LONG-TERM POTENTIATION OF TRANSMISSION AT NEOCORTICAL
SYNAPSES IN SLICES OF RAT SENSORIMOTOR CORTEX AND THE
INVOLVEMENT OF NMDA RECEPTORS.

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This thesis is dedicated to the memory of

Pat and Sean Murphy.

ABSTRACT.

1.   Long-term potentiation (LTP) is an enduring activity-dependent increase in synaptic efficacy which has been considered as a neural substrate for learning and memory.

2.   LTP was induced in 22% of postsynaptic potentials (p.s.p.s) and 87% of field potentials evoked by stimulation of the subcortical white matter and recorded in layers III, V & VI of slices of adult rat sensorimotor cortex. Conditioning paradigms used were either high frequency stimulation of an afferent pathway or the repetitive pairing of an afferent volley with a postsynaptic injection of depolarizing current. Intracellularly recorded LTP was found to be input specific, homosynaptic and associative.

3.   D-2-amino-5-phosphonopentanoic acid (AP5) was used to assess the involvement of N-methyl-D-aspartate (NMDA) receptors in neurotransmission and in the induction and expression of LTP in the neocortex. The majority of p.s.p.s (89%) and field potentials (80%) were mediated in part by NMDA receptors. A non-NMDA receptor mediated component always preceded a NMDA receptor mediated one.

4.   AP5 blocked the induction of LTP in field potentials recorded in 5 out of 7 slices; following washout of AP5, a second, identical set of conditioning stimuli induced LTP.

5.   AP5 applied 15-20 minutes after the induction of LTP, reduced the magnitude of potentiated field potentials in 13 out of 13 slices and 4 out of 4 potentiated p.s.p.s. A potentiated non-NMDA receptor mediated component preceded a potentiated NMDA receptor mediated one.

6.   In most cases, the onset latency of the potentiated component of p.s.p.s was delayed by ~2ms after the onset of synaptic activity. This delay was not voltage-dependent. LTP appeared to be a property of intrinsic neocortical connections but not of the fastest conducting afferents, possibly arising from outside the neocortex.

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LIST OF ABBREVIATIONS.

LTP	Long-Term Potentiation.
LTD	Long-Term Depression.
p.s.p.	Postsynaptic Potential.
e.p.s.p.	Excitatory Postsynaptic Potential.
i.p.s.p.	Inhibitory Postsynaptic Potential.
csf	Cerebrospinal Fluid.
E_m	Resting Membrane Potential.
R_{in}	Apparent input resistance.
AP5	2-Amino-5-Phosphonopentanoic Acid.
NMDA	N-Methyl-D-Aspartate.
GABA	γ-Amino-Butyric Acid
Lat	Lateral.
Med	Medial.
SD	Standard Deviation.
SEM	Standard Error of the Mean.

CHAPTER ONE

-- INTRODUCTION --

1.1 (i) Lear ning and memory.

The neocortex is indisputably involved in learning and memory. Yet, our understanding of the cellular mechanisms that underlie these cortical phenomena remains rudimentary. The aim of this thesis is to extend our knowledge of the cellular processes that are associated with learning events in the neocortex, at the level of the individual neurone, the unitary component of the brain.

1.1 (ii) The Hebbian synapse.

By the late nineteenth century, biologists had discovered that most mature neurones lose their capacity to divide, consequently models of learning and memory that evoked *de novo* synthesis of brain cells were abandoned. In the closing decade of the nineteenth century, Ramon y Cajal (1894) and Tanzi (1893) independently of each other, proposed the connectionist view of learning and memory i.e. learning involved the strengthening of connections amongst neurones, whilst memory was the product of such modification. At the time, however, there was no direct evidence to support this concept.

During the past century, the connectionist view has been restated several times, evolving the hypothesis that the synapse is the critical site of plastic change (Konorski, 1948; Hebb, 1949; Eccles, 1953 and Kandel, 1977). This was best stated by the psychologist Donald Hebb (1949) in what has today become known as Hebb's postulate:

> *"When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased"* (Hebb, 1949)

And restated in today's phraseology: the conjunction of excitatory activity
in both pre- and postsynaptic neurones, in some way, strengthens their
synaptic connection, such that the presynaptic cell can thereafter more
readily excite the postsynaptic cell. Such a theoretical connection is
known as a *Hebbian* synapse.

1.1 (iii) The discovery of long-term potentiation.

The discovery of long-term potentiation (LTP) in the early 1970's
(Bliss & Lømo, 1973; Bliss & Gardner-Medwin, 1973), provided biologists
with an electrophysiological correlate of learning, which has since been
shown to behave like a *Hebbian* synapse (for reviews see Bliss & Lynch,
1988; Brown *et al.*, 1988; Wigstrôm & Gustafsson, 1988).

LTP describes an activity-dependent, enduring increase in synaptic
efficacy and was first observed in the hippocampus (Bliss & Lømo, 1973). A
brief period of high frequency stimulation applied to the afferent nerve
fibres of the perforant path, induced an enduring increase in the amplitude
of field potentials recorded in the granule cells of the dentate gyrus.
This could last for many hours in the anaesthetized rabbit (Bliss & Lømo,
1973) and for days or weeks in unanaesthetized rabbits with chronically
implanted electrodes (Bliss & Gardner-Medwin, 1973).

LTP has since been demonstrated at all the major synaptic connections
of the hippocampal trisynaptic circuit (Schwartzkroin & Wester, 1975; Lynch
et al., 1977; Andersen *et al.*, 1977; Alger & Tyler, 1976, Yamamoto & Chujo,
1978) and other regions of the brain: subiculum and septum (Racine *et al.*
1983), medial geniculate (Gerren & Weinberger, 1983), cerebellar cortex
(Ito, 1983), visual cortex (Komatsu *et al.*, 1981; Lee, 1982; Artola & Singer,
1987), somatosensory cortex (Voronin, 1985; Bindman *at el.*, 1988), frontal

cortex (Sutor & Hablitz, 1989a), prefrontal cortex (Hirsch & Crepel, 1990)

and piriform cortex (Kanter & Haberly, 1990).

1.1 (iv) LTP: A neural substrate for learning and memory?

LTP is common to both the hippocampus and the cerebral cortex (for

review see Bindman et al., 1991), brain structures indisputably involved in

learning and memory. Bilateral removal or lesion of the hippocampus in man,

impairs the ability to lay down new memories (Scoville & Milner, 1957;

Milner, 1972; Zola-Morgan, Squire & Amaral, 1986). The possibility that a

connection might exist between hippocampal learning and LTP, generated

considerable interest in the phenomenon: was LTP a neural substrate for

learning and memory?

LTP shares many features that are characteristic of learning and

memory. Representational and declarative memory is known to be rapidly

induced, long-lasting, associative and contextually specific, likewise LTP

is rapidly induced (McNaughton, 1983), long-lasting (Bliss & Gardner-

Medwin, 1973; Racine et al., 1983), synapse-specific (Andersen et al., 1977)

and associative (Levy & Steward, 1979; Barrionuevo & Brown, 1983;

Gustafsson & Wigström, 1986ab; Kelso et al., 1986). The parallels are

compelling, though a direct link between LTP and learning has yet to be

established (McNaughton et al., 1986; Morris et al., 1989).

1.2 (i) Hippocampal LTP.

LTP has been best characterised in the hippocampus, mainly because its

neuronal circuitry is relatively simple and well understood. The cell body

layers of the pyramidal and granule cells can be discerned with the naked

eye! From animal to animal, brain slice to brain slice, it is possible to

stimulate a known afferent pathway and record monosynaptic events in a known population of postsynaptic hippocampal neurones.

1.2 (ii) *Conditioning paradigms.*

Variants of two conditioning paradigms have been used to induce LTP, both fulfilling the *Hebbian* requirement for the conjunction of pre- and postsynaptic activity. The first is high frequency stimulation of an afferent pathway (see Bliss & Lynch, 1988; also Teyler & DiScenna, 1987) and the second, is the conjunction of presynaptic release of neurotransmitter with depolarization of the postsynaptic neurone (Kelso *et al.*, 1986; Wigström & Gustafsson, 1986). Both paradigms are highly effective as inducers of hippocampal LTP with success rates of ≥80% (Gustafsson & Wigström, 1988; Bliss & Lynch, 1988).

1.2 (iii) *An induction threshold for LTP in the hippocampus.*

An important feature of hippocampal LTP is that it exhibits an intensity threshold for induction (Bliss & Lømo, 1973; Schwartzkroin & Wester, 1975; McNaughton *et el.*, 1978; Yamamoto & Sawada, 1981 and Lee, 1983), such that an increase in the intensity of the conditioning stimuli increases (i) the probability of inducing LTP, and (ii) its magnitude. This property is believed to reflect the requirement for co-activation or co-operativity between afferent nerve fibres and postsynaptic assemblies of neurones (McNaughton *et el.*, 1978). Co-operativity between afferent fibres determines the level of depolarization achieved in the postsynaptic neurones.

1.2 (iv) Postsynaptic depolarization and NMDA receptors in the induction of hippocampal LTP.

The induction of hippocampal LTP is dependent on the level of postsynaptic depolarization developed during conditioning, the greater the depolarization, the greater the likelihood for the induction of LTP, though this only holds as long as the depolarization is below the reversal potential for calcium ions (Douglas et al., 1982; Wigstrom & Gustafsson, 1985a,b; Wigstrom & Gustafsson, 1986; Malinow & Miller, 1986; Sastry et al., 1986; Wigstrom et al., 1986; Gustafsson et al., 1987 and Malenka et al., 1988)

Conversely, procedures that reduce or prevent postsynaptic depolarization during conditioning, such as coapplication of the inhibitory neurotransmitter, γ-Amino-Butyric Acid (GABA) (Scharfman & Sarvey, 1985) or postsynaptic injection of hyperpolarizing current (Malinow & Miller, 1986), block the induction of LTP.

For the Schaffer collateral/commissural input onto CA1 pyramidal neurones (Collingridge et al., 1983; Wigström & Gustafsson, 1985a,b) and for the perforant path input onto the granule cells of the dentate gyrus (Harris et al., 1984; Errington et al., 1987), the induction of LTP is dependent on the activation of postsynaptic N-methyl-D-aspartate (NMDA) receptors and the relief of the voltage-dependent Mg^{2+}-blockade of the ionophore associated with them. The NMDA channel is unique amongst directly ligand-gated channels in that it is voltage-sensitive (Mayer et al., 1984; Ascher & Nowak, 1986). Extracellular Mg^{2+} ions lodge within the channel pore and impede the flow of current. Current will only flow through the NMDA channel when the postsynaptic membrane is sufficiently depolarized to repel the Mg^{2+}-blockade of the channel pore (Mayer et al., 1984; MacDermott et al., 1986; Ascher & Nowak, 1986). Unlike the ionophores associated with the non-NMDA receptors, the NMDA channel is permeable to Ca^{2+} ions (McDermott et al., 1986). It is the entry of Ca^{2+} ions into the postsynaptic neurone via the NMDA channel that is thought to trigger the biophysical and/or structural changes that manifest LTP. Both

pharmacological blockade of the NMDA receptors or the injection of Ca^{2+}

ion buffers into the postsynaptic neurone, prevent the induction of LTP in

the dentate gyrus and CA1 region of the hippocampus (Collingridge et al.,

1983; Lynch et al., 1983; Harris et al., 1984; Errington et al., 1987).

Hence, for some hippocampal synapses, the activation of NMDA receptors

by the presynaptic release of neurotransmitter and a critical level of

depolarization of the postsynaptic membrane are coupled in a manner that

determines the efficacy of synaptic transmission. The interplay between

receptor activation and membrane potential confer associative and

threshold properties on the inductive processes for LTP (see Brown et al.,

1988 and Wigström & Gustafsson, 1988).

Interestingly, LTP induced in the mossy fibre input onto the neurones

of the CA3 region of the hippocampus, is not dependent on the activation

of NMDA receptors (Harris & Cotman, 1986).

1.2 (v) The expression of LTP in the hippocampus.

The expression of hippocampal LTP is not associated with lasting

changes in resting membrane potential or apparent input resistance of the

postsynaptic neurones (Andersen et al., 1980; Barrionuevo & Brown, 1983).

Neither is it due to a reduction in synaptic inhibition (Haas & Rose, 1984;

Wigström & Gustafsson, 1985a; Abraham et al., 1987; Taube & Schwartzkroin,

1987). A number of mechanisms have been suggested to mediate the

maintenance and expression of hippocampal LTP; some are described below:

(1) Enhanced release of neurotransmitter.

By monitoring the levels of neurotransmitter in the extracellular fluid,

prior to, and after conditioning, it has been shown that LTP is associated

with an increase in the absolute levels of neurotransmitter in the

extracellular fluid, suggesting that potentiation is maintained by an

increase in the presynaptic release of neurotransmitter (Skrede & Malthe-

Sorensen, 1981; Dolphin et al., 1982; Bliss et al., 1986; Lynch & Bliss,

1986; Errington et al., 1987). Conversely, it has been suggested that the

increase in the absolute levels of neurotransmitter is due to a decrease

in the rate of uptake of neurotransmitter into glia cells (Barbour et al.,

1989).

Recently, Bekkers & Stevens (1990) and Malinow & Tsien (1990),
employing whole cell voltage-clamp techniques, carried out a quantal
analysis of neurotransmission prior to and after the induction of
LTP in slice and culture preparations of hippocampus. Quantal
analysis provides estimates of the amplitude of response to a single
quantum of transmitter, the probability of release and the number of
release sites; the latter two are presynaptic phenomena whereas the
former is postsynaptic. Both Bekkers & Stevens (1990) and Malinow &
Tsien (1990) report that quantal analysis revealed that LTP was
associated with an increase in the probability of transmitter release
(i.e. decrease in synaptic failures). Thus, these findings support
the view that maintenance of LTP is due to an increase in
transmitter release (Bliss & Lynch, 1988). It should be noted that
an increase in the probability of transmitter release cannot be
explained by a decrease in the rate of removal of released
transmitter.

However, Malinow & Tsien

(1990) did not exclude the possibility of postsynaptic modification. In

order to improve the voltage control of the postsynaptic membrane, Malinow

& Tsien (1990) blocked the postsynaptic potassium currents by including

caesium ions in the electrolyte of the recording electrodes. Baranyi et al.

(1990) have recently reported that LTP recorded in the motor cortex of

awake cats is associated with the reduction of a postsynaptic potassium

current. Because of the presence of caesium ions, it is unlikely that

Malinow & Tsien (1990) would have been able to detect changes in

postsynaptic potassium currents. Clearly, the possibility that modification

of postsynaptic potassium currents may play a role in the maintenance of

hippocampal LTP needs further investigation.

(2) Increased number of postsynaptic receptors.

Baudry et al. (1980) proposed that LTP was maintained by an increase in

the number of postsynaptic glutamate receptors. However their assay for
measuring the density of glutamate receptors was flawed as it did not discriminate between
postsynaptic receptor and transmitter uptake sites (see Sastry &

Goh, 1984; Lynch et al., 1985). Indeed it has been reported that LTP is

associated with a reduced dendritic responsiveness to iontophoretically

applied glutamate in the dentate gyrus (Sastry & Goh, 1984; Lynch et al.,

1985) and a similar finding was reported for the CA1 region of the

hippocampus, though in the latter case the reduced responsiveness was only

seen during the first 30 minutes after the induction of LTP (Schwartzkroin

& Taube, 1986; Taube & Schwartzkroin, 1988). However, an increase in the

number or an increase in the responsiveness of postsynaptic receptors may

still play a role in the maintenance of LTP. Recently, Davies et al. (1989)

reported an increase in the responsiveness of hippocampal neurones to

iontophoretically applied α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic

acid (AMPA), a non-NMDA receptor agonist (AMPA/quisqualate receptor). This

effect was not immediate, but took at least one hour to reach its maximum,

suggesting that there is a succession of underlying processes responsible

for the maintenance of LTP; perhaps the earliest phase mediated by

presynaptic mechanisms, such as the increased release of neurotransmitter,

and the later by postsynaptic modification.

(3) Morphological modification.

LTP has been associated with morphological changes at the level of the

synapse and synaptic spine that are believed to result in the enhancement

of synaptic current and improved synapse-dendritic coupling (Lee et al.,

1980; Chang & Greenough, 1984; Brown et al., 1988; Desmomd & Levy, 1988;

Andersen, 1989).

1.3(i) The neocortex.

LTP of synaptic transmission in the neocortex, unlike LTP in the hippocampus, is poorly characterised. The complex neuronal circuitry and heterogeniety of neurones make it virtually impossible to (i) record reliably from a known population of cortical neurones and (ii) stimulate an identifiable, homogeneous afferent pathway in the *in vitro* neocortex. LTP in the hippocampus reflects a change in the strength of synaptic connections between pre- and postsynaptic neurones. It has yet to be established if LTP in the neocortex is also due to synaptic modification. Indeed, early investigations into enduring changes in neocortical synaptic efficacy suggested that potentiation was related to changes in neuronal excitability and not to specific modification at the level of the synapse or dendrite (see below).

1.3(ii) Long-term changes in membrane excitability.

Enduring changes in the electrical excitability of cortical neurones was first demonstrated in the cerebral cortex by Burns (1957). Polarizing current applied locally to the parietal cortex in anaesthetized cats, not only increased spontaneous electrical activity but induced a persistent increase in neuronal excitability that lasted for tens of minutes after the termination of the conditioning current.

Bindman *et al.* (1964) reported that field potentials recorded in the somatosensory cortex of anaesthetized cats, evoked by sensory stimulation of the contralateral forepaw, could be potentiated by the application of polarizing current to the surface of or locally within the cortex (also see Voronin, 1971). Enhancement of the evoked potentials was first observed during the passage of the polarizing current and if the current was

applied for 5 minutes or longer, the potentiation then endured for tens of minutes and in some cases for more than 3 hours once conditioning was terminated. It has since been shown (Landau *et al.*, 1964) that the main effect of polarizing current is to depolarize large numbers of cortical neurones, causing them to fire trains of action potentials. The enduring enhancement of the orthodromically evoked field potential reported by Bindman *et al.* (1964) appeared to exemplify the *Hebbian* synapse, though it remained to be shown if the enhancement was associative.

Bindman *et al.* (1962) often observed a lasting increase in spontaneous electrical activity within the neocortex, coincident with the lasting potentiation of evoked field potentials, suggesting that underlying changes wrought by conditioning were not confined to the synaptic pathway. Indeed it seemed that the conjunction of presynaptic with postsynaptic activity was not an essential requirement for the induction of the observed modifications in neocortical activity. However at this juncture, Bindman *et al.* (1962, 1964) were unable to identify the locus of modification or the underlying mechanisms. Moreover they were not certain if the modification was due to changes in the cortex itself, though Bliss *et al.* (1968) showed a few years later that changes in synaptic transmission could be elicited in undercut, neuronally isolated slabs of neocortex in   anaesthetized cats.

1.3 (iii) A postsynaptic locus.

A postsynaptic locus for the mechanisms that induce long-term changes in the excitability of neocortical neurones was suggested by Black-Cleworth, Woody & Nieman (1975) and O'Brien, Wilder & Steven (1977). Both groups employed *in vivo* associative models of learning; pairing sensory cues with direct stimulation of the cortex. They concluded that it

was postsynaptic depolarization evoked by direct cortical stimulation and not the synaptic potential elicited by the sensory cue that was the crucial inductive factor.

A postsynaptic locus for the mechanisms underlying changes in neuronal excitability was demonstrated by Bindman, Lippold & Milne (1979). A period of intense stimulation of the pyramidal tract of anaesthetized cats, during the blockade of synaptic transmission, induced an enduring enhancement of electrical activity in the somatosensory cortex. As conditioning, in the form of trains of antidromic action potentials was confined to the pyramidal neurones alone, Bindman *et al.* (1979) concluded that the location of the mechanisms responsible for the changes in neuronal excitability and the site of modification was the postsynaptic neurone.

1.3(iv) *Membrane properties and neuronal excitability.*

The suggestion that postsynaptic depolarization and firing alone was sufficient to induce long-lasting increases in membrane excitability was supported by an intracellular study performed by Bindman & Prince (1986) in anaesthetized rats. The membrane excitability of single cortical neurones was assessed as either the spontaneous activity or the number of action potentials elicited by a fixed pulse of depolarizing current injected directly into the postsynaptic neurone. Single neurones were conditioned with an intracellular injection of steady depolarizing current lasting many minutes, causing impaled neurones to fire action potentials at 20-40Hz. All neurones conditioned in this manner exhibited increases in membrane excitability that was not associated with any consistent change in membrane potential, but was associated with a significant increase in

input resistance. A gross increase in input resistance would potentiate in a non-specific manner all synaptic inputs.

It is tempting to suggest that changes in membrane excitability play a role in learning. Convergent excitatory synaptic input onto a single neocortical neurone could create the conditions that bring about gross changes in input resistance and thereby the potentiation of synaptic transmission. However, even though the neurone itself may be specifically targeted for potentiation and thereby conditioned in an associative manner, the potentiation itself would not be specific with regard to the synaptic input onto the potentiated neurone. From the study of LTP in the hippocampus, it is known that potentiation is selective for the synaptic input that is active during conditioning. It is homosynaptic i.e. pre- and postsynaptic elements are linked in a highly specific and associative manner. As such, hippocampal LTP is a phenomenon of synaptic transmission and not due to a gross change in membrane properties. Could this be also in the neocortex?

1.3 (v) LTP in the neocortex.

Baranyi & Fehér (1978) carried out the first intracellular study of LTP of neocortical e.p.s.p.s. Antidromic action potentials elicited by electrical stimulation of the pyramidal tract and orthodromic e.p.s.p.s evoked by stimulation of the ventro-lateral nucleus (VL-e.p.s.p.s) were recorded in single pyramidal neurones in the motor cortex of anaesthetized cats. One of two paradigms was used to condition the cells: the first was antidromic stimulation at 15-60Hz for 5-30 seconds and the second was 150 repetitive pairings of the VL-e.p.s.p. with a preceding antidromic action potential (10-300ms). Potentiation was judged to have been induced if there was (i) an increase in the magnitude and/or decrease in the onset

latency of the VL-e.p.s.p. or (ii) an increase in neuronal excitability. Plastic modifications were observed in 13.7% of the conditioned neurones, lasting from a few seconds to 27 minutes after the termination of conditioning. It was found that the pairing conditioning paradigm was more effective as an inducer of LTP than high frequency antidromic stimulation. This observation implied that in the neocortex pairing of presynaptic activity with postsynaptic activity was more efficacious as an inducer of LTP than postsynaptic depolarization alone.

Baranyi & Fehér (1978) then attempted to reproduce the conditions created by pairing pre- and postsynaptic activity by substituting antidromic action potentials with an injection of depolarizing current delivered via the recording electrode. However, the experiment was flawed as stimulation of the ventro-lateral nucleus was discontinued during the conditioning period, thus preventing the temporal pairing of presynaptic activity with the injection of depolarizing current. Though the conditioning did induce potentiation of the VL-e.p.s.p. and increase neuronal excitability in 8 out of 16 conditioned neurones, it never lasted more than 20 seconds. Of the three conditioning paradigms so far employed by Baranyi & Fehér (1978), the most effective had been that which allowed conjunction of presynaptic activity with postsynaptic depolarization.

In 1981, Baranyi & Fehér tested a fourth conditioning paradigm. They proposed that the convergence of excitatory input onto a neocortical neurone could create the conditions that lead to enduring changes in synaptic efficacy (heterosynaptic facilitation). Intracellularly recorded e.p.s.p.s were evoked from separate afferent pathways by stimulation of the VL-nucleus (VL-e.p.s.p.), the cortical surface (Call-e.p.s.p.) and the contralateral radial nerve (SS-e.p.s.p.: somatosensory). In 16 out of 34 pyramidal neurones it was possible to elicit e.p.s.p.s from two or more

different inputs. In each experiment, one input was selected as the control and the stimulus parameters set to evoke a subthreshold e.p.s.p., whilst a second input was used as the conditioning stimulus and the shock parameters set to evoke one or more orthodromic action potentials. The control e.p.s.p. was then repetitively paired 150 times with the conditioning stimulus that preceded the former by 0-100ms. After conditioning the control e.p.s.p. was then monitored for a number of minutes. If there was an increase in its magnitude or/and a decrease in the onset latency, the e.p.s.p. was judged to have been potentiated. Conditioning of SS-e.p.s.p.s with orthodromic action potentials evoked by VL-stimulation induced potentiation in 54% of trials. VL-e.p.s.p. conditioning of the Call-e.p.s.p. induced potentiation in 36% of trials. Call-e.p.s.p. conditioning of the VL-e.p.s.p. induced potentiation in 21% of trials. In most cases the potentiation lasted for only a few minutes, however in three cases it endured for 18, 23 and 55 minutes. In 30% of neurones, potentiation of an e.p.s.p. was associated with an increase in input resistance and a 4-6mv depolarization of the resting membrane potential. However changes in input resistance and membrane potential were also observed for neurones that failed to support potentiation of an input. Baranyi and Fehér (1981) concluded that concomitant changes in input resistance and/or membrane potential were not responsible for the underlying potentiation of synaptic potentials i.e. enhancement of synaptic potentials was not due to a non-specific increase in the membrane excitability of the postsynaptic neurone as had been previously implied by Bindman & Prince (1986).

Further evidence for a postsynaptic locus for the mechanisms that underlie changes in the potentiation of synaptic transmission was reported by Baranyi and Szente (1987). They made intracellular recordings from 533

neurones in the motor cortex of anaesthetized cats. Pairing of e.p.s.p.s with injections of depolarizing current into the postsynaptic neurone or with antidromic action potentials evoked by stimulation of the of pyramidal tract, successfully induced LTP, lasting 40-60 minutes, in 28% of conditioned neurones. The onset of the conditioning stimuli followed the test shock by 0-200ms, allowing a degree of temporal conjunction between presynaptic activity and postsynaptic depolarization. If the neurone was hyperpolarized by either an intracellular injection of hyperpolarizing current or exposure to the inhibitory neurotransmitter, GABA, LTP failed to be induced. Injection of the Ca^{2+} ion chelator, ethyleneglycol-bis-(β-aminoethyl-)-N,N'-tetraacetic acid (EGTA), into the postsynaptic neurone also prevented the induction of LTP, suggesting a postsynaptic role for Ca^{2+} in the induction of neocortical LTP. Furthermore, injection of the neuronal transport inhibitor, colchicine, into postsynaptic neurones that had previously shown potentiation, prevented the further induction of LTP, suggesting that operative neuronal transport is also a requirement for neocortical LTP. Baranyi and Szente (1987) concluded that the site for the mechanisms that underlie the induction of LTP was the postsynaptic membrane. Once again, some neurones that supported LTP also exhibited concomitant increases in input resistance, though increases in input resistance were also observed in neurones which failed to express LTP. It seemed likely that changes in gross membrane properties were not responsible for the maintenance of LTP. However, Baranyi et al., (1990) have recently reported that LTP recorded in neurones of the motor cortex of awake cats, is associated with a decrease in a postsynaptic potassium current.

The aim of the work described in this thesis was to investigate the induction and maintenance of LTP in the *in vitro* sensorimotor cortex. In the hippocampus, it is well established that LTP is homosynaptic, input specific and associative. These properties had yet to be fully characterised for LTP at neocortical synapses.

In the past, the most effective method for the induction of LTP has proved to be a brief period of intense high frequency stimulation applied to an afferent input. In the hippocampus, the experimenter is reasonably certain that the conditioning stimuli will be confined to the stimulated pathway, allowing the use of a second input as an independent control. Such confidence in the integrity of afferent pathways is not afforded to the experimentor using an *in vitro* slice preparation of neocortex. Because of its complex neuronal circuitry, it is unlikely that the effects of conditioning are confined to the afferent pathway stimulated by the test shock. Indeed, the neuronal excitation elicited by high frequency stimulation of the subcortical white matter spreads throughout the slice, recruiting vast numbers of neurones and possibly affecting the majority of neocortical synapses. Consequently, high frequency stimulation is not a suitable conditioning paradigm for the investigation of the input specificity and associativity of LTP at neocortical synapses.

A method for the induction of LTP exists, that allows the experimenter to selectively condition an afferent input by limiting the conditioning stimulus to the postsynaptic neurone. This paradigm was developed in the hippocampus by Wigström & Gustafsson, (1986) and also by Kelso *et al.,* (1986) independently of each other. LTP is induced in one input by repeatedly pairing single afferent volleys with injections of postsynaptic depolarizing current, thus ensuring the temporal conjunction of pre- and

postsynaptic activity. A second input, not paired with the injections of postsynaptic current, is then used to assess the specificity of the conditioning and its outcome. Baranyi and Szente (1987) employed a similar method to induce potentiation of p.s.p.s evoked by stimulation of a single afferent pathway in the motor cortex of anaesthetized cats. However, they did not use a second pathway to test the input specificity of the induced LTP. It was therefore not known if the potentiation was due to modification of the conditioned synapses or to non-specific changes in membrane properties.

In some of the experiments described in this thesis, an attempt was made to stimulate two separate afferent pathways; to evoke e.p.s.p.s with little or no shared afferent input. One input was conditioned using a conditioning paradigm modelled on that of Wigström & Gustafsson, (1986), whilst the second, non-conditioned input, was used as an independent control, to test the specificity of conditioning and expression of neocortical LTP.

The role of the NMDA receptor in the induction of LTP at some hippocampal synapses is well documented. However, little was known about the involvement of NMDA receptors in the induction and maintenance of LTP at neocortical synapses. The latter will be addressed in this thesis.

N,B, Some results arising from work described in this thesis have been published elsewhere: Aram, Bindman, Lodge & Murphy, 1987; Bindman, Murphy & Pockett, 1988; Bindman & Murphy, 1988a,b; Bindman & Murphy, 1989; Bindman & Murphy, 1990; Murphy & Bindman, 1990 and Bindman, Christofi, Murphy & Nowicky, 1991,

CHAPTER TWO

− METHODS AND MATERIALS −

2.1 (1) The preparation.

Adult male Sprauge-Dawley rats (160-220g) were anaesthetized with halothane (May and Baker) or killed by cervical dislocation and then decapitated. The skull was exposed by cutting the skin lengthwise with a scalpel blade, lifting aside the pelt and then scraping away the underlying soft tissue. With the use of a pair of flat-ended pliers the top of the skull was removed piece by piece, working from the caudal to the rostral end. Great care was taken not to compress the underlying brain tissue. The meninges were carefully lifted free of the brain with the aid of a sharp scalpel and forceps. With the brain still *in situ*, a coronal block of brain containing the sensorimotor cortex was then cut with a scalpel blade and transferred to a petri dish filled with ice-cold artificial cerebro-spinal fluid (csf) which had been bubbled with 95%O_2/5%CO_2 gas (British Oxygen Company). The operation from decapitation to immersion in csf usually took 1 to 3 minutes.

The block was trimmed to include the dorsal aspect of the cerebral cortex and the underlying white matter together with hippocampus and some thalamus. The trimmed block was lifted free of the csf with filter paper and dried, then the caudal side was stuck to a silicone cutting block with cyanoacrylate adhesive (Radio Spares). The cutting block and brain were then placed in the cutting carriage and covered with ice-cold csf. A vibroslice (Campden Instruments Ltd.) was used to cut four to six 400μM or 450μm thick coronal slices, the first slice was discarded as it was of unknown thickness. Each slice was individually placed, using a Number 4 watercolour brush (TVB), on filter paper saturated with ice-cold csf in a petri dish (slice dish) resting on ice. Once slicing had been completed, the slice dish was transferred to an incubation chamber (Alger et al. 1984). The chamber was a perspex (medical grade) box with a lid (17.5cm

high, 16.5cm wide and 16.5cm deep). The bottom of the chamber was filled

with tap water which covered a scintered glass pipe through which

$95\%O_2/5\%CO_2$ gas was bubbled. The slice dish rested on a central pedestal

some 6cm above the water and was protected from condensed water droplets

evolved by the bubbling with a perspex baffle. | As only one slice at
a time could be placed in the recording chamber, the role of the
incubation chamber was to maintain surplus slices until they were
required for experimentation. Slices kept in the incubation chamber
remained viable for upto twelve hours.

2.1 (ii) Artificial cerebrospinal fluid.

The csf was made freshly for each experiment and comprised (in mM):

NaCl, 125; $CaCl_2$, 2.5; KCl, 3.2; NaH_2PO_4, 1.2; $MgCl_2$, 1.1; $NaHCO_3$, 19; D-glucose,

10. When bubbled with $95\%O_2/5\%CO_2$ gas and heated to 37°C the csf had a

pH of 7.3.

2.1 (iii) The experimental chamber – the slice interface.

After the initial recovery period in the incubation chamber ()$\overset{1}{X}$hrs), a

slice of sensorimotor cortex was transferred to the interface recording

chamber (figure 1) and left to recover for at least one hour. The slice

was placed on lens tissue supported by a nylon mesh, the underside of the

slice being continuously perfused with warmed oxygenated csf. The lens

tissue drained surplus csf from the slice chamber to an outer well where

it was sucked away by a 21 gauge hypodermic needle that was attached to a

glass support tube and Portex tubing and the suction supplied by a 3

roller peristaltic pump (Crouzet, 60rpm). The uppermost side of the slice

was exposed to a constant stream of humidified $95\%O_2/5\%CO_2$ gas.

FIGURE 1: THE INTERFACE CHAMBER. (overleaf)

The brain slice (A) rested on lens tissue supported by a nylon mesh
stretched across the top of the inner well (B) and was continually
perfused with warmed and oxygenated csf, delivered to the underside of the
slice (csf denoted in orange and direction of flow indicated by large bold
arrowheads). The effluent csf was removed from the slice interface (A & B)
by tissue wicks that drained into an outer well (C), here the effluent was
removed under suction (F). The upperside of the slice was exposed to a
stream of warmed and humidified oxygen (95%) and carbon dioxide (5%) gas
(flow of gas denoted by open arrowheads). The gas, which had previously
passed through a warm water bath directly below the interface chamber,
entered the outer well via a series of gas vents that directed the flow
over the slice (one of eight vents illustrated).

A bubble trap (D) removed bubbles from the csf before it entered the inner well (B). The shaded
objects in the inner well (B) are glass rods which were used to reduce the csf dead space. The
reference electrode (E) is shown inserted in the bubble trap.
The thermistor was placed in the water bath (both not shown) below the inner well.
Scale: The internal diameter of the inner well was approximately 25mm.

THE SLICE PREPARATON (below).

Micrograph of a Nissl stained coronal section of sensorimotr cortex of adlt rat similar to those
used in this study. The position of recording and stimulating electrodes are shown. Two pairs of
bipolar stimulating electrodes are placed in the subcortical white matter, laterally and medially
with respect to the recording electrode in layer V (this configuration typical for the majority
of experiments).
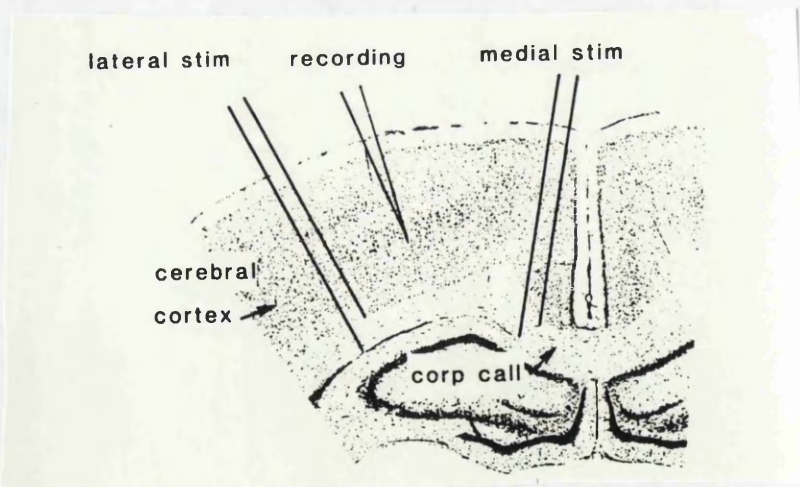
FIGURE 1: THE INTERFACE CHAMBER.
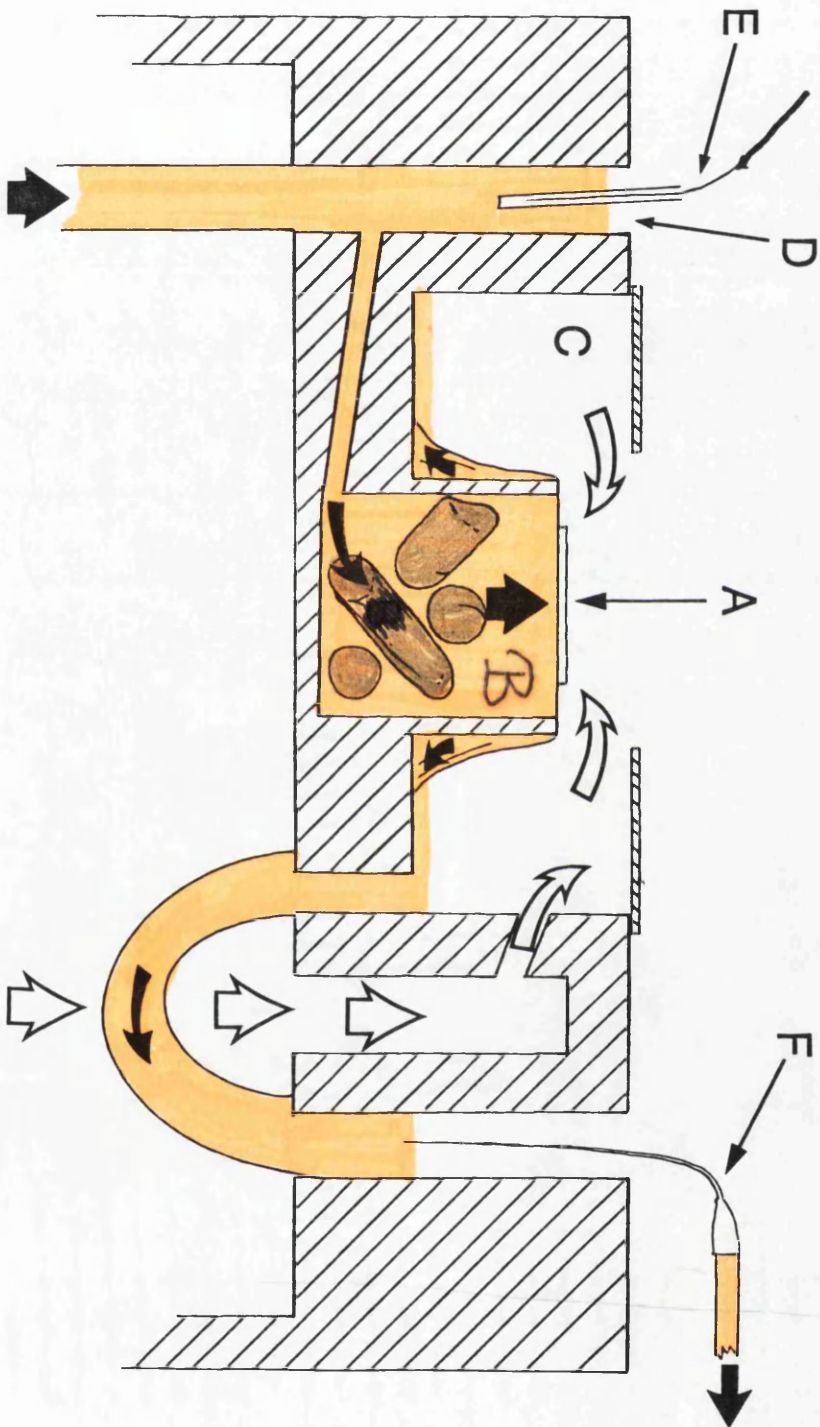
Both the csf and gas mixture were heated to 32-36°C prior to entry to the slice chamber by passage through a water bath immediately below the slice chamber. The water bath temperature was maintained within ±0.5°C during an experiment, by a heating element (Eureka wire, 12V) which was regulated by a thermistor-current feedback circuit (designed by G. Read, UCL).

2.1 (iv) Csf delivery system.

A gravity feed system was initially used to deliver csf to the slice chamber. This consisted of two 50ml syringes, each filled with 50mls of csf bubbled with 95%O_2/5%CO_2 gas and connected via portex tubing to the slice chamber. The delivery syringe was selected by a microvalve and the rate of flow determined by the height of the syringe above the slice chamber, usually set to give 1-2mls per minute. The surplus csf from the outer well was returned to the syringe in use, effectively forming a closed system. The dead space between the delivery syringe and the underside of the slice was 7mls.

