GSVS,'GH:',3)
        CALL CGL(GSWI,1)
157     CALL CGL(GNF)
        CALL CGL(GSWI,1)
        CALL CGL(GSO,0)
        X=XMAX
        Y=YMAX
        IF(IDBPLO.EQ.1)THEN
          YMAXS=YMAX/2.
        ELSE
          YMAXS=YMAX
        ENDIF
        XWIND=X*(-0.3)
        YWIND=Y*(-0.2)
        CALL CGL(GSW,XWIND,X,YWIND,Y)
        CALL CGL(GMA2,0.0,0.0)
        CALL CGL(GLA2,XMAX,0.)
        CALL CGL(GMA2,0.,0.)
        IF(IDBPLO.NE.1)THEN
          CALL CGL(GLA2,0.,YMAX)
        ELSE
          CALL CGL(GLA2,0.,YMAXS-.5)
          CALL CGL(GMA2,0.,YMAXS)
          CALL CGL(GLA2,0.,YMAX)
        ENDIF
        IF(IDBPLO.EQ.1)THEN
          CALL CGL(GMA2,0.,(YMAXS))
          CALL CGL(GLA2,XMAX,YMAXS)
        ELSE
          GOTO 11
11      CONTINUE
        ENDIF
C  ***  SET PENICILLIN LINE
        YLINE=YSET1/100.
        PLINE=YMAXS/60.0
        PLABE=Y*0.05
        IF(FF.GT.1.0)THEN
          GOTO 1570
         ELSE
          GOTO 1580
        ENDIF
1570    CALL CGL(GMA2,0.0,0.0)
        CALL CGL(GSLW,0.,PLINE)
        CALL CGL(GMA2,FF,YLINE)
        CALL CGL(GLA2,TT,YLINE)
        CALL CGL(GMA2,FF+((TT-FF)*0.33),YLINE+PLABE)
        CALL CGL(GMA2,FF+((TT-FF)*0.33)+INASHT,YLINE+PLABE)
        ENCODE(20,1571,CUMSTR)CUMSTR
1571    FORMAT(A20)
        CALL CGL(GT,CUMSTR,20)
1573    CALL CGL(GSLW,0.,0.)
        CALL CGL(GMA2,0.0,0.0)
C
```

```
C  ***  SET LINE
1580     IF(B.NE.0)THEN
           GOTO 158
         ELSE
           GOTO 159
         ENDIF
C
158      CONTINUE
         YLINE2=YSET2/100.
         CALL CGL(GSLW,0.,PLINE)
         IF(BZ.LE.-1)THEN
           CALL CGL(GSLS,4,10101010,4)
           CALL CGL(GMA2,BZ,YLINE2)
           CALL CGL(GLA2,0.,YLINE2)
         ELSE
           CALL CGL(GMA2,BZ,YLINE2)
         ENDIF
         CALL CGL(GSLS,1,11111111,4)
         CALL CGL(GLA2,EZ,YLINE2)
         CALL CGL(GMA2,BZ+((EZ-BZ)*0.4)+JNASHT,YLINE2+PLABE)
         ENCODE(20,1581,LUMSTR)LUMSTR
1581     FORMAT(A20)
         CALL CGL(GT,LUMSTR,20)
159      IF(IFLAGF)170,170,160
160      YLINE3=YSET3/100.
         CALL CGL(GSLW,0.,PLINE)
         CALL CGL(GMA2,B2Z,YLINE3)
         CALL CGL(GLA2,E2Z,YLINE3)
         CALL CGL(GMA2,B2Z+((E2Z-B2Z)*0.4)+KNASHT,YLINE3+PLABE)
         ENCODE(20,163,TUMSTR)TUMSTR
163      FORMAT(A20)
         CALL CGL(GT,TUMSTR,20)
170      CALL CGL(GSLW,0.0,0.0)
         CALL CGL(GMA2,0.0,0.0)
```

```
C  ***  INDICATE SCALES
       XLABE1=XWIND*0.065
       XLABE3=YWIND*0.18
       XLABE4=YWIND*0.3
       XLABE5=YWIND*0.6
       XLABE2=YWIND*0.1
       LIMITX=XMAX/8
       CALL CGL(GMA2,FF,0.0)
       CALL CGL(GLA2,FF,XLABE3)
       CALL CGL(GMA2,FF+XLABE1,XLABE4)
       IBEGIN=0.0
       ENCODE(2,203,UMSTR)IBEGIN
203    FORMAT(I2)
       CALL CGL(GT,UMSTR,2)
       IF(XMAX.GT.120)THEN
         LINC=4
         LZS=4
       ELSE
         LINC=2
         LZS=2
       ENDIF
       DO 210 K=LZS,LIMITX,LINC
       X1=7.5*FLOAT(K)+FF
       X=7.5*FLOAT(K)
       IXSCAL=X/15.0
       CALL CGL(GMA2,X1,0.0)
       CALL CGL(GLA2,X1,XLABE3)
       CALL CGL(GMA2,X1+XLABE1,XLABE4)
       IF(MOD(K,2).EQ.0)THEN
        GOTO 207
       ELSE
        GOTO 209
207    ENCODE(2,208,UMSTR) IXSCAL
208    FORMAT(I2)
       CALL CGL(GT,UMSTR,2)
209    CONTINUE
       ENDIF
210    CONTINUE
       YLABEL=XWIND*(0.95)
       YLABE2=XWIND*(0.8)
       YLABE3=XWIND*(0.35)
       XLABE7=YWIND*0.12
214    CONTINUE
       DO 220 K=1,YMAXS
       Y=1.0*FLOAT(K)
       IYSCAL=100*Y
       JMVY=Y
       IF(IDBPLO.EQ.-1)THEN
         CALL CGL(GMA2,0.0,Y+YMAXS)
         CALL CGL(GLA2,XLABE1,Y+YMAXS)
       ELSE
         CALL CGL(GMA2,0.0,Y)
         CALL CGL(GLA2,XLABE1,Y)
       ENDIF
       IF(MOD(K,2).NE.0)THEN
         GOTO 215
       ELSE
         GOTO 22
215    CONTINUE
```

