LEABHARLANN CHOLÁISTE NA TRÍONÓIDE, BAILE ÁTHA CLIATH | TRINITY COLLEGE LIBRARY DUBLIN
Ollscoil Átha Cliath | The University of Dublin

Terms and Conditions of Use of Digitised Theses from Trinity College Library Dublin

Copyright statement

All material supplied by Trinity College Library is protected by copyright (under the Copyright and Related Rights Act, 2000 as amended) and other relevant Intellectual Property Rights. By accessing and using a Digitised Thesis from Trinity College Library you acknowledge that all Intellectual Property Rights in any Works supplied are the sole and exclusive property of the copyright and/or other IPR holder. Specific copyright holders may not be explicitly identified. Use of materials from other sources within a thesis should not be construed as a claim over them.

A non-exclusive, non-transferable licence is hereby granted to those using or reproducing, in whole or in part, the material for valid purposes, providing the copyright owners are acknowledged using the normal conventions. Where specific permission to use material is required, this is identified and such permission must be sought from the copyright holder or agency cited.

Liability statement

By using a Digitised Thesis, I accept that Trinity College Dublin bears no legal responsibility for the accuracy, legality or comprehensiveness of materials contained within the thesis, and that Trinity College Dublin accepts no liability for indirect, consequential, or incidental, damages or losses arising from use of the thesis for whatever reason. Information located in a thesis may be subject to specific use constraints, details of which may not be explicitly described. It is the responsibility of potential and actual users to be aware of such constraints and to abide by them. By making use of material from a digitised thesis, you accept these copyright and disclaimer provisions. Where it is brought to the attention of Trinity College Library that there may be a breach of copyright or other restraint, it is the policy to withdraw or take down access to a thesis while the issue is being resolved.

Access Agreement

By using a Digitised Thesis from Trinity College Library you are bound by the following Terms & Conditions. Please read them carefully.

I have read and I understand the following statement: All material supplied via a Digitised Thesis from Trinity College Library is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of a thesis is not permitted, except that material may be duplicated by you for your research use or for educational purposes in electronic or print form providing the copyright owners are acknowledged using the normal conventions. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgement.

Characterisation of LTP induced by multiple spaced 200 Hz protocol in the rat dentate gyrus *in vitro*

by

Laurence O'Dwyer

TRINITY COLLEGE
LIBRARY DUBLIN

A dissertation submitted for the degree of Doctor of Philosophy at the University of Dublin, Trinity College.

This research was carried out in the Department of Physiology.

April 2004

TRINITY COLLEGE
0 2 JUN 2004
LIBRARY DUBLIN
THESIS
7450
7489.

A man sets himself the task of portraying the world. Over the years he fills a given surface with images of provinces and kingdoms, mountains, bays, ships, islands, fish, rooms, instruments, heavenly bodies, horses, and people. Shortly before he dies he discovers that this patient labyrinth of lines is a drawing of his own face.

- Jorge Luis Borges, October 31, 1960

Acknowledgements

Anyone who does three years of research ends up knowing at least one or two things about science. For my part, I know that the last page is always the first page, and that neither would ever be written without the help of others.

Prof. Roger Anwyl has guided me throughout my time in his lab and I would like to thank him very much for all his patience and support. His knowledge and reading have helped me enormously and given me an appreciation of the beauty of neuroscience. More importantly, he has helped me through difficult times in the lab and his understanding and commitment at these moments made my work a lot easier.

Prof. Michael Rowan has always encouraged me in my work and bought me a few rounds over the years – I want to thank him for his goodwill and generosity.

Thanks also to Prof. Chris Bell for allowing me to carry out research in his department.

In the lab I have worked with a disparate group of researchers, all talented, all entertaining. I had a lot to learn when I started and they taught me many things, from the making of electrodes, to strategies for arguing with the sales man from the California Fine Wire Company. I thank them for their friendship and for making the lab a colourful place to work – Dr. Jianqun Wu, Dr. Kevin Wang, Dr. Barabara Gisabella, Dr. Sarah Harney, Dr. Phil Welsby and Dr. Tony Rush.

Late-night experiments would have been a dull experience were it not for Tony Rush keeping equally poor hours as myself. As is the way, I remember these as the best experiments, with the department empty and endless conversations while we waited for recording to finish.

Not being able to think of anything myself, it was Frank Maher who picked up an LTP paper in UCC one day and that paper sent us both looking for work in this department. Thanks also to - Eric Downer, Maeve Barry, Mikel Egana, Jaimie O'Dwyer, Etain Tansey, Patricia Bergin, Marie Fogarty, Ivan Sullivan, Aedín Minogue, Aubrey Storey, Canice O'Brien, Kieran Walsh, Alice Jordan, Dr. Barry Boland, Dr. Yvonne Nolan, Dr. Darren Martin and Dr. Aileen Lynch - they have all

in their own way ensured that walking to work in the morning also meant looking forward to meeting old friends.

There are others who have helped me, but one I can't forget is Dr. Anne Harris in UCC – without her kindness and support I would never have had the opportunity to start this research.

Finally, for my mother and father, who have given me everything, who have driven to Dublin too many times, who have forgotten themselves in their wish to see me through, I hope that they can read the words that go between my words to say that I love them very much and it is to them that I dedicate this thesis.

For my mother and father

DECLARATION

This thesis has not been submitted as an exercise for a degree at any other university. Except where stated, the work described therein was carried out by me alone.

I give permission for the Library to lend or copy this thesis upon request.

Signed: Laurence O' Dwyer

Declaration

I declare that this work has not been submitted previously as a thesis for a degree at this or any other institution and that it is entirely my own work. The Trinity College Dublin Library may lend this thesis without restriction.

Laurence O'Dwyer

Laurence O'Dwyer BSc.

List of Illustrations

Fig. 1.1 Diagram of the hippocampus

Fig. 3.1 LTP induced by conventional HFS

Fig. 3.2 100 Hz LTP

Fig. 3.3 Single 200 Hz LTP

Fig. 3.4 Multiple spaced 200 Hz LTP

Fig. 3.5 Comparison of 3 HFS protocols

Fig. 3.6 No inhibition of 100 Hz LTP with propranolol

Fig. 3.7 No inhibition of single 200 Hz LTP with propranolol

Fig. 3.8 Propranolol causes a non-significant decrease in multiple spaced 200 Hz LTP

Fig. 3.9 Reduction of the late-phase of multiple spaced 200 Hz LTP with scopolamine

Fig. 3.10 Reduction of the late-phase of multiple spaced 200 Hz LTP with LY 341495

Fig. 3.11 No inhibition of multiple spaced 200 Hz LTP with MPEP

Fig. 3.12 Inhibition of multiple spaced 200 Hz LTP with emetine

Fig. 3.13 Inhibition of multiple spaced 200 Hz with anisomycin

Fig. 3.14 Anisomycin inhibits the late-phase of multiple spaced 200 Hz LTP

Fig. 3.15 Anisomycin has no effect on conventional LTP induced by 8 trains of 8 pulses at 200 Hz

Fig. 3.16 Reduction of induction and early and late-phase of multiple spaced 200 Hz LTP with PKC inhibitor BIS I

Fig. 3.17 Application of the PKA inhibitor, KT 5720, reduces the early and late-phases of LTP induced by the multiple spaced 200 Hz protocol

Fig. 3.18 Application of the PKA inhibitor after the 3rd 200 Hz reduces the early and late-phases of multiple spaced 200 Hz LTP

Fig. 3.19 A comparison between slices treated with KT 5720 after the 3rd 200 Hz and slices treated with KT 5720 throughout the experiment

Fig. 3.20 KN 62 reduces both the early and late-phases of LTP induced by the multiple spaced 200 Hz protocol

Fig. 3.21 KN 93 does not significantly reduce LTP induced by the multiple spaced 200 Hz protocol

Fig. 3.22 PD 98059 does not inhibit LTP induced by mild stimulation protocol

Fig. 3.23 PD 98059 inhibits large amplitude LTP induced by multiple spaced 200 Hz

Fig 3.24 PD 98059 inhibits the expression of LTP induced by multiple spaced 200 Hz protocol