The gravity feed system proved to be unsatisfactory when it was required to rapidly wash-in and washout pharmacological agents. During the exchange of csf it was necessary to discard the efflux from the outer well, this led to difficulties in maintaining a steady level of csf in the delivery syringe and hence a steady csf level and constant perfusion pressure at the slice interface; any sudden changes in level compromised the stability of the slice and the recording electrode(s). To overcome these problems the gravity feed system was replaced by a 8 roller variable-speed peristaltic pump (Watson-Marlow, 502S pump, 508MC2 pump head). This delivered csf to the slice chamber at a constant rate (1 to 4mls per minute) and allowed a smooth exchange of csf which was independent of the volume of csf in the delivery container. A 50ml

measuring cylinder was used as the delivery container and held 50mls of csf bubbled with 95%O_2/5%CO_2 gas. Prior to entering the slice chamber, the csf passed through a bubble trap (figure 1) which improved the smoothness of its flow to the underside of the slice. A smooth flow was essential for stable intracellular recording.

The experimental chamber (slice chamber, outer well and water bath) was supported by four rubber bungs and rested on a mild steel base plate on anti-vibration mountings.

2.1 (v) The recording electrodes.

Microelectrodes were pulled from capillary glass (Clarke Electro-medical Instruments, GC120F-15) on a horizontal one-stage puller (designed by Livingston and Duggar, 1934).

Intracellular microelectrodes were filled with 3M potassium chloride or 4M potassium acetate. In the later experiments only potassium acetate filled electrodes were used because they did not alter the reversal potential of chloride mediated i.p.s.p.s (Krnjević & Schwartz, 1967).

Only microelectrodes with series resistances between 60MΩ and 120MΩ when tested in csf were used. Experimentally it was found that microelectrodes with series resistance less than 60MΩ made poor neuronal penetrations while those with series resistances of more than 120MΩ were noisy and resisted the passage of applied current. Microelectrodes showing rectification on the passage of current were discarded.

Extracellular microelectrodes were pulled in the same manner as per the intracellular microelectrodes, except that once pulled the shaft of the microelectrodes were broken with a pair of pliers which shattered the microelectrode tip. The microelectrode tips were then examined under a

light microscope, only tips with a clean break and outside diameter between 5μm and 15μm were used. Extracellular microelectrodes were filled with 10% sodium chloride (10g NaCl/100mls distilled water) and had series resistances less than 10MΩ. The blunt end of the microelectrode was sealed with wax to prevent the leakage of electrolyte once the silver chloride electrode wire had been inserted. The tips of microelectrodes used for intracellular recording could not be resolved by light microscopy.

2.1 (vi) Recording and current injection circuits.

Intracellular microelectrodes were connected to the headstage (Hx0.1) of an Axoclamp 2A pre-amplifier (Axon Instruments) by a silver/silver chloride wire. The reference electrode consisted of another silver/silver chloride wire inserted into a 1.5cm length of Portex tubing filled with agar made with physiological saline and was placed in the bubble trap where it was in continuity with the csf bathing the slice. The agar interface between the silver and csf prevented any noxious interaction between the slice and the silver chloride. Prior to the experiment, the silver/silver chloride wires had been connected together to form two half-cells and left overnight in physiological saline to attain a stable iso-potential. This minimised the likelihood of d.c. drift between the wires during an experiment. The Axoclamp pre-amplifier had bridge balance (0-1000MΩ) and current injection (0-10nA) facilities. The voltage signal was amplified ten times by the Axoclamp before being passed to a d.c. input of a Tektronix 5A18N dual trace amplifier. The output was displayed on a Tektronix 511 storage oscilloscope and passed to a FM channel amplifier of a 4 channel tape recorder (Racal 4DS: 3 FM channels and 1 audio channel) and stored on FM tape (BSAF). The frequency bandwidth used to record the

voltage signal was either 0-1250Hz or 0-2500Hz. The input current signal from the Axoclamp was treated in a similar manner and stored on FM tape.

Extracellular microelectrodes were connected by silver/silver chloride wires to either an Axoclamp (1x) headstage and pre-amplifier or to a Neurolog d.c. pre-amplifier (NL102) and Neurolog headstage, the reference electrode was a silver/silver chloride wire inserted into an agar wick and placed into the bubble trap. The voltage signals were amplified ten times by both the Neurolog and Axoclamp pre-amplifiers and then treated in the same manner as described for the intracellular voltage signal.

Both Neurolog and Axoclamp pre-amplifiers had internal voltage calibration facilities. The Axoclamp also had an input current measurement facility. Calibration signals were routinely recorded and stored on FM tape at the end of each experiment.

2.1 (vii) Microelectrode support and drive systems.

Intracellular electrodes were clamped in a nylon holder with a light-weight plastic screw. The holder was connected to a Clarke digital microelectrode drive. The drive unit was supported by a mild steel arm clamped to a retort stand. This was firmly secured to a lathe-carriage attached to the mild steel base plate. The lathe-carriage allowed the microelectrode to be moved to any position within the slice chamber. The drive unit was driven by a remote control facility, allowing the microelectrode to be advanced or withdrawn in controlled steps as little as 2μm at a time.

Extracellular electrodes were held in a nylon holder which was attached to a micromanipulator bolted to the base plate. Coarse controls were used to position the extracellular electrode over the slice. The

microelectrode was advanced by hand using either coarse or fine

micromanipulator drive screws.

2.1 (viii) The stimulating electrodes.

Bipolar stimulating electrodes were made from a pair of tungsten wires

(Clarke Electromedical Instruments, TM50-5) which were electrically

(tip diameter of 50µm).
insulated except for their tips∧ Ensuring that their tips were within

0.5mm of each other, the wires were fixed with dental acrylate into a

glass support tube. The electrodes were connected to a isolated

stimulator (Devices, MK. IV) by light-weight sleeved wires. Each pair of

stimulating electrodes was held in a micromanipulator.

In the majority of experiments, two pairs of stimulating electrodes

were placed in the subcortical white matter, occasionally one pair of

stimulating electrodes was placed in the grey matter in layers III/IV.

A digitimer (Digitimer D4043) was used to govern the stimulation rate

and also to generate a trigger pulse which preceded each shock. The

trigger pulses were used to activate the oscilloscope beam and were stored

on FM tape to aid later analysis. The shock parameters set via the

stimulator were in the order of 0.05-0.2ms width and 0-90V voltage

strength.

2.1 (ix) Prevention of electrical interference and mechanical vibration.

The experimental chamber, recording and stimulating electrode

assemblies, drive units and headstages were secured to a heavy mild steel

base plate resting on three inflated car inner tubes (Austin Mini). This

was surrounded from beneath, above and on three sides by a shield of

aluminium sheeting. The shield, base plate, perfusion system, water bath

and electrical equipment were earthed through the common ground on the Tektronix amplifier (5A18N). These precautions ensured that the recordings were relatively free of artifactual voltage distortions.

The base plate, supported by the inflated tyres, was mounted on a wooden table standing on rubber blocks. This arrangement minimised the effects of incidental vibration at the slice-recording/stimulation interface.

2.2 (1) RECORDING & STIMULATION EXPERIMENTAL DESIGNS.

Three recording and stimulation protocols were employed in this investigation. Each is briefly explained:

2.2 (1).1 Lateral & Medial stimulation.

The vast majority of experiments were of this form: one pair of stimulating electrodes was placed in the subcortical white matter medially and a second pair placed laterally, with respect to the intended site of cell impalement. The stimulating electrodes had to be positioned before cell penetration. Any attempt to move them after cell impalement would ~~probably~~ have resulted in the loss of the cell. Intracellular impalements were made in either layer V/VI or layer III.

Throughout this thesis a postsynaptic potential evoked by a medially placed pair of stimulating electrodes is referred to as the medial postsynaptic potential. Likewise, a postsynaptic potential elicited by a laterally placed pair of stimulating electrodes is termed the lateral postsynaptic potential.

Postsynaptic potentials were evoked by each pair of stimulating electrodes at a rate of 0.1Hz. The lateral and medial shocks were separated by 5 seconds. During this interval the apparent input resistance of the

neurone was monitored by the injection of 80-150ms pulses of hyperpolarizing current (0.2-0.4nA), within the linear range of the voltage-current relationship for most neocortical neurones (Stafstrom, Schwindt, Flatman & Crill, 1984; Stafstrom, Schwindt, & Crill, 1984). The stimulation parameters were set to give stable subthreshold p.s.p.s.

The independence of the medial and lateral afferent pathways was tested by co-activating the medial and lateral stimulating electrodes. If the result was summation of the evoked responses, it was assumed that the stimulating electrodes activated separate pathways and the neurone accepted for further experimentation. However, if summation was not evident, the stimulus parameters of one or both pairs of stimulating electrodes were altered and then retested for summation. If summation failed to occur, it was assumed that the lateral and medial stimulating electrodes activated a common afferent pathway, consequently the impaled neurone was abandoned and the stimulating electrodes repositioned further apart.

2.2 (1).2 Grey & white matter stimulation.

Occasionally, a pair of stimulating electrodes was placed in the white matter ventrally and a second pair placed dorsally in layer III/IV with respect to the intended site of cell impalement. Otherwise the protocol was the same as that described for the lateral and medial stimulating electrodes.

2.2 (1).3 Extracellular experiments.

A single pair of stimulating electrodes was placed in the white matter. One extracellular microelectrode was then placed in layer V and a second extracellular microelectrode in layer III. These were placed above the stimulating electrodes. The slice was then stimulated at 0.1Hz and the

position of the recording electrodes adjusted until purely negative field

potentials were recorded, as changes in these are more readily

interpretable. The stimulus parameters were set to elicit approximately

1-2mV negative peak deflection field potentials. The slice was then

stimulated at 0.1Hz or 0.05Hz.

2.2 (ii) Positioning of the microelectrodes.

Intracellular and extracellular microelectrodes were either placed in

layer III or layer V of adult rat sensorimotor cortex. Layer III was

estimated to be some 500μm below the pial edge and layer V   in a zone

spanning 40 to 60% of the distance from the pial edge to the border of

the white matter (Paxinos & Watson, 1882). At the end of each recording,

the width of the grey matter (pial edge to white matter) and the location

of the recording site(s) was always measured to check that the recording

electrodes had been in the correct layer, sometimes an electrode that had

been thought to be in layer V was found to be in the dorsal region of

layer VI.

Extracellular electrodes were advanced using the fine drive of the

micromanipulator until the recording circuit was closed. They were then

advanced a further 20μm to ensure a good electrical contact with the

surface of the slice.

2.2 (iii) Intracellular microelectrode cell penetration.

Intracellular electrodes were positioned above the slice surface using

the lathe-cariage. The intracellular microelectrode was advanced using the

microdrive until it touched the surface of the slice and closed the

recording circuit. The bridge was then balanced, the maximum capacitance

neutralisation applied without positive feedback and the voltage offset set to 0mV or +30mV. The microelectrode was then advanced 2µm at a time in concert with a brief passage of depolarizing current (~60nA) evoked by tweaking the Clear switch on the Axoclamp. The passage of positive current aided cell penetration, although the underlying changes wrought by this procedure are not known. The microelectrode was gradually advanced until it reached ~350µm below the surface of the slice, at this point it was withdrawn and repositioned for a fresh track through the slice.

On penetration of a neurone there was a sudden negative shift in potential which was sometimes accompanied by action potentials. To prevent the loss of polarization and the generation of action potentials, hyperpolarizing current (0.5-1.5nA) was rapidly applied to the cell and maintained for at least 10 minutes. This was found empirically to lead to good long-term recordings. At the end of this time the hyperpolarizing current was gradually reduced to zero. For a cell to be acceptable for further experimentation, it had to have a resting membrane potential more negative or equal to -65mV, input resistance of >15MΩ and an overshoot action potential. If a neurone failed to meet any of these conditions, it was abandoned.

Occasionally a glial cell was impaled, these had stable membrane potentials about -80mV and did not show membrane charging during the passage of an intracellular current pulse (±0.4nA). The depth of all penetrations below the surface of the slice was recorded.

did not show any signs of activity or appeared granular when viewed through the dissecting microscope, it was abandoned and replaced with a fresh one from the incubation chamber.

2.2 (iv) Characterisation of membrane properties and synaptic responses.

The resting membrane potential of each neurone was monitored throughout an experiment with the use of a digital volt meter supplied with the Axoclamp pre-amplifier. Occasionally there was a small d.c. drift between the silver/silver chloride wires. To estimate the amount of d.c. drift and to check the validity of the volt meter readings, the apparent membrane potential was always measured at the end of an experiment. This involved stepping out of the cell and observing the change in potential.

As previously mentioned, the input resistance was continually monitored throughout the experiment with the passage of hyperpolarizing current (80-150ms, 0.2-0.4nA, 3-4 pulses/10s). By applying hyperpolarizing and depolarizing current of different magnitudes it was also possible to examine the current-voltage (I/V) relationship of the neurone. This method of altering the membrane potential was also used to examine the voltage-dependency of the p.s.p.s. These were evoked once the membrane had become fully charged, usually 30-50ms after the onset of the current pulse.

At some point during an experiment the neurone was made to fire action potentials, either synaptically or by injection of depolarizing current. This enabled measurement of the action potential amplitude and the threshold membrane potential.

2.2 (v) Pyramidal neurones identified by antidromic stimulation.

Neocortical neurones can be divided into two groups, (i) interneurones which are confined to the neocortical grey matter and (ii) pyramidal cells which have axons that project into the underlying white matter. It should be noted that within each division there are a number of sub-types of neurone in terms of their morphology, connectivity, neurotransmitters and

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electrophysiology (McCormick *et al.*, 1986; Mason & Larkman, 1990; Larkman & Mason, 1990).

Neurones in which antidromic action potentials were elicited on direct stimulation of their axons in the white matter (action potential upshoot directly from resting membrane potential and followed at >100Hz) were classified as pyramidal cells. It was possible that a large number, if not all of the cells that failed to elicit an antidromic action potential were pyramidal cells. These cells may not have had axons in the vicinity of the stimulating electrodes, consequently it was not possible to directly stimulate their axons.

2.2 (vi) Pharmacology.

Experiments were performed, using intracellularly recorded p.s.p.s to assess the pharmacological effects and time course of action of the $GABA_A$ antagonist, bicuculline methiodide (0.5-2μM, Sigma); the NMDA receptor antagonist, D-AP5 (10-20μM, Cambridge Research Biochemicals) (Watkins & Evans, 1981) and the non-specific glutamate antagonist, kynurenic acid (0.5-2.5mM, Sigma) (Perkins & Stone, 1982; Ganong *et al.*, 1983).

All drugs were made up in csf and bath applied via the perfusion system. Attempts to apply AP5 by dropping it directly onto the surface of the slice were abandoned as this tended to dislodge the recording electrode. The I/V relationship of the neurone and the voltage-dependency of the evoked p.s.p.s were assessed during (i) the control, (ii) maximum drug effect and (iii) once the drug had been washed from the slice.

In about half of the intracellular experiments the slices were exposed to 0.5-1μM bicuculline methiodide. This was bath applied an hour before recording commenced and was maintained throughout the length of these experiments.

Extracellular experiments were also performed to assess the action of AP5 and kynurenic acid.

2.2 (vii) Magnesium-reduced csf.

Intracellular and extracellular experiments were performed to examine the effects of reduced concentrations of extracellular Mg^{2+} ions on evoked synaptic responses.

Magnesium reduced csf was prepared in the same manner as for normal csf, except for the omission of magnesium chloride.

After an initial control period of 10-20 minutes in normal csf, the csf was temporarily exchanged for reduced Mg^{2+} csf. AP5 was bath applied to the slice in both the presence and absence of extracellular Mg^{2+}.

2.3 (i) Induction of LTP.

Two separate paradigms were used to induce LTP. The first, termed · pairing   conditioning, was the pairing of synaptic activity with intracellular depolarization, and was based on the inductive paradigm developed in the hippocampus by Gustafsson & Wigström, 1986 and Kelso *et al.*, 1986. The second conditioning paradigm, high frequency stimulation of an afferent pathway, was developed in the hippocampus by Bliss & Lømo, 1973 and Bliss & Gardner-Medwin, 1973.

2.3 (ii) Pairing condiditioning paradigm and the induction of LTP.

An intracellularly recorded p.s.p. was paired with an intracellular injection of depolarizing current sufficient to make the neurone fire 8 to 12 action potentials (200-300ms, 0.5-2.0nA). This was repeated once every 10 seconds for 25-50 trials and the onset of the depolarizing current was timed to correspond with the peak of synaptic activity. Only one pathway at a time was conditioned, the second, unconditioned, pathway, was used to monitor the specificity and the associativity of the effects of conditioning.

After conditioning, the evoked responses were monitored for at least 15 minutes. Only those conditioned responses which showed a sustained increase in amplitude and/or depolarizing slope were considered to have undergone long-term potentiation.

After 20 minutes the conditioning procedure was either repeated, often with a different onset latency, current strength and duration, or AP5 was bath applied to assess the involvement of NMDA receptors in the expression of LTP. Sometimes the conditioning stimuli was applied to the previously unpaired p.s.p..

The I/V relationship of a neurone and voltage-dependency of the p.s.p.s were assessed prior to and after conditioning.

2.3 (iii) High frequency stimulation of an afferent pathway and the induction of LTP.

High frequency stimulation of an afferent input was used to elicit LTP in both extracellular and intracellular experiments. After a stable control period of 10-20 minutes, high frequency stimulation was applied to one afferent pathway. This conditioning procedure consisted of 2 to 6 trains

of shocks at 100 to 500Hz, lasting for 0.1 to 1.0 seconds, applied every 10
s. The width of the conditioning shocks was usually twice that of the
control shock. The voltage was rarely altered. To minimise the likelihood
of polarization of the stimulating electrodes, the shock polarity was
alternated between trains.

After conditioning, the evoked responses were monitored for at least
15 minutes. Only those conditioned responses which showed a sustained
increase that lasted more than 15 minutes were considered to have
undergone long-term potentiation. At this point high frequency stimulation
conditioning was either repeated, sometimes with a stronger shock or AP5
was applied to the slice to assess the involvement of NMDA receptors in
the expression of LTP.

2.3 (iv) AP5 and its effects on the induction of LTP.

Extracellular experiments were performed to assess the involvement of
NMDA receptors in the induction of LTP. The high frequency stimulation
conditioning paradigm was used as the inductive method.

After a 10-20 minute control period, AP5 was bath applied to the slice
for 15 minutes prior to conditioning and its effects noted. Immediately
after conditioning, the AP5 was washed from the slice. Once the evoked
responses had recovered from the effects of the drug, the conditioning
procedure, identical to that applied during exposure to AP5, was then
repeated.

2.4 (i) Analysis of experimental data.

Records were analysed off-line using a Gould digital storage oscilloscope (Gould 1604) and waveform processor (Gould 160). The amplitude or duration of evoked responses were either measured individually or as electronic averages from the oscilloscope work face. The amplitude and threshold of action potentials and the voltage deflections produced by injected intracellular current were measured from single records.

Statistical analysis and graphical representation of data was assisted with the use of a Del Corporation AT computer and Lotus 123 software. Graphs and record traces were plotted on a digital XY plotter (Gould Colorwriter 1620) or Epson dot matrix printer.

CHAPTER THREE

-- RESULTS --

3.1 Introduction.

Both intracellular and extracellular electrophysiological techniques were used to investigate: (i) activity-dependent modifications in synaptic efficacy and (ii) the involvement of excitatory amino acids in neocortical neurotransmission and cortical plasticity.

The data arising from these experiments is presented in three parts. The first describes the intracellular experiments; detailing the electrophysiological properties of neocortical neurones, the types of evoked postsynaptic potentials, actions of $GABA_A$ and excitatory amino acid antagonists on p.s.p.s and membrane properties.

Part two describes the induction and maintenance of neocortical LTP in intracellularly recorded p.s.p.s. It also describes the action of bath applied AP5 on the expression of LTP.

The third part covers the extracellular experiments and is primarily concerned with the role of the NMDA receptor in the induction and expression of neocortical LTP.

**PART ONE: RESULTS OF INTRACELLULAR EXPERIMENTS –
ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES.**

3.I.1 (i) Electrophysiological properties of neocortical neurones.

Long-term (1-5hr) intracellular recordings were obtained from 67

neocortical neurones. The mean resting membrane potential and input

resistance of this sample was -79mV and 42MΩ respectively. The majority of

neurones were located in layer V of the neocortex (56 out of 67) and the

remainder in layer VI (6 neurones) and layer III (5 neurones). The

electrophysiological properties of this sample are presented in table 1.

3.I.1 (ii) Classification of neocortical neurones.

The frequency distribution of resting membrane potential, input

resistance, action potential threshold and action potential amplitude is

unimodal (figure 2).

Sixty-eight percent of cells tested (see methods) were positively

identified as pyramidal neurones (table 2). There was no significant

difference between the electrophysiological properties of layer V pyramidal

neurones and those layer V neurones not positively identified as such

(table 3).

All 67 neurones exhibited adaptation of action potential frequency in

response to depolarizing current pulses. Some layer V neurones appeared to

be of the *burster* type (McCormick *et al.*, 1985; Mason & Larkman, 1990)

though the majority were of the *regular spiking* variety (figure 3)

(McCormick *et al.*, 1985; Mason & Larkman, 1990). No systematic

investigation of action potential frequency and adaptation was undertaken.

TABLE 1: ELECTROPHYSIOLOGICAL PROPERTIES OF NEOCORTICAL NEURONES.

TOTAL SAMPLE.	Mean.	SD.	Range.	n.
E_m (mV)	-79.0	7.85	-(68-100)	66
[+]Action Potential (mV)	97.7	8.72	75-117	67
[+]Action Potential (mV)	74.2	6.72	60-90	64
*Firing Threshold (mV)	23.6	7.29	13-45	63
R_{in} (MΩ)	42.1	28.90	13-152	67

LAYER III NEURONES.	Mean.	SD.	Range.	n.
E_m (mV)	-82.4	7.57	-(72-92)	5
[+]Action Potential (mV)	106.8	13.20	88-116	4
[+]Action Potential (mV)	74.0	10.80	60-83	4
*Firing Threshold (mV)	32.8	8.81	25-45	4
R_{in} (MΩ)	23.4	6.91	13-30	5

LAYER V NEURONES.	Mean.	SD.	Range.	n.
E_m (mV)	-78.9	8.22	-(68-100)	55
[+]Action Potential (mV)	97.2	8.28	75-117	54
[+]Action Potential (mV)	74.4	6.51	60-90	54
*Firing Threshold (mV)	23.0	6.90	13-44	53
R_{in} (MΩ)	43.3	29.90	18-152	56

LAYER VI NEURONES.	Mean.	SD.	Range.	n.
E_m (mV)	-76.5	2.74	-(73-80)	6
[+]Action Potential (mV)	96.2	7.33	88-107	6
[+]Action Potential (mV)	72.7	6.98	66-84	6
*Firing Threshold (mV)	23.5	6.80	16-34	6
R_{in} (MΩ)	46.5	27.60	20-95	6

[+]: action potential measured from resting membrane potential to peak.
[+]: action potential measured from threshold to peak.
*: action potential threshold measured from E_m.
E_m: resting membrane potential.
R_{in}: apparent input resistance measured in the linear range of the current-voltage relationship.
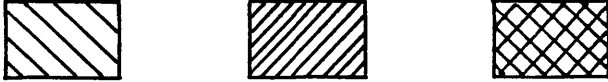
FIGURE 2: DISTRIBUTION OF ELECTROPHYSIOLOGICAL PROPERTIES OF NEOCORTICAL NEURONES.

Key to histograms: LAYER V   LAYER VI   LAYER III

                     NEURONES.   NEURONES.   NEURONES.

FREQUENCY HISTOGRAM OF: –

A: Resting membrane potential.

B: Input resistance.

C: Firing threshold   (mV above resting membrane potential).

D: Action potential amplitude measured from resting membrane potential to peak.
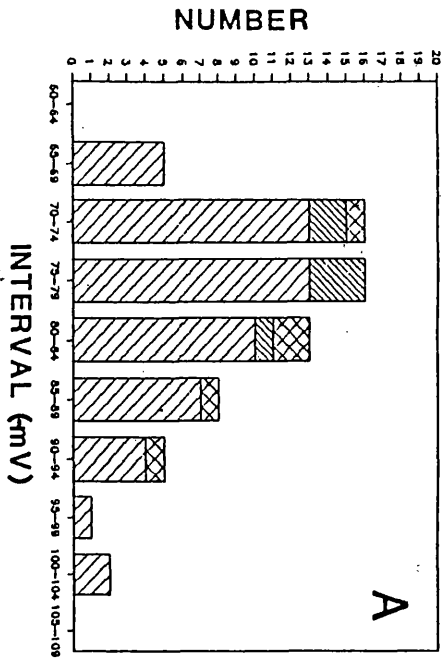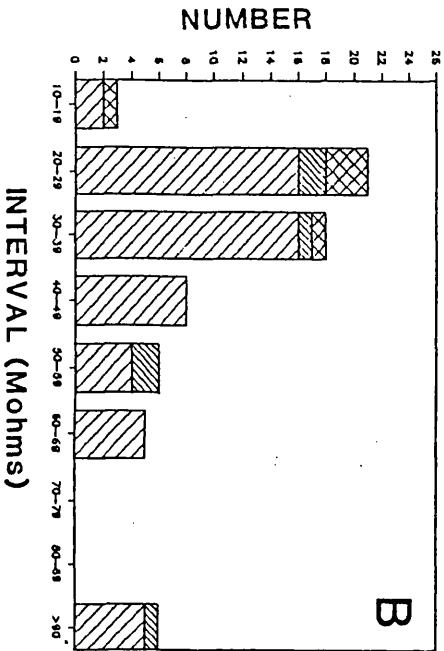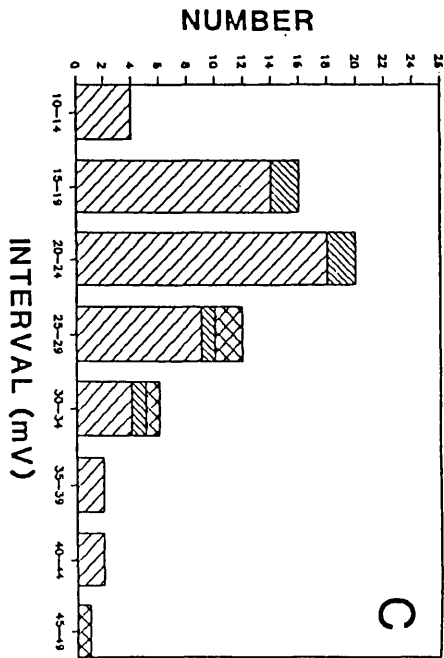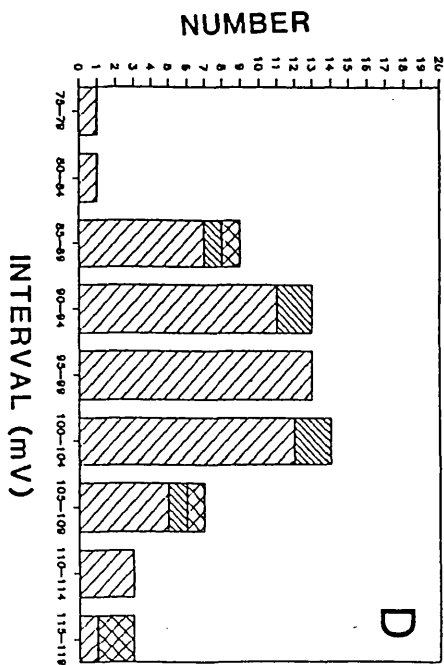
TABLE 2: ANTIDROMIC ACTION POTENTIALS EVOKED FROM WHITE MATTER STIMULATION.

NEURONES:	--PYRAMIDAL--		--UNIDENTIFIED--	TOTAL
	Tested A/D Positive.	Tested A/D Negative.	Not Tested.	
LAYER V	22 (71)	9 (29)	25	56
LAYER VI	2 (50)	2 (50)	2	6
LAYER III	2 (66)	1 (34)	2	5
TOTAL	26 (68)	12 (32)	29	67

Bracketed values are percentages.

A/D: antidromic action potential .

N.B. Stimulating electrodes were placed on the white matter either laterally and medially or directly below the recording site.

TABLE 3: ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER V PYRAMIDAL NEURONES COMPARED WITH THOSE OF UNIDENTIFIED LAYER V NEURONES.

IDENTIFIED PYRAMIDAL NEURONES (antidromic action potential elicited by white matter stimulation).

	Mean.	SD.	SEM.	Range.	n.
E_m (mV)	-78.0	9.20	2.01	-(68-100)	21
Threshold(mV)	22.5	6.78	1.52	14-42	20
†A.P. (mV)	74.0	5.25	1.15	65-82	21
‡A.P. (mV)	96.1	8.04	1.75	81-112	21
R_{in} (MΩ)	44.0	27.53	5.87	18-152	22

UNIDENTIFIED NEURONES (white matter stimulation failed to elicit an antidromic action potential).

	Mean.	SD.	SEM.	Range.	n.	Significance
E_m (mV)	-82.7	9.84	3.28	-(70-100)	9	0.5>P>0.1
Threshold(mV)	22.7	10.38	3.46	14-44	9	P>>0.5
†A.P. (mV)	70.0	7.73	2.58	60-80	9	0.5>P>0.1
‡A.P. (mV)	92.7	8.57	2.86	75-104	9	0.5>P>0.1
R_{in} (MΩ)	57.7	45.07	15.02	20-150	9	0.5>P>0.1

NEURONES NOT TESTED FOR ANTIDROMIC ACTION POTENTIALS.

	Mean.	SD.	SEM.	Range.	n.	Significance
E_m (mV)	-78.4	6.54	1.31	-(68-94)	25	P>>0.5
Threshold(mV)	23.3	5.63	1.15	13-36	24	P>0.5
†A.P. (mV)	76.3	6.44	1.31	67-90	24	0.5>P>0.1
‡A.P. (mV)	99.8	7.76	1.58	86-117	24	0.5>P>0.1
R_{in} (MΩ)	37.5	24.40	4.88	19-140	25	0.5>P>0.1

†A.P.: Action Potential Amplitude measured from threshold to peak.

‡A.P.: Action Potential Amplitude measured from RMP to peak.

Statistical significance values for unpaired Student's t-test; pyramidal neurones compared with unidentified and non-tested neurones. *THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THESE PROPERTIES OF IDENTIFIED PYRAMIDAL NEURONES AND THE UNIDENTIFIED NEURONES OF LAYER V.*

FIGURE 3: SUPRATHRESHOLD RESPONSES TO INJECTION OF DEPOLARIZING CURRENT IN TWO LAYER V NEURONES.

Example of two neocortical neurones which had different firing characteristics in response to intracellular injections of depolarizing current.

In each panel the upper trace is input current (in all figures the resting current is zero) and the lower trace is membrane potential.

A: Example of a *burster* neurone. Note the depolarizing afterpotential and late afterhyperpolarization.

Layer V pyramidal neurone: resting membrane potential, −77mV and input resistance, 37MΩ. Calibration as in B.

B: Example of a *regular spiking* neurone. In response to a suprathreshold depolarizing current pulse, this neurone fired a train of action potentials which rapidly adapted.

Layer V pyramidal neurone; resting membrane potential, −88mV and input resistance, 40MΩ.

N.B. Records in A were obtained using a Neurolog 102 pre-amplifier.

A

B

2nA
40mV

40ms
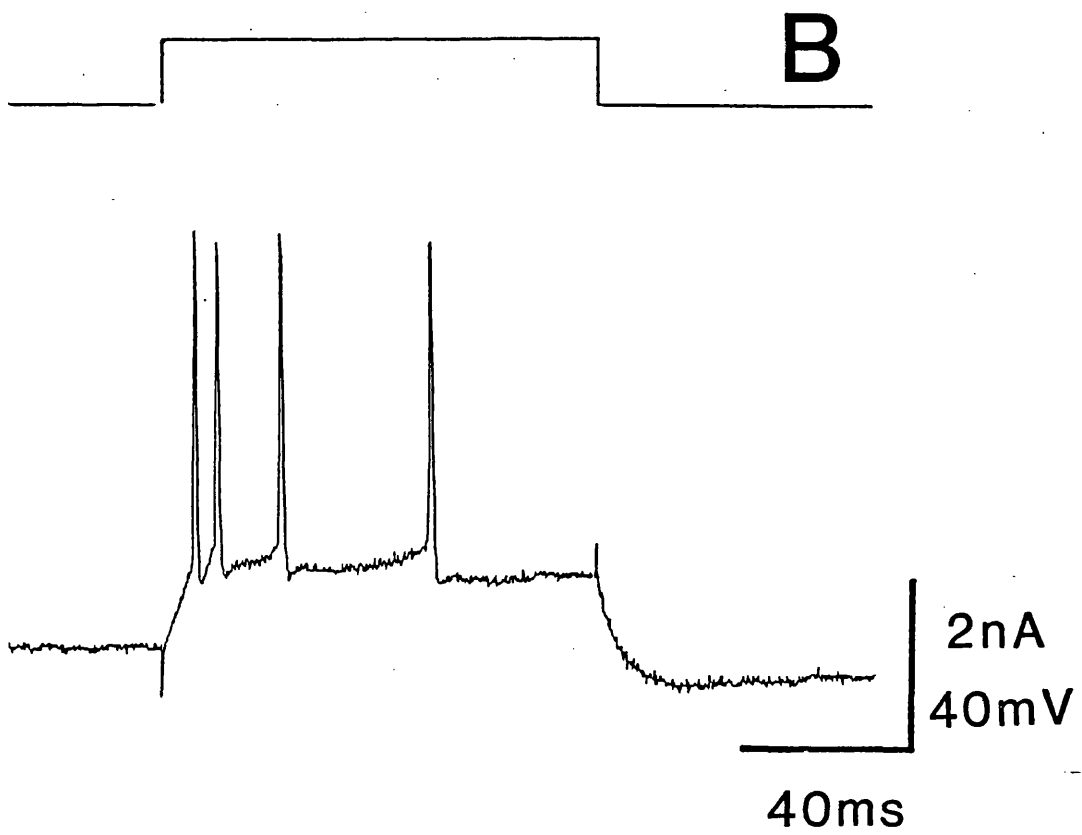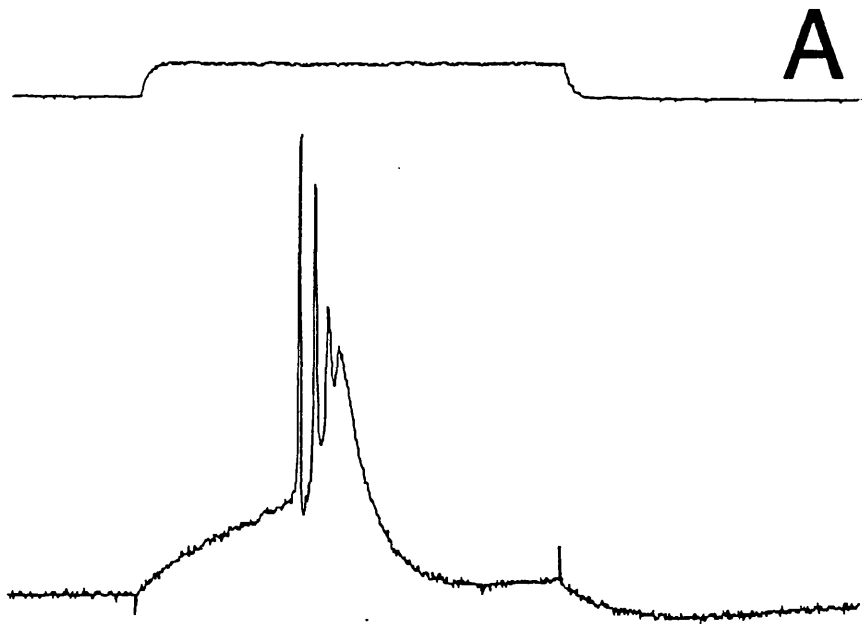
Occasionally, an impalement was made in a neurone which exhibited high frequency, non-adapting, action potential activity. These impalements never lasted for more than a few minutes and their characteristics were similar to those of the *fast spiking* cells described by McCormick et al., (1985)

3.I.1 (iii) Bicuculline methiodide and electrophysiological properties of neocortical neurones.

It has been reported that inclusion of a $GABA_A$ receptor antagonist in the bathing medium can uncover synaptic potentials mediated by NMDA receptors and also increase the probability of inducing LTP in both the hippocampal and neocortical slice preparations (Wigström & Gustafsson, 1985a; Artola & Singer, 1987, 1990; Collingridge & Davies, 1990). To find out if these observations held for sensorimotor cortex, the $GABA_A$ receptor antagonist, bicuculline methiodide (Scholfield, 1982), was added to the bathing medium in about half of the experiments.

Forty-one of the 67 neurones were exposed to micromolar concentrations of bicuculline methiodide (0.5-2µM). To ensure that the drug had reached an equilibrium concentration within the slice, it was added to the perfusing csf 40 minutes or more before recording commenced. There was no significant difference in the electrophysiological properties of layer V neurones exposed to and those not exposed to bicuculline methiodide (table 4), suggesting that the drug did not alter the passive membrane properties of the neurones (see section 3.I.2 (ii) for effects of bicuculline methiodide on evoked p.s.p.s).

TABLE 4: BICUCULLINE METHIODIDE AND ELECTROPHYSIOLOGICAL PROPERTIES OF LAYER V NEURONES.

LAYER V NEURONES NOT EXPOSED TO BICUCULLINE METHIODIDE.

	Mean.	S.D.	S.E.M.	Range.	n.
E_m (mV)	-80.8	8.28	1.81	-(70-100)	21
Threshold (mV)	23.3	7.20	1.61	13-44	20
$^+$A.P. (mV)	76.1	8.09	1.81	60-90	20
*A.P. (mV)	98.9	8.66	1.89	88-117	21
R_{in} (MΩ)	41.4	32.69	7.13	18-150	21

LAYER V NEURONES EXPOSED TO BICUCULLINE METHIODIDE.

	Mean.	S.D.	S.E.M.	Range.	n	Significance.
E_m (mV)	-77.8	8.09	1.39	-(68-100)	34	0.5>P>0.1
Threshold (mV)	22.8	6.82	1.19	14-42	33	P>>0.5
$^+$A.P. (mV)	73.4	5.26	0.90	61-80	34	0.5>P>0.1
*A.P. (mV)	96.1	7.99	1.39	75-112	33	0.5>P>0.1
R_{in} (MΩ)	44.5	28.53	4.82	20-152	35	P>>0.5

$^+$: action potential amplitude measured from threshold to peak.

*: action potential amplitude measured from RMP to peak.

Statistical significance values from unpaired Student's t-test.

3.I.1 (iv) Subthreshold responses to injections of intracellular current.

Lasting changes in the input resistance of neocortical neurones have been associated with enduring changes in neuronal excitability induced as a direct result of conditioning (Bindman & Prince, 1964). Consequently the current-voltage (*I-V*) relationship was assessed for each neurone, prior to and after conditioning.

In response to subthreshold injections of square pulses of depolarizing and hyperpolarizing current, the majority of neurones, 41 of 67, elicited non-rectifying voltage responses. Their membranes were fully charged within 35ms of the onset of a current pulse and the charge maintained without appreciable decrement (≤200ms). The *I-V* relationship of these cells tended to be near linear in   the hyperpolarizing

direction,   often the voltage change for a given current was greater in the depolarizing than hyperpolarizing direction (Connors *et al.*, 1982).

The remaining 19 neurones exhibited pronounced time-dependent rectification in their membrane responses to injected current. Figure 4 illustrates the membrane responses and *I-V* relationships of two rectifying neurones. Two cells displayed a pronounced voltage- and time-dependent increase in inward rectification on depolarization whilst the remaining 17 cells exhibited a pronounced voltage- and time-dependent increase in the hyperpolarizing direction. .

FIGURE 4: CURRENT-VOLTAGE RELATIONSHIP OF 2 NEOCORTICAL NEURONES.

A: Superimposed specimen records showing response to hyperpolarizing and subthreshold depolarizing current pulses of variable amplitude, (upper trace is input current; lower trace is voltage).

Layer V pyramidal neurone: resting membrane potential of -70mV; input resistance of 26MΩ. (action potential attenuated).

The symbols ● & O mark the measurement latencies used for the current-voltage plot in B.

B: Plot of change of voltage measured at 26ms (●) and 118ms (O) after the onset of the current injection (see A). Each data point is a mean calculated from 6 consecutive records evoked by the same current intensity, the standard error of the mean is lost within each symbol. Note the time- and voltage-dependence of membrane potential in response to hyperpolarizing current at 26ms and 118ms. The voltage difference in response to -0.4 & -0.8nA are statistically significant; P<0.001 in both cases (unpaired Student's t-test).