```
      IF(IDBPLO.EQ.-1)THEN
      CALL CGL(GMA2,YLABE3,YMAXS+Y-(XLABE7))
      ELSE
        CALL CGL(GMA2,YLABE3,Y-(XLABE7))
      ENDIF
      IF(IFLAGB.EQ.1)THEN
        ENCODE(4,219,NUMSTR) IYSCAL
219     FORMAT(I4)
        CALL CGL(GT,NUMSTR,4)
      ELSE
        ENCODE(4,21,NUMSTR)JMVY
21      FORMAT(I4)
        CALL CGL(GT,NUMSTR,4)
22    CONTINUE
      ENDIF
      ENDIF
220   CONTINUE
      CALL CGL(GMA2,0.,YMAXS)
      IF(IDBPLO)222,222,221
221   IDBPLO=-1
      GOTO 214
222   ALFX=XMAX*0.4
      CALL CGL(GMA2,ALFX,XLABE5)
      CALL CGL(GT,'Time (min)',10)
      ALFY=YMAX*0.6
      ALFY1=YMAX*0.5
      CALL CGL(GMA2,YLABEL,ALFY)
      CALL CGL(GT,'Amplitude',9)
      IF(IFLAGB.GE.1.0) THEN
        CALL CGL(GMA2,YLABEL,ALFY1)
        CALL CGL(GT,'(% control)',11)
      ELSE
        CALL CGL(GMA2,YLABE2,ALFY1)
        CALL CGL(GT,'(mV)',4)
      ENDIF
      CONTINUE
C
C  ***  CALL THE PLOTTING SUBROUTINE
      NA=1
      M=0
      CALL SHOW(ARA,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BY,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
      NA=2
      IF(IDBPLO.EQ.-1)IDBPLO=-2
      IF (NGRAPH-1) 230,230,240
240   CALL SHOW(ARB,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BY,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
      NA=3
      IF (NGRAPH-2) 230,230,250
250   CALL SHOW(ARC,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BY,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
      NA=4
      IF (NGRAPH-3) 230,230,260
260   CALL SHOW(ARD,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BY,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
      NA=5
      IF (NGRAPH-4) 230,230,270
270   CALL SHOW(ARE,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BY,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
```

```
C
230     IPLOT=0
        WRITE(5,1300)
        READ(5,2010)IPLOT
        WRITE(5,99)
        READ(5,2010)IDBPLO
        IF(IPLOT)600,600,156
600     CALL CGL(GDVS,'GH:',3)
        CALL CGL(GIVS,'TI:',3)
        CALL CGL(GSVS,'TI:',3)
        GOTO 10
C
C  ***  CLEAR UP AND EXIT TO POS
50      CALL EXIT
C
C  ***  FORMAT STAMENTS
99      FORMAT(/'$TYPE 1 FOR DOUBLE GRAPH PLOT OR RETURN FOR NORMAL    ')
100     FORMAT(/'$PENICILLIN START?, OR TYPE -1 TO KEEP OLD AND GET AVE  ')
105     FORMAT(/'$PENICILLN END?    ')
109     FORMAT(/'$LABEL FOR CURRENT    ')
110     FORMAT(/'$PENICILLIN CURRENT?     ')
113     FORMAT(/'$SHIFT TO PUT nA LABEL(INTEGER, eg 30)     ')
116     FORMAT(/'$TYPE 1 TO DO AGAIN, RETURN TO CARRY ON     ')
180     FORMAT(/'$ENTER START OF ANY OTHER LINES      ')
190     FORMAT(/'$ENTER END OF ANY OTHER LINE       ')
200     FORMAT(/'$TYPE 1 FOR ANY MORE LINES     ')
1000    FORMAT(//' PLOT CALCULATIONS'/'        ')
1010    FORMAT(/'$XMAX=?    ')
1020    FORMAT(/'$YMAX=?     ')
1030    FORMAT(/' End of File')
1040    FORMAT(/'$ WHICH PLOT; 1,2,3 OR 4 ?     ')
1080    FORMAT(/'$Scaling factor-decimal?    ')
1090    FORMAT(/'$Filename for reading?     ')
1100    FORMAT(/'$ TYPE -1 TO EXIT      ' )
1120    FORMAT(/'$TYPE 1 FOR AVERAGING CONTROLS      ')
1130    FORMAT(/'$TYPE 1 FOR ABSOLUTE VALUES      ')
1150    FORMAT(/'$DO LINES NEED INDENTING;1,2,3,4,5     ')
1160    FORMAT(/'$BY HOW MUCH ?      ')
1190    FORMAT(/'$ARE ANY FILES ALTERNATE STIMULI? 1,2,3      ')
1200    FORMAT(/'$ANY MORE? 1,2,3      ')
1230    FORMAT(/'$TYPE 1 TO CHANGE ORDER OF PLOTTING       ')
1300    FORMAT(/'$TYPE 1 FOR PLOTTER OR RETURN TO PLOT ON SCREEN      ')
1500    FORMAT(/'$ENTER INTEGER VALUE FOR Y PLACING OF LINE     ')
1600    FORMAT(/'$ENTER VALUE FOR VERTICAL ADJUST OF LINE     ')
C
2000    FORMAT(12A1)
2010    FORMAT(I3)
2020    FORMAT(F10.0)
2030    FORMAT(4F8.3)
2040    FORMAT(I3)
        END
```