Fig. 3.25 No significant difference between PD 98059 added after the 3rd 200 Hz or when present throughout the experiment

Fig. 3.26 PD 98059 does not block the induction of multiple spaced 200 Hz LTP

Fig. 3.27 Inhibition of the expression of LTP by MAP kinase inhibitor is reversible

List of Abbreviations

ACh	Acetylcholine
AMPA	α-Amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid
AMPAR	AMPA receptor
cAMP	cyclic 3',5'-adenosine monophosphate
D-AP5	D-(-)-2-amino-5-phosphonopentanoic acid
CaM	Calmodulin
CaMKII	Calcium/calmodulin-dependent protein kinase II
DAG	Diacylglycerol
E-LTP	Early-phase LTP
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
ERK	Extracellular signal-regulated kinase
GABA	Gamma-aminobutyric acid
HFS	High-frequency stimulation
ICSs	Intracellular Ca^{2+} stores
IP_3	Inositol-1,4,5-trisphosphate
LFS	Low-frequency stimulation
L-LTP	Late-phase LTP
LTD	Long-term depression
LTP	Long-term potentiation
mAChR	Muscarinic acetylcholine receptor
MAP kinase	Mitogen-activated protein kinase
MCPG	(+)-α-Methyl-4-carboxyphenylglycine
mGluR	Metabotropic glutamate receptor
NMDA	N-methyl-D-aspartic acid
NMDAR	NMDA receptor
PI	Phosphatidylinositol
PI3K	Phosphatidylinositol 3-kinase
PKA	cAMP-dependent protein kinase A (Protein kinase A)
PKC	Protein kinase C
PLC	Phospholipase C
PP-1	Protein phosphatase 1

PP-2A	Protein phosphatase 2A
PPF	Paired-pulse facilitation
PSD	Postsynaptic density

Summary

Long-term potentiation (LTP) in the hippocampus is believed to be a cellular correlate of learning and memory in the mammalian brain. Four different high-frequency stimulation (HFS) protocols were developed which induced distinct forms of LTP in the medial perforant path of the dentate gyrus. The magnitude of LTP and the time course of its decline were found to be critically dependent on the duration and intensity of the HFS protocol employed. In particular, a multiple spaced 200 Hz protocol with a 10 min spacing between each HFS was found to induce a large amplitude, non-decremental LTP.

The characteristics of the different forms of LTP were investigated pharmacologically using inhibitors of protein synthesis, neurotransmitter receptors and protein kinases. The β-adrenergic receptor antagonist, propranolol, was found to cause a non-significant reduction in 200Hz LTP and multiple spaced 200 Hz LTP and to have no effect on 100 Hz LTP, indicating that β-adrenergic receptors have a modulatory role in LTP induced by stronger stimulation protocols. Muscarinic acetylcholine receptors were found to have a more important role in multiple spaced 200 Hz LTP with the muscarinic acetylcholine receptor antagonist, scopolamine, causing a significant reduction in the late-phase of this robust form of LTP. The late-phase of multiple spaced 200 Hz LTP was also significantly reduced with the mGluR antagonist LY341495. $mGluR_5$ was not responsible for this reduction in late-phase LTP as the $mGluR_5$ selective antagonist, MPEP, had no effect on multiple spaced 200 Hz LTP.

The role of protein synthesis in the different forms of LTP was investigated using anisomycin and emetine. Mild amplitude LTP induced by 8 trains of 8 stimuli at 200 Hz was not affected by anisomycin indicating that it is independent of protein synthesis. In contrast, large amplitude LTP induced by the multiple spaced 200 Hz protocol was completely inhibited by both emetine and anisomycin. Application of anisomycin directly after the final HFS in the multiple spaced 200 Hz protocol caused a reduction in late-phase LTP. Together, these results indicate that protein synthesis is required for the induction and also for the maintenance of multiple spaced 200 Hz LTP.

PKC was found to have an important role in the induction and maintenance of multiple spaced 200 Hz LTP as the PKC inhibitor, Bis-1, significantly reduced LTP immediately after the first train of 200 Hz. LTP was maintained at this reduced level for the duration of the experiment indicating that PKC activation is required for the full expression of multiple spaced 200 Hz LTP.

The role of PKA in multiple spaced 200 Hz LTP was investigated using the PKA inhibitor, KT 5720. Application of KT 5720 both before and directly after the multiple spaced 200 Hz protocol caused a reduction in the early and late phase of LTP indicating that PKA is required for the maintenance of multiple spaced 200 Hz LTP. The induction of LTP was not however inhibited when KT 5720 was present throughout the experiment.

The CaMKII inhibitor, KN-62, was also found to cause a significant reduction in both the early and late phases of multiple spaced 200 Hz LTP. Another CaMKII inhibitor, KN-93, caused a non-significant reduction in the early and late phases of multiple spaced 200 Hz LTP.

The involvement of mitogen-activated protein kinase (MAP kinase) was investigated in the induction, expression and maintenance of LTP induced by the conventional 8 trains of 200 Hz protocol and also by the multiple spaced 200 Hz protocol. Activation of MAP kinase was found to be involved in the large amplitude non-decremental LTP induced by the multiple spaced 200 Hz protocol, although not in LTP induced by the brief 200 Hz protocol. Specifically, MAP kinase was found to be necessary for the expression but not the induction or maintenance of the LTP induced by the multiple spaced 200 Hz protocol. Thus, a selective MAP kinase inhibitor, PD98059, did not inhibit induction of LTP when applied only prior to HFS and washed out concurrent with HFS. However, the MAP kinase inhibitor did inhibit LTP expression when applied directly after HFS. Moreover, the inhibition of LTP expression by the MAP kinase inhibitior was fully reversible, even at 90 min post-HFS, showing that MAP kinase is persistently active for at least 90 min after the induction of LTP and that MAP kinase is not involved in the maintenance of LTP.

Table of Contents

Acknowledgements	ii
Declaration	v
List of Illustrations	vi
List of Abbreviations	viii
Summary	x
Table of Contents	xii
1 Introduction	***1***
1.1 Hippocampus	2
1.1.1 Hippocampal neurons	3
(1) Granule cells in the dentate gyrus	3
(2) CA3 pyramidal cells	4
(3) CA1 pyramidal cells	4
(4) Interneurons	4
(5) Trisynaptic circuit	5
1.2 Ionotropic glutamate receptors	7
1.2.1 AMPA receptors	7
1.2.2 Kainate receptors	8
1.2.3 NMDA receptors	10
1.3 Metabotropic glutamate receptors	12
1.4 Other neurotransmitter receptors	16
1.4.1 Adrenergic receptors	16
1.4.2 Muscarinic acetylcholine receptors	18
1.5 Synaptic plasticity	20
1.5.1 Long-term potentiation (LTP) in the hippocampus	20
1.5.2 Characteristics of LTP	20
1.5.3 Methods of induction of LTP	21

1.5.4 The role of NMDA receptors in LTP 22
1.5.5 The role of Ca^{2+} in LTP 23
1.5.6 The role of silent synapses and AMPA receptors in LTP 25

1.6 The locus of expression of LTP 28

1.7 The role of adrenergic, muscrainic acetylcholine
and metabotropic glutamate receptors in LTP 31
1.7.1 The role of adrenergic receptors in LTP 31
1.7.2 The role of muscarinic acetylcholine receptors in LTP 35
1.7.3 The role of metabotropic glutamate receptors in LTP 39

1.8 The role of protein synthesis in LTP 42

1.9 The role of protein kinases in LTP 46
1.9.1 Ca^{2+}/calmodulin-dependent kinase (CaMKII) 46
1.9.2 Protein kinase C (PKC) 50
1.9.3 Protein kinase A (PKA) 55
1.9.4 Mitogen-activated protein kinase (MAP kinase) 59

2 Materials and methods ***68***

2.1 Animals 69
2.2 Artificial cerebrospinal fluid (ACSF) 69
2.3 Preparation of slices 69
2.4 Experimental set-up 70
2.5 Electrodes 69
2.6 EPSP recording 71
2.7 High-frequency stimulation protocols 72
2.8 Data analysis and statistics 72
2.9 Compounds 73