C: Superimposed specimen records showing response to hyperpolarizing and depolarizing current pulses of variable amplitude (from top to bottom input current was +0.6, +0.4, +0.2, -0.2, -0.4 & -0.8nA). The symbols ● & O mark the measurement latencies used to generate the current-voltage plot in D.

Layer V pyramidal neurone: resting membrane potential of -90mV; input resistance of 60MΩ.

D: Plot of change of voltage measured at 38ms (●) and 160ms (O) after the onset of the current (see C). Each data point is a mean calculated from 6-12 consecutive records evoked by the same current intensity, the standard error of the mean is lost within each symbol. Note the time-dependent difference in the depolarizing direction. The difference between 38ms and 160ms at +0.4 & +0.6nA is statistically significant; P<0.001 in both cases (unpaired Student's t-test).
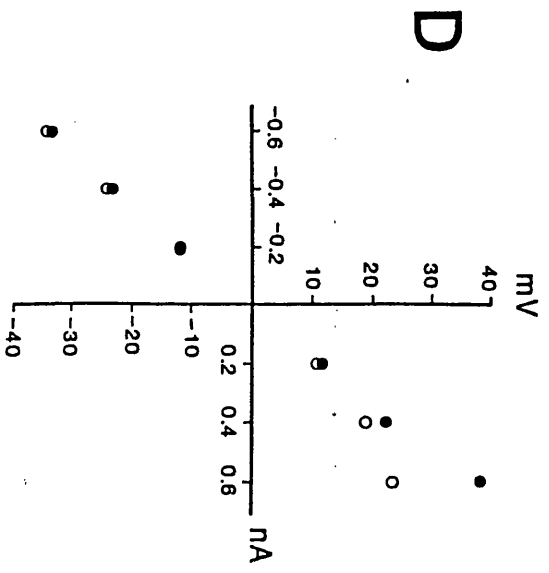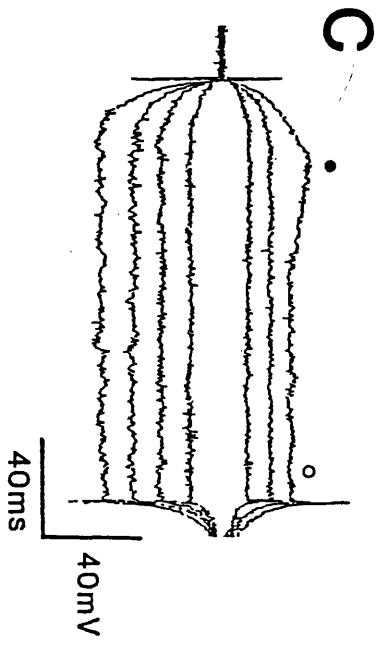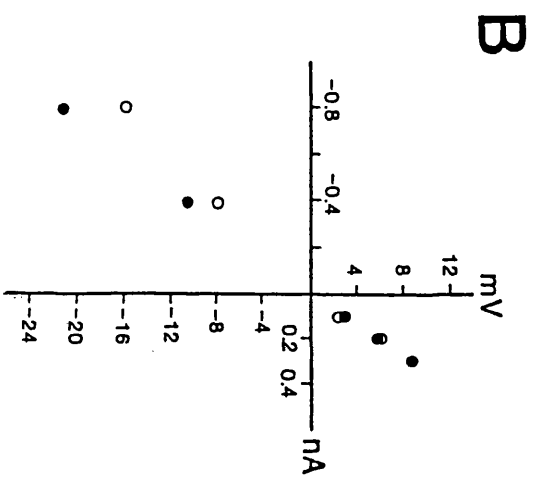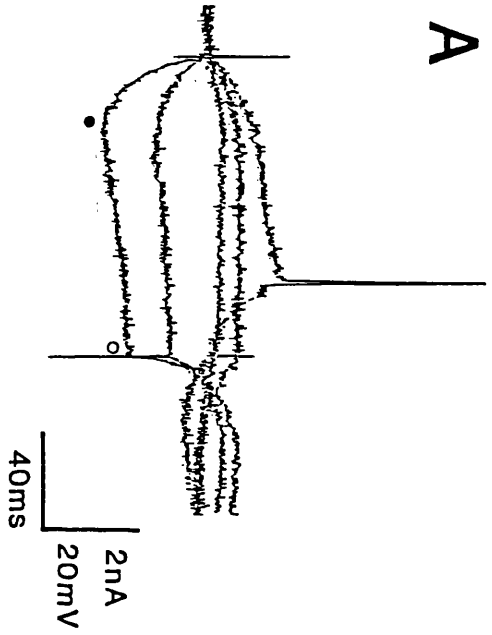
Twenty-seven percent of the layer V neurones exhibited pronounced anomalous rectification. The mean resting potential for this group was -78.8mV and the mean input resistance 44.4MΩ and were not significantly different when compared with the 73% of layer V neurones which did not exhibit pronounced anomalous rectification.

In 10 out of 11 neurones in which LTP was induced, the expression of LTP was not associated with a change in their $I-V$ relationships. In one neurone, the expression of LTP was coincident with a small but highly signficant increase in input resistance. It should be noted that for most cells, the $I-V$ relationships were constructed using only hyperpolarizing currents.

3.1.2 (i) Postsynaptic potentials recorded in neocortical neurones.

Long-term (1-5hr) recordings were obtained for 119 evoked postsynaptic potentials of which 108 were identified as e.p.s.p.s and 11 as i.p.s.p.s. (table 5).

Inhibitory potentials were distinguished from e.p.s.p.s in one of two ways: (i) subthreshold depolarizing postsynaptic potentials which reversed to become a hyperpolarizing potential when the postsynaptic neurone was depolarized above -70mV, were identified as chloride-mediated (GABA$_A$) i.p.s.p.s (Krnjević & Schwartz, 1967; Scholfield, 1978) and (ii) those which exhibited pronounced hyperpolarization when evoked at resting membrane potential were presumed to be potassium-mediated (GABA$_B$) i.p.s.p.s (Newberry & Nicoll, 1985; Deisz & Prince, 1989). Nine inhibitory potentials were characterised as GABA$_A$ mediated i.p.s.p.s and the remaining 2 as GABA$_B$ mediated i.p.s.p.s.. Both types of inhibitory potential are illustrated in figure 5.

TABLE 5: POSTSYNAPTIC POTENTIALS AND AFFERENT INPUT.

Table showing the number of each type of evoked postsynaptic potential against the afferent input to which the test shock was applied.

---TYPE OF EVOKED POSTSYNAPTIC POTENTIAL---

AFFERENT INPUT	SUBTHRESHOLD E,P,S,P,	FIRING DISCHARGE PRECEDED BY SUBTHRESHOLD E,P,S,P,	LATE DISCHARGE NOT PRECEDED BY SUBTHRESHOLD E,P,S,P,	I,P,S,P,
LATERAL[†]	38	6	4	5
MEDIAL[‡]	48	2	0	4
WHITE MATTER[#]	7	0	0	1
GREY MATTER[*]	3	0	0	1
TOTAL	96	8	4	11

†: potentials evoked via stimulating electrodes placed laterally on the subcortical white matter with rspect to the site of cell impalement.

‡: potentials evoked via stimulating electrodes placed medially on the subcortical white matter.

#: potentials evoked via stimulating electrodes placed directly below the site of cell impalement on the subcortical white matter.

*: Potentials evoked via stimulating electrodes placed in layer III/IV of the grey matter.

Excitatory potentials were sub-divided into three categories. The largest category of 96 postsynaptic potentials consisted of graded subthreshold e.p.s.p.s (i.e. size of subthreshold potential increased with stronger stimulus shocks until firing threshold breached, at which point the e.p.s.p. gave rise to an orthodromic action potential). During recording the stimulus shock was always fixed to evoke subthreshold e.p.s.p.s. Examples of graded e.p.s.p.s are illustrated in figures 6, 7, 9, 11, 13, 15, 17, 20, 22, 24 & 25.

The second category of e.p.s.p. consisted of 8 short latency subthreshold e.p.s.p.s coupled to a late latency firing discharge superimposed on a depolarizing wave (figure 5C). Often it proved impossible to elicit the early subthreshold e.p.s.p. alone. The late discharge was usually an-all-or-nothing event.

The last category of 4 excitatory potentials were all-or-nothing paroxysmal depolarizing shifts in membrane potential with variable onset latencies of more than 40ms (figure 5D).

3.I.2 (ii) Action of bicuculline methiodide on postsynaptic potentials.

All ♂ neurones which had short latency e.p.s.p.s coupled to late firing discharges and 3 out of the 4 neurones in which paroxysmal depolarizing shifts were observed were bathed in bicuculline methiodide. Conversely, 6 out of the 9 neurones which exhibited $GABA_A$ mediated i.p.s.p.s were not exposed to bicuculline methiodide. It is likely that the presence of a firing discharge was a consequence of exposure to bicuculline methiodide.

It has been reported that in the adult rat visual cortex, the presence of a $GABA_A$ antagonist is essential for the induction of LTP recorded in the majority of layer III neurones (Artola & Singer, 1987).

FIGURE 5: NEOCORTICAL POSTSYNAPTIC POTENTIALS.

A: Example of a chloride-mediated i.p.s.p. recorded in a layer V
neurone; resting membrane potential, -80mV; input resistance,
100MΩ.

Records are superimposed traces of an i.p.s.p. evoked at
different membrane potentials; voltage (*lower records*); input
current (*upper records*). Once the membrane was fully charged,
the i.p.s.p. was evoked by stimulating the lateral white matter.
Note that at resting membrane potential the i.p.s.p. appeared as
a depolarizing potential, however on depolarization it reversed
and became a hyperpolarizing potential. The reversal potential
for the i.p.s.p. was near -71mV and is similar to the equilibrium
potential for chloride ions (see Krnjevic & Schwartz, 1967;
Scholfield, 1978). Calibration as in D (action potential
attenuated by frequency bandwidth of tape recorder).

B: Example of a composite postsynaptic potential recorded in a
layer VI pyramidal neurone; resting membrane potential, -79mV;
input resistance, 95MΩ. The record is an electronic average of
16 successive responses evoked at 0.1Hz by stimulation of the
medial white matter. The *closed star* indicates an e.p.s.p. which
was followed by hyperpolarizing potential marked by the *open
star*. The late hyperpolarizing potential was an i.p.s.p., however
unlike that shown in A, this i.p.s.p. was presumbably mediatd by
potassium ions (Deisz & Prince, 1989). Calibration as in D.

C: Example of a subthreshold e.p.s.p. (●) coupled to a late firing
discharge recorded in a layer V neurone; resting membrane
potential, -80mV; input resistance, 30MΩ. The subthreshold
e.p.s.p. was a graded potential whereas the late firing discharge
was an all or nothing event. The records show two superimposed
e.p.s.p.s evoked by medial white matter stimulation at 0.1Hz.
O/caisionally the late firing discharge was superimposed on two
depolarizing waves (as shown). Bathing medium contained 1μM
bicuculline methiodide. Calibration as in D (action potential
attenuated by frequency bandwidth of tape recorder).

D: Example of a late onset paroxysmal depolarizing shift recorded
in a layer V neurone; resting membrane potential, -87mV; input
resistance, 33MΩ. The records show three superimposed
responsess evoked by lateral white matter stimulation at a rate
of 0.1Hz. The evoked potentials were of variable latency and
accompanied with an action potential discharge. Bathing medium
contained 2μM bicuculline methiodide (action potential
attenuated by frequency bandwidth of tape recorder).
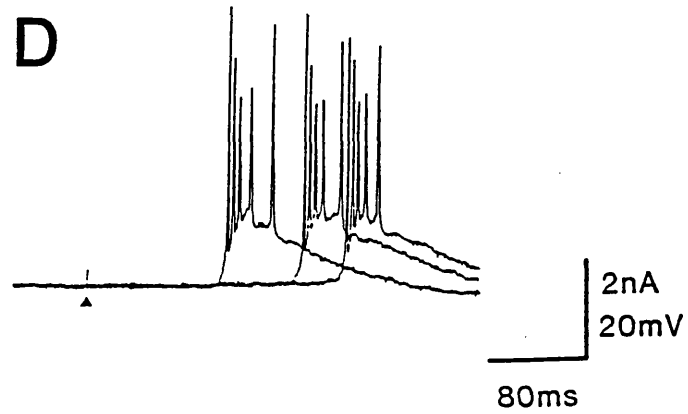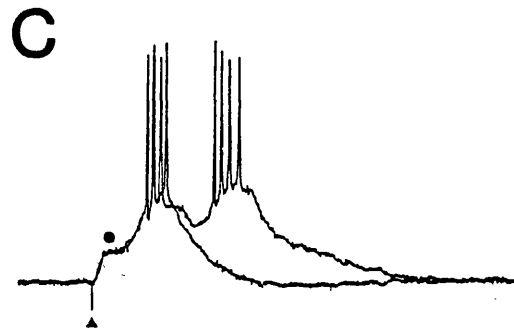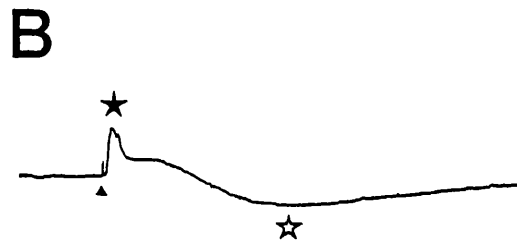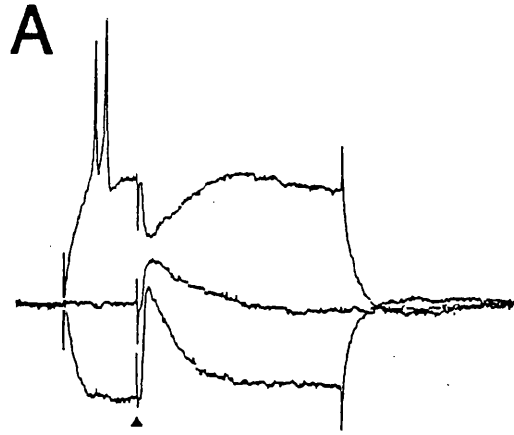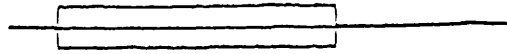
However, the concentration of GABA$_A$ antagonist used, in itself caused a progressive growth in the evoked responses. For the majority of neurones described in this thesis that were exposed to bicuculline methiodide (39 cells), the concentration of bicuculline methiodide was in the range of 0.5-1.5μM and the p.s.p.s recorded in them were stable i.e 0.5-1.5μM bicuculline methiodide did not induce instability in evoked p.s.p.s.

The remaining two neurones exposed to bicuculline methiodide, were exposed to a concentration of 2μM. In one cell, the evoked p.s.p.s remained subthreshold. However in the other, 2μM bicuculline methiodide transformed a subthreshold e.p.s.p. into a paroxysmal depolarizing shift (figure 6). This last experiment was an exception, as the bicuculline methiodide was added to the bathing medium during the recording period and not 40 minutes or more prior to recording. Clearly the concentration of bicuculline methiodide was critical with respect to the stability of evoked responses (see figure 6).

3.1.2 (iii) Onset and peak latencies of neocortical e.p.s.p.s.

The mean values for onset and peak latencies of subthreshold e.p.s.p.s were approximately 4ms and 13ms respectively . Table 6 lists the onset and peak latencies of e.p.s.p.s evoked by different inputs.

3.1.2 (iv) Action of AP5 and kynurenic acid on neocortical postsynaptic potentials.

Activation of NMDA receptors is an essential prerequisite for the induction of LTP at some hippocampal synapses (Collingridge et al., 1983; Errington et al., 1987), yet the expression of hippocampal LTP is mediated by non-NMDA receptors (Davies et al., 1990; Malinow & Tsien, 1990). At the

FIGURE 6: ACTION OF BICUCULLINE METHIODIDE ON NEUROTRANSMISSION IN THE ADULT NEOCORTEX.

A: Postsynaptic potential evoked at 0.1Hz, recorded in a layer V neurone; resting membrane potential, -87mV; input resistance, 27MΩ.

(i): PRIOR TO EXPOSURE TO BICUCULLINE METHIODIDE.

Electronic average of 8 successive e.p.s.p.s.

(ii): ACTION OF BICUCULLINE METHIODIDE.

Specimen record illustrating the effects of 2μM bicuculline methiodide after 15 minutes exposure.

(iii): EXCITATORY AMINO ACID ANTAGONIST BLOCKS BICUCULLINE METHIODIDE INDUCED CONVULSIVE ACTIVITY.

Specimen record showing the effect of bath applied 1mM kynurenic acid, an excitatory amino acid antagonist. The action of kynurenic acid was reversible (data not shown).

B: Record shown here is A(ii) replotted on a longer timescale. Note the duration of the depolarizing afterpotential ()1sec).

40mV

80ms

800ms
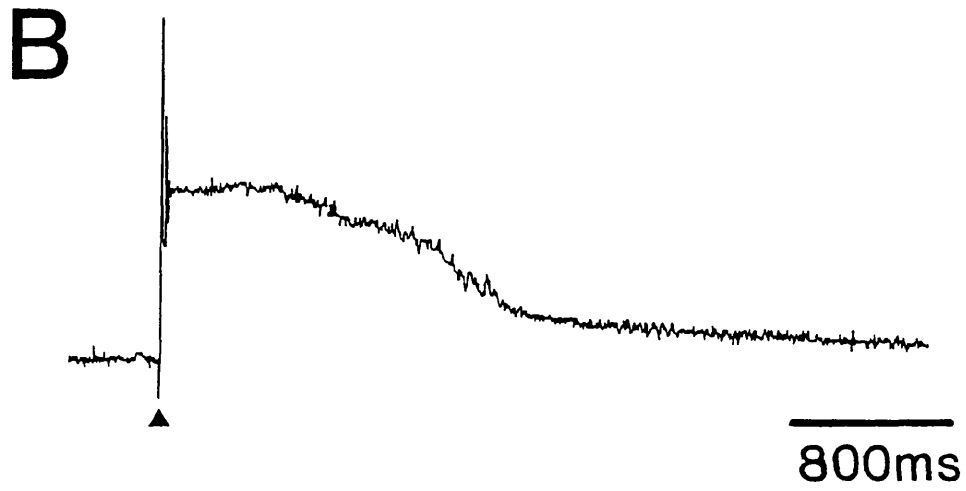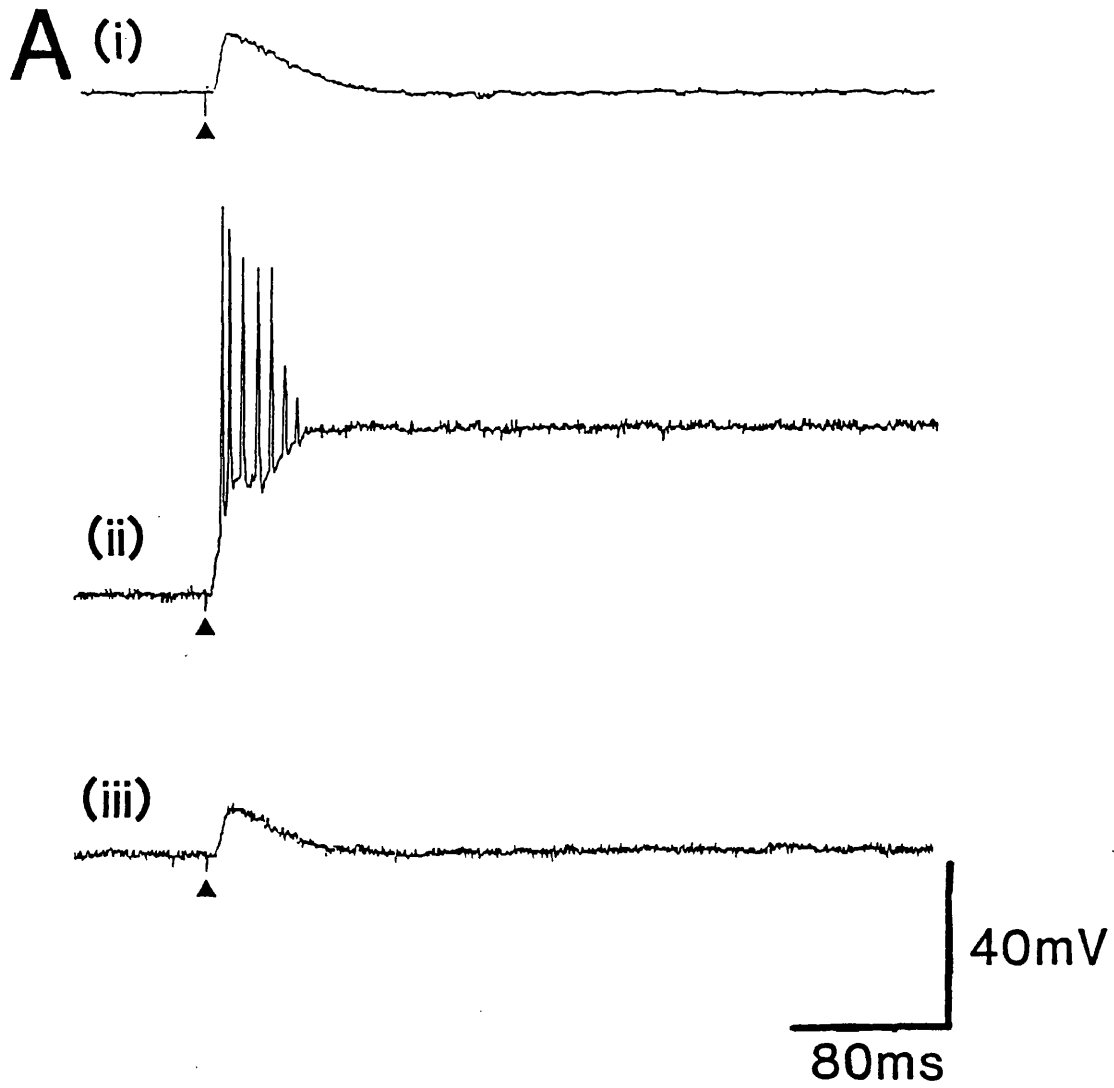
TABLE 6: ONSET AND PEAK LATENCIES OF SUBTHRESHOLD
E. P. S. P. S (including those coupled to late firing dischages for
calculation of onset latencies).

Units in milliseconds,

SUMMARY OF ONSET AND PEAK LATENCIES OF E. P. S. P. S FOR ALL LAYERS.

Input	Mean Onset	SD,	Range,	n,	Mean Peak,	SD,	Range,	n,
LATERAL	3,9	1,47	1,5-6,9	42	13,9	6,13	4,5-36,6	37
MEDIAL	4,1	1,86	1,2-9,8	48	12,5	4,67	5,2-31,4	47
WHITE MATTER	4,0	1,49	2,0-6,6	7	12,7	4,48	7,6-20,0	7
GREY MATTER	2,7	0,70	2,0-3,4	3	8,0	1,32	6,5-9,2	3

SUMMARY OF ONSET AND PEAK LATENCIES OF E. P. S. P. S RECORDED IN LAYER V
NEURONES.

Input	Mean Onset	SD,	Range,	n,	Mean Peak,	SD,	Range,	n,
LATERAL	3,9	1,3	1,6-6,5	33	13,9	6,36	4,5-36,6	31
MEDIAL	4,0	1,80	1,2-9,8	40	12,7	4,92	5,2-31,4	39
WHITE MATTER	3,4	1,04	2,0-4,6	5	10,5	2,83	7,6-15,0	5
GREY MATTER	2,8	-	-	1	6,5	-	-	1

SUMMARY OF ONSET AND PEAK LATENCIES OF E. P. S. P. S RECORDED IN LAYER VI
NEURONES.

Input	Mean Onset	SD,	Range,	n,	Mean Peak,	SD,	Range,	n,
LATERAL	4,0	2,30	1,5-6,9	4	14,9	7,02	1,9-4,5	3
MEDIAL	3,0	1,08	1,9-4,5	4	9,2	1,85	6,7-10,7	4
WHITE MATTER	4,8	-	-	1	20,0	-	-	1
GREY MATTER	2,0	-	-	1	8,2	-	-	1

SUMMARY OF ONSET AND PEAK LATENCIES OF E. P. S. P. S RECORDED IN LAYER III
NEURONES.

Input	Mean Onset	SD,	Range,	n,	Mean Peak,	SD,	Range,	n,
LATERAL	4,1	1,77	2,7-6,7	4	11,9	3,70	7,7-14,7	3
MEDIAL	5,4	2,67	3,5-9,3	4	13,0	3,02	10,7-17,3	4
WHITE MATTER	6,6	-	-	1	16,2	-	-	1
GREY MATTER	3,4	-	-	1	9,2	-	-	1

time that the work described in this thesis was commenced, little was known about the involvement of NMDA and non-NMDA receptors in neurotransmission in sensorimotor cortex. Given the importance of NMDA receptors for models of neural plasticity, the NMDA receptor antagonist, AP5, was used to assess their involvement in neurotransmission at neocortical synapses. Also, the broad spectrum glutamate receptor antagonist, kynurenic acid, was used to assess the involvement of non-NMDA receptors.

The action of AP5 was examined on 53 postsynaptic potentials recorded from 30 neurones in the presence of 1.1mM Mg^{2+} and evoked at resting membrane potential (mean for sample -80mV). In addition, 4 of these neurones were exposed concurrently to kynurenic acid and AP5 and a further 3 exposed to kynurenic acid alone. Half of the neurones were also bathed in micromolar concentrations of bicuculline methiodide.

Bath application of D-AP5 (10-20µM) or DL-AP5 (17-40µM) produced a reduction in amplitude and/or duration in 47 out of 53 postsynaptic potentials (89%) (see table 7 for summary). Kynurenic acid (0.5-2.5mM) applied alone or in the presence of AP5, reduced or abolished 11 out of 11 postsynaptic potentials. In general, AP5 reduced a late component of postsynaptic potentials (see figure 7, 8 & 11A) whereas kynurenic acid reduced all components (see below and figures 7 & 8). Both drugs exerted their full effect within 10-15 minutes of exposure and were differentially reversible, AP5 within 15-30 minutes and kynurenic acid within 20-40minutes.

Figure 7 illustrates the typical action of AP5 and the combined effect of AP5 and kynurenic acid on a subthreshold e.p.s.p. recorded in a layer V neurone. 10µM D-AP5 reduced the magnitude of the e.p.s.p., the greatest effect seen in the late portion whilst the onset latency and the initial

TABLE 7: NEURONES AND POSTSYNAPTIC POTENTIALS EXPOSED TO 10–20µM D–AP5.

	No. of Neurones,	No. of p.s.p.s,	No. of e.p.s.p.s	No. of AP5-sensitive e.p.s.p.s	No. of i.p.s.p.s	No. of AP5-sensitive i.p.s.p.s,
LAYER V;	25	43	37	35	6	4
LAYER VI;	3	6	6	4	0	0
LAYER III;	2	4	4	4	0	0
TOTAL	30	53	47	43	6	4

The 4 AP5-insensitive e.p.s.p.s had peak amplitudes <5mV.
All AP5-sensitive e.p.s.p.s had peak amplitudes >5mV.

FIGURE 7: ACTION AND TIME COURSE OF AP5 AND KYNURENIC ACID ON A LAYER V E. P. S. P.

A: Electronic averages of 8 successive e.p.s.p.s evoked by lateral
      white matter stimulation at a rate of 0.1Hz recorded in a layer
      V neurone. This cell had an action potential threshold 32mV
      above the resting membrane potential, an action potential
      amplitude of 106mV (measured from resting membrane potential
      to peak) and an input resistance of 55MΩ. The bathing medium
      contained 1μM bicuculline methiodide. Calibration as in B.

(i):   Control response, prior to bath application of AP5.

(ii): Effect of bath applied 10μM D-AP5 after 15 minutes exposure.

(III): Combined effect of bath applied 10μM D-AP5 and 1mM kynurenic
       acid, 18 minutes after the addition of kynurenic acid.

(iv): Partial recovery of evoked response 60 minutes after removal of
       D-AP5 and kynurenic acid.

B: Superimposed tracings records (i), (ii) & (iii) in A.
      Control (1), D-AP5 (2) and D-AP5 & kynurenic acid (3).
      Symbols (●) & (O) mark the latencies used to plot the graph
      below.

C: Graph showing the differential action of D-AP5 and kynurenic
      acid. Y-axis is amplitude of e.p.s.p. and X-axis is time. Each
      data point is the mean of 8 successive e.p.s.p.s measured at
      latencies of 12ms (●) and 25ms (O).
      AP5 has a greater effect at 25ms than 12ms whereas kynurenic
      acid in the presence of AP5 reduces the potential at both
      latencies. Note the actions of AP5 reached equilibrium within 10
      minutes of application and the combination of AP5 with
      kynurenic acid by 15 minutes after the onset of exposure to
      kynurenic acid.
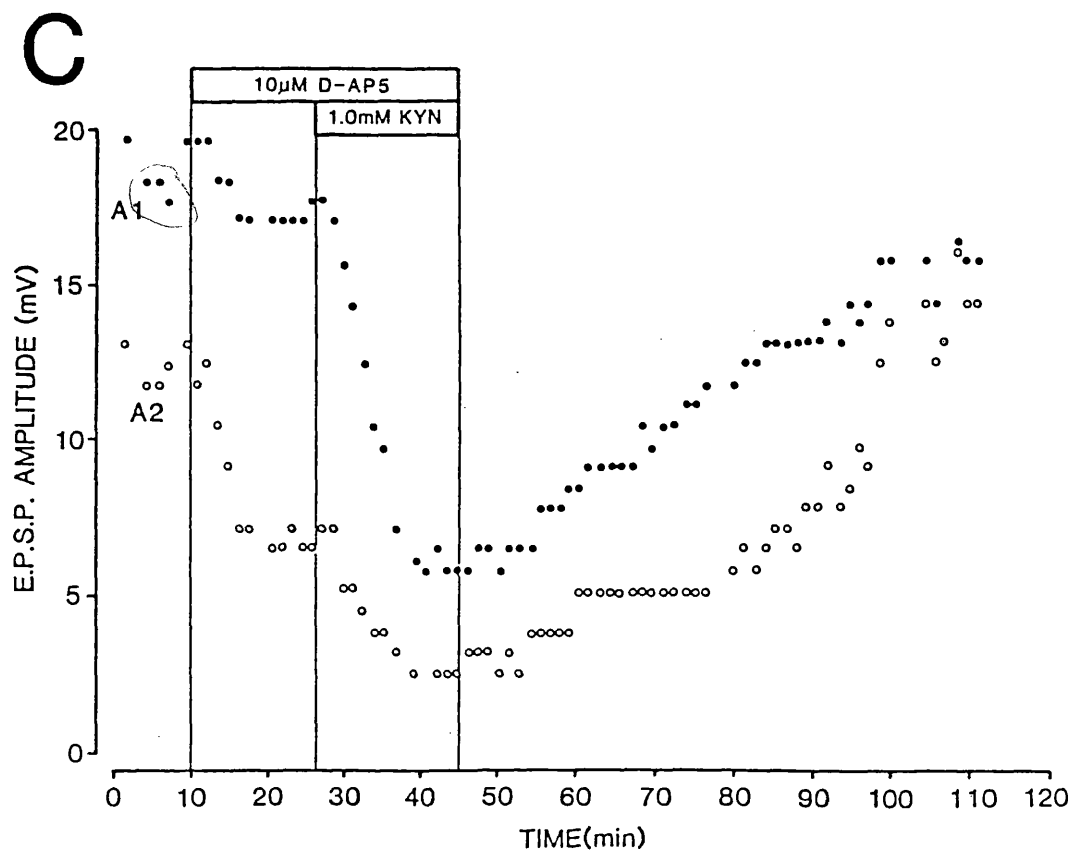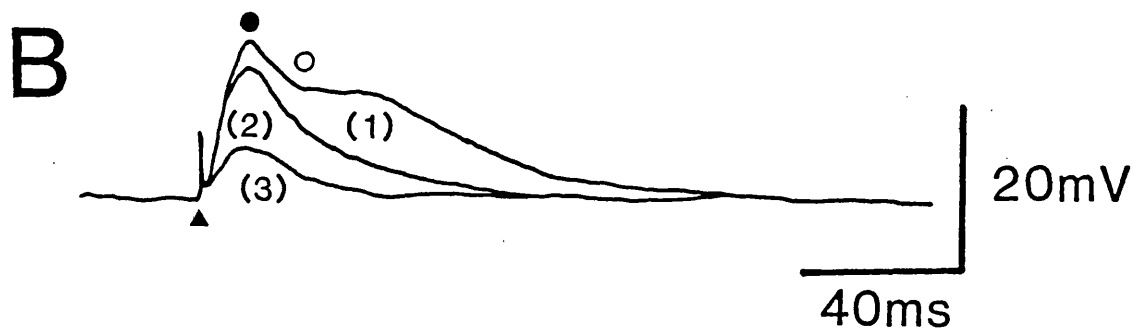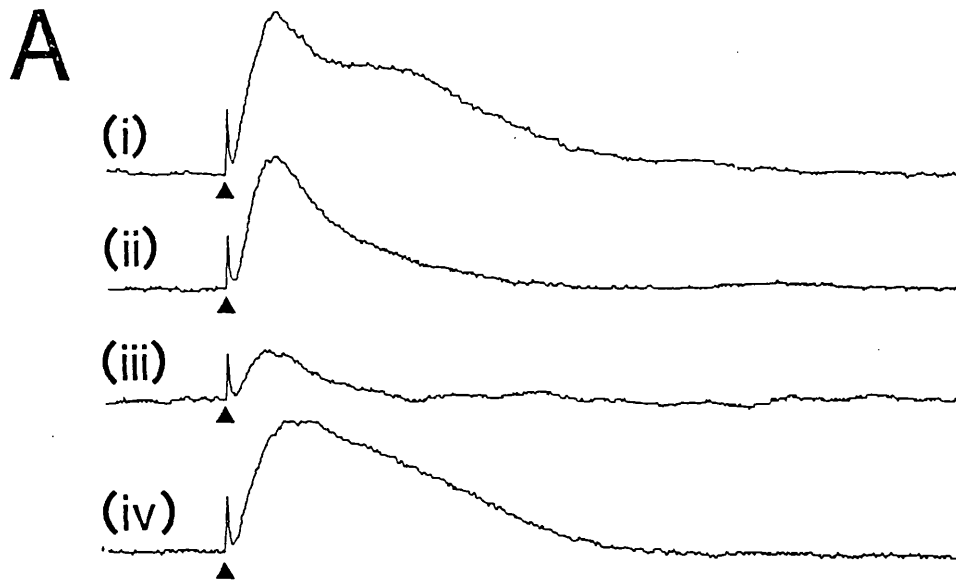
slope of the e.p.s.p. were unaffected. Together, 10μM D-AP5 and 1mM

kynurenic acid produced a further reduction in the e.p.s.p.. The action of

both drugs was readily reversible.

Figure 8 shows the action of AP5 and kynurenic acid on a subthreshold

e.p.s.p. coupled to a late firing discharge. 20μM D-AP5 abolished the late

firing discharge without affecting the subthreshold e.p.s.p.. The addition

of 2mM kynurenic acid to the bathing medium abolished the e.p.s.p..

In all cases where AP5 had an effect, the AP5-sensitive component of

the e.p.s.p. was always preceded by an AP5-insensitive component. Table 8

lists the mean onset and peak latencies of e.p.s.p.s recorded in layer V

neurones: (i) prior to the application of AP5 and (ii) the earliest latency

at which AP5 had an effect and the latency where it produced the maximum

reduction in amplitude. In 90% of cases, the onset of the action of AP5

preceded the peak of the e.p.s.p.

The presence of bicuculline methiodide was not a prerequisite in order

to show the effect of AP5 (compare figures 7 & 9).

Bath application of AP5 and kynurenic acid did not induce any

consistent changes in resting membrane potential or input resistance (see

table 9 and figures 9B & 9C) .

3.1.2 (v) Voltage-dependency of neocortical e.p.s.p.s.

In an attempt to demonstrate a postsynaptic action of NMDA receptors,

attributable to the voltage-dependent Mg^{2+}-blockade of the NMDA channel,

the voltage-dependency of 43 e.p.s.p.s recorded in layers III, V & VI of rat

sensorimotor cortex were examined prior to and during the application of

AP5. Of this sample, 39 e.p.s.p.s recorded in layer V/VI exhibited a

conventional voltage-dependency (figures 9 & 10).

FIGURE 8: ACTION OF AP5 & KYNURENIC ACID ON AN EARLY SUBTHREHOLD E. P. S. P. COUPLED TO A LATE FIRING DISCHARGE.

Specimen records of postsynaptic potentials recorded in a layer V neurone, evoked by lateral white matter stimulation at a rate of 0.1Hz. The neurone had a resting potential of -80mV and input resistance of 30MΩ. The bathing medium contained 1μM bicuculline methiodide.

Upper record: two superimposed traces, the upper trace is the control response consisting of an early subthreshold e.p.s.p. coupled to a late firing discharge, the lower trace shows the effect of bath applied 20μM D-AP5 after 50 minutes exposure. Note that the depolarizing slope and peak of the early e.p.s.p. is unaffected by D-AP5 whereas the late firing discharge is abolished.

Lower record: combined action of 20μM D-AP5 and 2mM kynurenic acid, 35 minutes after addition of kynurenic acid to bathing medium. Note that the synaptic potential is entirely abolished.

Neither AP5 or kynurenic acid induced changes in either resting membrane potential or input resistance.

40mV

40ms
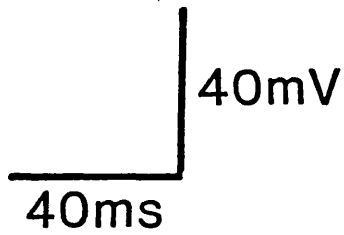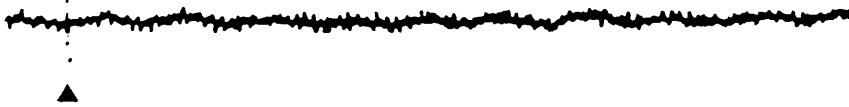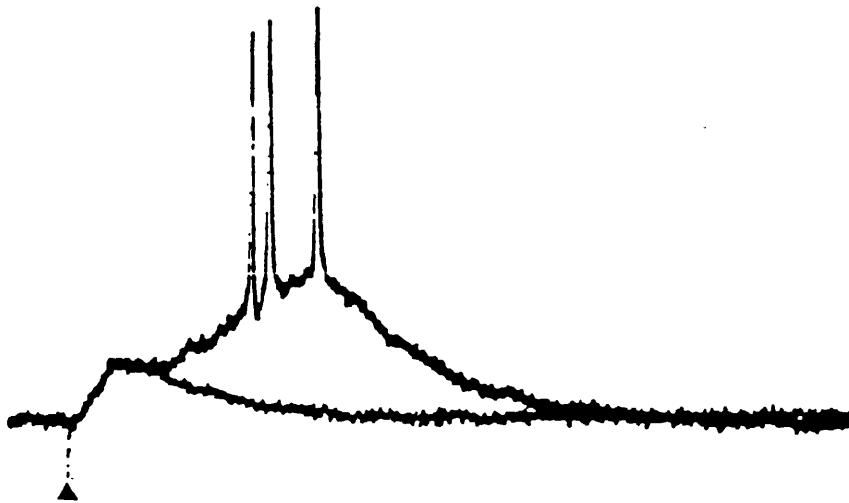
TABLE 8: ONSET LATENCY AND PEAK EFFECT OF AP5 ON E. P. S. P. S RECORDED IN LAYER V NEURONES.

LAYER V MEDIAL E.P.S.P.S (only subthreshold e.p.s.p.s included) .

Units (ms).	Onset of e.p.s.p.	Peak of e.p.s.p.	Onset of AP5 effect.	Peak effect of AP5.
Mean:	3.8	12.6	9.3	20.4
SD.:	2.04	3.77	4.62	9.74
Range:	1.2-8.7	7.7-18.6	2.9-19.0	8.0-36.0
n:	12	12	12	12

5 e.p.s.p.s excluded: 1 subthreshold e.p.s.p. coupled to firing discharges, 2 late latency suprathreshold e.p.s.p.s, 1 early latency suprathrehold e.p.s.p. and 1 small e.p.s.p. (amplitude >5mV) where AP5 had no effect.

AP5 abolished all firing discharges.

LAYER V LATERAL E.P.S.P.S. (only subthreshold e.p.s.p.s included)

Units (ms).	Onset of e.p.s.p.	Peak of e.p.s.p.	Onset of AP5 effect.	Peak effect of AP5.
Mean:	3.7	14.0	8.1	27.3
SD.:	1.30	3.86	1.72	14.67
Range:	1.6-5.1	7.9-20.6	5.8-10.6	8.0-50
n:	10	10	10	10

5 e.p.s.p.s excluded: 3 no effects, 1 subthreshold e.p.s.p. coupled to late firing discharge and 1 late latency firing discharge.