```
C       ***READ FOM WINCHESTER THE CALCULATIONS.
        SUBROUTINE FETCH (ARRAY,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BZ,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
        DIMENSION ARRAY (4,500)
        BYTE FILNAM(14)
        DATA FILNAM(13), FILNAM(14) /0,0/
        WRITE (5,1090)
1090    FORMAT(/'$ FILENAME FOR READING?    ')
        READ (5,2000) (FILNAM(I),I=1,12)
2000    FORMAT (12A1)
        OPEN (NAME=FILNAM,UNIT=1,TYPE='OLD',ACCESS='SEQUENTIAL')
        READ (1,2030) ARRAY
2030    FORMAT (4F8.3)
        CLOSE (UNIT=1)
        RETURN
        END


C       MORE DATA FROM WINCHESTER?
        SUBROUTINE MORE (IM)
        WRITE (5,2100)
2100    FORMAT('$TYPE 1 FOR MORE    ')
        READ (5,3030) IM
3030    FORMAT(I3)
        RETURN
        END

C       ***PLOT THE DATA
        SUBROUTINE SHOW(ARPL,XMAX,IFLAGB,IFLAGC,ICH,YAVE,NGRAPH,M,VA,
     *NA,MA,FF,IFLAGD,IAL1,IAL2,BZ,B2Z,EZ,E2Z,IIZ,IPLOT,IDBPLO,YMAXS)
        INCLUDE 'LB:[1,5] CGL.FTN'
C
        DIMENSION ARPL(4,500)
C  ***  INDENTING THE LINES
        L=0
        IF(NA.EQ.MA)L=VA
C  ***  CHANGING FOR ALTERNATE STIMULI
        IAL=1
        IF(NA.EQ.IAL1)IAL=2
C
        IF(NA.EQ.IAL2)IAL=2
C
C  ***  CHANGING PLOTTING ORDER
        IF(IIZ.EQ.1)ZZZ=4.0-FLOAT(K)
C  ***  AVERIGING THE VALUES
        IF(IDBPLO.EQ.-2)THEN
          ICH=ICH
          YX=YMAXS
        ELSE
          ICH=ICH
          YX=0.0
        ENDIF
140     K=ICH
        YAVE=0.0
        YAV=0.0
        DO 142 J=1,FF
        YAV=YAV+ABS(ARPL(K,J))
142     CONTINUE
        YAVE=YAV/FF
C
```

```
        YY=2.0-FLOAT(K)
C
195     M=M+1
        IF(IDBPLO.EQ.-2)M=1
        IF(IPLOT)197,197,450
450     CALL CGL(GDVS,'TI:',3)
        CALL CGL(GIVS,'GH:',3)
        CALL CGL(GSVS,'GH:',3)
        CALL CGL(GSWI,2)
197     CALL CGL(GSMKS,M,M)
        CALL CGL(GMA2,10.0,Y)
        IMAX=INT(XMAX)
        DO 110 J=1,IMAX
        X=FLOAT(J*IAL)+L
        IF(IFLAGC.EQ.1)THEN
          Y=YX+ZZZ+YY+ABS(ARPL(K,J))
        ELSEIF(IFLAGB.EQ.1)THEN
          Y=YX+ZZZ+YY+ABS(ARPL(K,J)/(YAVE))
        ELSE
          Y=YX+ZZZ+YY+ABS(ARPL(K,J)/(ARPL(K,1)))
        ENDIF
        CALL CGL(GMKA2,X,Y)
110     CONTINUE
        READ (5,2010)ITES
        IF(ITES.GE.1)ICH=ITES
        IF (ITES)52,53,140
52      CALL EXIT
53      RETURN
C
C  ***  FORMAT STATEMENTS
2010    FORMAT(I3)
        END
```

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