3 Results *74*

3.1 Different stimulation protocols induce multiple forms of LTP 75

3.2 Blockade of β-adrenergic receptors does not significantly inhibit LTP induced by three different protocols 76

3.3 Antagonism of muscarinic acetylcholine receptors significantly reduces the late phase of multiple spaced 200 Hz LTP 77

3.4 Antagonism of mGluRs significantly reduces the late phase of multiple spaced 200 Hz LTP 77

3.5 The role of protein synthesis in LTP induced by different stimulation protocols 78

3.5.1 Emetine inhibits the induction of multiple spaced 200 Hz LTP 78

3.5.2 Anisomycin inhibits the induction of multiple spaced 200 Hz LTP 79

3.5.3 Anisomycin inhibits the late phase of multiple spaced 200 Hz LTP 79

3.5.4 Anisomycin does not inhibit mild LTP induced by 8 trains of 8 pulses at 200 Hz 80

3.6 Antagonism of PKC reduces both the early and late phases of multiple spaced 200 Hz LTP 80

3.7 The PKA inhibitor, KT 5720, reduces early and late phases of multiple spaced 200 Hz LTP 81

3.8 The role of CaMKII in multiple spaced 200 Hz LTP 83

3.8.1 The CaMKII inhibitor, KN-62, reduces the early and late phases of multiple spaced 200 Hz LTP 83
3.8.2 The CaMKII inhibitor, KN-93, causes a non-significant reduction in multiple spaced 200 Hz LTP 84

3.9 The role of MAP kinase in LTP induced by different stimulation protocols 84
3.9.1 The MAP kinase inhibitor, PD 98059, does not inhibit LTP induced by 8 trains of 8 pulses at 200 Hz 84
3.9.2 PD 98059 inhibits multiple spaced 200 Hz LTP 85
3.9.3 PD 98059 inhibits the expression of multiple spaced 200 Hz LTP 85
3.9.4 PD 98059 does not block the induction of multiple spaced 200 Hz LTP 86
3.9.5 Inhibition of the expression of multiple spaced 200 Hz LTP by PD 98059 is reversible 86

4 Discussion ***87***

4.1 Different stimulation protocols induce different types of LTP 88

4.2 Adrenergic receptors 95

4.3 Muscarinic acetylcholine receptors 97

4.4 Metabotropic glutamate receptors 100

4.5 Protein synthesis 105

4.6 PKC 112

4.7 PKA 116

4.8 CaMKII 121

4.9 MAP kinase 127

5 References ***136***

1 Introduction

1.1 Hippocampus

Guilio Cesare Aranzio first coined the term "hippocampus" in 1564. He was described as a "dull pupil" of Andreas Vesalius and from his original description it is unclear whether he was comparing its appearance to that of a seahorse, a white silkworm, a horse caterpillar, or a bottlenose dolphin (Lewis, 1923). Whatever he was trying to describe, the etymology of the word is derived from the Greek - hippos, meaning horse and kampos meaning sea monster. The first evidence that damage to the hippocampus leads to deficits in memory was presented to the Royal Society of London by Brown and Schafer in 1887. They found that monkeys with lesions of the temporal lobes where the hippocampus is located examined objects with which they were previously familiar as if they had never seen them before. The experimenters also placed monkeys with lesions of the temporal lobes into a cage with a wild monkey and when a lesioned monkey approached the stranger it was savagely attacked. However, after the attack the monkeys went straight back to the stranger as if they had completely forgotten what had happened. This behaviour was not seen after lesions of the occipital lobes.

Towards the end of the 19th century the detailed anatomy of the hippocampus was worked out by Meynert, Golgi and Cajal (see Mayer, 1971) but neither the temporal lobes nor the hippocampus attracted much attention in the memory literature following Brown and Schaffer's initial discovery. Greater understanding of the role of the hippocampus in memory formation did not come until the middle of the 20th century with the studies of the epileptic patient H.M. In 1953 this patient had surgery to remove a part of each temporal lobe in order to alleviate his epilepsy. Two thirds of his hippocampus was destroyed in the operation and following the operation he had no knowledge of the year, the names of his friends or the rooms in which he lived. The follow up studies showed that he had an inability to retain new factual information and a retrograde amnesia that extended back for years prior to the surgery. Studies also revealed differences between declarative and procedural memory. Whereas H.M. was unable to learn new facts he was capable of learning simple procedural tasks such as mirror writing, puzzles and stylus mazes (Scoville and Milner, 1957).

Subsequent studies in rats revealed similar findings. O'Keefe *et al.* (1975) showed that lesions of the fornix, which has afferent and efferent connections to the

hippocampus, led to rats being unable to complete a water-finding task when place-learning was required but found that the same rats were successful with cue-learning tasks.

Since the first paper concerning long-term potentiation (LTP) in the rabbit hippocampus in 1973 (Bliss and Lomo, 1973), the hippocampus has been extensively studied in the field of neurophysiology.

In the present study slices of the rat brain were used for investigation. In the rat the hippocampus extends almost from the septum dorsally to the caudal part of the amygdala ventrally, resembling the shape of a cashew nut. In the rat, as in all mammals, the hippocampus consists of two C-shaped, interlocking principal cell layers: the granule cell layer of the dentate gyrus (DG) and the pyramidal cell layer of Ammon's horn. Cajal (1911) subdivided Ammon's horn into an area of large pyramidal cells (*regio inferior*) and a region of more densely packed smaller pyramidal cells (*regio superior*). Lorente de No (1934) categorized Ammon's horn further into four subfields: CA (*cornu Ammonis*) 1 to CA4.

1.1.1 Hippocampal neurons

(1) Granule cells in the dentate gyrus

The dentate gyrus has three cell layers, a granule cell layer, a molecular cell layer which is largely acellular and a polymorphic cell layer known as the hilus. The granule cell layer consists of small spherically shaped cell bodies which are less than 10μm wide and are 4-6 cells deep. Dendrites extend to the molecular layer where they receive inputs from several sources including a major projection from layer II of the entorhinal cortex which sends glutamatergic fibres to the lateral and medial perforant paths. Many unmeylinated axons called mossy fibres emerge from the hilar region and enter the stratum lucidum of CA3 where they form synaptic connections. There are other mossy fibre collaterals which synapse with polylmorphic cells and project to other layers of the dentate gyrus.

(2) CA3 pyramidal cells

The pyramidal neurons in CA3 can be identified by the presence of large branched spines (known as excrescences) on their proximal dendrites. The mossy fibres from the dentate gyrus synapse with the proximal apical dendrites of CA3 pyramidal cells in stratum lucidum and the varicosities which surround the large branced dendritic spines of CA3 cells enable granule cells to make multiple synaptic connections with pyramidal cells at a single point of contact. Mossy fibres synapse with relatively few pyramidal cells in contrast to pyramidal cells which receive input from approximately 50 dentate granule cells. The CA3 also receives input from the entorhinal cortex, septal nucleus and the locus coeruleus.

(3) CA1 pyramidal cells

The CA1 receives innervation from CA3 cells, layer III of the entorhinal cortex, a cholinergic projection from the septal nucleus, and noradrenergic and serotonergic inputs. The cell bodies in the pyramidal layer are 3-6 cells deep and they are multi-polar neurons with an elaborate dendritic tree and the dendrites travel through the stratum oriens, stratum radiatum and stratum lacunosum-moleculare, where they synapse with afferent fibres. The axon and basal dendrites originate from the base of the cell body and extend within the alveus or stratum oriens to the subiculum or deep layers of the entorhinal cortex, terminating predominantly in layer V.

(4) Interneurons

GABAergic interneurons are found in the hippocampus and dentate gyrus. In the dentate gyrus they are mainly pyramidal basket cells which innervate the cell bodies of granule cells. There are three types of hippocampal interneuron – axo-axonic, basket cells and bistratified cells. Axo-axonic cells synapse onto the initial segment of the pyramidal cells and inhibit action potential initiation. Basket cells synapse onto the soma and bistratified cells synapse with the apical and basal dendrites. All interneuron subtypes synapse with the excitatory Schaffer collaterals and commissural-associational fibres in the strata radiatum and oriens (Patton and McNaughton, 1995).