AP5 abolished all firing discharges.

The onset of the effect of AP5 preceded the peak of the e.p.s.p. (measured prior to the application of AP5) in 10 out of 12 medial and 9 out of 10 lateral e.p.s.p.s.

TABLE 9: THE EFFECT OF EXCITATORY AMINO ACID ANTAGONISTS ON APPARENT INPUT RESISTANCE.

Significance values (control versus AP5, kynurenic acid & washout) obtained using unpaired Student's t-test.

Neurone A.   ---- Units in MΩ ----

Drug	Mean	SD.	SEM.	n	
Control	55.7	2.27	0.66	12	
10µM D-AP5	55.0	2.85	0.79	13	P>0.5
1mM Kyn +AP5	54.6	3.1	0.86	13	0.5>P>0.1
Washout	54.9	2.8	0.77	13	0.5>P>0.1

Neurone B.   ---- Units in MΩ ----

Drug	Mean	SD.	SEM.	n	
Control	49.8	1.76	0.46	15	
10µM D-AP5	50.0	1.33	0.26	26	0.5>P>0.1
1mM Kyn +AP5	49.9	0.88	0.18	26	0.5>P>0.1
Washout	49.9	1.25	0.28	20	0.5>P>0.1

Input resistance calculated from steady-state voltage deflection from resting membrane potential produced by intracellular injections of -0.4nA.

Input resistance measured in AP5 after 15 minutes exposure to the drug. Measurements made in the presence of kynurenic acid (Kyn) were also in the presence of AP5. Washout measurements were made once the e.p.s.p.s evoked in each neurone had returned to >70% of their control size.

Neurone A is the same cell as in figure 6.

Neurone B had a resting membrane potential of -80mV.

FIGURE 9: AP5-INSENSITIVE AND AP5-SENSITIVE COMPONENTS OF E. P. S. P. S RECORDED IN A LAYER V NEURONE.

All records in A, B & C were recorded in a layer V neurone with a resting membrane potential of -70mV and input resistance of 26MΩ. The bathing medium did not contain bicuculline methiodide.

A: *Upper records:* superimposed electronic averages of 8 successive e.p.s.p.s.; uppermost record is a control response and the lower record shows the effect of bath applied 20μM D-AP5 after 12 minutes exposure. The action of AP5 was fully reversible (washout record not shown).

Bottom record: An electronic subtraction of the upper two averages showing the AP5-sensitive component.

B: The upper two traces are superimposed specimen records showing the passive membrane response to a -0.4nA current pulse except that during one pulse an e.p.s.p. was elicited. The e.p.s.p. shown is a control response.

The bottom trace is an electronic subtraction of the upper two traces. This cancels out the underlying change in the membrane potential due to anomalous rectification.

C: The records shown here have been processed in the same as in B except in this case the e.p.s.p. had been exposed to 20μM D-AP5 for 14 minutes.

Note that in B and C the voltage deflection and anomalous rectification are identical. AP5 did not alter the resting membrane potential or input resistance in the hyperpolarizing direction.
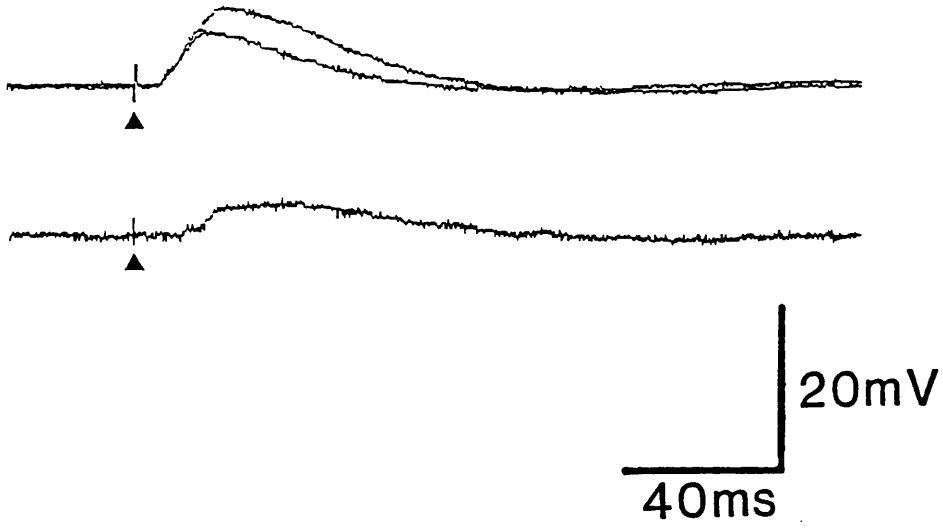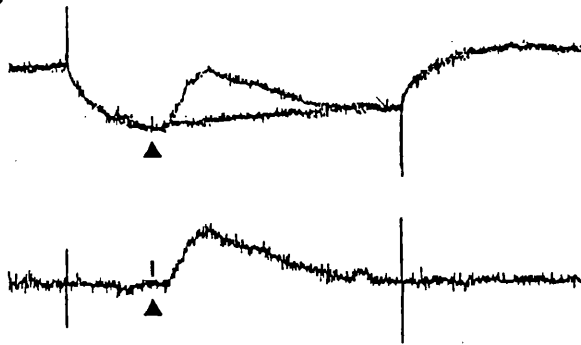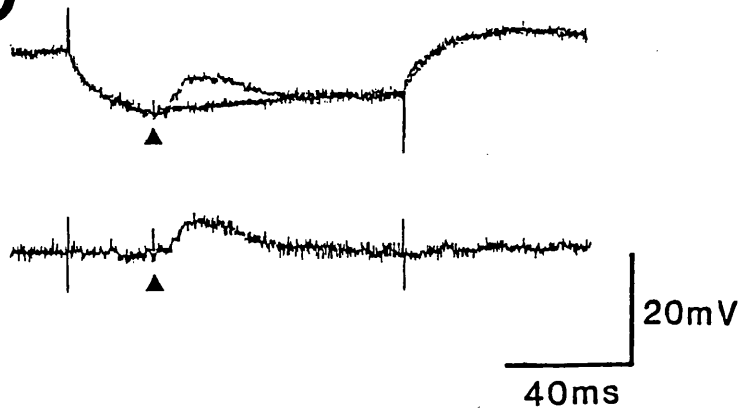
FIGURE 10: VOLTAGE-DEPENDENCY OF AP5-SENSITIVE AND AP5-INSENSITIVE COMPONENTS.

Same neurone as in figure 9.

A: Specimen records showing the control e.p.s.p. after subtraction of the underlying passive voltage changes induced by the input current (see figure 9B). The input current (in nA) is noted to the left of each record.

B: Records showing the effect of 20μM D-AP5 on the e.p.s.p. after 14-15 minutes exposure, i.e. the AP5-insensitive component of the e.p.s.p.. Records were treated as in A. Note the classical nature of the AP5-insensitive component; initial slope and magnitude increasing with hyperpolarizing current.

C: Electronic subtractions of the AP5 treated e.p.s.p. from the control e.p.s.p. at each input current. The resultant trace is the AP5-sensitive component. Note the conventional voltage-dependency of this component.

Note that the control e.p.s.p., AP5-insensitive component and AP5-sensitive component are all relatively independent of changes in somatic membrane potential induced by input current.
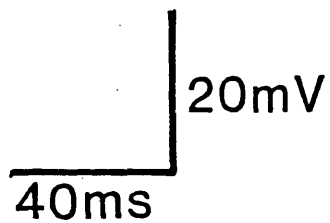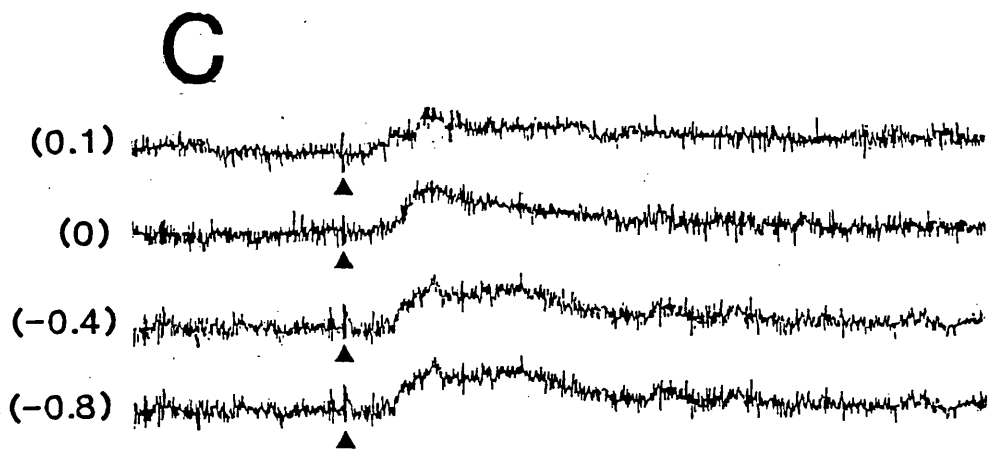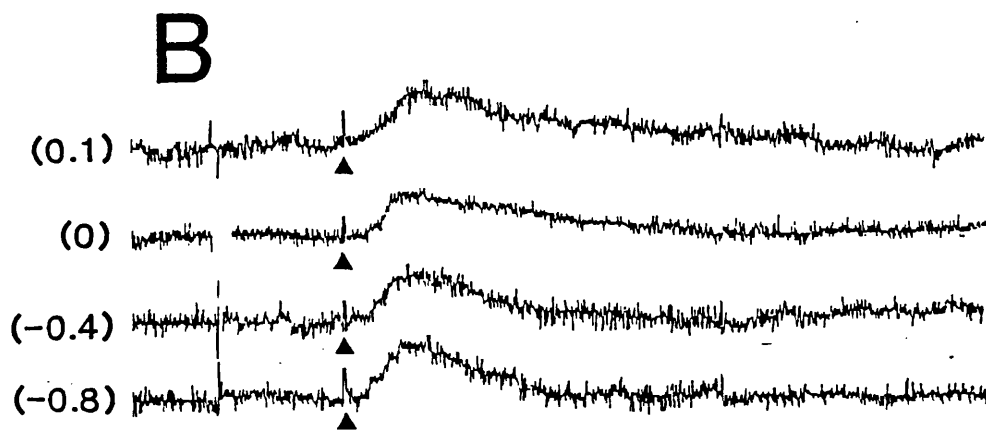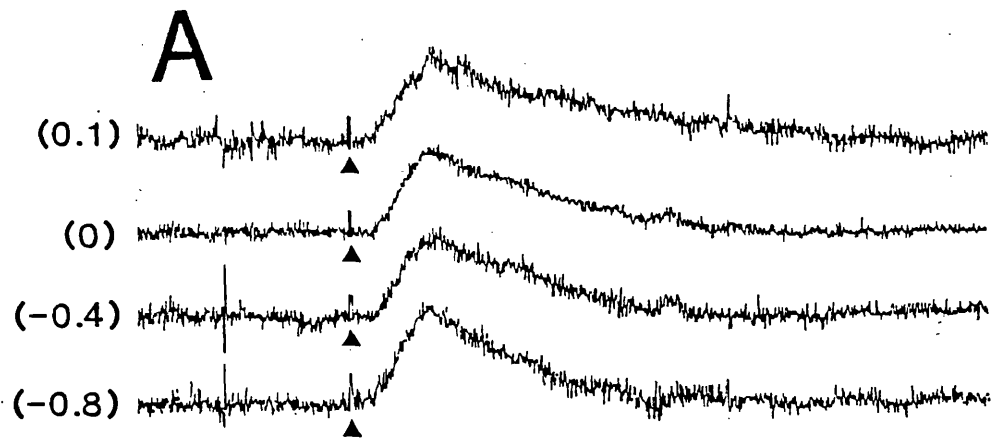
AP5 reduced the amplitude and/or duration of these e.p.s.p.s at all membrane potentials and the voltage-dependency of both AP5-insensitive and AP5-insensitive components were found to be conventional (figure 10), both in the absence and presence of bicuculline methiodide.

Two layer V neurones had e.p.s.p.s which had a non-conventional voltage-dependency, i.e. the amplitude of the e.p.s.p.s increased with membrane depolarization and decreased with hyperpolarization. However, both neurones were lost before the action of AP5 on the voltage-dependency of their e.p.s.p.s could be established.

Two layer III neurones had an non-conventional e.p.s.p. evoked by one of their two inputs (i.e. one input gave rise to a conventional e.p.s.p. whereas the other input elicited an non-conventional e.p.s.p.). In both neurones bath application of 20µM D-AP5 reduced the magnitude of their e.p.s.p.s but did not abolish non-conventional voltage-dependency.

3.1.2 (vi) Action of reduced concentration of extracellular Mg^{2+} ions on e.p.s.p.s recorded in layer V neurones.

The voltage-dependency of the NMDA channel is dependent on the concentration of extracellular Mg^{2+} ions (Mayer et al., 1984). To see if the concentration of Mg^{2+} ions (1.1mM) contained in the bathing medium was sufficient to confer voltage-dependency on the NMDA channels, evoked responses recorded in two slices of neocortex were monitored firstly in normal csf, and then in magnesium-reduced csf. AP5 was used to assess the degree of NMDA receptor mediated activity.

In both slices, perfusion with magnesium-reduced csf caused a progressive growth of the evoked potentials. In one slice, this resulted in the development of spreading depression (Leao, 1944). In the other slice, the enhancement of the e.p.s.p. was abolished by the addition of AP5 to the magnesium-reduced csf and is illustrated in figures 11 and 12. Exposure to magnesium-reduced csf did not effect resting membrane potential or input resistance, suggesting that the observed enhancement of the e.p.s.p. in magnesium-reduced csf was due to an increase in the activity of NMDA receptors.

FIGURE 11: ENHANCEMENT OF A LAYER V E.P.S.P. IN Mg^{2+}-REDUCED CSF.

All records shown were evoked by lateral white matter stimulation at 0.1Hz and recorded in a layer V pryamidal neurone; resting membrane potential, -75mV; input resistance, 20MΩ. The bathing medium contained 0.5 μM bicuculline methiodide. Calibration as in B.

A: Each record is an average of 16 successive e.p.s.p.s.

A(i):   Control e.p.s.p. in the presence of normal csf.

A(ii):   E.p.s.p. after 12 minutes exposure to Mg^{2+}-reduced csf .

A(iii):   E.p.s.p. after 52 minutes exposure to Mg^{2+}-reduced csf and 30 minutes exposure to 10μM D-AP5.

A(iv):   Effect of AP5, 22 minutes after the re-introduction of normal csf.

B: Superimposition of averages in A(ii),(iii) & (iv) showing subthreshold enhancement of the e.p.s.p. (1), the effect of 10μM D-AP5 on enhanced e.p.s.p. in Mg^{2+}-reduced csf (2), the additional reduction of the e.p.s.p. produced by the re-introduction of 1.1mM Mg^{2+} whilst in the presence of AP5.

The symbols (● & O) mark the latencies at which amplitudes were measured for the graph in figure 12.

C: Specimen example of a suprathreshold e.p.s.p. after 15 minutes exposure to Mg^{2+}-reduced csf.
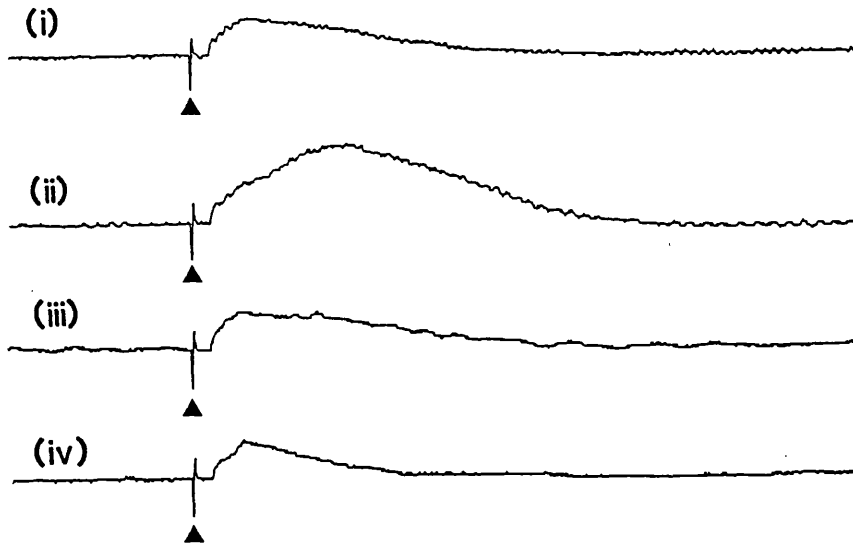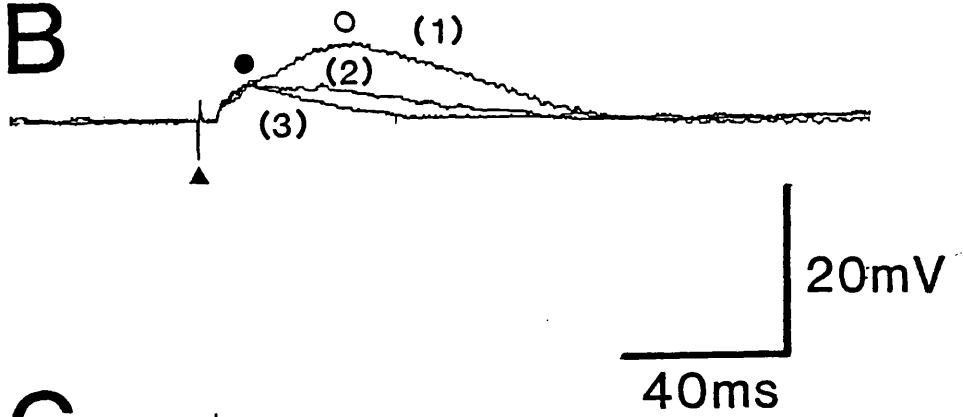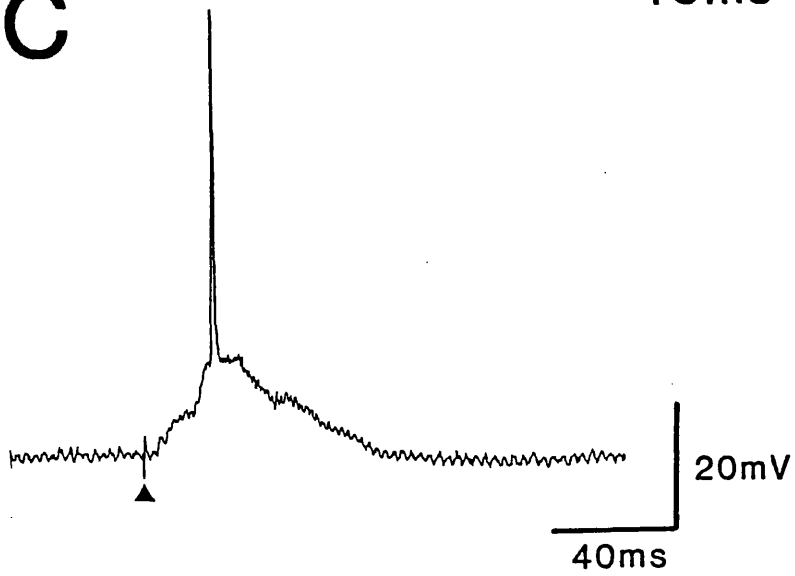
FIGURE 12: TIME COURSE ILLUSTRATING THE EFFECT OF Mg^{2+}-REDUCED CSF AND AP5 ON AN E. P. S. P. RECORDED IN A LAYER V NEURONE.

Graph shows the time course of action of Mg^{2+}-reduced csf and the antagonistic effect of AP5 on the e.p.s.p. shown in figure 11.

The data points are measurements of e.p.s.p. amplitude made from electronic averages of 8 successive e.p.s.p.s at latencies of 12ms (+) and 36ms (x). The horizontal bar of squares denotes the period when the slice was perfused with Mg^{2+}-reduced csf. The row of diamonds denotes the period when the slice was exposed to 10 μM D-AP5. The inverted triangles denote the presence of ≯1 synaptically evoked action potentials during the capture of the electronic average which is in vertical register.

AMPLITUDE OF EPSP. (mV).

TIME (min).
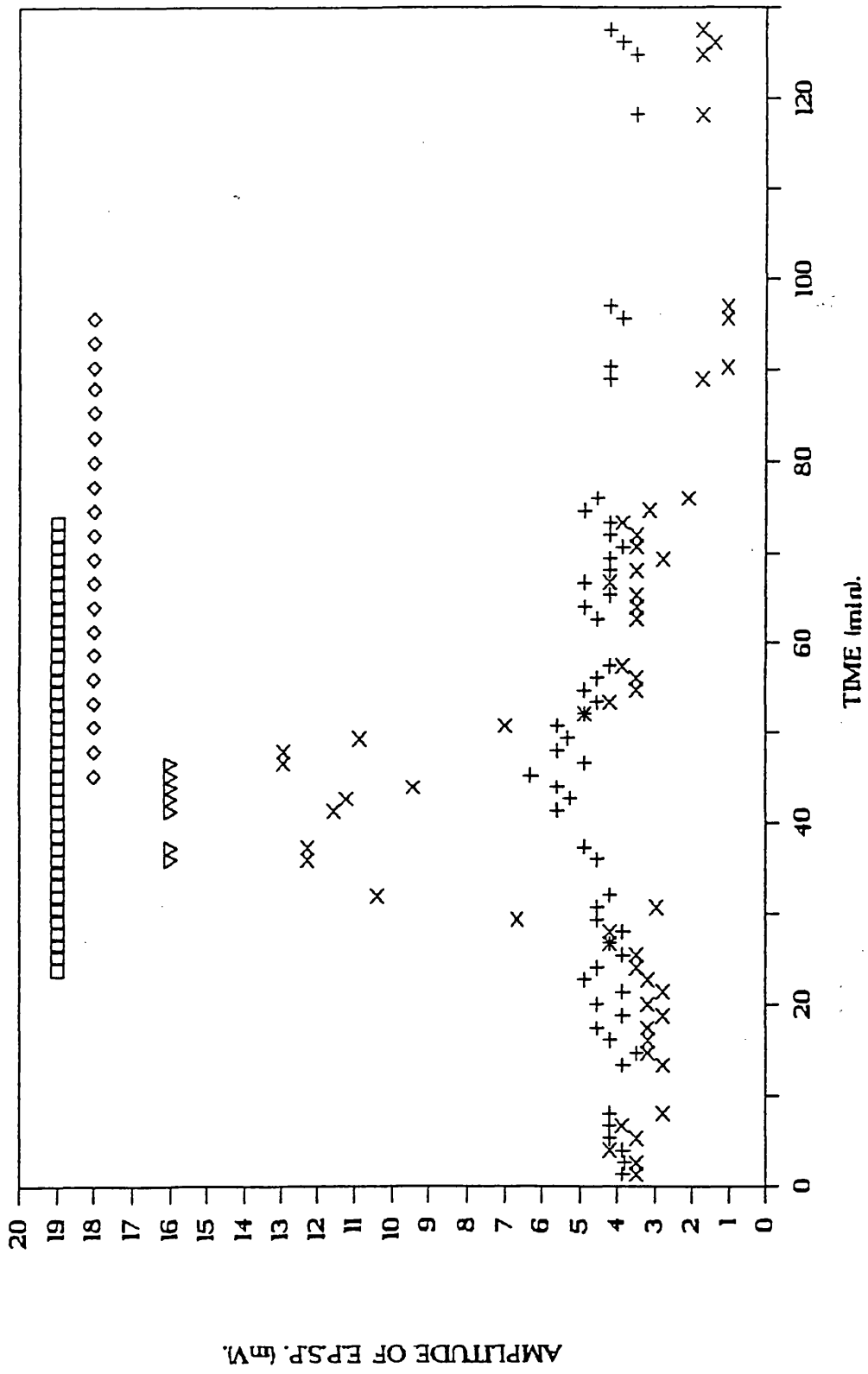
PART TWO: RESULTS OF INTRACELLULAR EXPERIMENTS - INDUCTION AND MAINTENANCE OF NEOCORTICAL LTP.

3.II.1 (i) Intracellularly recorded LTP in adult rat sensorimotor cortex.

LTP was successfully induced in 11 out of 50 neurones (22%) using either the pairing paradigm or high frequency stimulation of an afferent pathway.

3.II.2 (i) Conditioning using the pairing paradigm.

Fifty postsynaptic potentials recorded in 42 neocortical neurones were conditioned using the pairing paradigm. LTP was successfully induced in 6 out of 42 of these neurones (14%) and in 7 out of 50 inputs (see table 10). Potentiation became evident during and/or immediately after the end of the conditioning period. In all cases the potentiation was highly significant and lasted for more than 15 minutes. In two neurones, the same input was potentiated further by a second conditioning period: in one of these neurones, a second separate input was subsequently potentiated.

The first successful induction of LTP, observed during the work undertaken for this thesis is illustrated in figures 13 & 14 (*Expt. LTP1*). In this layer V neurone, LTP was induced in a subthreshold e.p.s.p. after being paired 23 times with conditioning current (conditioning was interrupted for 1 minute after the 8th pairing). Potentiation was first observed during the conditioning period and continued to increase once conditioning was terminated. Potentiation of the conditioned e.p.s.p. was manifest in 3 ways: (i) transformation from a subthreshold to suprathreshold potential, (ii) an immediate and maintained increase in amplitude of the earliest component of the e.p.s.p. (measured at 10ms) and

TABLE 10: INDUCTION OF LTP IN THE NEOCORTEX BY PAIRING P.S.P.S WITH PULSES OF DEPOLARIZING CURRENT.

Forty-two neurones were subjected to conditioning using the pairing paradigm. The summaries below list the number of separate pathways conditioned, the bracketed value is the total number of attempts.

NEURONES IN WHICH THE PAIRING PARADIGM SUCCESSFULLY INDUCED LTP.

| | No. of Neurones | *INPUTS POTENTIATED* | | | |
		No. of Lateral Inputs	No. of Medial Inputs	No. of White Matter.	No. of Grey Matter.
Layer V	4	3 (5†)	1 (1)	0 (0)	0 (0)
Layer III	2	1 (3†)	1 (1)	0 (0)	1 (2)

†: LTP induced on 2 separate occaisions in lateral input in the same neurone.

4 out of 6 neurones in which LTP was induced by pairing were in the presence of bicuculline methiodide.

NEURONES IN WHICH THE PAIRING PARADIGM FAILED TO INDUCE LTP*.

| | No. of Neurones | *INPUTS WHICH DID NOT POTENTIATE* | | | |
		No. of Lateral Inputs	No. of Medial Inputs	No. of White Matter.	No. of Grey Matter.
Layer V	32	22 (33)	15 (22)	1 (1)	1 (2)
Layer VI	4	2 (4)	0 (0)	0 (0)	2 (3)

*: LTD was induced by pairing in 3 neurones included in this sample.

17 of the neurones which failed to   express LTP after pairing were later conditioned by high frequency stimulation of an afferent input, this induced LTP in 3 neurones and LTD in one other.

23 out of 36 of neurones in which LTP failed to be induced by the pairing paradigm were bathed in medium containing bicuculline methiodide.

FIGURE 13: LTP INDUCED BY THE PAIRING PARADIGM IN A LAYER V NEURONE.

Records evoked by lateral white matter stimulation at a rate of 0.1Hz and recorded in a layer V neurone with a resting membrane potential of -68mV and input resistance of 31MΩ. Triangle marks the stimulus artefact. Calibration for A to E is in E.

A: Representative record of the control e.p.s.p..

B: Example of a pairing trial during the second conditioning train (2nd pairing of 25). The conditioning current pulse was 2nA in strength, 100ms in duration and timed to start 36ms after the test shock and was repeated 25 times. Upper trace input current, lower trace membrane voltage.

C: Single record showing potentiation of the e.p.s.p. 1 minute after conditioning..

D: Single record showing the continuing growth in the e.p.s.p. 11 minutes after conditioning.

E: Single record showing established (and increasing) potentiation in the e.p.s.p. 16 minutes after conditioning.
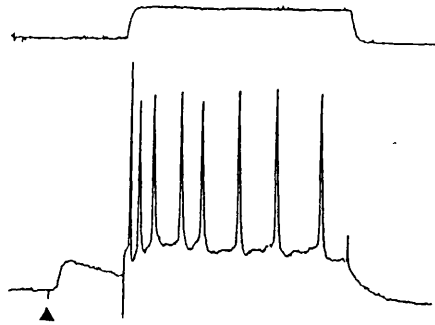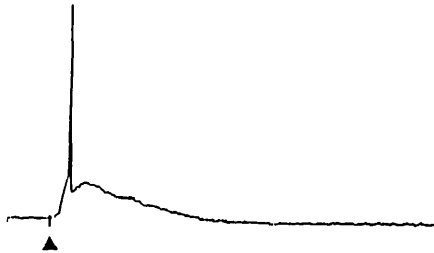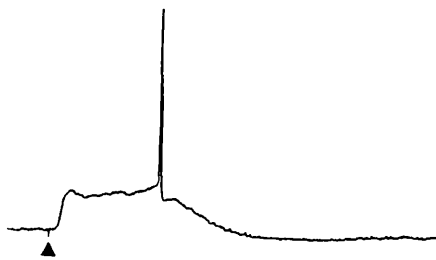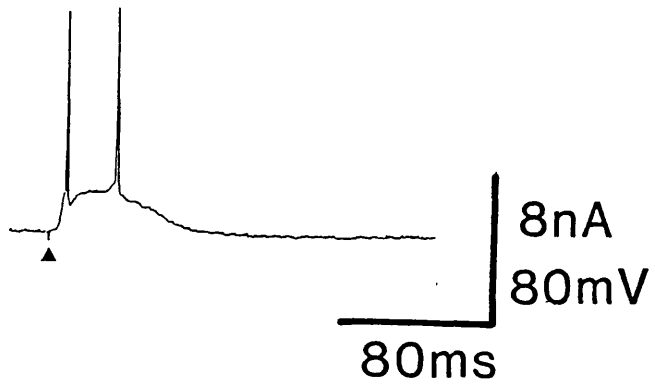
FIGURE 14: TIME COURSE OF SUPRATHRESHOLD LTP IN A LAYER V NEURONE.

Time course of the LTP induced in the lateral e.p.s.p. shown in figure 13.

In both A and B the two vertical bars denote the conditioning period when successive e.p.s.p.s, evoked by the test shock at 0.1Hz, were paired temporally with postsynatic injection of depolarizing current, sufficient to make the postsynaptic neurone fire a train of action potentials (see figure 13B).

Two attempts were made to induce LTP. During the first train (lesser bar) the e.p.s.p. was paired 8 times with a current pulse of 100ms duration timed to start 36ms after the test shock. The current strength was 1nA for the first 3 trails and 2nA thereafter. During the second train (larger bar) the e.p.s.p. was paired 25 times, using the same current parameter as in the latter part of the first train.

In both graphs the inverted triangle mark the e.p.s.p.s which had a short latency action potential (see figure 13C), a simple triangle marks a late latency action potential (see figure 13D) and superimposition of inverted and simple triangles mark e.p.s.p.s with two action potentials (see figure 13E).

Graph A: Data points represent the amplitude of each e.p.s.p. at a latency of 10ms. Note that potentiation is seen following the first condition period (lesser bar).

Graph B: Amplitude measured at a latency of 30ms. At this latency, potentiation is not evident until the end of the second condtioning train.
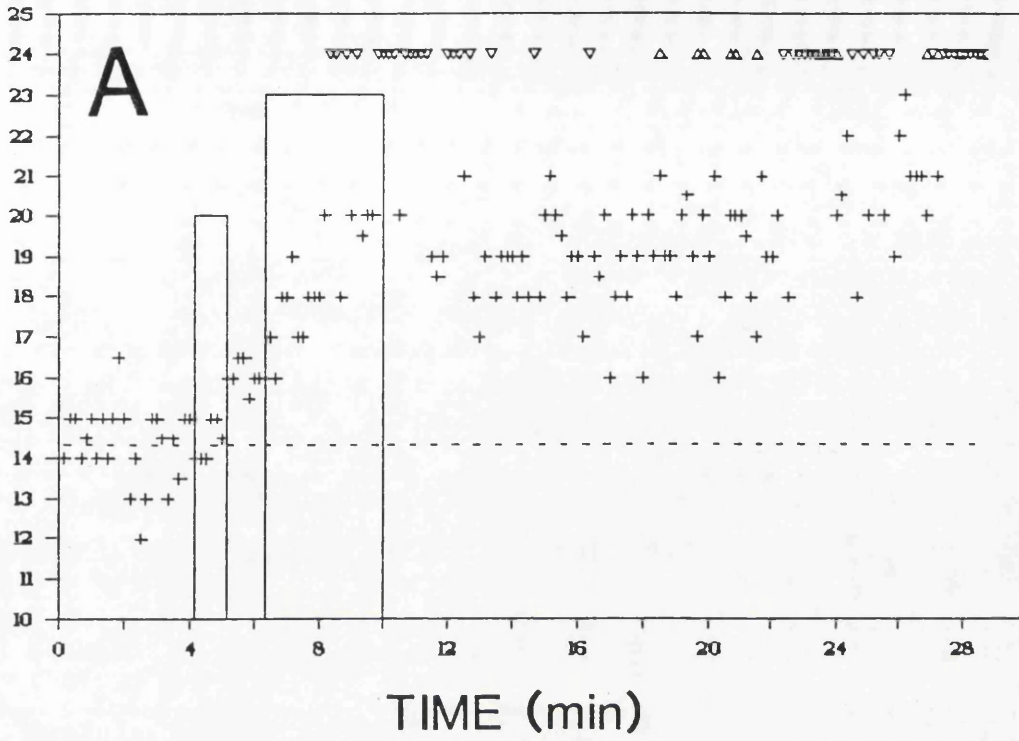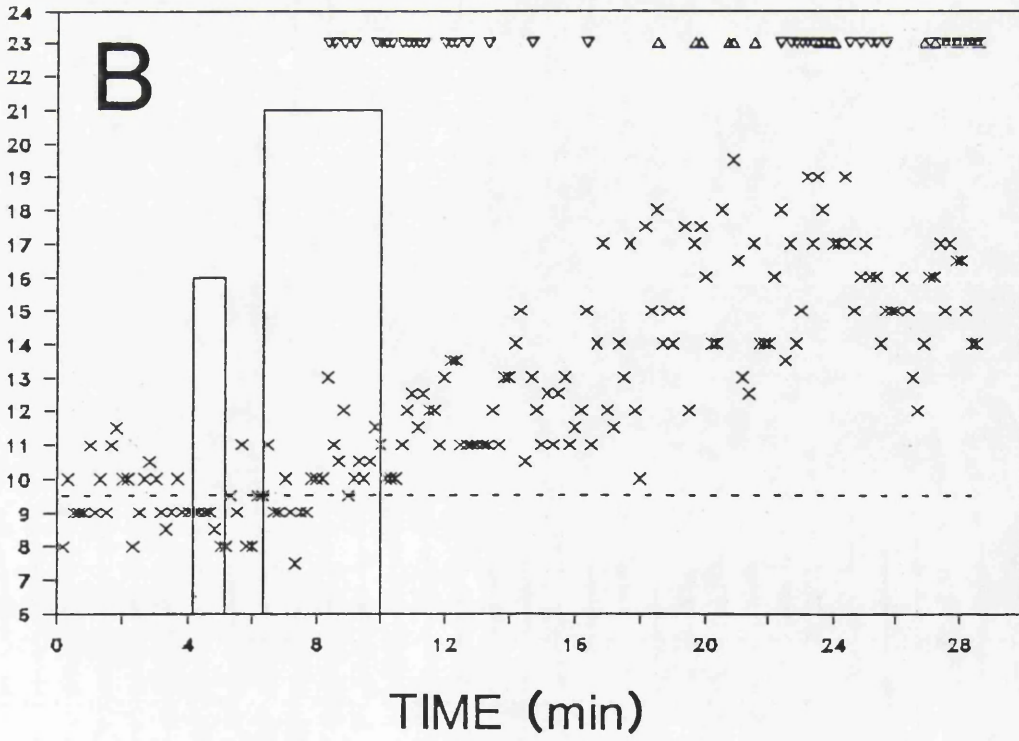
(iii) the onset of a slow progressive growth in a later component of the e.p.s.p. (measured at 30ms). Neither the conditioning procedure or the development of LTP affected the resting membrane potential or input resistance (apparent input resistance prior to conditioning (mean±SEM), $30.7\pm0.45M\Omega$, n=7; 15 minutes after conditioning, $30.6\pm0.55M\Omega$, n=25; P>>0.5, unpaired Student's t-test). The time course of this experiment illustrated in figure 14, shows a pre-conditioning control period of only 4 minutes. In fact, the actual control period lasted for 30 minutes, during which the e.p.s.p. remained stable. Unfortunately, due to a technical hitch (human error!), the recording of the first 26 minutes of the control period was lost.

It is clear from *Expt. LTP1* that the conjunction of synaptic activity with postsynaptic depolarization can result in an enduring increase in the efficacy of neocortical neurotransmission. However what is not so clear is whether the LTP was due to a general change in the postsynaptic neurone such that all synaptic inputs would have been potentiated or whether the change was localised to those synapses which were active during the presentation of conditioning current? To answer this question a series of experiments was performed in which two separate inputs were used to evoke postsynaptic potentials recorded in a single neurone; one input was conditioned whilst the other acted as a non-conditioned control.

3.II.2 (ii) Site of induction and synaptic specificity of LTP induced by the pairing paradigm..

To test the input specificity of LTP induced by the pairing paradigm, postsynaptic potentials were evoked by two independent inputs in 34 neurones. One postsynaptic potential to undergo conditioning and the other to act as an unconditioned control. LTP was induced in 5 of these

neurones, In each case only the postsynaptic potential which was paired with conditioning current developed LTP; the second unconditioned postsynaptic potential was not potentiated.

Figures 15 & 16 illustrate laterally and medially evoked postsynaptic potentials recorded in a layer V neurone (*Expt. LTP2*). The lateral response was identified as a composite postsynaptic potential, consisting of both an e.p.s.p. and i.p.s.p. whereas the medial response was an e.p.s.p.. Both potentials were evoked once every 10 seconds, 5 seconds apart. In this experiment only the lateral response was conditioned. Conditioning induced a highly significant and enduring increase in the size of the lateral response whereas the medial e.p.s.p., the unconditioned response, was not potentiated, i.e. only those synapses which were active during the presentation of the conditioning current exhibited potentiation. The unconditioned synapses, those which were out of phase during conditioning did not develop LTP.

The potentiation observed in the lateral response of *Expt. LTP2* may have been due to an increase in the e.p.s.p. or a decrease in the i.p.s.p. or both. The resting membrane potential was unaffected by conditioning and the establishment of LTP, however there was a slight increase in input resistance (prior to conditioning 34.2MΩ, during LTP 35MΩ; $0.05 > P > 0.002$. Student's unpaired t-test).

Figures 17 & 18 illustrate another experiment (*Expt. LTP3*) where LTP was induced in the paired input and not in the unpaired one. E.p.s.p.s were evoked by lateral and medial stimulation of the white matter. The lateral e.p.s.p. had two depolarizing peaks, one at 10ms and a larger one at 20ms. Pairing of the lateral e.p.s.p. with conditioning current timed to start just prior to the second peak induced a highly significant and enduring increase in the magnitude of the second peak of the lateral e.p.s.p..

FIGURE 15: LTP OF A COMPOSITE POSTSYNAPTIC POTENTIAL INDUCED BY THE PAIRING PARADIGM.

LTP induced in a laterally evoked synaptic potential in a layer V pyramidal neurone, resting membrane potential, -81mV; input resistance, 35MΩ. The medially evoked e.p.s.p. was not conditioned and therefore can be considered as an independent control of the specificity of conditioning within a single neurone. Bathing medium contained 1μM bicuculline methiodide.

Left: averages started 6 minutes before start of conditioning; *right*: averages started 1 minute after the end of conditioning.

Central panel is a single conditioning trial, the lateral p.s.p.s was paired 50 times with depolarizing current.