(5) Trisynaptic circuitry

The entorhinal cortex is considered to be the starting point of the trisynaptic circuit. Starting in layer II of the entorhinal cortex, the perforant pathway projects through the subiculum to the dentate gyrus and CA3. From the medial entorhinal cortex, fibres extend to the middle one-third (medial perforant path) and fibres from the lateral entorhinal cortex terminate in the outer one-third (lateral perforant path) of the molecular layer of the dentate gyrus (McNaughton, 1980). The dentate granule cells distribute their mossy fibres to the entire transverse or proximodistal extent of CA3. The proximal portions of the CA3 are preferentially reached by fibers that originate in the infrapyramidal blade, the crest and adjacent portion of the suprapryamidal blade. Cells in the tip of the suprapyramidal blade interact with more distal portions of the CA3 field. The mossy fibres that terminate on the basal dendrites of the more proximally located CA3 pyramidal cells arise mainly from the infrapyramidal blade of the dentate gyrus. Therefore Claiborne *et al.* (1986) concluded that "the influence of different poplulations of granule cells upon the cells in field CA3 is a function of their location within the granule cell layer."

The pyramidal cells in field CA3 give rise to the Schaffer collaterals that distribute to the CA1 region. The Schaffer collaterals synapse in the stratum radiatum and stratum oriens with the dendrites of CA1 pyramidal cells. The CA1 pyramidal cells, in turn, give rise to powerful projections to the subiculum that terminate in the deep half of the molecular layer and in the pyramidal cell layer. The connections between CA3 and CA1 and those from CA1 to the subiculum show a columnar organization perpendicular to the cell layer. Both intrinsic systems exhibit the same gradual topology: neurons in CA3 and CA1 form azonal columns in CA1 and the subiculum, respectively, with a reversed order. Proximal parts of CA3 and CA1 distribute fibres to distal parts of CA1 and the subiculum, respectively, and more distal parts of CA3 and CA1 interact with more proximal parts of CA1 and the subiculum respectively.

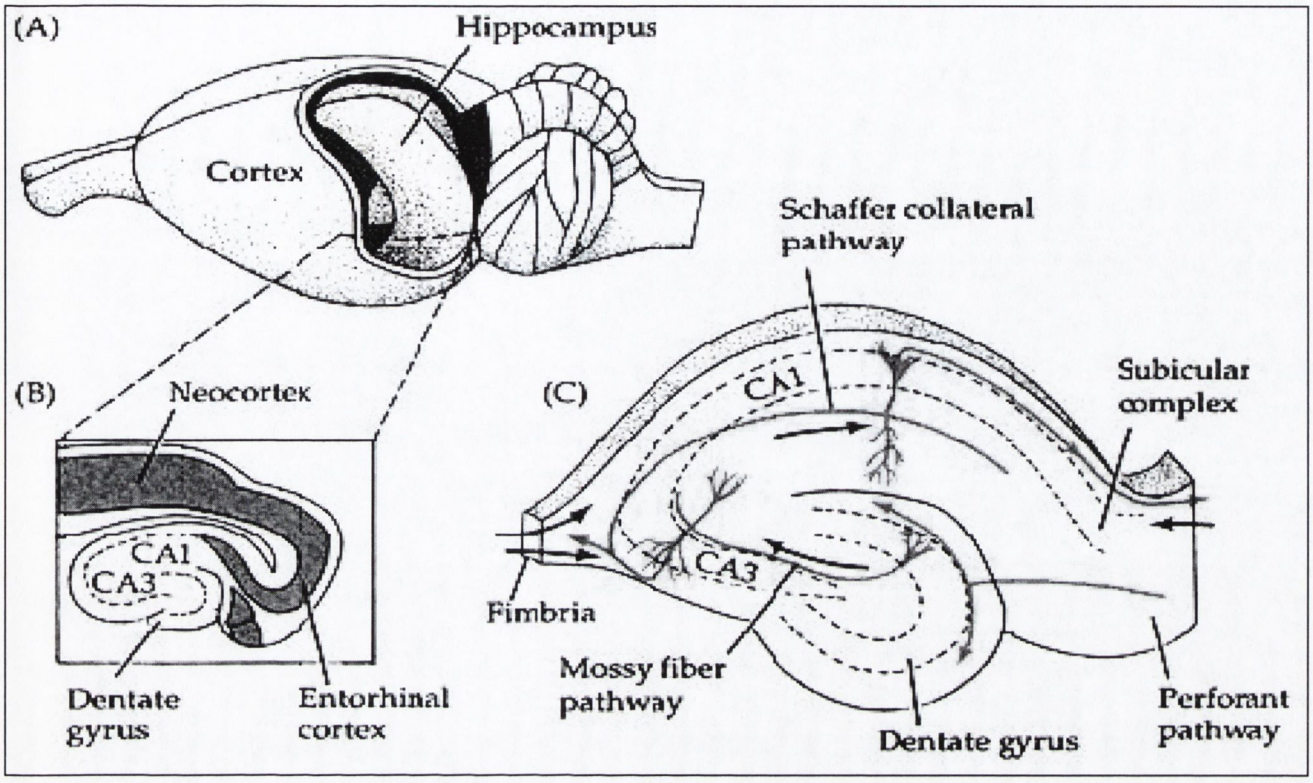

Fig. 1.1 Diagram of the hippocampus.

(a) The gross structure of the hippocampus in the rat brain.

(b) Transverse section through the hippocampus

(c) Detailed anatomy of the slice

1.2 Ionotropic glutamate receptors

1.2.1 AMPA receptors

The AMPA receptors are named after their specific agonist - α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid. AMPA receptors form heteromeric assemblies of subunits encoded by four genes (GluR1, GluR2, GluR3 and GluR4). Each gene can code for two splice variants termed "flip" and "flop" which show regional distribution in the brain. Dentate granule cells have more flop than flip forms and CA3 pyramidal cells contain only the flip form (Sommer *et al.*, 1990). The different combinations of subunits give rise to channels with different conductance properties. For example, recombinant expression studies demonstrate that the GluR2 subunit confers heteromeric receptors with the low Ca^{2+} permeability characteristic of most native AMPA receptors (Mayer and Westbrook, 1987; Jonas and Sakmann, 1992). In contrast to this the homomeric and heteromeric receptors assembled from the GluR1/-3/-4 subunits are significantly permeable to Ca^{2+}. Some inhibitory interneurons possess Ca^{2+} -permeable AMPA receptors, which have inwardly rectifying I/V relationships (Koh *et al.*, 1995; Isa *et al.*, 1996). AMPA receptors (AMPAR) mediate fast excitatory neurotransmission in the hippocampus through activation of a ligand-gated ion channel and they are primarily selectively permeable to Na^{+}. AMPAR exhibit linear current/voltage relationships with reversal potentials around 0mV (Collingridge *et al.* 1988a; Forsythe and Westbrook, 1988; Gallagher and Hasuo, 1989) and at the resting membrane potential (-60mV to -80mV) they provide most of the synaptic current needed to generate synaptic responses. The stereotypical AMPAR has a long extracellular N-terminal region, a shorter intracellular C-terminal and four transmembrane domains (designated MD1 to MD4) (Keinanen *et al.*, 1990).

AMPAR have a rise time of less than 1ms and decay time constants of 2-8ms in excised patches as opposed to NMDA receptors which were found to have rise times of 5-20ms and decay time constants of around 50 and 200ms in excised patches (Lester *et al.*, 1990; Hestrin *et al.*, 1990; Stern *et al.*, 1992). The affinity of AMPAR for glutamate is low (EC_{50} 480μM) (Patneau and Mayer, 1990) and the deactivation and desensitisation properties of AMPAR are determined by the

subunit composition, for example GluR1 and GluR4 deactivate and desensitize more quickly than GluR2. It is thought that the desensitisation of AMPAR contributes to the decay of synaptic currents (Trussell and Fischbach, 1989; Isaacson and Nicoll, 1991; Tang *et al.*, 1991; Vyklicky *et al.*, 1991; Yamada and Tang, 1993), but deactivation is normally much more rapid than desensitisation (Spruston *et al.*, 1995). There is widespread but differential distribution of GluR1-GluR4 mRNAs in the brain as has been revealed by *in situ* hybridization studies and AMPAR have been shown to be located postsynaptically, mainly in postsynaptic denstities, and within the dendritic and somatic cytoplasm by immunostaining (Hampson *et al.*, 1992; Petralia and Wenthold, 1992; Blackstone *et al.*, 1992). The most common antagonists of AMPAR include 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 2,3-dihydroxy-6-nitro7-sufamoyl-benzo (F) quinoxaline (NBQX) (Honore *et al.*, 1988; Sheardown *et al*, 1990).