The (*) in the lateral control record marks the presence of two hyperpolarizing potentials. It was likely that these were potassium mediated i.p.s.p.s.
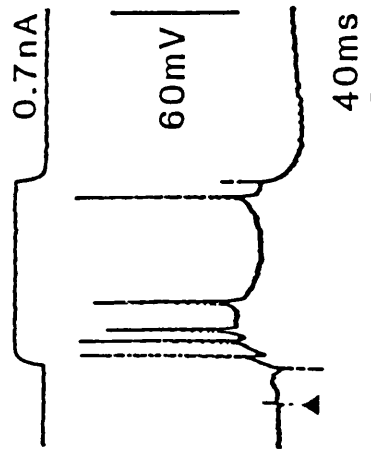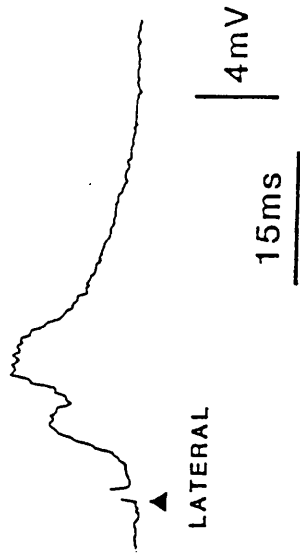
FIGURE 16: TIME COURSE OF LTP INDUCED BY THE PAIRING PARADIGM IN A COMPOSITE POSTSYNAPTIC POTENTIAL.

Time course of the experiment is shown in figure 15. LTP was induced in the lateral response by pairing it repetitively, 50 times, with depolarizing current (100ms long pulses, first 14 trials preceded the p.s.p., the remainder were timed to start 16ms after the test shock). The medial e.p.s.p. was not conditioned on this occasion.

Data points: minute averages of amplitude.

A: .   Lateral amplitude measured at 10ms latency after test shock.

B: Lateral amplitude at 20 ms, negative values reflect hyperpolarization.

C: Medial amplitude measured at 10ms.

Conditioning of the lateral p.s.p. (*between vertical lines*) induced profound and lasting changes in the lateral response. The medial e.p.s.p. was unaffected.

50 mins
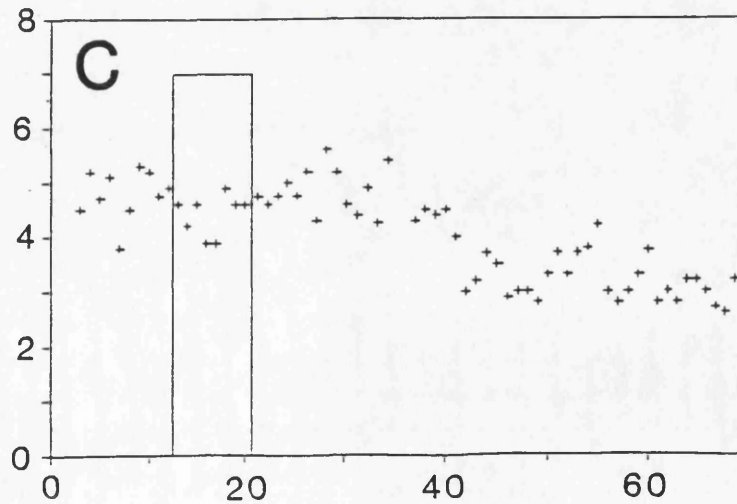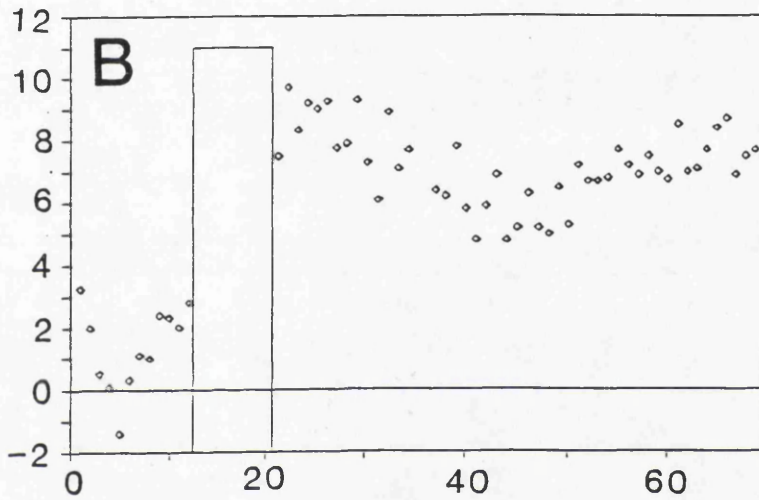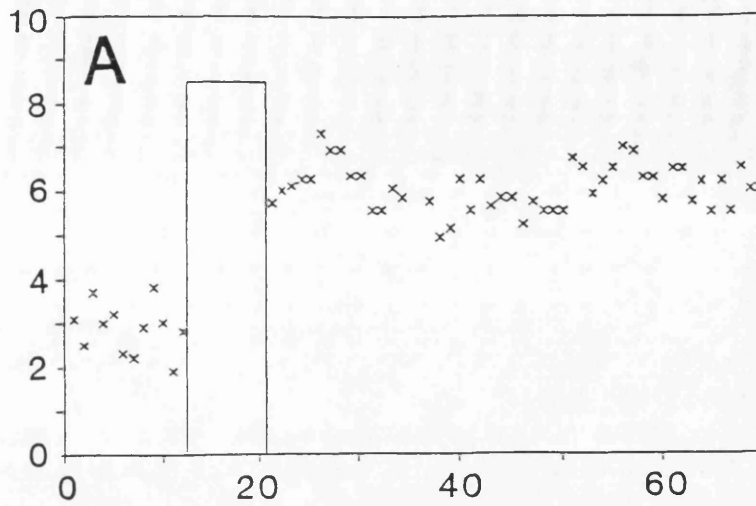
FIGURE 17: LTP OF A LATE COMPONENT OF AN E. P. S. P. INDUCED BY THE PAIRING PARADIGM.

Lateral and medial e.p.s.p.s were evoked alternately at 5sec intervals by white matter stimulation in a layer V neurone, resting membrane potential, −81mV; input resistance, 13MΩ. The bathing medium contained 1μM bicuculline methiodide.

Left: electronic averages of 16 successive e.p.s.p. started 10 minutes before the lateral e.p.s.p. was conditioned.

Right: electronic averages of 16 successive e.p.s.p.s started 10 minutes after the end of conditioning of the lateral e.p.s.p.. Note that the second peak of the lateral e.p.s.p. has been potentiated whereas the first peak of the lateral e.p.s.p. and the medial e.p.s.p. are not potentiated.

Central: single conditioning trial, the lateral e.p.s.p. was paired 34 times with a 2nA depolarizing pulse of 100ms duration, timed to start just after the second peak in the lateral e.p.s.p..

BEFORE

AFTER

LATERAL

MEDIAL

LATERAL

MEDIAL

2nA

40mV

40ms

15ms

8mV
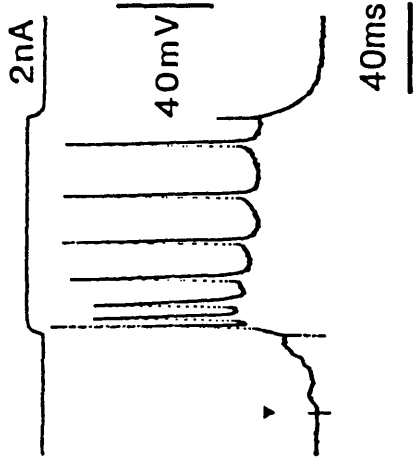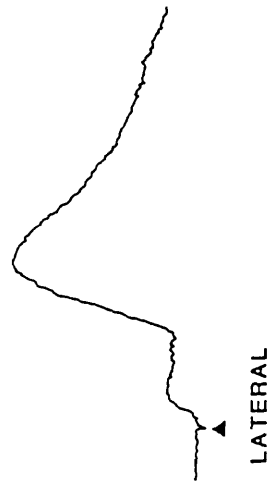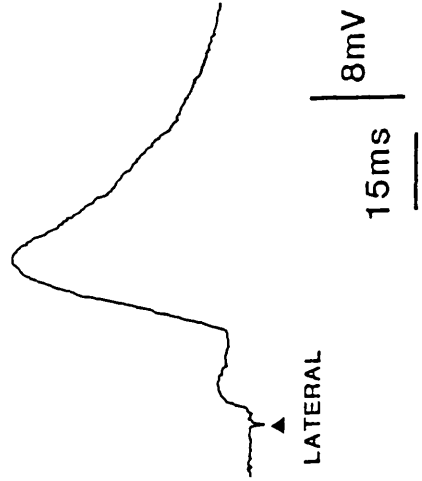
FIGURE 18: TIME COURSE OF LTP IN A LATE COMPONENT OF AN E. P. S. P. INDUCED BY THE PAIRING PARADIGM.

Time course of the experimental records shown in figure 17.

A: Minute averages of the amplitude of the lateral e.p.s.p. measured at latencies of 34ms (x) and 5ms (+) plotted against time. After a stable control period the lateral e.p.s.p. was conditoned, (*between vertical lines*). The conditoning depolarizing current was timed to start just after the second peak of the lateral e.p.s.p. (see figure 17, central panel) and was repeated 34 times (2nA, 100ms duration).

Conditioning induced a lasting increase in the second peak of the lateral e.p.s.p., the first peak was unaffected.

B: Minute averages of the medial e.p.s.p. measured at a latency of 11ms and plotted against time. The *vertical lines* drawn through the graph denote the period when the lateral e.p.s.p. underwent conditioning. Note that the medial e.p.s.p. was unaffected by the conditioning and subsequent potentiation seen in the lateral e.p.s.p..
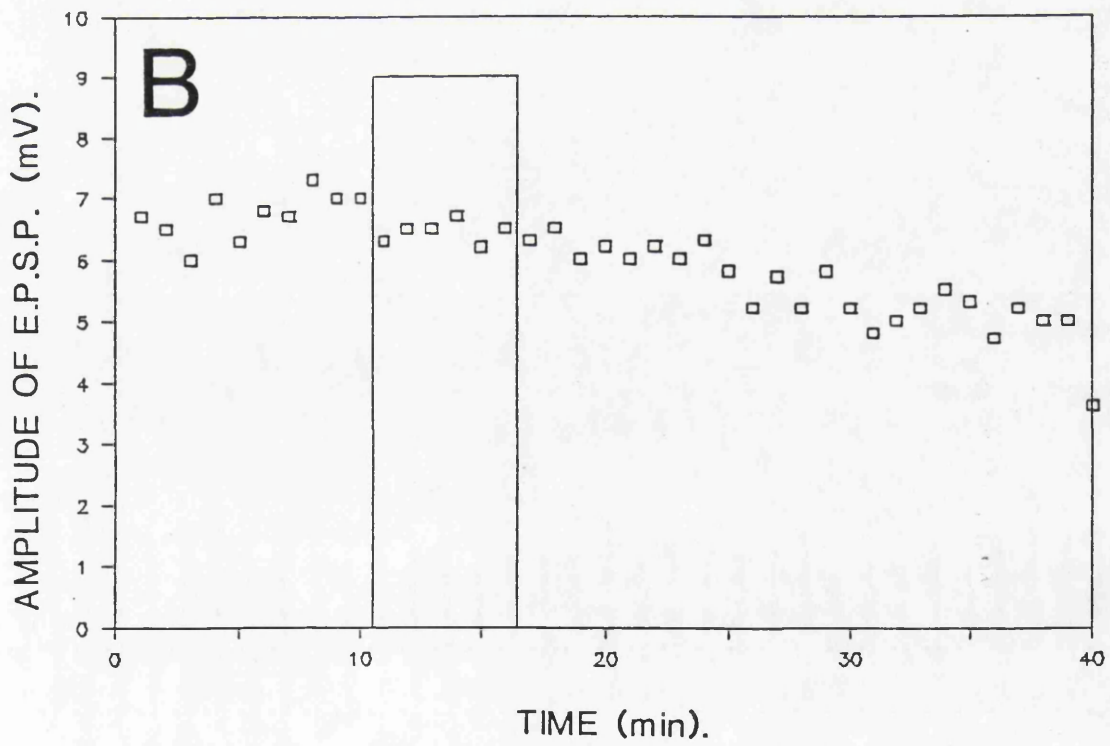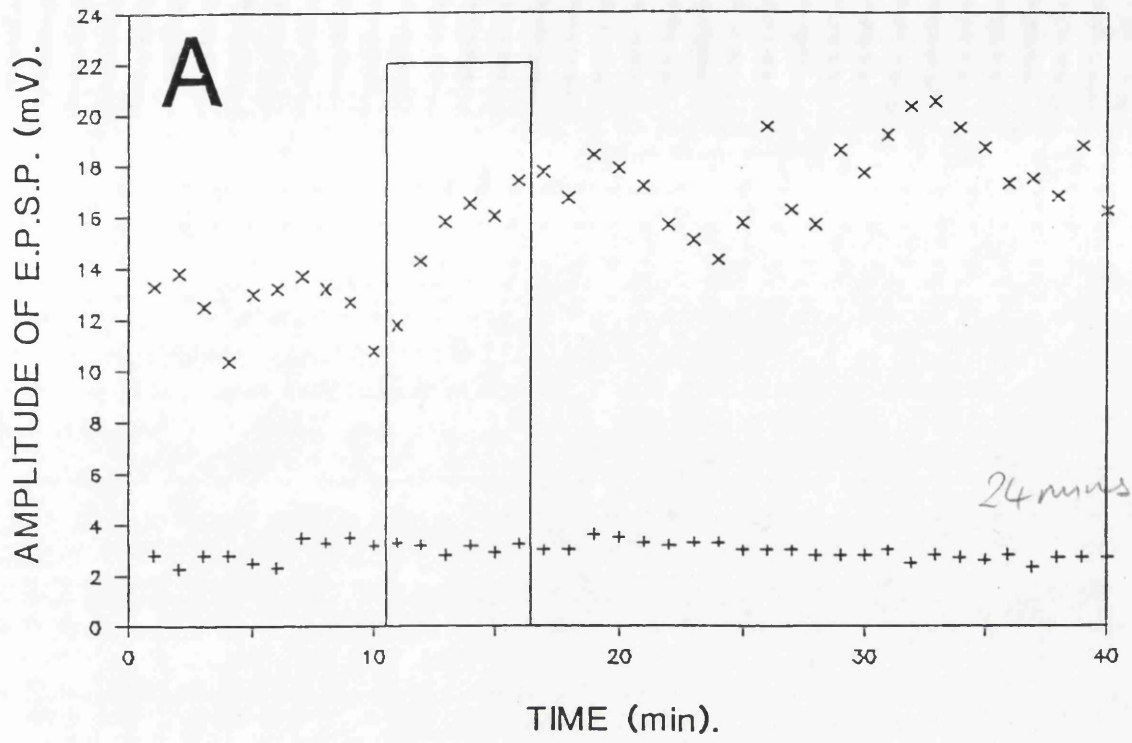
The first peak of the lateral e.p.s.p. and the medial response were not potentiated. Potentiation of the lateral e.p.s.p. persisted without decrement for 25 minutes, at which point the lateral e.p.s.p. was again paired with conditioning current. This produced a small but significant and enduring increase in the second peak of the lateral e.p.s.p., both the first peak of the lateral e.p.s.p. and the medial e.p.s.p. were again unpotentiated. Sixteen minutes later the medial e.p.s.p. was itself paired with conditioning current (figure 19). The medial pairing induced a small but highly significant increase in the medial e.p.s.p. but did not potentiate the lateral e.p.s.p.. It is possible that conditioning of the medial e.p.s.p. may have induced partial extinction of the established LTP of the lateral e.p.s.p.. Neither the resting membrane potential or input resistance was affected by conditioning and the development of LTP.

Figures 20 & 21 illustrate an example of LTP induced by the pairing paradigm of conditioning (*Expt. LTP4*) in a layer V neurone which exhibited a number of unusual features. Firstly, the unconditioned input, the lateral e.p.s.p., gave rise to a short latency e.p.s.p. coupled to a firing discharge. Secondly, the conditioned input, the medial e.p.s.p., was transformed by conditioning from a short latency subthreshold e.p.s.p. to a short latency e.p.s.p. of greater amplitude coupled to a *de novo* late firing discharge (figure 20C). Thirdly, conditioning induced a temporary 5mV depolarization of the resting membrane.

The depolarization of the membrane started half way through the conditioning period and persisted for 5 minutes after conditioning had ceased. During this period the lateral e.p.s.p. temporarily lost its late firing discharge (figure 21A) and the *de novo* firing discharge of the medial e.p.s.p. was greatly enhanced (figure 21B).

FIGURE 19: INDUCTION OF LTP IN ONE INPUT AND EXTINCTION IN ANOTHER.

Graphs of amplitude (minute averages) of e.p.s.p.s against time for the same neurone in figures 17 & 18, starting 16 minutes after end of graph in figure 18.

Previously LTP had been induced in the lateral e.p.s.p. (see figure 17 & 18). An attempt was then made to condition the medial e.p.s.p. by pairing it with depolarizing current (31 x 2.5nA, 100ms duration, timed to start 10ms after medial test shock). This was successful; coincident with the potentiation seen in medial e.p.s.p. was decremental change in the previously potentiated element of the lateral e.p.s.p..

A: Graph of minute averages of the lateral e.p.s.p. measured at latencies 5ms (+) and 34ms (x).

            The medial e.p.s.p. was conditioned during the period denoted by the *vertical bar*. The first peak of the lateral e.p.s.p. (at 5ms, see figure 18A) was unaffected by the medial conditioning, however the second peak (x) began to decline after conditioning.

B: Graph of minute averages of the medial e.p.s.p. measured at 11ms. The medial e.p.s.p. was postsynaptically conditioned during the period denoted by the *vertical bar*. This induced a highly significant increase in the e.p.s.p. (P<0.001, Student's t-test).
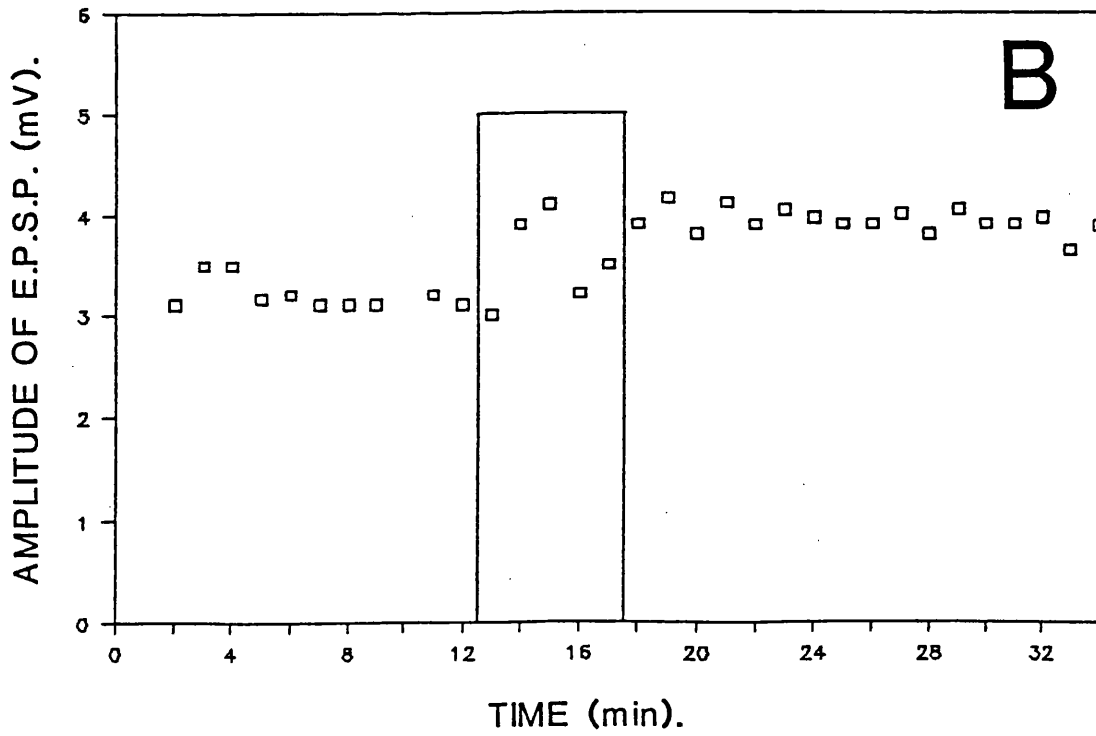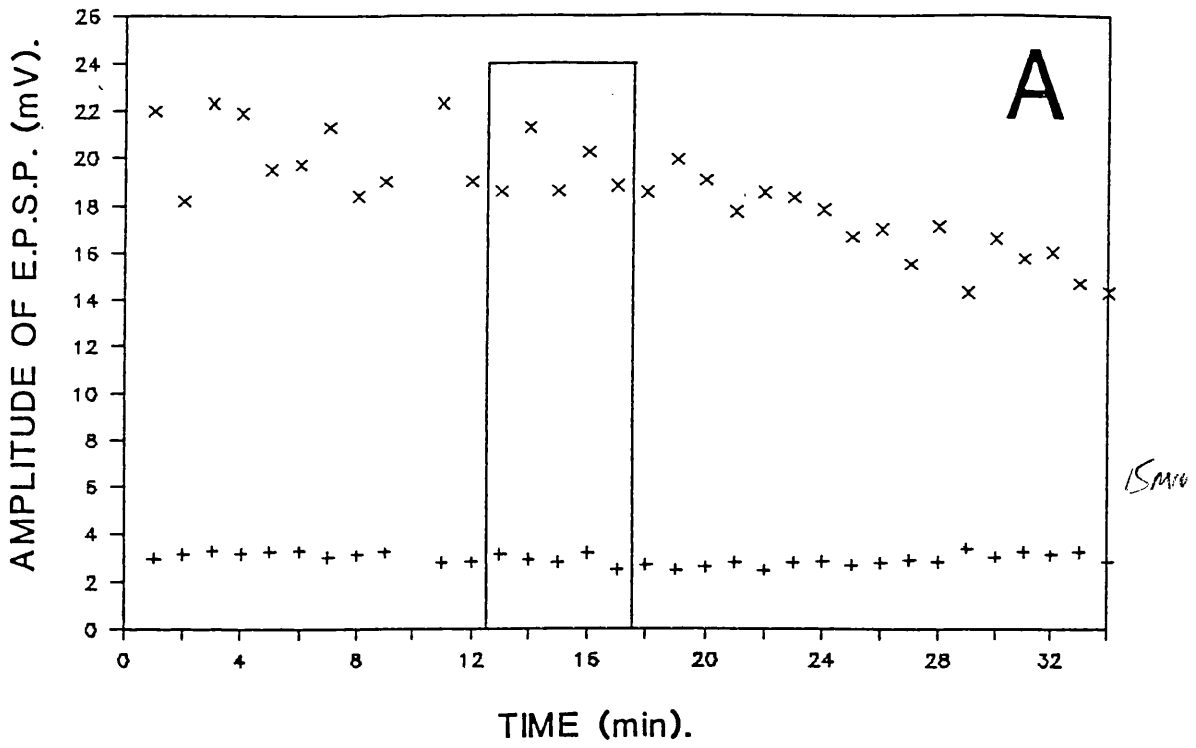
FIGURE 20: LTP INDUCED BY THE PAIRING PARADIGM IN A NEURONE WITH A SUPRATHRESHOLD E. P. S. P. AS THE UNCONDITIONED CONTROL.

Records recorded in a layer V neurone with a resting membrane potential of −70mV and input resistance of 45MΩ. Bathing medium contained 2μM bicuculline methiodide.

A: Electronic average of 8 successive responses evoked by stimulation of the medial white matter at 0.1Hz, prior to postsynaptic conditioning. Calibration bar is 40mV and 40ms.

B: Record showing a pairing trial. Upper trace conditioning current (+1nA), lower trace membrane voltage. Calibration bar is 40mV and 80ms.

C: Electronic average of e.p.s.p. 15 minutes after the conditioning period. Action potentials are distorted by averaging. Note that the e.p.s.p. has been transformed to an early subthreshold e.p.s.p. coupled to a firing discharge. Calibration bar as in A.
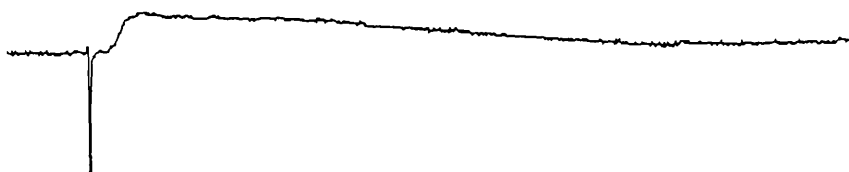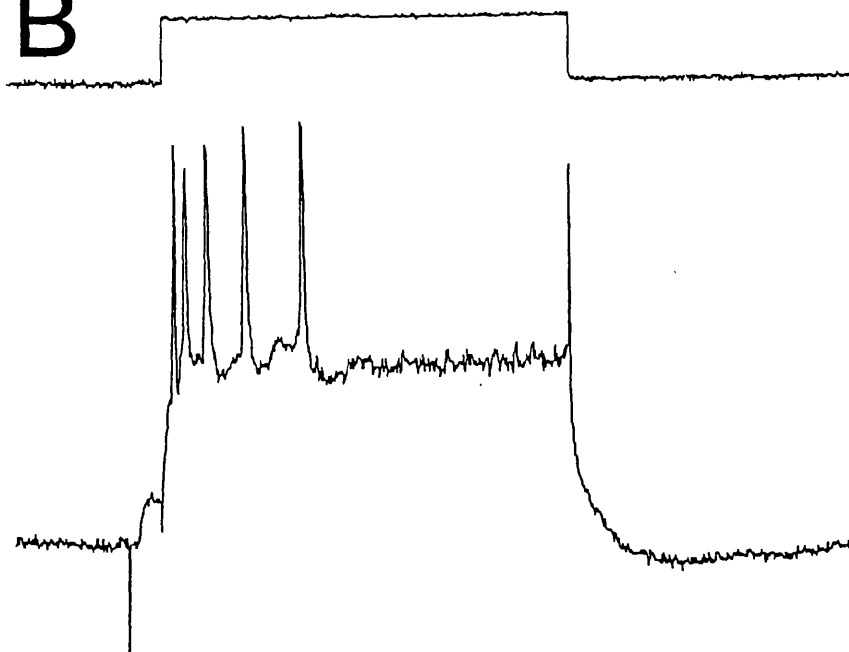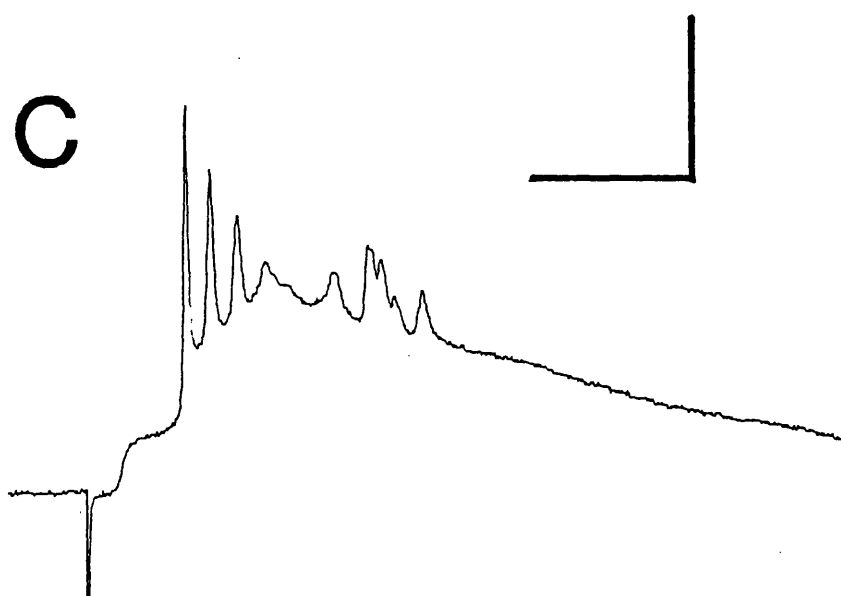
FIGURE 21: TIME COURSE OF LTP INDUCED BY THE PAIRING
PARADIGM IN A NEURONE WITH A SUPRATHRESHOLD E. P. S. P. AS
THE UNCONDITIONED CONTROL.

Induction of LTP in a medial e.p.s.p.; tranformation of a subthreshold
e.p.s.p. into an early subthreshold e.p.s.p. coupled to a firing discharge.
The unconditioned e.p.s.p.; the independent control, was a short latency
subthreshold e.p.s.p. coupled to a firing discharge.

Same neurone as in figure 20.

A: *UNCONDITIONED E.P.S.P..* Time course of the lateral e.p.s.p.. Data
points: (+) amplitude of the early subthreshold e.p.s.p. at 10ms
latency measured from electronic averages of 4 successive
responses, (inverted triangle) denotes the presence of a late
firing discharge.

The medial e.p.s.p. was conditioned during the period marked by
the vertical bar. Halfway through conditioning and for 5 minutes
afterwards there was a 4-5mV depolarization in the resting
membrane potential. The period of depolarization corresponds
with the temporary loss of late firing discharge ($\blacktriangledown=0$),
otherwise the lateral response was unaffected by the
conditioning of the medial e.p.s.p..

B: *CONDITIONED E.P.S.P..* Time course of the medial e.p.s.p.. Data
points: (+) minute averages of the amplitude of the e.p.s.p.
measured at latency of 10ms, (inverted triangle) minute averages
of action potentials per e.p.s.p.

After a stable control period of 22 minutes the lateral e.p.s.p.
was paired repetitively with injections of depolarizing current
(27x200ms, +1nA, onset latency of 20ms after test shock).
Conditioning induced a significant change in the form of the
lateral e.p.s.p. (see figure 20C). Not only was the early
subthreshold e.p.s.p. potetentiated, but it also developed a late
firing discharge. The increase in the early e.p.s.p. was highly
significant, mean control value prior to conditioning,
10.3mV ±0.30SEM (n=12), mean value 15 minutes after
conditioning, 14.5mV ±0.38SEM (n=12), 0.001>P (Student's t-test).

The decrement in the discharge rate immediately after
conditioning corresponds with the period of membrane
depolarization (5mV for 5 min post conditioning).

23
mvs
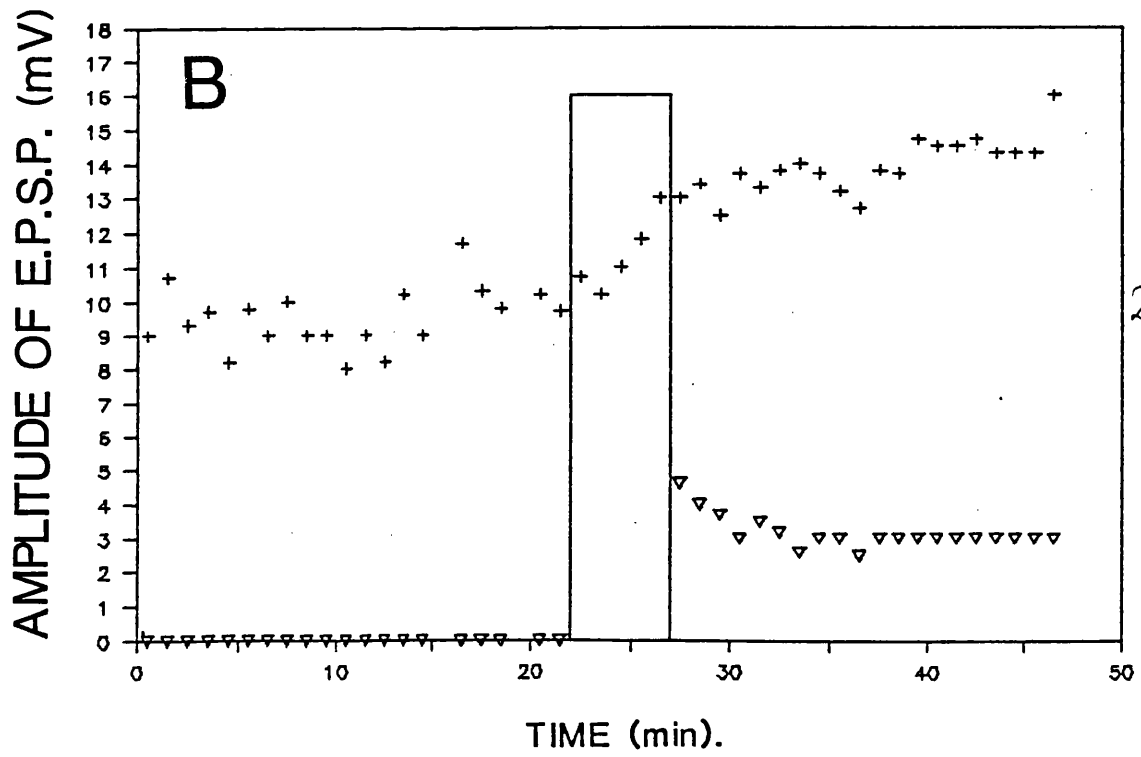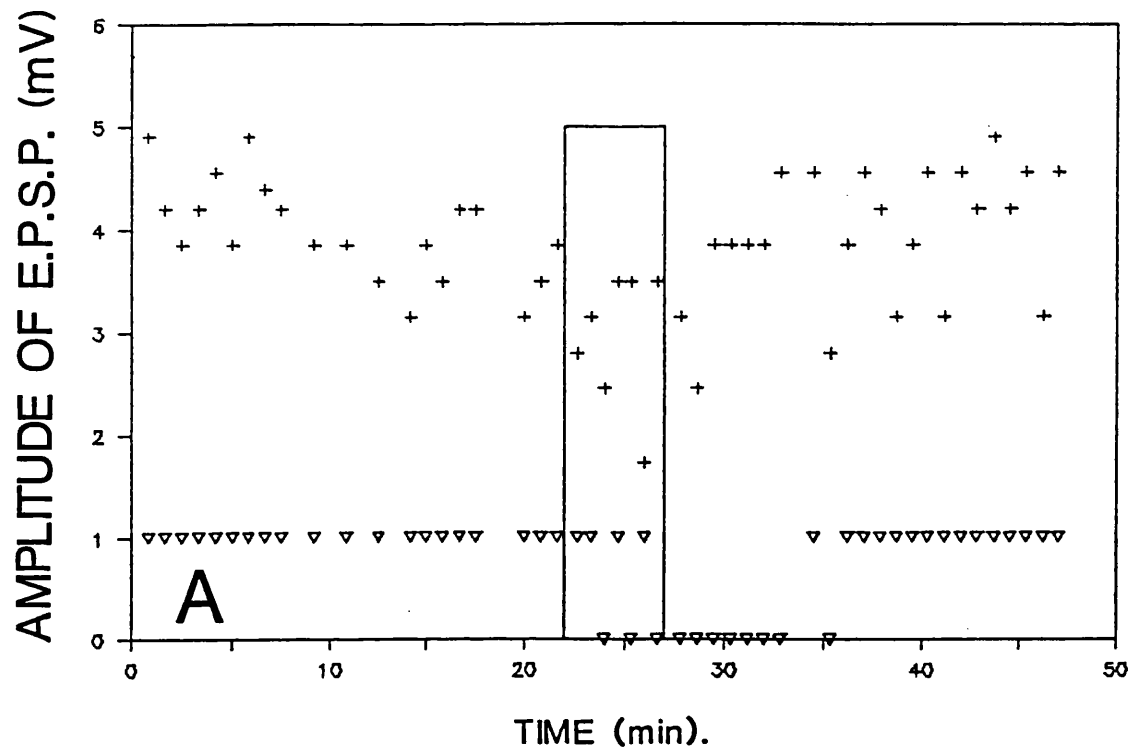
3.II.2 (iii) NMDA receptors participate in the expression of LTP induced by the pairing paradigm.

Figures 22 & 23 illustrate an experiment (*Expt. LTP5*) in which a potentiated e.p.s.p. was exposed to AP5. Subthreshold e.p.s.p.s evoked by lateral and medial stimulating electrodes were recorded in a layer V neurone and LTP was induced in the lateral e.p.s.p. using the pairing paradigm of conditioning. Unlike *Expt. LTP2* and *Expt. LTP3* where potentiation was seen as a maintained increment in amplitude (i.e. a step function increase), the potentiation induced in the lateral e.p.s.p. of *Expt. LTP5* exhibited a *de novo* burst of action potentials and continued to grow progressively in magnitude. Bath application of AP5 significantly reduced the magnitude of the potentiation and completely abolished the *de novo* burst firing. Partial recovery of the potentiated lateral e.p.s.p. was seen as AP5 was washed from the slice, but the cell was lost before full recovery could be ascertained (see below for expanded section on participation of NMDA and non-NMDA receptors in the expression of LTP). Once again resting membrane potential and apparent input resistance were unaffected by conditioning and the expression of LTP (input resistance prior to second conditioning period (mean±SEM), 21.1±0.42MΩ, n=8; after conditioning, 22.4±0.46MΩ, n=8; P>0.5, unpaired Student's t-test).

3.II.2 (iv) Neurones in which the pairing paradigm did not induce LTP.

The pairing paradigm of conditioning failed to induce LTP in 36 neurones out of 42 (43 inputs out of 50). In 29 of these neurones, conditioning did not induce any changes in the magnitude of postsynaptic potentials, resting membrane potential or input resistance. In two neurones conditioning induced a 4-5mV depolarization which was maintained once conditioning was terminated. In a further 3 neurones conditioning induced a

FIGURE 22: ACTION OF AP5 ON LTP INDUCED BY THE PAIRING PARADIGM IN A LAYER V NEURONE.

Effect of AP5 on expression of LTP in a layer V neurone with a resting membrane potential of -86mV and input resistance of 22MΩ.

From A to D: representative pairs of traces of e.p.s.p.s evoked by alternate stimulation of the lateral and medial white matter at 0.2Hz. Lateral and medial e.p.s.p.s are denoted by "LAT" and "MED" respectively. Calibration in D. Bath medium did not contain bicuculline methiodide.

A: During control (between 1st and 2nd conditioning attempts, see figure 23).

B: After the establishment of LTP in the lateral e.p.s.p. (figure 23 for details of conditioning parameters).

C: Maximum effect of bath applied 17µM DL-AP5, after the establishment of LTP.

D: Partial recovery of potentiation in the lateral e.p.s.p. after the removal of AP5.
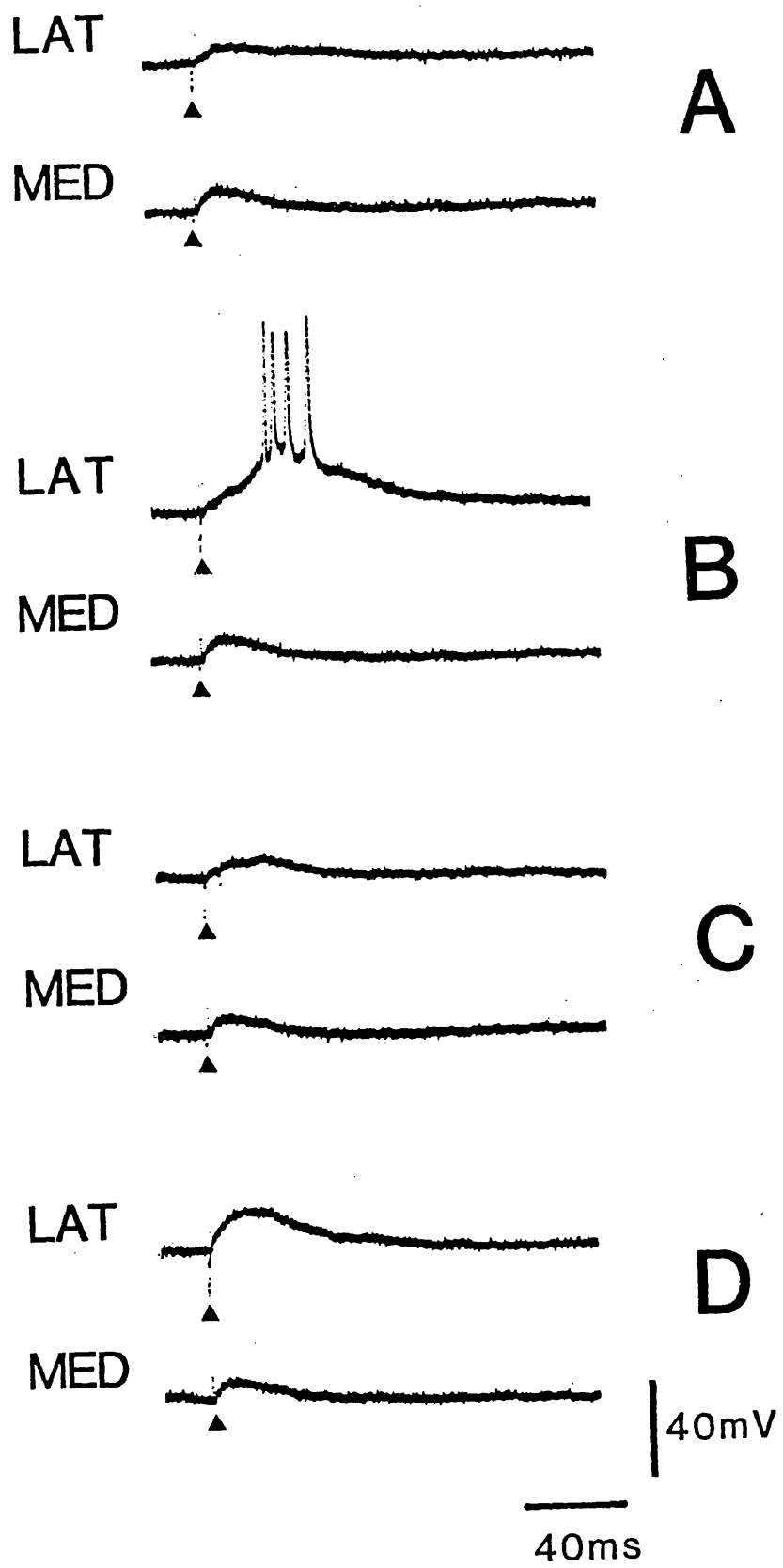
FIGURE 23: ACTION OF AP5 ON THE MAINTENANCE OF LTP INDUCED BY THE PAIRING PARADIGM.