1.2.2 Kainate receptors

Kainate receptors first came to be distinguished from AMPAR when C-fibre afferents in the spinal cord showed differential sensitivity to kainate and quisqualate (Davies *et al.*, 1979) but because there were no specific antagonists or agonists available they were often grouped together with AMPAR and collectively referred to as non-NMDA receptors. However, in recent years improved pharmacological tools have led to a greater understanding of kainate receptors in the brain.

Kainate receptors are encoded by two gene families, GluR5-7 and KA1 and KA2, both of which have significant structural homology to AMPA receptors. The subunits can be divided into two groups on the basis of their structural homology and affinity for [H^3]kainate. GluR5-GluR7 are low affinity subunits and display 75% homology with each other but only 40% homology with GluR1-GluR4. KA1 and KA2 are high affinity subunits and are 68% homologous. Kainate receptors are similar to AMPAR in that that each subunit comprises ~900 amino acids with and M_r ~100kDa (Bettler *et al.*, 1990; Herb *et al.*, 1992; Hollmann and Heinemann, 1994; Bettler and Mulle, 1995) and are thought to have the same topology (Wo and Oswald, 1995). Splice variants have been described for GluR5 and GluR7, and regulation of single channel conductance, current-voltage relations and Ca^{2+}-

permeability of the channel have been described by post-translational editing of GluR5 and GluR6. Kainate receptors are widely distributed throughout the cortex, limbic system, hippocampus and cerebellum. They elicit a fast onset and rapidly desensitising response. The study of kainate receptors has been hindered by the fact that AMPAR and kainate receptors have similar pharmacological profiles and similar biophysical properties in heterologous expression systems.

Kainate receptors have been shown to contribute to the EPSC at mossy fibre synapses onto CA3 (Castillo *et al.*, 1997; Vignes and Collingridge, 1997), hippocampal interneurons (Frerking *et al.*, 1998; Cossart *et al.*, 1998), retina (Devries and Schwartz, 1999), amygdala (Li and Rogawski, 1998) and cortex (Kidd and Isaac, 1999). Kainate receptor-mediated EPSC (EPSC KA) has a much smaller peak amplitude and much slower decay kinetics than AMPA receptor-mediated EPSCs. Given that kainate receptors desensitise rapidly it is surprising that they show slow decay times for EPSCs. This might be because kainate receptors are located extrasynaptically and so the glutamate transient is substantially slowed by diffusion and the decay of the EPSC matches the clearance of glutamate (Kullmann *et al.*, 1999). The small size of the EPSC KA compared to the EPSC AMPA may be due to a smaller number of release sites with kainate receptors, or a smaller quantal response per release site (Frerking *et al.*, 1998). Kainate receptor single channel conductances can range from values similar to AMPAR (~10pS) to much smaller values (<1pS). The conductance depends on the post-transcriptional editing of the channel (Cossart *et al.*, 1998). Similar to EPSC AMPA, EPSC KA has a reversal potential near 0mV, which suggests that the receptor is permeable to multiple cations. The current-voltage relationship of kainate receptors in the hippocampus is linear (Frerking *et al.*, 1998), but in the thalamocortical synapses it shows strong rectification (Kidd and Isaac, 1999). It was initially thought that AMPAR and kainate receptors responded in parallel to release of glutamate from single presynaptic axons but studies in the retina, spinal cord and cortex suggest that the EPSC KA can be elicited without AMPA EPSCs (DeVries and Schwartz, 1999; Kidd and Isaac, 1999) and the EPSC AMPA can be generated independently of EPSC KA (Li *et al.*, 1999; Kidd and Isaac, 1999).

In situ hybridisation and immunostaining studies have indicated the relative distributions of the kainate receptor subunits in the brain. GluR5 mRNA is found in the subiculum, CA1 and Purkinje cell layer of the cerebellum. GluR6 mRNA is

found in high levels in the dentate gyrus and CA3. GluR7 is widely distributed at a relatively low level. KA1 expression is restricted exclusively to the CA3 pyramidal and dentate granule neurons and KA2 is found at a uniform concentration in all neurons (Wisden and Seeburg, 1993; Bahn *et al.*, 1994; Good *et al.*, 1993; Lomeli *et al.*, 1992).

Kainate, domoate and ATPA are agonists of kainate receptors. (s)-5-iodowillardiine is the most selective of the willardiine derivatives for kainate receptors with EC_{50} values of 0.14μM in DRG (kainate receptors) and 19μM in hippocampal neurons (AMPA receptors) (Wong *et al.*, 1994). SYM2081 is also a potent agonist with EC_{50} values at homomeric GluR5 and GluR6 of 0.12μM and 0.23μM, respectively, whereas at homomeric GluR1 and GluR3, the EC_{50} values are 132μM and 453μM, respectively (Donevan *et al.*, 1998). NS-102 was the first kainate selective antagonist to be developed and it exhibits a 20-fold selectivity for kainate versus AMPA receptor-mediated response (Wilding and Huettner, 1996). LY293558 and LY294486 are also selective inhibitors of kainate receptors (Bleakman *et al.*, 1996; Clarke *et al.*, 1997).

1.2.3 NMDA receptors

NMDA receptors cluster on the heads of dendritic spines (Luo *et al.* 2002) and they have also been shown to colocalise with AMPA receptors (Bekkers and Stevens, 1989, Takumi *et al*, 1999). The NMDA receptor is composed of 7 subunits – NR1, NR2A-D and NR3A and NR3B. NR1 was discovered in 1991 (Moriyoshi *et al.*, 1991), NR2A-D were subsequently identified (Ikeda *et al.*, 1992; Kutsuwada *et al.*, 1992; Meguro *et al.*, 1992; Monyer *et al.*, 1992) and most recently NR3A and NR3B were identified (Chatterton *et al.*, 2002; Matsuda *et al.*, 2002; Belenikin *et al.*, 2003). In order for a receptor to function the NR1 subunit must be co-expressed with at least one of the other subunits (Ikeda *et al.*, 1992; Kutsuwada *et al.*, 1992; Meguro *et al.*, 1992; Monyer *et al.*, 1992). However in *Xenopus* oocytes NR1 can form a functional receptor by itself (Moriyoshi *et al.*, 1991). It is not clear whether the receptor subunits coassemble to form a tetrameric (Laube *et al.*, 1998) or a pentameric (Premkumar and Auerbach, 1997) stoichiometry. The different arrangements of subunits confers different characteristics on the receptor such as rates of desensitisation and ionic conductance. The varied conductance levels of

NMDA receptors observed in native neurons (Cull-Candy *et al.*, 1988; Momiyama *et al.*, 1996) closely match the multiple conductance levels of heteromeric combinations of NR1 and various NR2 subunits. Conductance values range from 50 to 75pS depending on the subunit arrangement (Stern *et al.*, 1992, 1994; Wyllie *et al.*, 1996; Das *et al.*, 1998). The overall structure of the NMDA receptor is the same as that of non-NMDA receptors (Wood *et al.*, 1995; Kuner *et al.*, 1996) and NR1 has 25-29% homology with non-NMDA glutamate receptor. The M2 segment in NMDA receptors is thought to be a re-entrant loop based on the pattern of accessibility from both sides of the membrane of charged sulfhydryl reagents to cysteines substituted for M2 residues (Kuner *et al.*, 1996). NR2A-D subunits have a 42-56% homology between themselves and a 21-26% homology with other GluRs. NR3A and NR3B subunits have a 57% homology between themselves.