Graph showing the time course of lateral and medial e.p.s.p.s shown in figure 22.

Data points are minute averages of the amplitude of lateral (●) and medial (■) e.p.s.p.s measured at 20ms and 10ms respectively.

Test shocks were applied at 0.2Hz, alternating between lateral and medial stimulating electrodes.

LATERAL E.P.S.P.

Two attempts (double vertical lines) were made to induce LTP in the lateral e.p.s.p. by repetitively pairing it with postsynatic injections of depolarizing current (30x300ms, +0.8nA, timed to start 15ms after lateral test shock). The first attempt failed to induce LTP. Stronger current was used during the second attempt and successfully induced LTP (30x300ms, 1nA, 20ms latency).

Potentiation of the lateral e.p.s.p. was evident by the 13-18th pairing trial of the 2nd conditioning period. Immediately after conditioning, potentiation of the lateral response was manifest by the development of a suprathreshold e.p.s.p. (early e.p.s.p. coupled to a late firing discharge). Inverted triangles (▼) denote a lateral suprathreshold response.

Bath application of 17μM DL-AP5 (horizontal bar) reversibly reduced the expression of LTP (washout not shown).

MEDIAL E.P.S.P.

The medial e.p.s.p. was evoked at 0.1Hz throughout the experiment and was unaffected by (i) the conditioning current and (ii) changes in the lateral e.p.s.p..
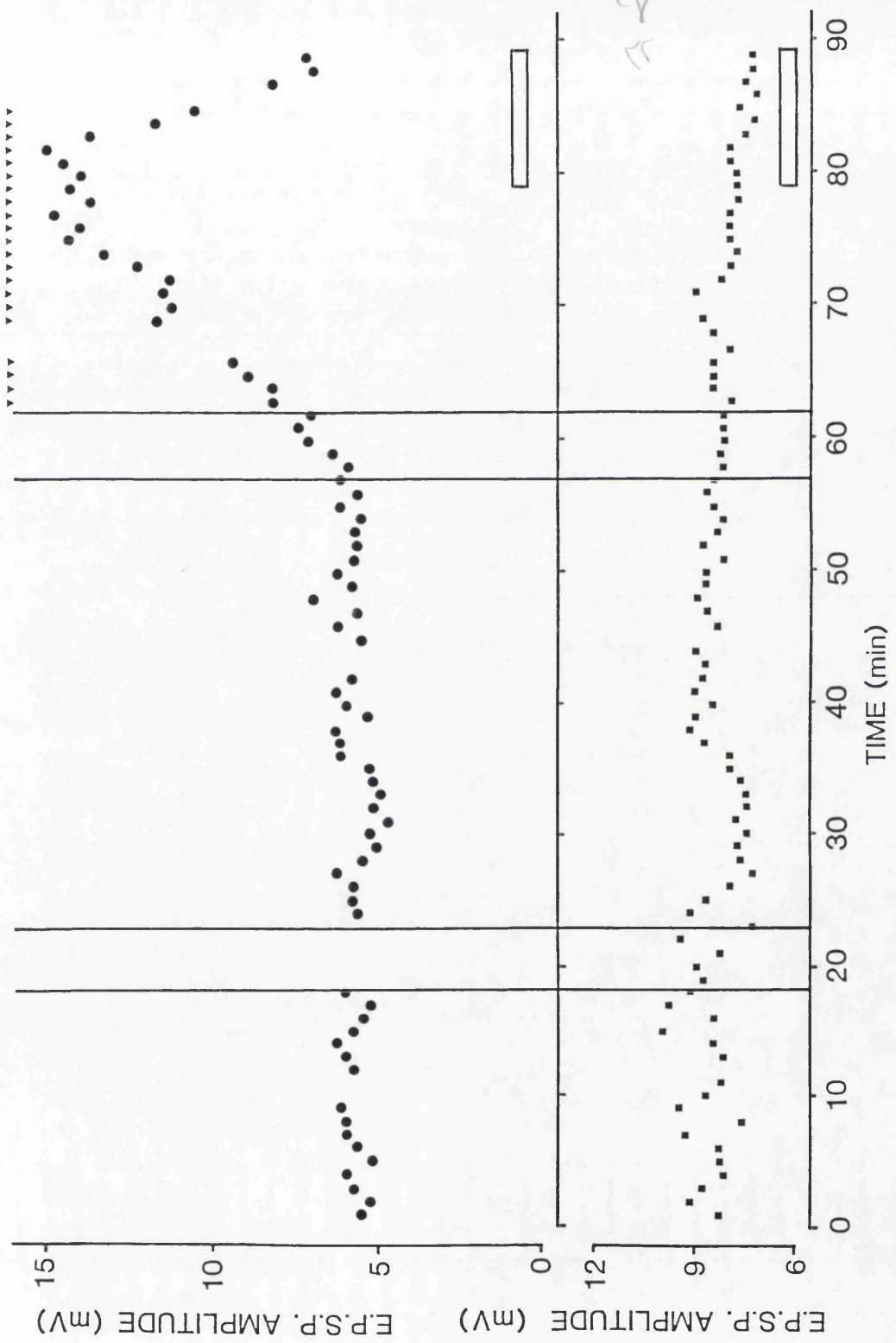
highly significant and enduring decrease in the conditioned postsynaptic potential which was not associated with changes in resting membrane potential or input resistance. Such activity-dependent decreases in synaptic efficacy are described as associative long-term depression (LTD) (Bindman, Murphy & Pockett, 1988; Stanton & Sejnowski, 1989).

Figure 24 illustrates an example of LTD of an e.p.s.p. induced by the pairing paradigm of conditioning and recorded in a layer VI neurone. E.p.s.p.s were evoked laterally and medially. Conditioning of the lateral e.p.s.p. induced a profound and enduring decrease in its magnitude while the medial response, the unconditioned e.p.s.p. was unaffected. After 15 minutes the decrease in the lateral e.p.s.p. was reversed by increasing the strength of the lateral test shock and after a 10 minute control period the lateral e.p.s.p. was again conditioned. This induced a second LTD of the lateral e.p.s.p. (not shown). The lateral test shock was once again increased to reverse the depression of the lateral e.p.s.p. and subsequent bath application of AP5 showed that an appreciable component of the e.p.s.p. was AP5-sensitive (not shown).

Seventeen of the 36 neurones which did not develop LTP or LTD were subjected to brief high frequency stimulation of one or more inputs. High frequency stimulation induced LTP in three neurones and LTD in another (see section 3.II.3(i)).

FIGURE 24: LONG-TERM DEPRESSION INDUCED BY THE PAIRING PARADIGM.

Induction of LTD in a layer VI neurone; resting membrane potential, -80mV and input resistance, 20MΩ.

Lateral and medial e.p.s.p.s were evoked alternately at 0.2Hz. The bathing medium contained 1μM bicuculline methiodide.

A: Graph of mean peak amplitudes of lateral (+) and medial (x) e.p.s.p.s. measured from electronic average of eight successive responses. From the 7th to 8th minute the lateral e.p.s.p. gave rise to an action potential and lateral stimulation was discontinued for 2 minutes. The lateral e.p.s.p. was conditioned during period denoted by the *vertical bar:* (32x1.4nA, timed to start20ms prior to the peak of the e.p.s.p.).

Conditioning produced a lasting depression in the lateral e.p.s.p.. The medial e.p.s.p. was unaffected by conditioning.

B: Records are electronic averages of eight successive responses. *Left:* averages taken 1 minute before the onset of postsynaptic conditioning of the lateral e.p.s.p..
Right: averages started ~3 minutes after the end of conditioning.
Central panel: single traces, with intracellularly applied condtioning current above and voltage below.

A

B

BEFORE   AFTER

LATERAL

MEDIAL

1.4nA

40mV

100ms

LATERAL

50ms   10mV

MEDIAL
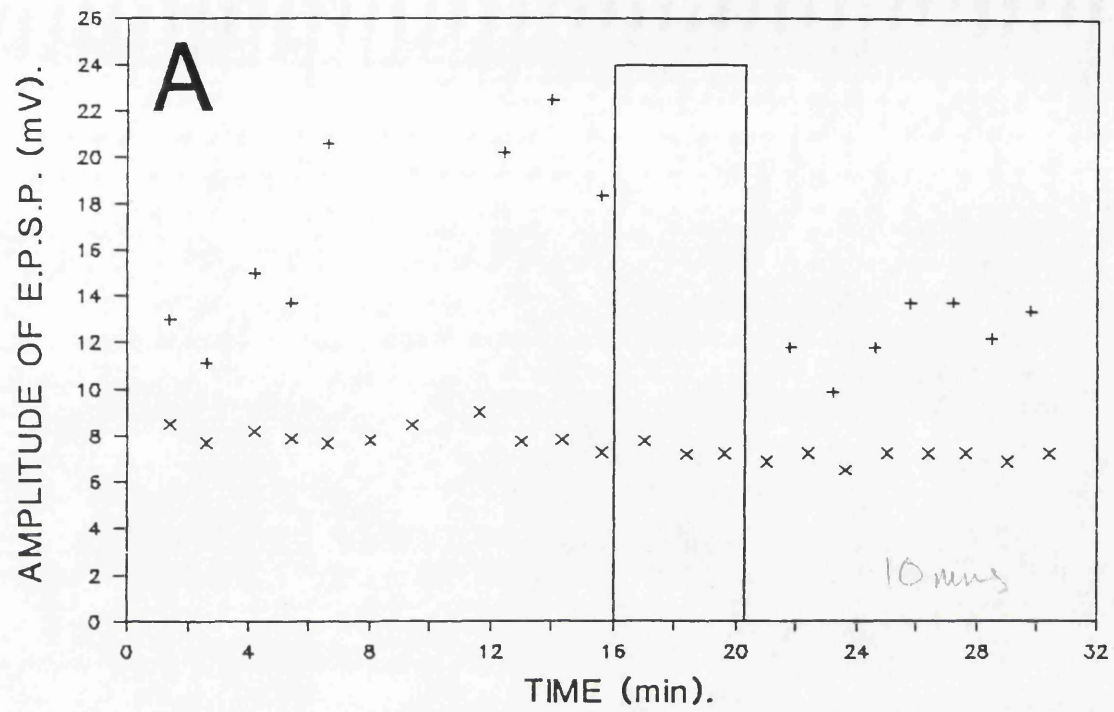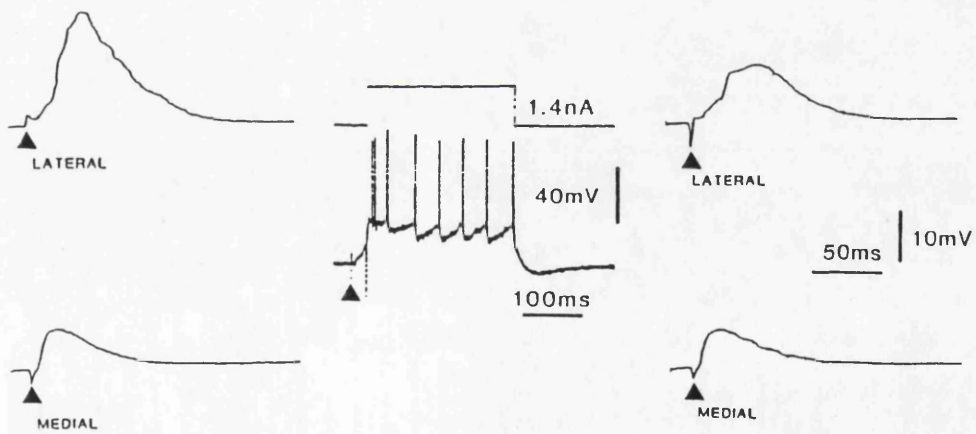
3.II.2 (v) Comparison of the electrophysiological properties of neurones in which long-term changes in synaptic efficacy were or failed to be induced.

Table 11 lists the mean values for resting membrane potential, action potential threshold, action potential amplitude and input resistance for neurones that exhibited: (i) LTP induced by the pairing paradigm ; (ii) LTD induced by the pairing paradigm; or (iii) were unaffected by conditioning using the pairing paradigm – hereafter each category is referred to as the LTP, LTD and fail groups respectively. Statistical analysis (Student's unpaired t-test) showed that no significant difference existed between the electrophysiological properties of the LTP, LTD and fail groups. The large disparity in the mean input resistance and standard deviation between the LTP and LTD groups when compared with the fail group necessitated confirmation of the t-test result with a second test of significance, the analysis of the variance (ANOVA). ANOVA confirmed that no significant difference existed for input resistance, between the three groups (ANOVA: P=0.25, 40 degrees of freedom).

3.II.2 (vi) Do conditioning parameters determine the outcome of the pairing paradigm?

Table 12 lists the postsynaptic conditioning parameters applied to each neurone. In the case of the neurones that exhibited LTP, only those conditioning parameters which evoked LTP are shown. In one instance the same input was successfully potentiated on two separate occasions and both sets of conditioning parameters are listed. For the neurones which manifested LTD, only those parameters that elicited LTD are shown, here again one input was depressed on two separate occasions and both sets of parameters are shown. The neurones that failed to elicit LTP or LTD are treated differently, only one set of conditioning parameters is listed per

TABLE 11: COMPARISON OF ELECTROPHYSIOLOGICAL PROPERTIES OF
NEURONES IN WHICH PAIRING FAILED TO OR INDUCED LONG-TERM
CHANGES IN SYNAPTIC EFFICACY.

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONES IN WHICH
PAIRING INDUCED LTP:

	Resting Membrane Potential (mV)	Action Potential Threshold (mV)	Action Potential Amplitude (mV)	Input Resistance (MΩ)
Mean:	-79.7	23.2	77.3	29.5
SD:	±9.22	±5.98	±6.19	±10.65
SEM:	±3.77	±2.44	±2.52	±4.35
n:	6	6	6	6

LTP vs FAIL (unpaired Student's t-test):

P>0.5	P>0.5	0.5>P>0.1	0.5>P>0.1

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONES IN WHICH PAIRING INDUCED LTD:

	Resting Membrane Potential (mV)	Action Potential Threshold (mV)	Action Potential Amplitude (mV)	Input Resistance (MΩ)
Mean:	-80.0	19.3	69.0	26.7
SD:	±4.00	±4.62	±7.55	±11.55
SEM:	±2.31	±2.67	±4.36	±6.67
n:	3	3	3	3

LTD vs FAIL (unpaired Student's t-test):

P>0.5	P>0.5	0.5>P>0.1	0.5>P>0.1

ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONES IN WHICH PAIRING FAILED TO
INDUCE LONG-TERM CHANGES IN SYNAPTIC EFFICACY:

	Resting Membrane Potential (mV)	Action Potential Threshold (mV)	Action Potential Amplitude (mV)	Input Resistance (MΩ)
Mean:	-78.9	22.3	73.3	47.9
SD:	±8.60	±7.37	±6.63	±33.52
SEM:	±1.52	±1.35	±1.19	±5.92
n:	32	30	31	32

TABLE 12: THE PAIRING PARADIGM: CONDITIONING PARAMETERS.

CONDITIONING PARAMETERS WHICH INDUCED LTP:

Cell	Afferent Input	No of Pairings	Current Strength (nA)	Length of Pulse (ms)	Onset Latency (ms)
1)	lat	28	2	100	20
2)	lat	50	0.7	100	16[+]
3[a])	lat	34	2	100	30
3[b])	lat	32	2.5	100	30
3)	med	31	2.5	100	20
4)	med	27	1	200	20
5)	lat	30	1	300	20
6)	grey	50	2	200	5

CONDITIONING PARAMETERS WHICH INDUCED LTD:

Cell	Afferent Input	No of Pairings	Current Strength (nA)	Length of Pulse (ms)	Onset Latency (ms)
1)	med	33	1.4	100	24
2)	med	32	1.4	170	46
3[c])	lat	30	1.6	250	25
3[d])	lat	30	1.4	350	25

CONDITIONING PARAMETERS WHICH FAILED TO INDUCE LTP:

Cell	Afferent Input	No of Pairings	Current Strength (nA)	Length of Pulse (ms)	Onset Latency (ms)
1)	lat	25	0.2	200	10
1)	med	32	0.15	200	5
2)	lat	25	0.7	100	20
3)	lat	25	1.3	100	10
4)	lat	30	0.8	100	30
5)	med	33	1	100	5
6)	med	25	1	100	20
7)	lat	25	0.7	100	60
8)	med	26	0.65	100	30
9)	lat	25	0.7	150	15
10)	lat	25	1.5	200	40
11)	lat	30	1.2	120	25

CONDITIONING PARAMETERS WHICH FAILED TO INDUCE LTP *(continued)*.

Cell	Afferent Input	No of Pairings	Current Strength (nA)	Length of Pulse (ms)	Onset Latency (ms)
12)	lat	25	1	250	80
13)	med	25	1	240	0
14)	lat	32	0.5	100	16
15)	lat	30	1	100	35
16)	lat	34	1	100	40
17)	med	30	1.4	300	15
18)	lat	25	0.8	300	20
19)	lat	30	0.8	300	10
19)	med	30	0.8	300	10
20)	lat	25	0.8	200	20
21)	med	30	1.2	200	15
21)	lat	30	1.4	200	10
22)	med	30	0.8	200	10
23)	med	30	0.9	200	15
24)	med	40	1	200	10
24)	lat	40	1	300	10
25)	lat	30	0.8	200	25
26)	lat	30	0.8	200	0
26)	lat	30	0.5	200	0
27)	med	40	1	200	10
27)	lat	40	1	200	10
28)	grey	34	1.4	300	10
29)	grey	30	0.8	300	10
30)	white	30	1.8	100	6
31)	lat	35	1	200	0
32)	white	50	0.9	200	40

[a], [b]: same input, conditioned twice, LTP induced on both trials.

[c], [d]: same input, conditioned twice, LTD induced on both trials.

conditioned input, where an input was conditioned more than once, only the more vigorous set of conditioning parameters (i.e. that with the strongest conditioning current) is listed.

The data shown in table 12 was used to test the possibility that postsynaptic induction of LTP was related to one or more variable in the set of conditioning parameters. The Student's t-test was used to test the statistical significance of each parameter, comparing the mean values for the fail group with those for the LTP and LTD groups respectively (table 13). No statistical significance was found for (i) the latency of onset of the conditioning current pulse, (ii) the length of the current pulse and (iii) the number of pairings per conditioning period. However when the mean values for the strength of the conditioning current for both the LTP and LTD groups were compared with that for the fail group, they were found to be highly statistically significant. This was confirmed by ANOVA, for the three groups: $P<0.001$. The strength of the conditioning current was significantly greater for both LTP and LTD groups when compared with the fail group. However this observation must be interpreted with caution. The mean input resistances for the LTP and LTD groups were smaller than that for the fail group, 29.5MΩ 26.7MΩ and 47.9MΩ respectively. Given that the main criterion for selecting the strength of the conditioning current was that each pulse of current elicited 8-12 action potentials, the possibility exists that the difference seen in the strength of conditioning current for the three groups is simply a consequence of cable properties i.e. Ohm's law (voltage = resistance x current).

TABLE 13: THE PAIRING PARADIGM: STATISTICAL ANALYSIS OF CONDITIONING PARAMETERS.

-- (mean ± SEM) --

Group	No of conditioning trials	Latency of Pulse (ms)	No of Pairings	Pulse Length (ms)	Current Strength (nA)
LTP	8	20.1±2.81	35.3±3.31	150±75.59	1.7±0.71
LTD	4	28.8±5.85	31.3±0.75	217±53.75	1.5±0.05
FAIL	38	18.1±2.71	30.6±0.91	188±11.56	0.9±0.05

Results of Student's t-test (unpaired):

LTP vs FAIL	P>0.5	0.1>P>0.05	0.5>P>0.1	P<0.001*
LTD vs FAIL	0.5>P>0.1	P>0.5	0.5>P>0.1	0.01>P>0.001*

LTP: Conditioning parameters which induced LTP (one input was successfully potentiated on two separate ocaisions)

LTD: Conditioning parameters which induced LTD (in one input LTD was induced on two separate ocaisions)

FAIL: Conditioning parameters from neurones which were not potetentiated, where an input was conditioned on more than one oc aision, only the strongest set of conditioning parameters was used   for t-test analysis.

3.II.3 (1) Induction of LTP by high frequency stimulation of an afferent input.

Inputs of 26 neurones were conditioned by the application of a series of brief high frequency trains of shocks via the stimulating electrodes used to apply the test shock (see table 14).

LTP was successfully induced in 5 out of 26 neurones, 19.2% (17 of which had previously failed to develop LTP after conditioning with the pairing paradigm). The conditioning parameters that induced LTP were not dissimilar to those that failed to induce LTP in other neurones (table 15). In a minority of cases (n=4), conditioning induced short lasting changes (<5 minutes) in synaptic efficacy, either as an increase or a decrease in the size of the conditioned e.p.s.p. (post-tetanic potentiation and post-tetanic depression respectively). In a small number of experiments (n=5), the effects of the conditioning trains were not confined to the conditioned input i.e. the unconditioned input was also affected. In these neurones the changes in synaptic efficacy were short-lived (<5 minutes).

LTP in the 5 neurones that were successfully potentiated was initially masked by a period of post-tetanic depression (see figure 26).

Figures 25 & 26 illustrate an example of LTP recorded in a layer V neurone, induced by high frequency stimulation of an afferent pathway. Conditioning consisted of 4 trains of shocks (duration of each train was 200ms, shocks evoked at 100Hz, each shock twice the test shock width, intertrain interval of 10 sec) and induced a short-lived depression of the e.p.s.p. (about 10 minutes), followed by potentiation manifest as an increase in the depolarizing slope, an increase in the probability of eliciting an action potential and an increase in the duration of the e.p.s.p.. Once LTP was fully established the slice was exposed to 10μM and

TABLE 14: INDUCTION OF LTP BY HIGH FREQUENCY STIMULATION OF AFFERENT INPUTS RECORDED IN SINGLE NEOCORTICAL NEURONES. .

Conditioning by high frequency stimulation of an afferent pathway was attempted in 26 neurones. The summaries below list the number of inputs conditioned by neocortical layer (bracketed values are number of conditioning attempts applied to input).

NEURONES IN WHICH HIGH FREQUENCY STIMULATION INDUCED LTP.

	No of Neurones	No of Lateral Inputs	No of Medial Inputs	No of White Matter	No of Grey Matter
Layer V	5	3 (3)	1 (3)	1 (1)	1 (1)

Postsynaptic conditioning failed to induce LTP in 3 of these neurones.

2 out of 5 of the cells in which high frequency stimulation conditioning induced LTP were exposed to bathing medium containing bicuculline methiodide.

NEURONES IN WHICH HIGH FREQUENCY STIMULATION FAILED TO INDUCE LTP.

	No of Neurones	No of Lateral Inputs	No of Medial Inputs	No of White Matter	No of Grey Matter
Layer V	18	8†(9)	5 (8)	5 (15)	1 (2)
Layer VI	2	1 (1)	1 (2)	-	-
Layer III	1	-	1 (1)	-	-

Postsynaptic conditioning had failed to induce LTP in 14 of these neurones.

10 out of 21 of the neurones in which high frequency stimulation failed to induce LTP were exposed to bathing medium containing bicuculline methiodide.

†: LTD induced in one input after second high frequency stimulation conditioning attempt.

TABLE 15: HIGH FREQUENCY STIMULATION PARAMETERS USED IN CONDITIONING TRAINS.

HIGH FREQUENCY STIMULATION PARAMETERS WHICH INDUCED LTP.

Cell	No of Trains	Shock Frequency (Hz)	Train Length (s)	Increased Shock
1)	3	400	2	No
2)	3	400	1	No
3)	3	400	1	Yes
4)	6	100	0.1	Yes
5)	4	100	0.5	Yes

HIGH FREQUENCY STIMULATION PARAMETERS WHICH FAILED TO INDUCE LTP.

Cell	No of Trains	Shock Frequency (Hz)	Train Length (s)	Increased Shock
1)	6	400	0.125	No
2)	3	400	2	Yes
3)	3	400	0.1	Yes
4)	3	400	1.2	Yes
5)	4	400	2	No
6)	3	400	1	Yes
7)	3	400	1	Yes
8*)	3	400	0.5	Yes LTD
9)	6	100	0.5	Yes
10)	4	200	0.5	No
11)	6	400	0.1	Yes
12)	6	100	0.2	Yes
13)	6	100	0.13	Yes
14)	3	100	0.1	Yes
15)	4	100	0.14	No
16)	6	100	0.2	Yes
17)	6	100	0.1	Yes
18)	4	400	1	Yes
19)	4	400	0.5	Yes
20)	4	100	0.5	Yes
21)	4	100	0.1	Yes

*: Conditioning induced LTD.

FIGURE 25: LTP INDUCED BY HIGH FREQUENCY STIMULATION IN A LAYER V NEURONE.

LTP induced by high frequency stimulation in an e.p.s.p. recored in a layer V neurone (resting membrane potential, -90mV; input resistance, 33MΩ). The bathing medium did not contain bicuculline methiodide.

Records in A, C & D are electronic averages of 4 successive e.p.s.p.s evoked by stimulation of the underlying white matter at 0.1Hz.

A: E.p.s.p. prior to high frequency stimulation. Calibration in D.

B: The slice was conditioned by high frequency stimulation of the white matter; 4 trains at 100Hz, each lasting 500ms and twice the test shock stimulus width.
Record shows the first conditioning train (denoted by horizontal bar). Note the two late paroxysmal events after the conditioning train. Action potential height is attentuated by the frequency response of the tape recorder.

C: Potentiated e.p.s.p. 18 minutes after conditioning (e.p.s.p.s that gave rise to action potentials were excluded from average).

D: Effect of bath applied 20µM D-AP5 on the potentiated e.p.s.p. 38 minutes after conditioning.
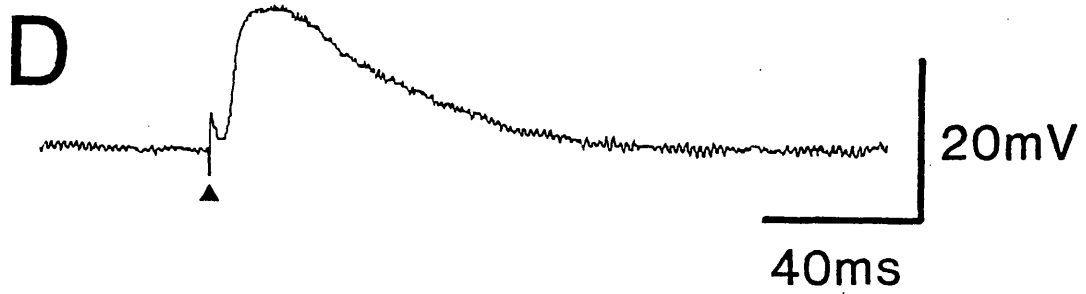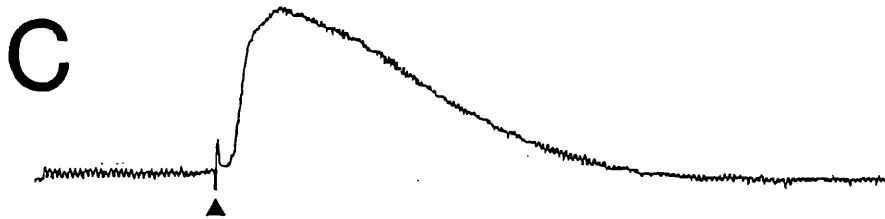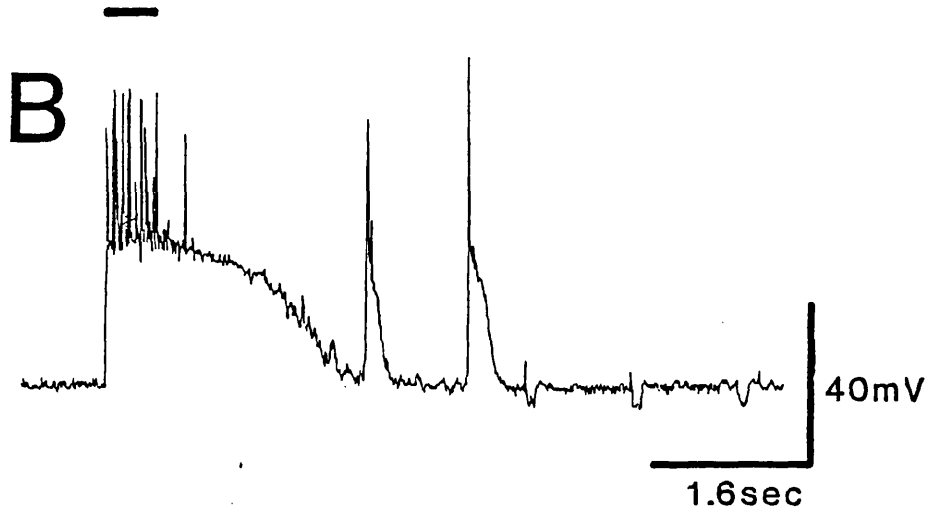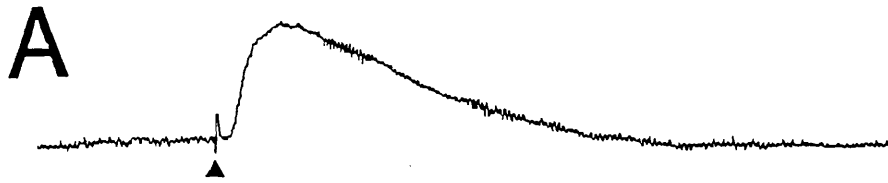
FIGURE 26: TIME COURSE OF INTRACELLULARLY RECORED LTP INDUCED BY HIGH FREQUENCY STIMULATION.

Time course of the experiment shown in figure 26.

A: Graph of minute averages of peak amplitude of e.p.s.p. (x)
      against time. After stable control, high frequency stimulation
      was applied via the test shock stimulating electrodes to the
      white matter (conditioning denoted by vertical line). Immediately
      after conditioning there was a period of post-tetanic
      depression, followed by the development of LTP.
      LTP was expressed as (i) an increase in peak amplitude and (ii)
      generation of action potentials accompanying the e.p.s.p.. 10μM
      D-AP5 (narrow bar) was bath applied 22 minutes after high
      frequency stimulation and did not appear effect the
      potentiation. 20μM D-AP5 (thick bar) abolished action potential
      firing and caused a small, but highly significant decrease in
      the peak amplitude; prior AP5: 19.9mV ±0.1SEM, n = 6 (six
      minutes); during 20μM D-AP5: 18.1mV ±0.2SEM, n=6 (six minutes);
      P<0.001 (Student's t-test).

B: Graph of minute averages of decay time to ½ peak amplitude (+).
      Vertical line denotes period of conditioning. Inverted triangles
      denote suprathreshold e.p.s.p.(s) in minute interval. AP5 as in A.
      Conditioning induced an increase in the duration of the e.p.s.p..
      10μM and 20μM D-AP5 reduced the duration of the e.p.s.p. as
      evinced by the decrease in decay time to ½ peak amplitude.
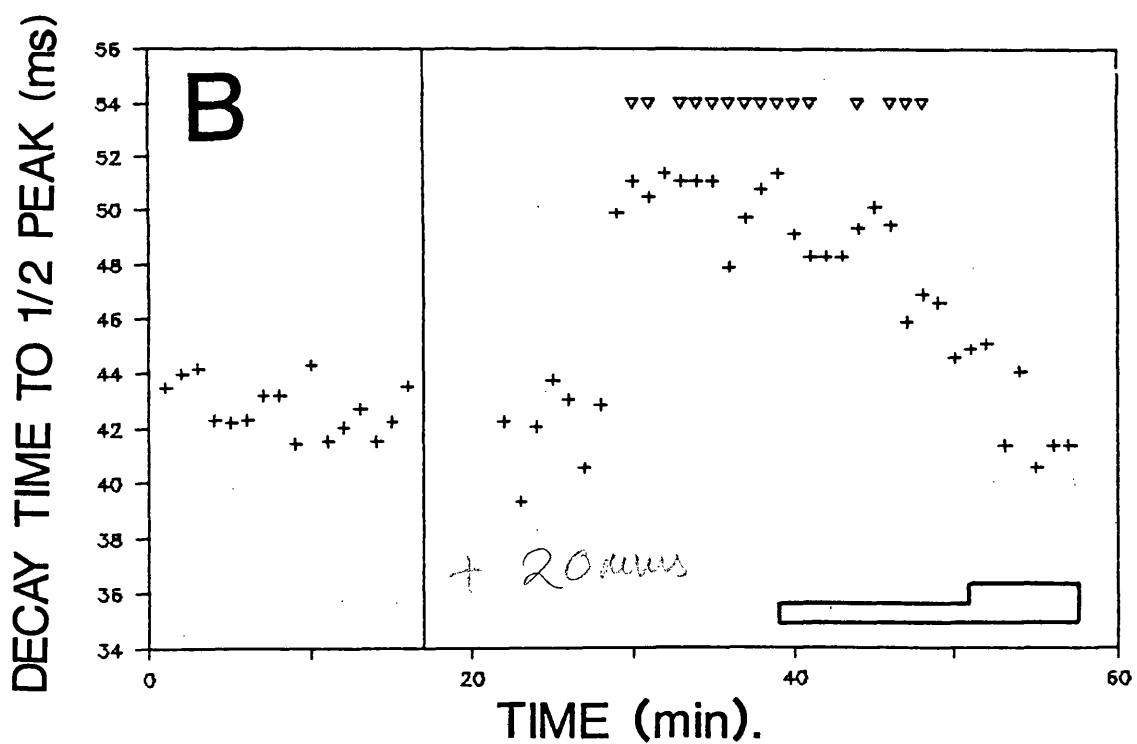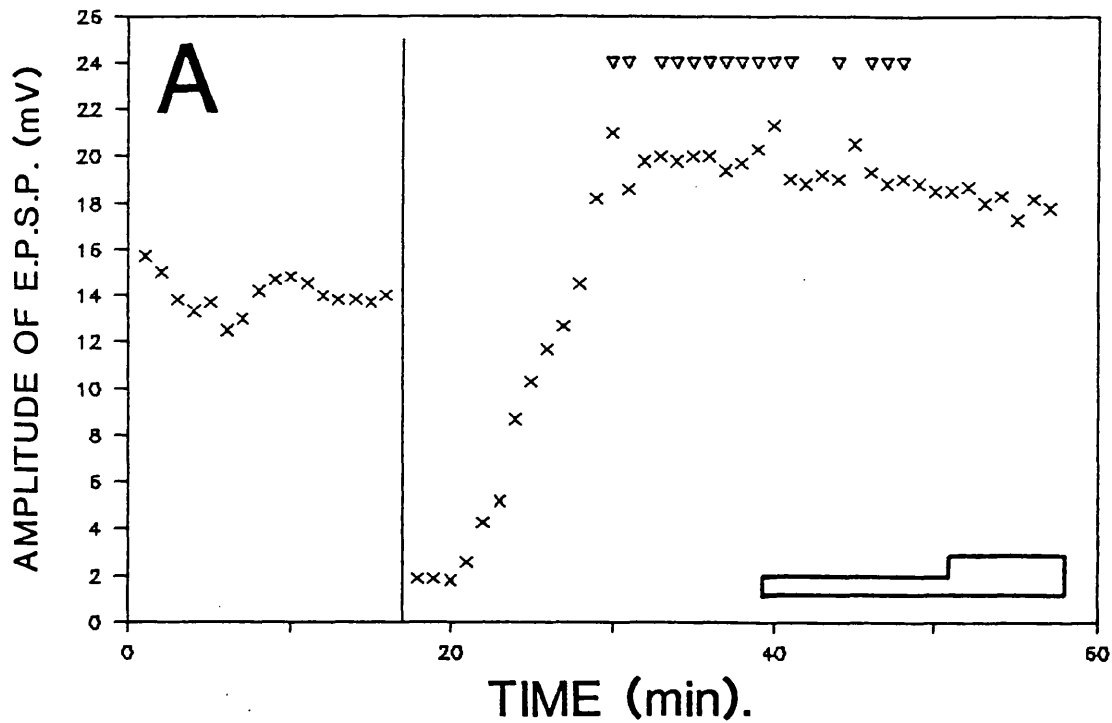
then 20μM D-AP5. 10μM AP5 had little effect on the magnitude and duration of the potentiated e.p.s.p. whereas 20μM AP5 reduced the probability of evoking an action potential and also reduced the peak amplitude and duration of the e.p.s.p. without altering its initial depolarizing slope. Neither resting membrane potential or input resistance were affected by conditioning or the development of potentiation.

The conditioning parameters which elicited LTP were indistinguishable from those those which failed to induce LTP (table 15).

3.II.4 (i) The nature of intracellularly recorded LTP.

Table 16 lists electrophysiological characteristics of the 11 neurones (12 inputs) that supported LTP, induced by either postsynaptic conditioning or high frequency stimulation. The majority of the e.p.s.p.s (11 out of 12) in these neurones had onset latencies that were less than 5ms and based on this criterion are defined as short latency e.p.s.p.s. The single remaining e.p.s.p. had an onset latency that was more than 40ms and is defined as a late latency e.p.s.p.. In 9 out of 11 short latency e.p.s.p.s, the onset of potentiation (measured from superimpositions of control and potentiated electronic averages of e.p.s.p.s) was not coincident with the onset of the e.p.s.p. but occurred many milliseconds later (see table 16). The remaining 2 short latency e.p.s.p.s were very different, in one the onset of potentiation was coincident with the onset of the e.p.s.p., whilst in the other, the onset of potentiation preceded the onset of the e.p.s.p. measured prior to conditioning (see figure 27).

Bath application of AP5 revealed that a significant proportion of the potentiated component of successfully conditioned e.p.s.p.s was mediated by NMDA receptors (see figures 23 & 27). In 3 out of 3 potentiated short

TABLE 16: ELECTROPHYSIOLOGICAL CHARACTERISTICS OF 10 NEURONES & 11 E.P.S.P.S IN WHICH LTP WAS INDUCED.

Cell & e.p.s.p.	Layer	BMI	E_m (mV)	Firing threshold (mV from E_m)	Pathway stimulated to evoke e.p.s.p.	Onset latency of e.p.s.p. (ms)	Latency of potentiated component in LTP (ms)	V_m of onset of potentiated component in LTP, below firing threshold (mV)	Method of induction of LTP	Onset of effect of APS (ms)
1a	III	Yes	-81	+25	Lat	3.6	20[b]	-15	Pair	-
1b[c]	III	Yes	-81	+25	Med	3.5	5.33	-20	Pair	-
2	III	No	-92	+33	GM	3.0	4.53	-32	Pair	-
3	V	Yes	-66	+22	Lat	3.2	4.5	-18	Pair	-
4	V	Yes	-81	+15	Lat	2.7	4.9	-13	Pair	-
5	V	Yes	-70	+24	Med	5.3	8.5	-18	Pair	-
6	V	Yes	-74	+18	Med	3.7	3.7	-18	HFS	-
7	V	No	-86	+20	Lat	2.1	9.6	-15	Pair	14.9
8	V	No	-70	+15	Lat	5.1	4.6	-15	HFS	11.9
9	V	No	-90	+22	WM	3.7	5.0	-16	HFS	7.7
10	V	No	-100	+18	Lat	>40[c]	>40[c]	c	HFS abolished e.p.s.p.	-
11	V	Yes	-73	+18	Lat	4.6	>35[d]	d	HFS	-
MEAN			-80.3	+21.25		3.62	5.4	-18.0		
±SEM			2.97	1.47		0.31	1.2	1.9		
n			12	12		11	10	10		

Key: BMI, bicuculline methiodide; Lat, lateral; Med, medial; GM, grey matter stimulation in layers II/III; WM, white matter stimulation below recording electrode; Pair, pairing paradigm of conditioning; HFS, high frequency stimulation of an afferent pathway; E_m, resting membrane potential; V_m, membrane potential.

Footnotes:
a the e.p.s.p.s from Lat and Med stimulation were potentiated independently.
b pairing pulse starting at latency of 35ms; value omitted from mean.
c long latency variable response; omitted from mean.
d prolongation of spiking.

FIGURE 27: NMDA & NON-NMDA RECEPTOR MEDIATED COMPONENTS OF POTENTIATED POTENTIALS PRIOR TO AND AFTER CONDITIONING.

A: Superimposed averages of 8 successive e.p.s.p.s evoked by stimulation of the lateral white matter and recorded in a layer V neurone: resting membrane potential, -86mV; action potential amplitude, 89mV and input resistance of 22Mohms. LTP induced by the pairing paradigm of conditioning.

Averages were taken (a) prior to conditioning, (b) during expression of LTP and (c) subsequent bath application of 17µM D-AP5. The bathing medium did not contain bicuculline methiodide. Same experiment as in figures 22 & 23.

B: Superimposed averages of 8 successive e.p.s.p.s evoked by white matter stimulation at 0.1Hz and recorded in a layer V neurone: resting membrane potential, -90mV; action potential amplitude, 103mV (80mV measured from threshold) and input resistance, 33Mohms. LTP induced by high frequency stimulation.

Averages were taken (a) prior to conditioning, (b) during the expression of LTP and (c) during subsequent bath application of 20µM D-AP5. The bathing medium did not contain bicuculline methiodide. Same experiment as in figures 25 & 26.

C: Superimposed averages of 4 successive e.p.s.p.s evoked by stimulation of the lateral white matter recorded in a layer V neurone; resting membrane potential, -70mV; action potential amplitude, 91mV and input resistance; 26MΩ. LTP was induced by high frequency stimulation.

Averages were taken (a) during control, (b) during bath application of 20µM D-AP5 during control, (c) during expression of LTP and (d) subsequent bath application of 20µM D-AP5. The bathing medium did not contain bicuculline methiodide.
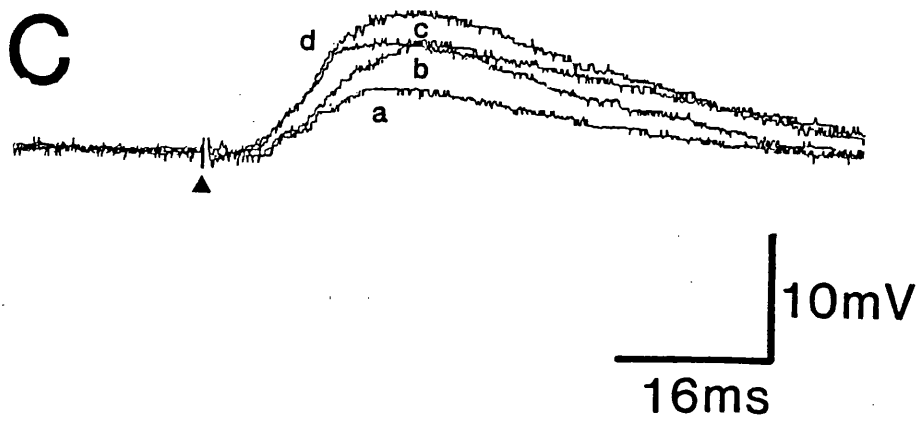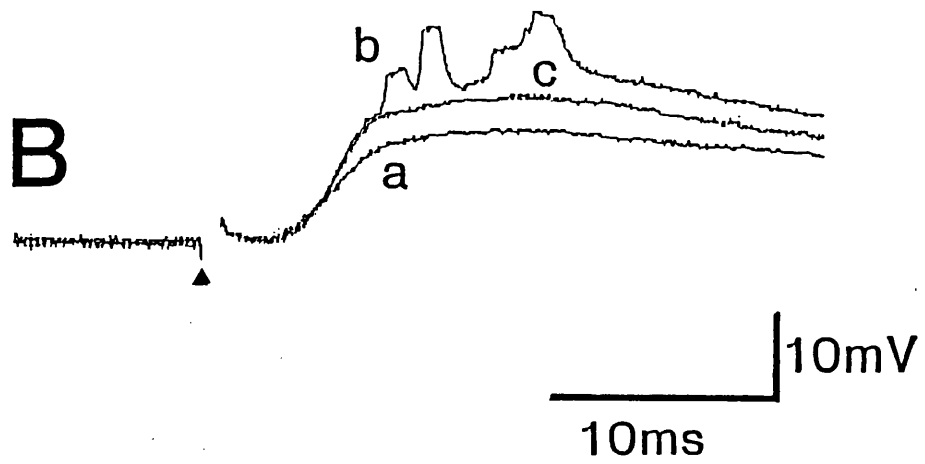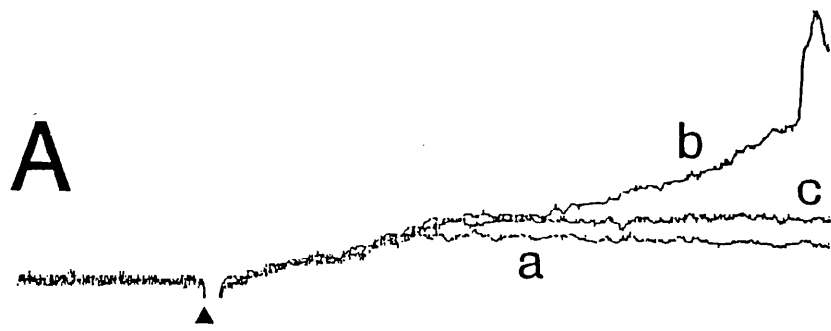
latency e.p.s.p.s, the AP5-sensitive potentiated component was preceded by a potentiated AP5-insensitive component. In the case of the late latency e.p.s.p. that was potentiated, the potentiated component was entirely abolished by AP5; i.e. it was mediated soley by NMDA receptors.

3.II.4 (ii) Is the expression of LTP voltage-dependent?

From superimposition of records of e.p.s.p.s evoked at different membrane potentials, prior to and after conditioning, it was possible to examine the voltage-dependency of the potentiated component (figure 28). In all potentiated postsynaptic potentials (n=12), LTP was evident at both hyperpolarized and depolarized membrane potentials and was not voltage-dependent.

FIGURE 28: DELAY IN POTENTIATION IS NOT VOLTAGE-DEPENDENT.

Same neurone as in figures 22 & 23.

Each pair of superimposed traces are control and potentiated lateral
e.p.s.p.s. evoked at the following membrane potentials: -86mV (resting
membrane potential), -90mV, -95mV, -99mV and -103mV. Membrane potential
was altered by injections of square pulses of hyperpolarizing current. At
all membrane potentials, the onset of potentiation was clearly delayed by
8-10ms with respect to the onset of the e.p.s.p.. The delay was independent
of the membrane potential.

-86mV

-90mV

-95mV

-99mV

-103mV

10mV

8ms
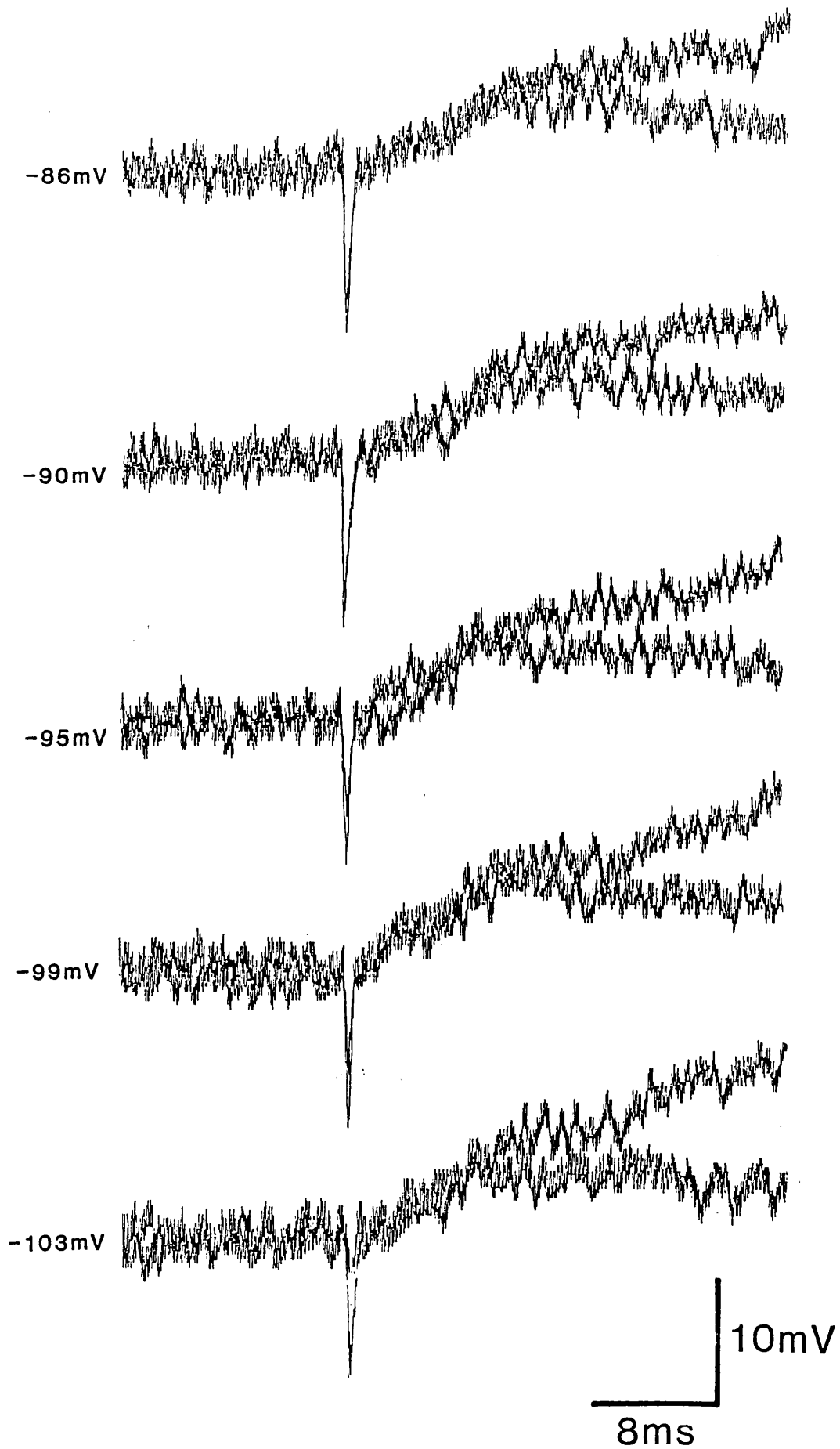
PART THREE: RESULTS OF EXTRACELLULAR EXPERIMENTS.

3.III.1 (i) Introduction.

Field potentials were recorded in layers III and V/VI from 15 slices of rat sensorimotor cortex. Layer III field potentials consisted of a negative monophasic wave with a peak latency of 8ms. Layer V/VI field potentials had 2 negative peaks, an early peak with an average latency between 7-8ms and a later peak with an average latency of 20ms (figures 29 & 30)

3.III.1 (ii) Action of AP5 on neocortical field potentials.

Bath application of 10-20μM D-AP5 reversibly reduced the amplitude and duration of field potentials recorded in both layer III and Layer V/VI in 8 of 10 slices. AP5 reduced the amplitude of the monophasic peak of layer III potentials and only reduced the late peak in layer V/VI field potentials. Bicuculline methiodide was not required to uncover the presence of AP5-sensitive components in both layer III and layer V/VI field potentials.

AP5 had no effect on field potentials recorded in 2 slices, in one slice the potential was small (<55μV) and in the other slice AP5 increased the amplitude of the field potential, presumably because the recording electrode was near a source of non-NMDA receptor mediated current.

3.III.1 (iii) The induction of extracellularly recorded LTP.

High frequency stimulation successfully induced LTP in 13 out of 15 slices (in 87% of slices). Three of the 15 slices were bathed in 1μM bicuculline methiodide and conditioning induced LTP in 2 of these slices.

The remaining 12 slices were not exposed to bicuculline methiodide and LTP was induced in 11 of them. The presence of micromolar concentrations of bicuculline methiodide in the bathing medium was not necessary for the induction of LTP.

Figure 29 illustrates the induction of LTP in a layer V field potential. After a 20 minute control period, during which the field potential remained stable, the slice was conditioned by application of 3 trains of high frequency shocks via the stimulating electrodes to the underlying subcortical white matter. A brief period of post-tetanic depression immediately followed conditioning, a phenomenon which was observed in the majority of experiments (see figure 30). By 12 minutes after conditioning, potentiation of the field potential was evident as a small increase in the amplitude of the early peak and a larger increase in the late peak (see also figure 30E).

Table 17 lists the mean peak values for field potentials measured prior to the induction of LTP, during established LTP and subsequent exposure to 10-20μM D-AP5 recorded in 13 slices (one field potential per slice). The mean increase in peak amplitude produced by LTP was 28% (±19.6% sample standard deviation; n = 13; range 8-76%).

In 5 slices, a second set of conditioning stimuli induced a further increase in the magnitude of LTP, in one slice a further enhancement was induced by a third set of conditioning stimuli.

3.III.1 (iv) Action of AP5 on the expression of LTP.

Bath application of 10-20μM D-AP5, applied 15-20 minutes after the induction of LTP, reversibly reduced the magnitude of potentiation in 12 out of 13 slices (mean reduction in peak amplitude 18%±15SD, n = 12). Both

FIGURE 29: INDUCTION OF LTP AND THE ACTION OF AP5 ON THE EXPRESSION OF LTP IN A LAYER V FIELD POTENTIAL.

Field potentials were recorded in layer V and evoked by stimulation of the underlying white matter at 0.1Hz.

GRAPH: Graph of field potential amplitude measured from electronic averages of 8 successive responses at 6ms (+) and 10ms (x) after the test shock. After a stable control the slice was conditioned by high frequency stimulation via the test shock stimulating electrodes (3x400Hz, each 500ms duration). Conditioning induced post-tetanic depression of the field potential followed by the development of LTP. Once LTP was fully established the slice ws exposed to 10μM D-AP5. AP5 selectively reduced field potential at 10ms and not at 6ms. The action of AP5 was reversible.

RECORDS: Electronic averages of 8 successive responses.

A: Control, prior to conditioning.

B: Established LTP (24 minutes after conditioning).

C: Action of 10μM D-AP5 on potentiated response.

D: Superimposition of the control and potentiated responses. Note the increase in initial slope and the development of two distinct peaks in the potentiated potential.

E: Superimposition of potentiated responses prior to and during exposure to AP5. Note that AP5 selectively reduced the late component of the potential, the slope and first peak were unaffected.
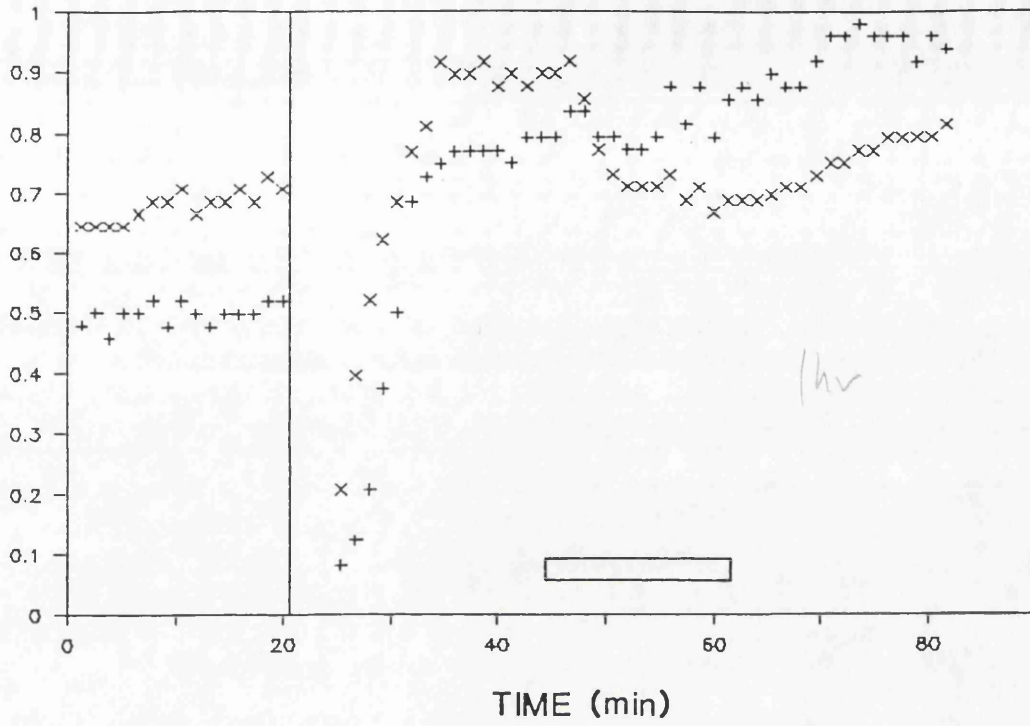
TABLE 17: LTP OBSERVED IN NEOCORTICAL FIELD POTENTIALS.

All values are mean peak amplitudes measured over 5 minutes.

Peak amplitudes during LTP and AP5 have been normalised as percentages of control values.

MEAN PEAK AMPLITUDES

LAYER	CONTROL (mV)	LTP (mV)	AP5 DURING LTP (mV)
III	1.14	1.76 (154%)	1.56. (137%)
III	0.74	0.88 (119%)	0.67 (90%)
III	0.94	1.04 (111%)	0.90 (96%)
III	0.61	0.77 (126%)	0.77 (126%)
III	1.30	1.49 (115%)	1.40 (108)%
III	0.77	0.91 (118%)	0.87 (113%)
III	0.71	1.05 (148%)	0.96 (135%)
III	1.26	1.44 (114%)	1.30 (103%)
V	0.75	0.95 (127%)	0.77 (103%)
V	0.85	0.92 (108%)	0.65 (76%)
V	0.71	0.89 (125%)	0.69 (97%)
VI	0.83	1.04 (125%)	0.94 (113%)
VI	0.93	1.64 (176%)	0.67 (72%)

MEAN±SD:	100%	128±19.6%	105±20.0%
		n = 13	n = 13

figures 29 and 30 illustrate the effect of AP5 on established LTP observed in layer V/VI field potentials, the predominant action of AP5 was to reduce the late peak in each potential.

Exposure to AP5 did not affect the underlying mechanism(s) responsible for the maintenance of LTP as evinced by recovery of potentiation on the removal of AP5 (figures 29 and 30).

3.III.1 (v) AP5 blocks the induction of neocortical LTP.

Bath application of 20μM D-AP5 10 minutes prior to and during conditioning blocked the induction of LTP in 5 out of 7 slices. Once AP5 had been washed from these slices, a second set of identical conditioning stimuli induced LTP in all 7 slices. In the 2 slices where AP5 failed to block induction of LTP, the potentiation produced by the second set of conditioning stimuli was 300% larger, suggesting that AP5, to a degree, had diminished the efficacy of the first set of conditioning stimuli in these slices.

Figure 30 shows the block of induction of LTP by AP5 in early (7ms) and late (20ms) peaks of a layer VI field potential. The early peak was AP5-insensitive while the later peak was significantly reduced by AP5. Application of the conditioning stimuli (4 trains, 100Hz, 100ms, 10sec apart) in the presence of AP5 did not induce LTP but did produce a period of post-tetanic depression. Following the washout of AP5, a second set of identical conditioning stimuli induced LTP in both the early and late components of the field potential. A third set of conditioning stimuli also induced a further enhancement of potentiation. Subsequent bath application of 20μM D-AP5 dramatically reduced the potentiated field potential, however it was still significantly larger when compared with the

FIGURE 30: AP5 BLOCKS THE INDUCTION OF LTP IN A LAYER V/VI FIELD POTENTIAL.

GRAPH: Graph of field potential amplitudes against time measured from electronic averages of 8 successive responses at fixec latencies of 7ms (x) and 20ms (+). Field potentials were evoked at 0.1Hz.

After a stable control period, 40μM DL-AP5 was bath applied to the slice (*horizintal bar*). After 10 minutes exposure the slice was conditioned (4x100Hz, each 100ms duration, intertrain interval 10s), conditioning denoted by *vertical line*. After conditioning the AP5 was washed from the slice. Once the field potential had returned to control values the conditioning was repeated. Bathing medium contained 1μM bicuclline methiodide.

RECORDS: Electronic averages of 8 successive responses.

A: Superimposition of control prior to application of AP5 and during exposure to AP5, just prior to the application of conditioning stimuli. Note that the main effect of AP5 is to reduce the late component of the field potential, the first peak was unaffected.

B: First average after the end of the first set of conditioning stimuli in the presence of AP5. Note that the response is severely depressed.

C: Recovery of the field potential after the removal of AP5.

D: First average after the end of the second set of conditioning stimuli in the absence of AP5. The response was severely depressed.

E: Superimposition of averages prior to and 26 minutes after the second conditioning.
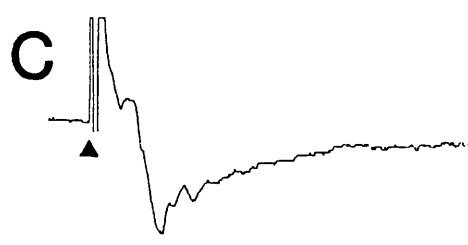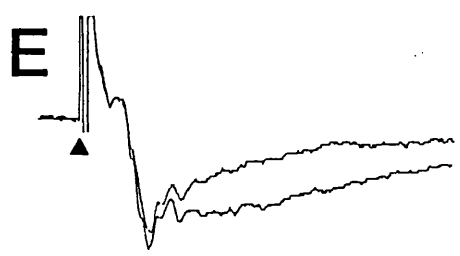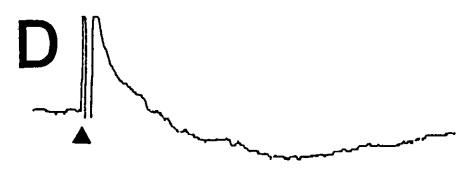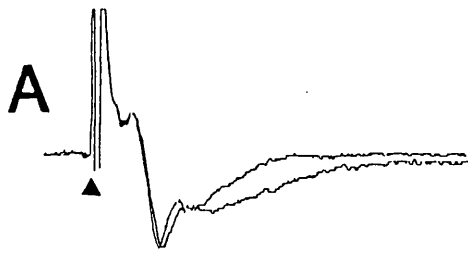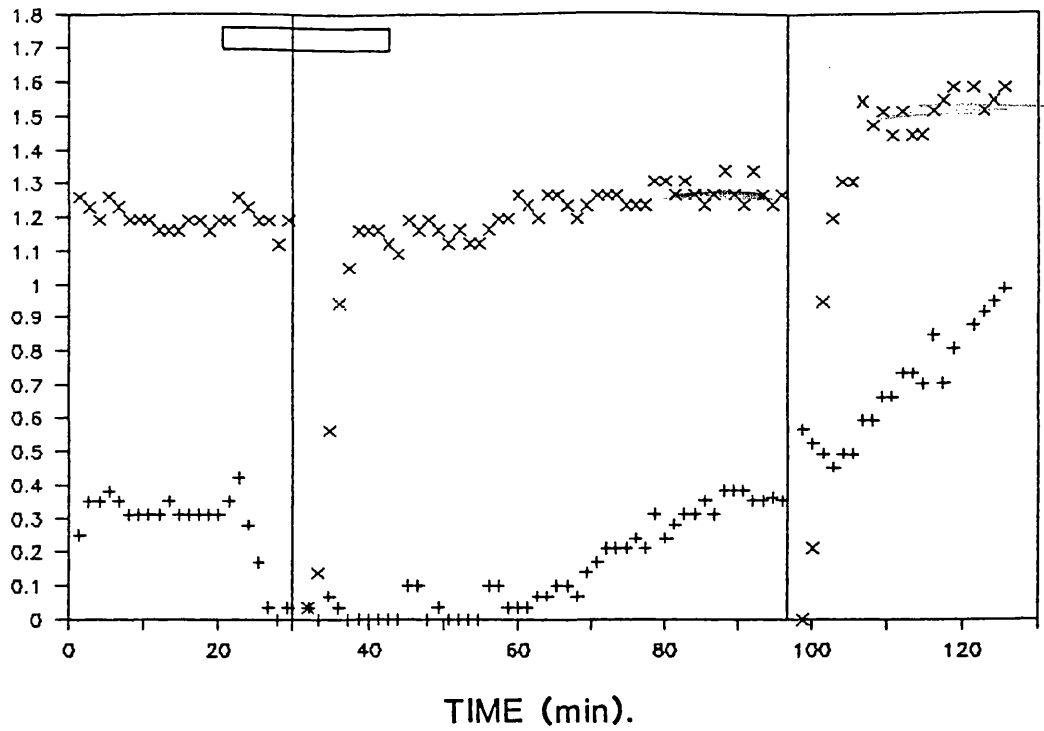
Graph from minute 90 to minute 126 and shows the development of LTP
after the second set of conditioning stimuli. A third set of conditioning
stimuli were applied to the slice (*vertical line*) induced a further
potentiation of the field potential. 40μM DL-AP5 was again bath applied to
the slice (*horizintal bar*) 20 minutes after the third conditioning. Decline
of amplitude measured at 7ms due to the fractionation of the field
potential.

F: Superimposed averages of the potentiated response prior to and
        during application of AP5. Both potentiated first and late
        components were reduced by AP5.

G: Recovery of the potentiated response after the removal of AP5.

F

G

2mV

16ms
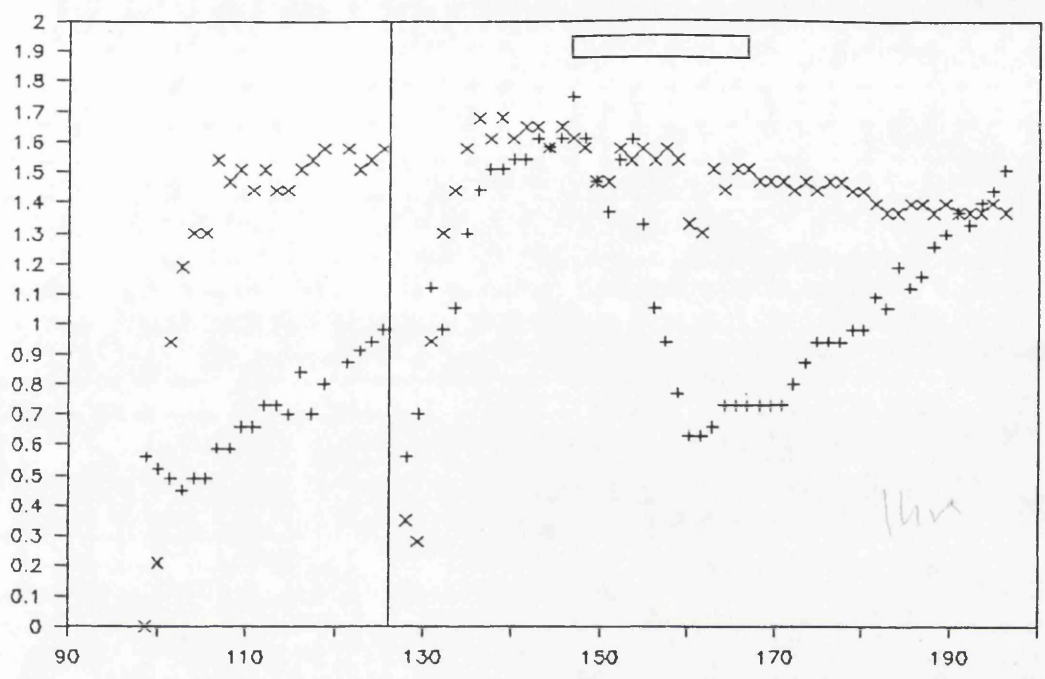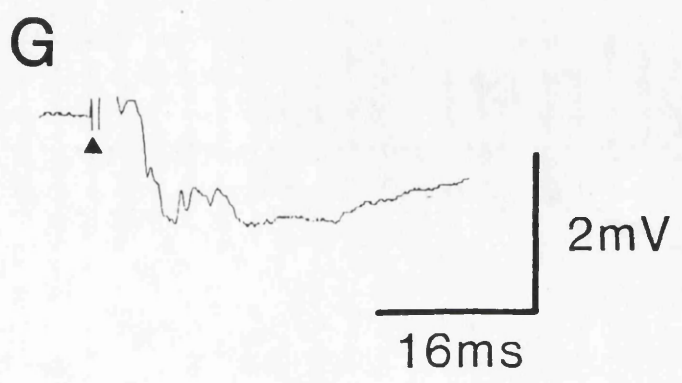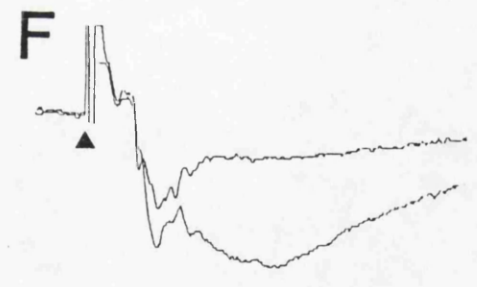
unpotentiated field potential prior to the first set of conditioning stimuli. Potentiation was seen to recover on the removal of AP5 from the bathing medium.

The voltage changes evoked by conditioning stimuli are illustrated in figure 31 for the experiment described in figure 30. The upper panel shows superimposed potentials elicited by the first and fourth trains of conditioning stimuli in the presence of AP5, and in the absenceof AP5 in the lower panel. In the absence of AP5, the potential evoked by the conditioning stimuli had a negative afterpotential which lasted >200ms and grew in both duration and amplitude with each successive train, whereas in the presence of AP5, not only was the initial response smaller than that seen in the absence of AP5, but there was also no successive growth in the afterpotential with each subsequent train of conditioning stimuli.

This action of AP5 on the potentials evoked by conditioning trains of stimuli was seen in all 5 slices where AP5 blocked the induction of LTP.

3.III.2 (1) Characterization of neocortical field potentials.

To determine the relative contributions of the afferent volley and synaptic currents to the recorded field potentials, synaptic currents were blocked in two slices by exposure to high extracellular concentrations of Mg^{2+} (final concentration 45-60mM) (Bindman et al., 1979).

The late peak of layer V field potentials recorded in the 2 slices were abolished by high Mg^{2+}, the early peaks were also significantly reduced, however in one potential there still remained a residual, but

FIGURE 31: ACTION OF AP5 ON CONDITIONING TRAINS.

Same experiment as in figure 30.

A: Superimposition of field voltage change recorded in layer V
         induced by the first and fourth trains of the conditioning
         stimuli (4x100Hz, 100ms duration at 0.1Hz) in the presence of
         40µM DL-AP5. Arrow denotes start of conditioning train.

B: As for A, except 69 minutes later and in the absence of AP5.
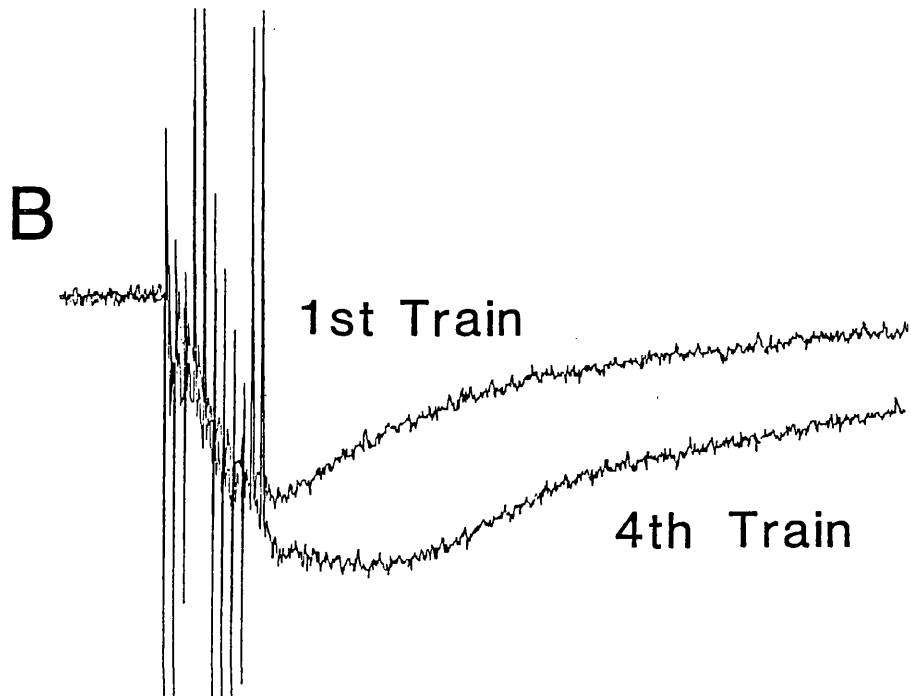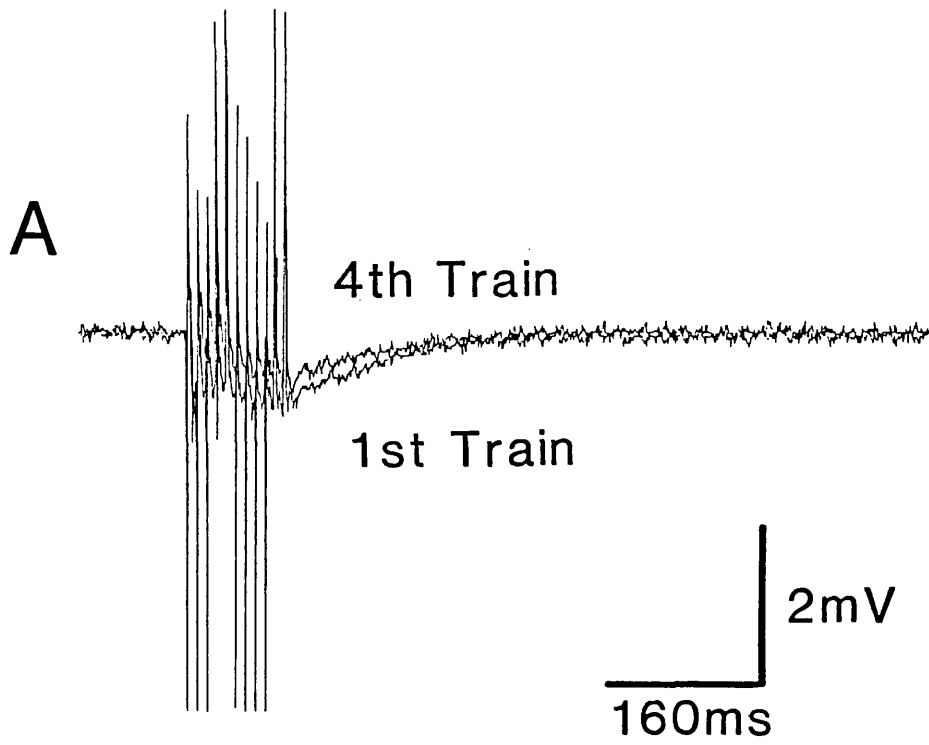
FIGURE 32: EFFECT OF HIGH EXTRACELLULAR Mg^{2+} ON NEOCORTICAL FIELD POTENTIALS.

A: superimposed electronic averages of 4 successive responses recorded in 1.1mM Mg^{2+} and after 20 minutes exposure to 45mM Mg^{2+}.
Upper pair of traces recorded in layer III.
Lower pair of traces in layer V.
Both layer V and III field potentials were recoreded simultaneoulsy in the same slice and evoked by the same test shock.

B: Superimposed averages of 8 successive responses (layer V field potential) in 1.1mM Mg^{2+} and after exposure to 60mM Mg^{2+}.
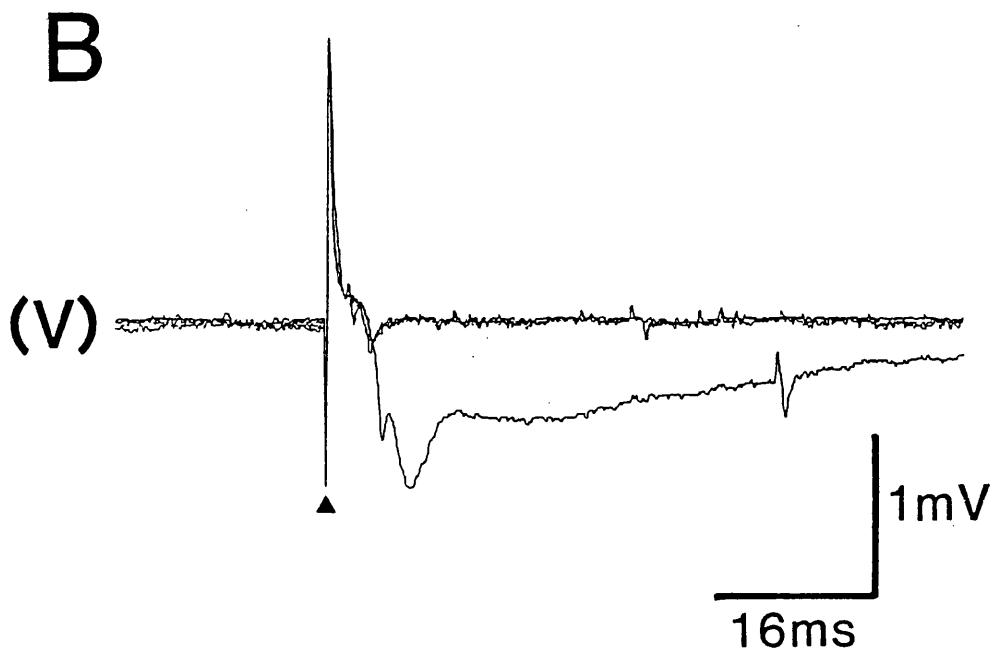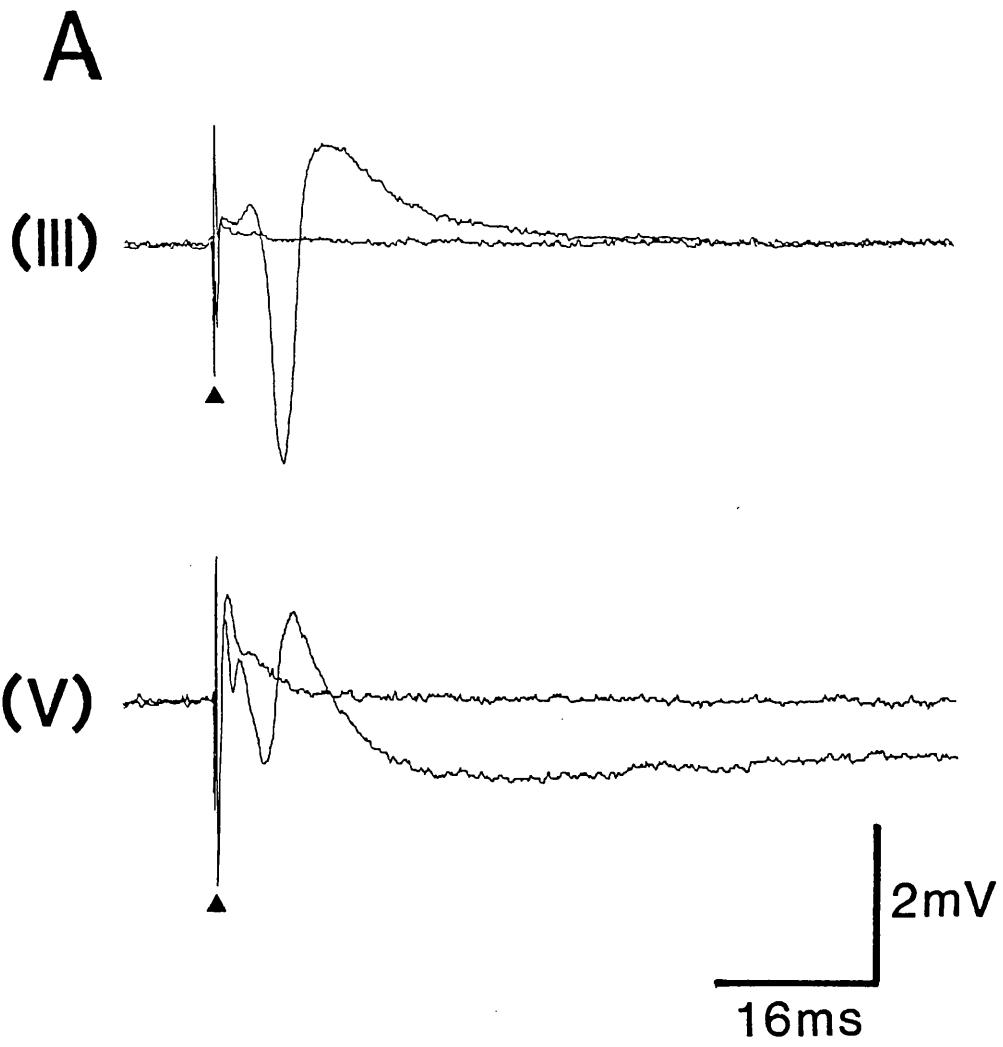
small peak, with a reduced peak latency whereas in the other layer V

potential, raised Mg^{2+} uncovered a small positive wave rising out of the

stimulus artefact (figure 32). The one layer III field potential exposed to

high Mg^{2+} was abolished entirely (figure 32). This data suggests that in

the main, field potentials reflected synaptic current flow.