NR1 RNA is found throughout the CNS but has a particularly high expression level in the hippocampus (Moriyoshi *et al.*, 1991). NR2A is widely distributed, NR2B is found only in the forebrain, NR2C is expressed predominantly in the cerebellum and NR2D is found at low levels throughout the brain. NR3B mRNA is expressed highly in pons, midbrain, medulla, whereas NR3A is more widely distributed (Matsuda *et al.*, 2002).

During normal synaptic activity NMDA receptors contribute little to the resting EPSP because the channel is normally blocked by Mg^{2+} and becomes unblocked when the postsynaptic cell is sufficiently depolarized by a strong input from many presynaptic neurons which relieves the Mg^{2+} blockade and allows glutamate to stimulate the influx of Na^{+} and Ca^{2+} into the cell (Mayer *et al.*, 1984). Under experimental conditions the Mg^{2+} blockade can also be overcome artificially if the postsynaptic membrane is directly depolarised with an intracellular recording electrode (Kelso *et al.*, 1986; Wigstrom *et al.*, 1986). The NMDA receptor is unique in that it is the only receptor that is gated by both a chemical agonist and a voltage sensor. The Mg^{2+} blockade can be overcome when cells are voltage clamped at depolarised potentials (Collingridge *et al.*, 1988a; Andreasen *et al.*, 1989), or when high frequency stimulation induces depolarisation (Collingridge *et al.*, 1988b; Gallagher and Hasuo, 1989). NMDA receptors can also be activated by lowering the Mg^{2+} in the bath in which slices are held and applying low frequency stimulation to the slices (Thomson, 1986; Coan and Collingridge, 1987; Blake *et al.*, 1988).

Glutamate bound NMDA channels have an N-shaped current-voltage curve with a region of "negative slope" conductance caused by relief of the Mg^{2+} block (Nowak *et al.*, 1984; Mayer and Westbrook, 1988, Hestrin *et al.*, 1990). In addition to Na^{+} and K^{+} ions NMDA receptors have been found to be permeable to Ca^{2+} (MacDermott *et al.*, 1986; Mayer and Westbrook, 1987, Jahr and Stevens, 1993).
In contrast to AMPA and kainate receptors, NMDA receptors have slow activation times with a τ(rise) of 10 to 50 ms (Wyllie *et al.*, 1998) and deactive with a much slower time-course. Because NMDA receptors have high affinity for glutamate, the peak synaptic glutamate concentrations are thought to be high enough at all times to fully activate these receptors. All NMDA receptor subunits have an asparagine residue which has been shown to be involved with the cation permeability and block of the channel (Mori *et al.*, 1992; Burnashev *et al.*, 1992; Sakurada *et al.* 1993).

N-methyl-D-aspartate (NMDA) is the specific agonist of NMDA receptors. The inhibitory neurotransmitter glycine can also act as a co-agonist of NMDA receptors and lead to potentiation of NMDA currents (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). It has been proposed that this potentiation may be due to a reduction in NMDA receptor desensitisation (Mayer *et al.*, 1989). NMDA receptors are competively and effectively blocked by (d)-2-amino-5-phosphonopentanoic acid (D-AP5) and (R)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (R)-CPP) (Watkins and Collingridge, 1989). The polycyclic amine, 5-methyl-10,11-dehydro-5H-debenzo[a,d]cyclohepten-5,10-imine (MK-801) is a potent, non-competitive, open-channel, NMDA antagonist (Wong *et al.*, 1986).

1.3 Metabotropic glutamate receptors

Besides fast activating ionotropic receptors, glutamate can also interact with G-protein coupled metabotropic glutamate receptors (mGluRs). It was first discovered in the mid 1980s that glutamate could modify excitatory synaptic transmission by acting on glutamate receptors linked directly to second messenger systems via GTP-binding proteins (Sladeczek *et al.*, 1985). Before this it was thought that cell excitability and synaptic transmission could only be modified by extrinsic afferents such as acetylcholine, serotonin and dopamine – receptors which are coupled to G-proteins (Hille, 1994; Conn and Pin, 1997). The existence of a novel receptor which

interacted with GTP-binding proteins was shown by Sugiyama *et al.*, in 1987 and subsequently it was also shown that glutamate leads to an increase in the intracellular calcium concentration in cultured hippocampal cells via G-protein activation (Furuya *et al.*, 1989).

mGluRs form a family of eight subtypes termed $mGluR_1$ to $mGluR_8$. The first mGluR was cloned by a functional expression screening procedure (Houamed *et al.*, 1991; Masu *et al.*, 1991). Seven other subtypes have been cloned to date (Pin *et al.*, 1992; Tanabe *et al.*, 1992; Abe *et al.*, 1992; Minakami *et al.*, 1993; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Saugstad *et al.*, 1994; Duvoisin *et al.*, 1995). The receptors are quite large (approximately 1000 amino acids) and they form part of the family of class C heptahelix G-protein-coupled receptors (GPCRs) called the family 3 GPCRs. These include all mGluRs, Ca^{2+}-sensing (Brown *et al.*, 1993) and $GABA_B$ receptors (Kaupmann *et al.*, 1997), and some putative olfactory, pheromone, and taste receptors.

mGluRs comprise two main domains separated, in most cases, by a cysteine-rich N-terminal extracellular domain where the agonist binding site is located (O'Hara *et al.*, 1993; Okamoto *et al.*, 1998; Han and Hampson, 1999; Peltekova *et al.*, 2000; Kunishima *et al.*, 2000). The mGluRs also have a C-terminal domain of variable length and a seven transmembrane domain (7TM) region, which shares very low sequence similarity with other G-protein-coupled receptors. The large extracellular domain has been shown to be structurally related to bacterial periplasmic amino-acid-binding proteins (O'Hara *et al.*, 1993; Costantino and Pellicciari, 1996; Hu *et al.*, 2000). Similar to bacterial periplasmic proteins, mGluRs are thought to contain two globular lobes in the extracellular domain which are separated by a cleft where the ligand binds (Kunishima *et al.*, 2000). G-protein activation is a complex protein-protein interaction and is not well understood but the intracellular loops between transmembrane domains are involved and the second intracellular loop (the longest and least conserved) determines G-protein-coupling specificity (Pin *et al.*, 1994; Gomeza *et al.*, 1996). The third intracellular loop is surprisingly short and highly conserved and this loop is thought to play a role in G-protein activation and in cooperation with the first intracellular loop and the C-terminal tail it controls the coupling efficacy (Mary *et al.*, 1998; Chang *et al.*, 2000).

On the basis of sequence homology, agonist and antagonist pharmacology, and coupling to signal transduction pathways in expression systems, the mGluR are classified into three groups (Nakanishi, 1992). Within each group there is a 70% homology between receptor subtypes and between each group there is a 45% homology of receptors. Class I comprises $mGluR_1$ and $mGluR_5$ and is activated by 3,5-dihydroxyphenylglycine (DHPG). Group I mGluRs are positively coupled to phospholipase C (PLC) which in turn leads to hydrolysis of phosphoinositide (PI) and the production of IP_3 and DAG (Birse *et al.*, 1993). IP_3 binds to IP_3R on intracellular stores releasing sequestered calcium into the cytoplasm (Murphy and Miller, 1988; Irving *et al.*, 1990). DAG production leads to the activation of PKC which can trigger many internal cascades through phosphorylation of many substances. Group II mGluRs consist of $mGluR_2$ and $mGluR_3$ and are negatively coupled to adenylyl cyclase. Group III receptors consist of $mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$ and are also negatively coupled to adenylyl cyclase. However the situation has been complicated by studies that show that the group I and II agonist, (1*S*,3*R*)-ACPD, can increase basal cAMP levels in the rat hippocampus (Schoepp and Johnson, 1993; Winder and Conn, 1993; Wright and Schoepp, 1996). This effect may be due to potentiation of endogenous adenosine-stimulated cAMP formation. Also an increase in cAMP produced by a mixture of mGluR agonists was found to be greater than the sum of the agonists applied singly suggesting that there is an interaction between the G-proteins and/or the 2nd messengers controlled by these mGluRs (Reid *et al.*, 1996).