3.III.2 (ii) Test shock parameters used in extracellular and intracellular studies.

Given the very different rates of induction of LTP for the

extracellular and intracellar studies, induced by high frequency

stimulation of an afferent pathway, 19.2% and 87% respectively, the

conditioning parameters were examined to look for any differences which

might account for the induction rate disparity. The duration and stimulus

frequency of the conditioning trains were similar for both studies. However

it was not possible to determine the relative strengths of the

conditioning shocks by examining the shock parameters. Variables such as the

position and depth of penetration of the stimulating electrodes within the

white matter, together with their age and condition made a comparison

unreliable. As the shocks used for the conditioning trains were based on

the test shocks, it was decided to perform a number of experiments where

field potentials and e.p.s.p.s were simultaneously evoked by the same test

shock and recorded in the same neocortical layer to allow a direct,

comparison of the effectiveness of the test shock.

Figure 33 shows field potentials and e.p.s.p.s recorded simultaneoulsy

in the same slice and evoked by the same test shock (two slices in total).

In every case, the field potential was significantly smaller than those

evoked during the extracellular study (see figures 29 & 30), whereas the

FIGURE 33: SIMULTANEOUS RECORDING OF SUBTHRESHOLD E. P. S. P. S AND FIELD POTENTIALS.

A: Simultaneous electronic averages of 8 successive responses.
Upper: subtheshold e.p.s.p. recoreded in a layer V neurone; resting membrane potential, -90mV; input resistance, 33MΩ.
Lower: field potential recorded in layer V.
The tips of the recording microelectrodes were within 200μm of each other.

B: Simultaneous electronic averages of 4 successive responses.
Upper: subthrehold e.p.s.p. recorded in a layer V neurone; resting membrane potential, -80mV; input resistance, 25MΩ.
Lower: accompanying field potential, recording electrode within 200 μm of intracellular electrode (on surface of slice).

Bicuculline methiodide was not used in either experiment.
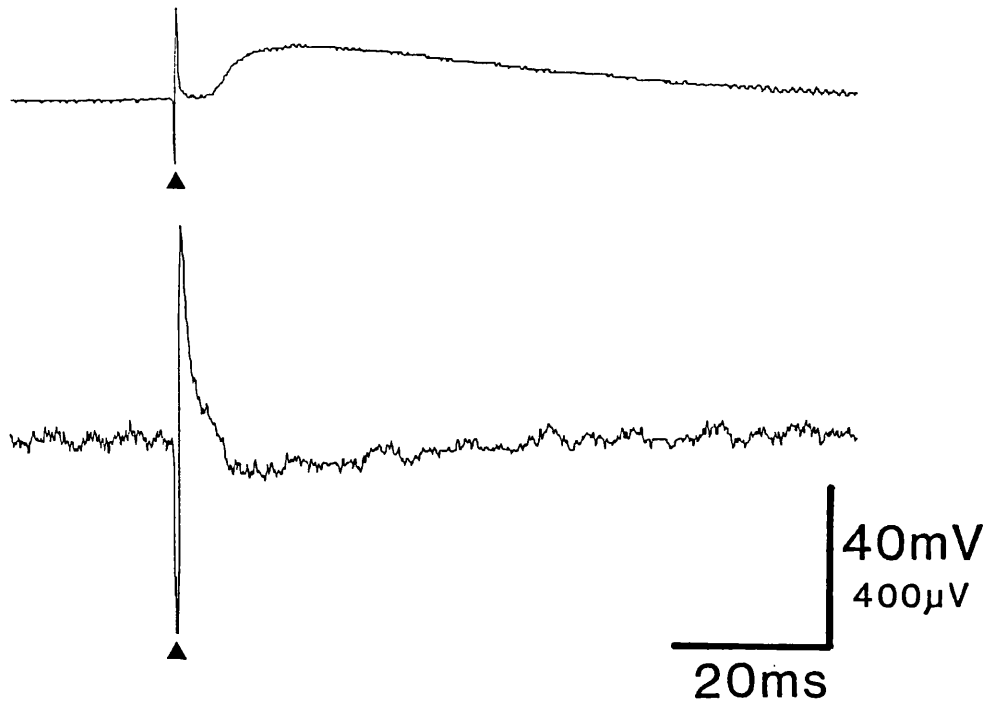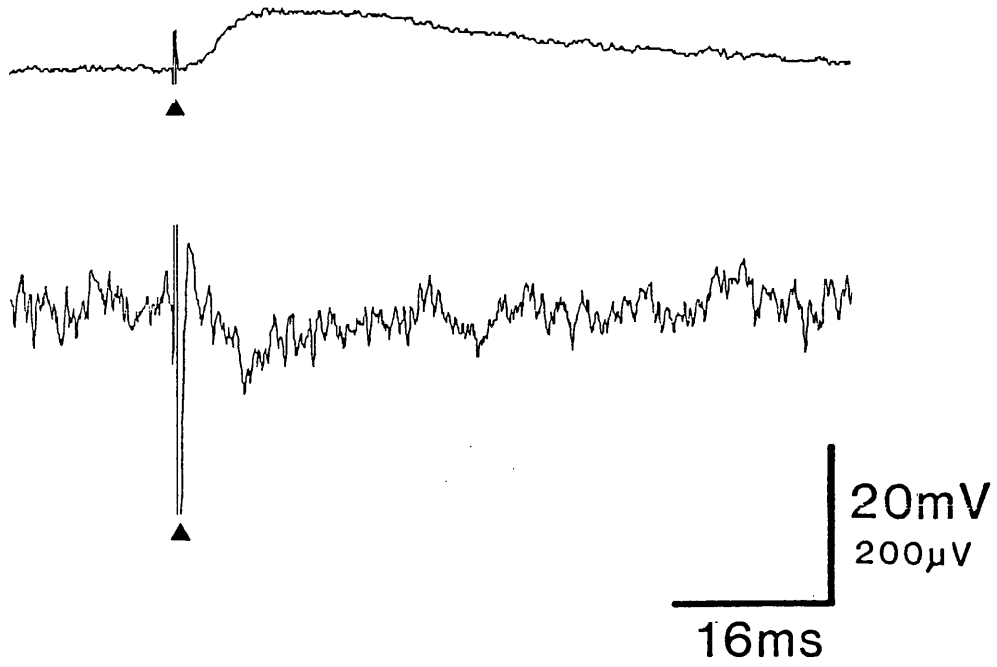
e.p.s.p.s were consistent with those seen in the intracellular study. These experiments imply that the test shocks used for the extracellular study were of greater magnitude than those used for the intracellular one. Likewise, the shocks used during high frequency stimulation in the extracellular study were probably stronger than those used for the intracellular study and might have been an important factor in determining the outcome of condtioning.

CHAPTER FOUR

– DISCUSSION –

4.1 (i) Long-term potentiation - A neocortical phenomenon?

The main finding of this thesis is that long-term potentiation can be induced in the isolated sensorimotor cortex by repetitively pairing intracellularly injected current pulses with e.p.s.p.s evoked by stimulating the subcortical white matter. The effect is selective to the paired e.p.s.p. since unpaired e.p.s.p.s in the same cell were not potentiated and the expression of potentiation was not associated with changes in neuronal input resistance or membrane potential. This is evidence that LTP in the neocortex can be induced by a postsynaptic mechanism and is additional evidence that in the neocortex as in the hippocampus (Baranyi & Fehér, 1981; Kelso *et al.*, 1986; Wigström & Gustafsson, 1986) neocortical LTP does have the conjunctive property required by the Hebb (Hebb, 1949) model of associative learning, that only e.p.s.p.s which occur while the cell is firing are potentiated. Thus far, other studies of neocortical LTP in the *in vitro* slice preparation have employed high frequency stimulation of an afferent input as the conditioning stimulus (Artola & Singer, 1987; Sutor & Hablitz, 1989; Kimura *et al.*, 1989; Hirsch & Crepel, 1990; Kanter & Haberly). While these studies confirm the finding of this thesis that LTP is a neocortical phenomenon, they were unable to determine the input specificity and associativity of the observed potentiation.

Another important finding of this thesis is that as for some hippocampal synapses (Collingridge *et al.*, 1983; Errington *et al.*, 1987) activation of NMDA receptors is a requirement for the induction of neocortical LTP, suggesting that the underlying inductive mechanisms of LTP may be similar in these two brain regions. However in contrast to hippocampal LTP, NMDA receptors are involved in the expression of LTP at neocortical synapses.

4.2(i) Nature of intracellular recordings.

The electrophysiological properties of the neurones described in this thesis are similar to those reported for neocortical pyramidal neurones by other laboratories: visual cortex (Artola & Singer, 1987; Jones and Baughman, 1988; Kimura *et al.*, 1989; Mason & Larkman, 1990); motor and sensorimotor cortex (McCormick *et al.*, 1985; Thomson, 1986; Sutor & Hablitz, 1989a).

The suprathreshold properties of the neurones in response to injections of depolarizing current were in agreement with the findings of McCormick *et al.* (1985) and Mason & Larkman (1990). All layer III neurones were of the *regular-spiking* variety, whereas the layer V neurones were either *regular-spiking* or *bursting* cells. Occasionally, an impalement was made in a cell characteristic of an interneurone, the *fast-spiking* cell-type described by McCormick *et al.*, (1985), though such cells were never held form more than a couple of minutes and are excluded from this study. It is therefore probable that the majority of cells from which intracellular recordings were made were pyramidal neurones.

4.3 Characteristics of neurotransmission at neocortical synapses.
4.3(i) Both NMDA & non-NMDA receptors participate in transmission at neocortical synapses.

Synaptic potentials mediated by NMDA receptors can be distinguished from those mediated by non-NMDA receptors by their sensitivity to micromolar concentration of the NMDA receptor antagonist, AP5 (Watkins & Evans, 1981; Davies *et al.*, 1982). The vast majority of p.s.p.s (89%) and field potentials (80%) recorded in layers III and V/VI of sensorimotor cortex, evoked by white matter stimulation and in the presence of physiological concentrations of Mg^{2+} were in part, mediated by NMDA

-165-

receptors, thus extending the findings of Thomson (1986) for layer III e.p.s.p.s in motor cortex and the first demonstration that e.p.s.p.s recorded in layer V neurones are mediated by both NMDA and non-NMDA receptors (Aram *et al.*, 1987). The latter finding has since been confirmed by Jones & Baughman (1988) for layer V e.p.s.p.s recorded in visual cortex.

The NMDA and non-NMDA receptor mediated component of both e.p.s.p.s and field potentials exhibited a distinct temporal relationship with respect to each other: a NMDA receptor mediated component was always preceded by a non-NMDA receptor mediated one. A minority of potentials that tended to be of small amplitude were mediated entirely by non-NMDA receptors. None of the e.p.s.p.s were mediated soley by NMDA receptors.

4.3 (ii) What determines the temporal relationship between NMDA and non-NMDA receptor mediated components?

The most parsimonious explanation for the temporal relationship between non-NMDA and NMDA receptor mediated components is that expression of the NMDA receptor component is dependent upon and therefore subsequent to, the depolarization of the postsynaptic membrane mediated by the non-NMDA receptors: membrane depolarization must first occur in order to relieve the Mg^{2+}-blockade of the NMDA channel. If this is so, then both receptor-types must be located on the postsynaptic membrane. Such an assumption is supported by a number of lines of investigation: the iontophoretic action of NMDA and non-NMDA agonists on layer III and V neurones (Dingledine *et al.*, 1986; Flatman *et al.*, 1986; Thomson, 1986; Jones & Baughman, 1988), the demonstration of a non-conventional voltage relationship of e.p.s.p.s attributable to the Mg^{2+}-blockade of NMDA channels (Nowak *et al.*, 1984; Mayer & Westbrook, 1985) recorded in layer III (Thomson, 1986; Artola & Singer, 1990) and layer V neurones (Jones &

Baughman, 1988). Also there is good evidence to suggest that both NMDA and non-NMDA receptors are co-located at some glutamatergic synapses (Forsythe & Westbrook, 1988; O'Brien & Fischbach, 1986; Huettner & Baughman, 1988; Thomson *et al.*, 1989; Bekkers & Stevens, 1989).

However, an explanation for the temporal relationship based on the voltage-dependent Mg^{2+}-blockade of the NMDA channel is not substantiated by the findings of this thesis. Depolarization of the postsynaptic membrane by intracellular injection of positive current did not alter the temporal relationship between non-NMDA and NMDA receptor mediated components of layer III and layer V/VI e.p.s.p.s.. However, it is conceivable that the injected current failed to influence the NMDA receptors, hence the inability to demonstrate a voltage-dependency for the onset of the NMDA receptor mediated component of neocortical e.p.s.p.. Also the possibility exists that an i.p.s.p. may have shunted the injected depolarizing current. A further possibility is that the NMDA receptors were not located on the postsynaptic neurone, though this is unlikely (Thomson *et al.* 1989). (see section below on voltage-dependency of e.p.s.p.s).

Using pharmacological methods, Artola & Singer (1990) found that the expression of a NMDA receptor mediated component of e.p.s.p.s evoked by white matter stimulation and recorded in layer III neurones of adult rat visual cortex was not dependent on the presence of a non-NMDA receptor mediated component. They examined the actions of a selective non-NMDA receptor antagonist, 6-cyano-2,3-dihydroxy-7-nitroquinoxaline (CNQX) and the NMDA receptor antagonist, AP5, on the time of course of e.p.s.p.s. They found that the time course of the AP5-sensitive and CNQX-insensitive synaptic components were similar, suggesting that both were not only mediated by NMDA receptors, but that a priming depolarization via the non-NMDA receptors is not a prerequisite for the expression of NMDA receptor

activity. However, their experiments did not reveal the cause of the temporal relationship between non-NMDA and NMDA receptor activity.

A possible clue to the source of the temporal relationship is offered by the work of Jones and Baughman (1988). They examined the AP5-sensitive and AP5-insensitive components of e.p.s.p.s recorded in layer V neurones of visual cortex, evoked by either stimulation of the white matter or the grey matter of layer II/III. E.p.s.p.s evoked by white matter stimulation were typical of those shown in this thesis, however those evoked by grey matter stimulation were different in two respects: firstly they exhibited an AP5-sensitive non-conventional voltage-dependency which will be discussed below; and secondly the latency of onset for AP5-sensitive and AP5-insensitive components were coincident. The latter suggests that the temporal relationship seen for the NMDA and and non-NMDA receptor mediated components of white matter evoked e.p.s.p.s is a product of the afferent pathway i.e. the non-NMDA receptor component are mediated by early or monosynaptic inputs whilst the NMDA receptor component is mediated by late or polysynaptic inputs. The re-location of the stimulating electrodes onto the grey matter of layer II/III by Jones & Baughman (1988) probably circumvented the polysynaptic pathway that gave rise to the NMDA receptor component in layer V e.p.s.p.s following stimulation of the white matter.

Of course it is highly likely that NMDA receptors are located on intermediate synapses in the polysynaptic pathway activated by white matter stimulation. Given the slow rise time of NMDA receptor mediated component, this too might introduce a further element of synaptic delay (Davies, 1990).

4.3(iii) NMDA receptor activity and i.p.s.p.s.

Unlike the e.p.s.p.s described in this thesis, in the hippocampus, NMDA receptors do not contribute to the generation of e.p.s.p.s, when evoked at low frequency (<1Hz) and in the presence of physiological concentrations of Mg^{2+} (Collingridge & Davies, 1990). Under these conditions, a hippocampal postsynaptic potentials consist of a short latency e.p.s.p. tightly coupled to a late i.p.s.p. (Collingridge & Davies, 1990). The i.p.s.p. effectively clamps the later portions of the early e.p.s.p. so that the membrane is not sufficiently depolarized to relieve the Mg^{2+}-blockade of the NMDA channel. However, in the presence of a $GABA_A$ antagonist, the efficacy of the late i.p.s.p. is reduced and it becomes possible to depolarize the neurone into a range where the Mg^{2+}-blockade of the NMDA channel is removed, thus allowing current to flow through the NMDA channel. Removal of extracellular Mg^{2+} ions also uncovers the presence of a NMDA receptor mediated component in hippocampal postsynaptic potentials (Collingridge & Davies, 1990).

It has been claimed that $GABA_A$ antagonists are required for the expression of a NMDA receptor mediated component in neocortical e.p.s.p.s recorded in layers II, III & IV of rat visual cortex (Artola & Singer, 1987). This was certainly not the case for the e.p.s.p.s recorded in layers III, V & VI of rat sensorimotor cortex described in this thesis, of which the majority were mediated in part by NMDA receptors and this was not dependent upon the presence of a $GABA_A$ antagonist. Perhaps this was because there was little or no evidence of contamination by i.p.s.p.s.. Conversely, the e.p.s.p.s recorded by Artola & Singer (1987;1990) in layer III of rat visual cortex were coupled to a late i.p.s.p. (Diasz & Prince, 1989), hence the need for a $GABA_A$ antagonist in their experiments to unmask an AP5-sensitive component.

None of the layer V/VI e.p.s.p.s examined had an AP5-sensitive
component that exhibited the non-conventional voltage-dependency that has
been attributable to the relief of the Mg^{2+}-blockade of the NMDA channel
(Mayer & Westbrook,1985; Thomson, 1986). It is possible that the Mg^{2+} in
the bathing medium was ineffective, thereby conferring a conventional
voltage-dependency on current flow through the NMDA channel. This is
unlikely, as removal of extracellular Mg^{2+} ions greatly enhanced the
magnitude of the NMDA receptor mediated component (also Thomson 1986;
confirmed for layer V by Jones & Baughman, 1988). This observation
suggests that the concentration of Mg^{2+} ions in the bathing medium was
sufficient to regulate current flow through the NMDA channel. It is
therefore unlikely that the failure to demonstrate a non-conventional
voltage-dependency was due an insufficient concentration of Mg^{2+} ions in
the bathing medium.

Perhaps the NMDA channels were not affected by the depolarizing
current injected into the neurones via the recording microelectrode. It is
well established that voltage-clamp techniques employing microelectrodes
(i.e. *sharp* opposed to *patch* electrodes) are not capable of clamping
neocortical e.p.s.p.s, presumably because the origin of the postsynaptic
current is a considerable electrotonic distance from the soma. Some
neurones also exhibited anomalous rectification which could have
substantially modified the injected current, damping it as it spread
throughout the neurone and thus minimising its effect at the synapses.

Jones & Baughman (1988) successfully demonstrated a non-conventional
voltage-dependency for the AP5-sensitive component of e.p.s.p.s recorded in
layer V neurones. However, this was only shown for three e.p.s.p.s evoked
by stimulation of the grey matter in layer II/III. It was previously argued

that white matter and grey matter stimulation activated the same population of NMDA receptors, but at different sites on the afferent pathway. If this is so, why is a non-conventional voltage-dependency not seen for e.p.s.p.s evoked by white matter stimulation? Jones & Baughman (1988) report that grey matter stimulation was less likely to activate inhibitory inputs than stimulation of the white matter. The latter might explain the inability to demonstrate a non-conventional voltage-dependency of e.p.s.p.s evoked by white matter stimulation.

There is evidence from the work of Artola & Singer (1987, 1990), that inhibitory inputs can obscure an AP5-sensitive non-conventional voltage-dependency. E.p.s.p.s evoked by white matter stimulation and recorded in layer III neurones of rat visual cortex, were mediated in part by NMDA receptors but did not exhibit a non-conventional voltage-dependency. Subsequent exposure of the neurones to a GABA$_A$ antagonist revealed the presence of an AP5-sensitive non-conventional voltage-dependency.

Is it possible that contaminant i.p.s.p.s prevented the expression of a non-conventional voltage-dependency in the e.p.s.p.s studied for this thesis? It is unlikely, as the presence of a GABA$_A$ antagonist in the bathing medium did not uncover an AP5-sensitive non-conventional voltage-dependency. The reasons underlying the inability to show a non-conventional voltage-dependency of layer V e.p.s.p.s needs further investigation.

4.4 Long-term potentiation in the neocortex.

4.4 (i) Nature of LTP induced by the pairing paradigm.

The findings described in this thesis show that LTP in the neocortex induced by the paradigm of conditioning is associative, homosynaptic and input specific; only those synapses active during the presentation of the conditioning current undergo the changes that manifest LTP. As such, LTP induced by the pairing paradigm is a strong candidate as a model for learning and memory. It also conforms to the constraints of the *Hebbian* synapse.

In one instance, potentiation of the conditioned response was paralleled by a weakening of the unconditioned input. This suggests that in the neocortex, there exists a facility for associative heterosynaptic depresson. Such a mechanism has been invoked for some aspects of neural plasticity in the postnatal development of the visual cortex (Singer, 1990).

4.4 (ii) Conditioning current and membrane properties.

Early investigations into the modification of cortical excitability, suggested that depolarization alone, devoid of synaptic activity, was sufficient to induce long-term changes in neuronal excitability (Bindman *et al.*, 1979; Bindman & Prince, 1986). It is therefore important to note that the pairing paradigm of conditioning did not induce non-specific modifications in neuronal excitabilty, suggesting that the conditioning currents employed were below a critical threshold for such changes.

4.4 (iii) Why were so many inputs resistant to change?

An outstanding feature of LTP in the adult sensorimotor cortex, is that the vast majority inputs were resistant to plastic modification. Hippocampal studies have shown that LTP is a saturable process, therefore the most parsomonious explanation for the elusiveniss of LTP in the neocortex, is that the majority of inputs were in a saturated state. However this is improbable as LTP was seen in 87% of experiments during the extracellular study.

One problem with white matter evoked postsynaptic potentials in the *in vitro* slice preparation is that their origin and point of synaptic contact on the postsynaptic membrane is unknown. It is possible that majority of synapses are located at a site beyond the reach of the conditioning current; only those synapses whose postsynaptic membranes were directly depolarized by the conditioning current were susceptable to plastic modification.

Another possiblity is that neocortical e.p.s.p.s evoked by white matter stimulation are contaminated with i.p.s.p.s, capable of reducing the efficacy of the condioning current. It has been reported that the addition of a GABA$_A$ antagonist to the bathing medium can increase the probability of inducing of LTP (Wigström & Gustafsson, 1985; Artola & Singer, 1987; 1990). This was not found to be the case for LTP induced by either high frequency stimulation or the pairing paradigm in sensorimotor cortex. Indeed, potentiation was seen in a postsynaptic potential which exhibited a very obvious i.p.s.p. prior to conditioning (figure 15).

4.4 (iv) Rates of induction of intracellularly recorded LTP.

Both the pairing and high frequency stimulation paradigms of conditioning induced intracellularly recorded LTP in the *in vitro* neocortex with comparable rates of effectiveness, 14% and 19.2% respectively. These rates are similar to those reported by Baranyi and his associatates for LTP of e.p.s.p.s recorded in layer V neurones in the motor cortex of anaesthetized cats: 13.7% (Baranyi & Fehér, 1978), 20% (Baranyi & Fehér, 1981) and 28% (Baranyi & Szente, 1987). It would seem that a feature of intracellularly recorded LTP in the adult neocortex is its rarity (see Kimura *et al.*, 1989).

4.4 (v) Time course of LTP induced by the pairing paradigm.

The time course of the expression of LTP depended on the conditioning paradigm used. LTP induced by the pairing paradigm of conditioning was always evident immediately after the cessesion of conditioning and was often apparent during the conditioning period itself. Clearly the mechanisms that underlie neocortical LTP are rapidly operative, a property consistent with rapid associative learning, especially with respect to noxious stimuli.

LTP resulting in a subthreshold e.p.s.p., was always expressed as a step function increase in amplitude (figure 16), similar to that reported by Gustafsson & Wigström (1988) for postsynaptically induced LTP in CA1 pyramidal neurones of the hippcampus. Potentiation as a step function was found to a reproducible feature at neocortical synapses; the same population of synapses could be potentiated in a step-like manner on more than one occasion.

In a few instances, postsynaptically induced LTP was expressed as a suprathreshold response, transformed from a previously subthreshold e.p.s.p.. Often potentiation of these e.p.s.p.s was expressed not as a step function increase in amplitude, but as a progressive gowth in magnitude (figure 23), suggesting the activation of a positive feedback process, resulting in further potentiation (see below).

4.4 (vi) Time course of LTP induced by high frequency stimulation.

LTP induced by high frequency stimulation had a very different time course of expression. Conditioning was always followed by a period of post-tetanic depression that lasted for many minutes before the eventual expression of the induced potentiation. The depression was probably due to a temporary reduction in synaptic transmission and not to modification of the postsynaptic membrane. Post-tetanic depression was never a feature of LTP induced by the pairing paradigm.

The stimulus trains used for conditioning occasionally evoked depolarizing shifts and bursts of action potentials that outlasted the trains for hundreds of milliseconds (figure 25). It is probable that high frequency stimulation caused the recruitment of a large number of neurones that did not normally contribute to the generation of the postsynaptic potential. Often the recruitment resulted in the reverbration of electrical activity within the neuronal circuitry of the slice (figure 25), and sometimes this was powerful enough to induce spreading depression, an unequivocal demonstration of neuronal recriutment. The pairing paradigm never induced spreading depression.

4.4 (vii) Site of the induction of intracellularly recorded LTP - Pairing
paradigm of conditioning.

By pairing, repeatedly, presynaptic activity with postsynaptic
depolarization, it was possible to induce long-term changes in synaptic
efficacy at neocortical synapses. The conditioning current was confined to
the conditioned neurone and is therefore compelling evidence that a
postsynaptic mechanism is involved in the induction of LTP. A similar
conclusion was reached by Baranyi & Szente (1987), using an *in vivo*
preparation. It would appear that as in the hippocampus, the induction of
LTP in the neocortex is voltage-dependent (see section on role of NMDA
receptor in LTP). This is supported by the observation made in this thesis
that there was a correlation between the outcome of conditioning by the
pairing method and the strength of the conditioning current i.e. the
stronger the depolarizing current, the greater the probability for the
induction of LTP.

4.4 (viii) Site of the induction of intracellularly recorded LTP - High
frequency stimulation conditioning

Unlike the pairing paradigm of conditioning, the effects of high
frequency stimulation were not likely to be confined to the postsynaptic
neurone. Indeed on a few occasions, high frequency stimulation induced
spreading depression, a wave of depolarization that invaded the whole
slice. It is therefore improbable that intracellularly recorded LTP, induced
by high frequency stimulation of the white matter, is entirely the product
of plastic modification of synaptic connections at the postsynaptic cell,
as claimed by Artola & Singer (1987; 1990). It is more probable that the
observed LTP is the postsynaptic integration of modified synaptic efficacy
in both mono- and polysynaptic inputs.

Given that conditioning of an afferent pathway by high frequency stimulation could evoke depolarizing events of considerable magnitude e.g. spreading depression, it is puzzling that it was only marginally more effective as an inducer of intracellularly recorded LTP than the pairing paradigm. This is even more puzzling when one compares the rate of 19.2% for the induction of LTP by high frequency stimulation of an afferent pathway for intracellularly recorded LTP, with a rate of 87% for extracellularly recorded LTP. The possible reasons for such a disparity in the probability of inducing LTP will be discussed later.

4.4 (ix) What determines the expression of LTP as either a step function or a progressive growth?

E.p.s.p.s potentiated as a step function did not elicit action potentials (figure 15), whereas they were a feature of e.p.s.p.s which continued to growth in magnitude after conditioning i.e progressive LTP (figure 23). It is possible that at synapses susceptible to potentiation, a recurring suprathreshold e.p.s.p., might depolarize the neurone sufficiently to trigger the mechanisms that induce LTP, thereby consolidating the potentiation and maintaining the depolarizing pressure for further potentiation. Conversely, the potentiation seen for subthreshold e.p.s.p.s was remarkably stable, if anything the amplitude of these e.p.s.p.s tended to decrease slightly with time. The stability of step function potentiated e.p.s.p.s may have been because of saturation of the inductive mechanism. However, this is unlikely as it proved possible to potentiate the same e.p.s.p., in a step function manner, on two consecutive occasions. The most likely explanation for the stability of step function LTP, is that the potentiated e.p.s.p.s themselves, did not breach the voltage threshold for the induction of LTP.

4.4 (x) NMDA receptors and the induction of LTP.

At some hippocampal synapses, the activation of NMDA receptors is a prerequisite for the induction of LTP (Collingridge *et al.*, 1983; Errington *et al.*, 1987). Indeed, the addition of NMDA to the bathing medium will enhance hippocampal synaptic potentials, though the effect gradually reverses on the removal of NMDA (Kauer *et al.*, 1988; Collingridge & Davies, 1990).

Depolarization of the postsynaptic membrane is a crucial event for the induction of LTP; it relieves the Mg^{2+}-blockade of the NMDA channels, allowing current to flow through them (Mayer & Westbrook, 1985, 1987). The NMDA channel is highly permeable to Ca^{2+} ions (Dingledine, 1983; Mayer & Westbrook, 1985, 1987; Mayer *et al.*,1987) and it is the entry of Ca^{2+} ions into the postsynaptic neurone during conditioning that initiates the events leading to the induction of LTP. In both the hippocampus and neocortex, injection of a Ca^{2+}-buffer into the postsynaptic neurone, prior to conditioning, will prevent the induction of LTP (Lynch *et al.*, 1983; Baranyi & Szente, 1987), suggesting once again that the postsynaptic inductive mechanisms for LTP may be similar in both cortical regions.

It is apparent from the field potential study, that pharmacological blockade of NMDA receptors, did prevent the induction of LTP in the sensorimotor cortex (also in visual cortex (Artola & Singer 1987; Kimura *et al.*, 1989) and motor cortex (Sutor & Hablitz, 1989c)). This observation certainly suggests that NMDA receptors are involved in the induction of neocortical LTP, though it is not clear if their activation is an essential element of the inductive process. It is not known if it is the passage of Ca^{2+} ions through the NMDA channels or their passage through voltage-dependent Ca^{2+} channels activated by NMDA receptor-mediated depolarization that is the trigger for the induction of neocortical LTP. The findings of

Bindman *et al.* (1979) and Bindman & Prince (1986), that depolarization of the postsynaptic alone could induce long-term changes in neuronal excitability has yet to be reconciled with current models of neocortical neural plasticity.

One can speculate that the pairing of e.p.s.p.s with injections of depolarizing current, facilitated the relief of the Mg^{2+}-blockade of the NMDA channels, thereby increasing the pressure for plastic modification. However, even in neurones where the postsynaptic induction of LTP was successful, it still proved impossible to demonstrate an AP5-sensitive non-conventional voltage-dependency of their e.p.s.p.s. This may be because the input currents used to examine the voltage-dependency of e.p.s.p.s were not as powerful as those employed during conditioning. The stronger currents used for conditioning would have infiltrated the dendritic tree more effectively than the weaker currents used to examine voltage-dependency. There is certainly a suggestion that the more powerful the conditioning current, the greater the likelihood that the outcome would be the induction of LTP.

4.4 (xi) GABA$_A$ antagonists and the induction of LTP.

Artola & Singer (1987, 1990) report that the addition of a GABA$_A$ antagonist to the bathing medium is essential for the induction of LTP in the *in vitro* adult rat visual cortex (also Teyler, 1990). They show that the main action of the GABA$_A$ antagonist is to enhance NMDA receptor activity (see section on voltage-dependency of e.p.s.p.) and thereby facilitate the inductive process for LTP. In contrast, it was found that in the *in vitro* adult rat sensorimotor cortex, the induction of LTP was not dependent on the addition of a GABA$_A$ antagonist to the bathing medium (also confirmed in frontal cortex by Sutor & Hablitz (1989a,c). Indeed, the

antagonist

addition of a $GABA_A$ did not markedly increase the likelihood of successfully inducing LTP.

Why should LTP be dependent on the relief of synaptic inhibition in visual cortex and not in sensorimotor cortex? Comparison of the e.p.s.p.s described in this thesis with those published by Artola & Singer (1987, 1990) for visual cortex, recorded in the absence of a $GABA_A$ antagonist and in the presence of physiological concentrations of Mg^{2+} ions, clearly shows that (i) the NMDA receptor mediated component of e.p.s.p.s is larger for the sensorimotor cortex than those seen in visual cortex, and (ii) the e.p.s.p.s of the visual cortex are contaminated to a greater degree with i.p.s.p.s. Both of these factors would have some bearing on the outcome of conditioning, clearly favouring sensorimotor cortex over the visual cortex. Perhaps this difference in the susceptibility for LTP reflects the differential development and function of these two cortical regions.

4.4 (xii) NMDA receptors and the expression and maintenance of LTP.

The maintenance of LTP at hippocampal synapses is not dependent on the continuing activation of NMDA receptors, nor is its expression (Collingridge et al., 1983; Gustaffson et al., 1987). This is also true for the maintenance of LTP in the neocortex; exposure to AP5 did not effect the underlying mechanisms responsible for its maintenance (figure 30). However, in contrast to the hippocampus, NMDA receptors do participate in the expression of neocortical LTP (figure 27) (Artola & Singer, 1987, 1990; Sutor & Hablitz, 1989a,c). However, the latter observation was not unexpected, as NMDA receptors were involved in the mediation of neocortical e.p.s.p.s prior to conditioning.

Hippocampal LTP is mediated by non-NMDA receptors (Muller & Lynch, 1988; Davies et al., 1989). For the experiments described in this thesis, it was found that both the NMDA and non-NMDA receptor mediated components of e.p.s.p.s and field potentials were potentiated during the expression of LTP and that the potentiated NMDA receptor mediated component was preceded by a potentiated non-NMDA receptor component. It is conceivable that potentiation of the NMDA receptor mediated component was consequent on the preceding potentiation of non-NMDA receptor activity, though this was certainly not the case in one instance, when potentiation was manifest as a *de novo* late latency firing discharge that was abolished entirely by subsequent exposure to AP5. Similar findings have been reported by Artola & Singer (1987, 1990) and Sutor & Hablitz (1989a,c). However, such late latency firing discharges are probably mediated by a polysynaptic pathway and is therefore possible that the observed potentiation is due to an increase in receptor activity at relay synapses. Clearly, the latter requires further investigation.

4.4 (xiii) Delayed onset of potentiation in neocortical e.p.s.p.s.

For the majority of neocortical e.p.s.p.s, the onset of potentiation was not coincident with the onset of the e.p.s.p., but was delayed for a few milliseconds. The delay in potentiation was not voltage-dependent, suggesting it was not due to a change in a voltage-dependent conductance. However, Baranyi et al. (1990) have reported that LTP of e.p.s.p.s recorded in layer V cells in the motor cortex of awake cats was accompanied by a decrease in a potassium conductance (I_A) and that this modification was Ca^{2+}-dependent, input specific and associative.

In the neocortex, e.p.s.p.s evoked by white matter stimulation are mediated by mono-, di- and polysynaptic afferents. The delay in

potentiation is likely to reflect the heterogenous nature of the synaptic input onto pyramidal neurones. It is tempting to suggest that the earliest inputs, probably monosynaptic, do not support LTP, whereas the later inputs, probably di- or polysynaptic afferents or even slower conducting monosynaptic afferents, are capable of supporting LTP. As the delay in onset of potentiation was a feature of LTP induced by both pairing and high frequency stimulation conditioning paradigms, it is likely that the delay in potentiation was attributable to modification of synapses located on the impaled neurone. Given that AP5 blocked the induction of LTP in the neocortex, it can assummed that the neocortical synapses that support LTP are glutamatergic. It is therefore likely that neocortical LTP is a feature of the local glutamatergic neuronal circuitry (also Sutor & Hablitz, 1989a,c; Hirsch & Crepel, 1990).

4.4 (xiv) What is the cause of the disparity in the induction rates for the intracellular and extracellular studies?

It was found experimentally, that high frequency stimulation conditioning was more efficacious in the extracellular than the intracellular study, with induction rates of 87% and 19% respectively. This disparity may reflect the recording technique employed. Field potentials are an aggregate of the electrical activity in a population of cells, consequently they are more likely to detect changes in synaptic efficacy than an intracellular recording which is limited to a single neurone. However, even if the latter is correct, it does not adequately account for the considerable magnitude of the LTP observed in potentiated field potentials. It is therefore more likely that the probability for the induction of LTP was greater for the extracellular study, suggesting that

there was a significant difference in the conditioning parameters used between the two studies.

By comparing field potentials and e.p.s.p., recorded simultaneoulsy in the same slice and layer, it became apparent that stronger conditioning shocks had been used in the extracellular study (see figure 33). Stronger conditioning shocks would recruit a larger number of afferents, improving the co-operativity between inputs and thereby produce a greater level of depolarization in the postsynaptic cells, the end result being an increased probability for the induction of LTP.

In addition to this, stronger shocks might recruit the smaller diameter afferents, such as extrinsic adrenergic and cholinergic fibres, that would not have necessarily been activated by the weaker shocks used in the intracellular study. This could account for the greatly increased induction rate seen in the extracellular study. Intact nor-adrenergic and cholinergic systems are essential for the neural plastic modification that occurs in the development of the post-natal visual cortex (Bear & Singer, 1986). These neurotransmitters may play an important role in the induction of neocortical LTP. Indeed, it has been reported that micromolar concentrations of nor-adrenaline can increase the probability of inducing LTP in the *in vitro* visual cortex (Conners & Bear, 1988). Futhermore, it was found that nor-adrenaline enhanced neurotransmission in both the *in vitro* visual cortex (Nowicky *et al.*, 1990ab) and sensorimotor cortex (Bindman *et al.*, 1990). Also, the monoamine, serotonin, greatly enhances NMDA induced currents in the neocortex (Nedergaard *et al.*, 1987). Interestingly, the pairing of evoked bursts of action potentials in response to injections of depolarizing current in neurones of the motor cortex of awake cats with the extracellular iontophoretic application of acetylcholine, caused an increase in neuronal input resistance which

persisted for tens of minutes (Woody *et al.* 1978; Woody & Gruen, 1987). The possible role of these transmitters in the induction and maintenance of LTP needs further investigation.

4.4 (xv) Associative long-term depression in the neocortex.

Paradoxically, the same conditioning precedures used in the pairing and high frequency stimulation paradigms that induced LTP, could also induce long-term depression (LTD). Like LTP, LTD was also input specific, homosynaptic and associative. In the hippocampus, it has been shown that the repetitive pairing of an e.p.s.p. with an injection of hyperpolarizing current, could induce associative LTD (Stanton & Sejnowski, 1989). It therefore appears that both depolarizing and hyperpolarizing currents can induce LTD, though the mechanism remains obscure.

A recent paper by Artola *et al.* (1990) suggests that both LTP and LTD in the neocortex are depolarization-dependent processes with different voltage thresholds. LTP is induced when postsynaptic depolarization is strong enough to activate a critical number of NMDA channels, whereas LTD is induced when the postsynaptic depolarization is below the threshold for the induction of LTP. Given this capacity for the up or down regulation of transmission at neocortical synapses, it is remarkable that evoked neocortical synaptic potentials remain stable for many tens of minutes and hours.

4.5 (1) Future considerations.

It is compelling to invoke LTP as a mnemonic device for learning and memory in the neocortex. However our understanding of the underlyiing processes is crude and it still remains, for the neuroscientist, to show that LTP is of physiological importance.

Having demonstrated that LTP is a neocortical phenomenon, it is now essential to elucidate and ascribe a function for the actual synaptic connections that undergo plastic modification. To this end, more information is required about the nature and output of the postsynaptic neurones and the origin and neurotransmitter of the afferent inputs onto them. The experimenter will also need to access the dendritic tree where most afferents make their synaptic contact.

Techniques have recently been developed that allow the experimenter to achieve most of the above. Perhaps the most significant are the synthesis of potent and selective agonists and antagonists for excitatory amino acid receptor/channels (Lodge & Johnson, 1990; Watkins et al., 1990), the demonstration that it is possible to record simulataneously from synaptically coupled neocortical neurones (Mason et al., 1990) and the development of whole cell voltage-clamp recording in the in vitro slice (Blanton et al., 1989). With such techniques it will be possible to manipulate better and therefore characterise the biophysical events that lead to changes in synpatic efficacy in the neocortex.

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