Group I mGluRs are widely expressed in the brain and are localized in the peripheral parts of postsynaptic densities (Baude *et al.*, 1993). In the pyramidal cell layer of CA2/CA3 there is high level of RNA expression for $mGluR_1$ and $mGluR_5$ (Kerner *et al.*, 1997) and in CA1 cells there is only expression of $mGluR_5$ (Lujan *et al.*, 1997). In the dentate gyrus the granule cells show expression of both $mGluR_1$ and $mGluR_5$ (Masu *et al.*, 1991; Shigemoto *et al.*, 1992). Group II mGluRs have been localised using immunohistochemical studies to the stratum lacunosum moleculare of CA1, the inner layer of the stratum lacunosum moleculare in the CA3 and the dentate gyrus (Petralia *et al.*, 1996; Shigemoto *et al.*, 1997). Group II mGluRs are found mainly at sites corresponding to perforant path and mossy fibre projections and are predominantly presynaptic (Shigemoto *et al.*, 1997). Of the

group III mGluRs, $mGluR_4$ is only expressed in the perforant pathway, $mGluR_7$ is strongly expressed in the dentate gyrus and $mGluR_8$ shows prominent staining in the lateral perforant path (Shigemoto *et al.*, 1997). All of these receptors are located presynaptically (Bradley *et al.*, 1996). $mGluR_6$ is exclusively expressed by ON bipolar cells in the retina and it has a role in the amplification of visual inputs (Nakanishi, 1995). Group III mGluRs are predominantly restricted to axon terminals close to release sites in both glutamatergic and non-glutamatergic synapses (Shigemoto *et al.*, 1997). This is in contrast to group II mGluRs which are located in the preterminal region of the axon.

(*RS*)-3,5-dihydroxyphenylglycine (DHPG) is a selective agonist of group I mGluRs (Ito *et al.*, 1992; Gereau and Conn, 1995a). (2*S*,1'*R*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine (DCG-IV) is an agonist of group II mGluRs and NMDA receptors (Hayashi *et al.*, 1993; Ishida *et al.*, 1993). (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3*R*)-ACPD) (ACPD) activates both group I and group II mGluRs (Masu *et al.*, 1991; Houamed *et al.*, 1991; Tanabe *et al.*, 1992; Abe *et al.*, 1992). Group III mGluRs are activated by L-2-amino-4-phosphonobutyrate (L-AP4) (Thomsen *et al.*, 1992; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Duvoisin *et al.*, 1995).
(*S*)-α-methyl-4-carboxyphenylglycine ((*S*)-MCPG), is an antagonist at group I and II mGluRs (Eaton *et al.*, 1993; Jane *et al.*, 1993; Kemp *et al.*, 1994; Hayashi *et al.*, 1994). (*S*)-4-carboxyphenylglycine (4CPG) is a specific antagonist of group I mGluRs (Hayashi *et al.*, 1994) and (*RS*)- α-methyl-4-tetrazolylphehylglycine (MTPG) is an antagonist at group II mGluRs (Jane *et al.*, 1995). (RS)-α-methyl-4-phosphono-phenylglycine (MPPG) is an antagonist at group III receptors (Jane *et al.*, 1995) and (*RS*)-α-cycloproplyl-4-phosphonophenylglycine (CPPG) inhibits both group II and III receptors (Toms *et al.*, 1996). A new drug 2S-2-amino-2-(1*S*,2*S*-2-carboxycyclopropan-1-yl)-3-(xanth-p-yl)propanoic acid (LY341495) has been developed recently which is selective for group II at low nanomolar concentrations but inhibits all groups of mGluRs at higher concentrations (Kingston *et al.*, 1998).

1.4 Other neurotransmitter receptors

1.4.1 Adrenergic receptors

More than a century ago it was discovered that an injection of extracts from the adrenal glands into an animal would produce many of the physiological effects of sympathetic nerve stimulation (Lewandowsky, 1898; Langley, 1901). Later, it was demonstrated that sympathetic nerves released an excitatory transmitter (Cannon and Uridil, 1921; Loewi, 1921) and a quarter of a century after this discovery direct chemical analysis revealed this neurotransmitter to be noradrenaline (von Euler, 1946). Adrenergic receptors were then differentiated into α and β-adrenergic receptors by Ahlquist (1948) who demonstrated two competitive hierarchies for various amines which produced predictable response in certain tissues.

The β_2-adrenergic receptor was the first G-protein coupled receptor to be cloned (Dixon *et al.*, 1986) and since then several other labs have isolated and cloned cDNAs for other adrenergic receptor subtypes (Svensson *et al.*, 1993; Hirasawa *et al.*, 1995). To date, nine receptor subtypes have been cloned and seven of these are found in the rat CNS: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C} and β_1 (Bylund *et al.*, 1994; Ford *et al.*, 1994). All of the receptors are members of the superfamily of seven transmembrane domain, G-protein-coupled receptors. α_1-adrenergic receptors are coupled to PLC and they produce their effects through the release of intracellular Ca^{2+} (Nichols, 1991). α_2-adrenergic receptors are negatively coupled to adenylyl cyclase and reduce cAMP formation while the β-adrenergic receptors act by stimulation of adenylyl cyclase (Kobilka *et al.*, 1988). Adrenergic receptors and G-proteins have been shown to interact through the third intracellular loop of the receptors. Chimeric receptor studies have shown that if the third intracellular loop of the β_2-adrenergic receptor is replaced with that of the α_{1B}-adrenergic receptor, then the chimeric β_2/α_{1B} receptor will be coupled to the second messenger system of the α_{1B} receptor, namely PLC (Cotecchia *et al.*, 1990). Point mutations in the carboxyl terminus of the third intracellular loop have also been shown to produce both α- and β-adrenergic receptors that do not need agonist binding to produce their effects via activation of G-proteins (Kjelsberg *et al.*, 1992; Samama *et al.*, 1993).

Desensitisation of adrenergic receptors occurs when the serine and threonine residues in the cytoplasmic tail or the third intracellular loop are phosphorylated by kinases such as β-adrenergic kinase (βARK) or PKA (Liggett *et al.*, 1992; Pippig *et al.*, 1993). Binding of a protein termed β-arrestin to the phosphorylated receptor sterically inhibits further G-protein activation (Freedman and Lefkowitz, 1996). Desensitised receptor-arrestin complexes are targeted for sequestration through clathrin-coated pits and are either dephosphorylated and recycled to the cell surface or targeted for degradation (Ferguson *et al.*, 1996; Goodman *et al.*, 1996; Yu *et al.*, 1993). Recent studies have also shown that stimulation of β_2-adrenergic receptors causes the assembly of a protein complex containing activated Src tyrosine kinases and the receptor which is mediated by β-arrestin (Luttrell *et al.*, 1999). It has been proposed that while β-arrestin binding can terminate receptor-G-protein coupling it is also responsible for initiating a second wave of signal transduction whereby the "desensitised" receptor functions as a critical component of a mitogenic signalling complex (Luttrell *et al.*, 1999).

Noradrenergic innervation of the hippocampus arises exclusively from neurons of the locus coeruleus. Noradrenergic fibres travel in the dorsal noradrenergic bundle and they enter the hippocampus by three pathways – the ventral amygdaloid bundle-ansa, the ipsilateral fasciculus cinguli and the fornix (Moore and Bloom, 1979; Loy *et al.*, 1980). Stimulation of the locus coeruleus has been shown to enhance the release of noradrenaline in the hippocampus and also to increase neuronal excitability in the dentate gyrus (Klukowski and Harley, 1994), a region which receives extensive noradrenergic innervation (Milner *et al.*, 2000). Dense plexuses of preterminal and terminal elements are to be found in the infragranular hilus of the dentate gyrus, the stratum lucidum of CA3 and the molecular layer of the subiculum (Loy *et al.*, 1980). The CA1 has the lowest overall density of noradrenergic innervation (Oleskevich *et al.*, 1989). Innervation of the dentate gyrus is greater than that of the rest of the hippocampus and in general the hippocampus receives greater innervation in the temporal rather than the septal ends (Loy *et al.*, 1980). Studies have also revealed the presence of adrenergic receptors on a variety of cell types in the hippocampus including principal excitatory neurons (Madison and Nicoll, 1982), interneurons (Bergles *et al.*, 1996), and glial cells (Duffy and MacVicar, 1995). More recent studies using antibodies for the β-adrenergic

receptors revealed dense β-adrenergic immunoreactivity (β-AR-I) in the somata of granule cells and a few hilar interneurons in the dentate gyrus (Milner *et al.*, 2000). Quantitatively, the greatest proportion of β-AR-I was found in the molecular layer and this immunocreactivity was further localised to dendrites and astrocytes (Milner *et al.*, 2000). β-AR-I was also observed in a few unmyelinated axons and axon terminals which formed synapses with dendritic spines (Milner *et al.*, 2000). Studies examining the distribution of α_1 adrenergic receptors by autoradiography have found a low overall expression of the receptor in the hippocampus but with a relatively homogenous regional and laminar distribution (Zilles *et al.*, 1991). α_{2B}-adrenergic receptor mRNA is found exclusively in the thalamus (Scheinin *et al.*, 1994) while both α_{2A} and α_{2C} mRNA is found in the hippocampus (McCune *et al.*, 1993; Rosin *et al.*, 1996). In the hippocampus $\alpha_{1A/B}$ labelling is present in the hilar and granular neurons of the dentate gyrus (Pieribone *et al.*, 1994; Day *et al.*, 1997). Similar to α_2-adrenergic receptors, the expression and distribution of α_1-adrenergic receptors as revealed by the binding of [^{3}H]prazosin, was found to be relatively low and homogeneous in the hippocampus (Zilles *et al.*, 1991).

1.4.2 Muscarinic acetylcholine receptors

Five genes encode the muscarinic acetylcholine receptors (mAChRs) and the five subtypes that result can be broken down into two broad functional categories: the M_1, M_3, and M_5 receptors which couple to the G_q family of G-proteins and the M_2 and M_4 receptors which preferentially couple to the G_i family of G-proteins (Nathanson, 1987; Wess, 1996; Caulfield and Birdsall, 1998). The M_1, M_3, and M_5 receptors activate PLC via the pertussis toxin-insensitive G-protein of the G_q family and the M_2 and M_4 receptors inhibit adenylyl cyclase and do not activate PLC. When both the G_i- coupled receptor and the G_q-coupled receptor are expressed at high levels they can couple to G_s and lead to an increase in cAMP (Migeon and Nathanson, 1994). PKA, PKC and CaMKII can regulate different isoforms of adenylyl cyclase (Hurley, 1999) and mAChRs can regulate each of these kinases, so it is to be expected that new mechanisms for the regulation of cAMP levels by mAChRs will be found in the future.

Acetylcholine (ACh) was the first neurotransmitter to be identified and until about 50 years ago it was the only one recognized in the brain. Although we now know that there are more than 50 neurotransmitters, the cholinergic system is still one of the most important neuromodulatory systems in the brain. Since the first study showing that mAChR activation causes an increase in protein tyrosine phosphorylation (Stratton *et al.*, 1989) it has been shown that mAChRs can activate tyrosine kinase and MAP kinase by multiple mechanisms. mAChRs can also influence ion channel function. Activation of mAChRs can in turn activate inwardly rectifying potassium channels and inhibit N- and P/Q-type Ca^{2+} channels via $G\beta\gamma$ subunits released from G_i-family proteins (Clapham and Neer, 1997).

Deafferentation of cholinergic input to the hippocampus and application of cholinergic antagonists have both been found to lead to deficits in memory and attention (Shannon *et al.*, 1990; Nilsson *et al.*, 1992; Bymaster *et al.*, 1993; Callahan *et al.*, 1993). However, the mechanisms by which mAChRs are involved in learning and memory are not known.

mAChRs have been shown to have an autoregulatory role in the release of ACh in the hippocampus (Raiteri *et al.*, 1989; Segal, 1989). But the identity of this autoreceptor still remains elusive. One possible candidate is the M_2 receptor which is highly expressed in the cell bodies of basal forebrain cholinergic neurons (Levey *et al.*, 1991). Studies using an antibody for the vesicular acetylcholine transporter (VAChT) which is located exclusively in cholinergic vesicles found that the M_2 receptor distribution in the hippocampus is similar to that of VAChT (Rouse *et al.*, 1999). Also electron microscope studies found that M2 is found predominantly in the presynaptic area (Rouse *et al.*, 1999). These findings provide the first clear evidence that the M_2 might be an autoreceptor on cholinergic nerve terminals in the hippocampus. There have also been reports that the M_4 receptor acts as an autoreceptor in the mouse cortex (Iannazzo and Majewski, 2000).

1.5 Synaptic plasticity

1.5.1 Long-term potentiation (LTP) in the hippocampus

The idea of LTP was pre-empted by the work of Hebb and Konorski in the 1940's who proposed that if two cells are active at the same time then the synapse linking these two cells will be strengthened through a coincidence detection rule (Hebb, 1949; Konorski, 1948). Hebb's often quoted postulate states that: W*hen an axon of cell A is near enough to excite cell B or repeatedly or consistently takes part in firing it, some growth process or metabolic changes take place in one or both cells such that A's efficiency, as one of the cells firing B, is increased.* The first experimental evidence for this was provided by Bliss and Lømo in 1968 when they stimulated the perforant path of the dentate gyrus of anaesthetized rabbits. Trains of stimuli at 20 Hz for 15 s were applied every hour for five hours and this stimulation protocol caused a progressive increase in the amplitude of the population spike of discharging granule cells. No potentiation occurred in the opposite hippocampus which they used as a control. The control pathway was tetanised at the end of the experiment which then gave an increase in the amplitude of the population spike. Their results were not published until 1973 (Bliss and Lømo, 1973), and this seminal paper describes all the basic phenomena of LTP – including pathway specificity, saturation, and an increase in the coupling of the synaptic potential to the discharge of the granule cells.

1.5.2 Characteristics of LTP

LTP has three basic characteristics – cooperativity, associativity and input-specificity. Cooperativity refers to the fact that weak tetani do not trigger LTP (McNaughton *et al.*, 1978) and so a threshold intensity is required in order for LTP to be successfully induced. Between weak tetani which induce a brief post-tetanic potentiation and strong tetani which induce LTP there are a number of intermediate frequencies which induce short-term potentiation which is defined as potentiation that returns to baseline levels within 1 hour (Lovinger *et al.*, 1988; Malenka, 1991; Bliss and Collingridge, 1993).

Associativity refers to the fact that LTP can be induced at a weak input if it is active at the same time as a strong tetanus to a separate but convergent input (McNaughton *et al.*, 1978; Levy and Steward, 1979).

Input-specificity means that inputs that are not active at the time of the tetanus are not affected by the high-frequency stimulation and are therefore not potentiated. (Anderson *et al.*, 1977; Lynch *et al.*, 1977). However, it has been discovered that the input-specificity of LTP need not imply a true synapse specificity (Engert and Bonhoeffer 1997). Engert and Bonhoeffer found that focal application of high-calcium solution led to the potentiation of inactive synapses close to the active synapses (<70μm away). This discovery may be important in the formation of cortical columns (Montague and Sejnowski, 1994; Stetter *et al.*, 1998).

1.5.3 Methods of induction of LTP

There are two main methods employed for the induction of LTP. The first is high-frequency (tetanic) stimulation applied to the afferent input at a stimulus intensity above the threshold (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973). The frequencies used are usually between 100 and 200 Hz. Short bursts of 100-Hz stimulation at intervals of 200 ms can induce LTP *in vivo* that lasts for several weeks (Larson *et al.*, 1986; Rose and Dunwiddie 1986; Staubli and Lynch, 1987). Under *in vitro* conditions in the slice preparation, the same stimulation protocol leads to LTP that last for hours rather than weeks. Stimulation elicited during the positive phase of the theta rhythm in urethane-anaesthetised rats has been reported to induce LTP (Pavlides *et al.*, 1988). The theta rhythm is a prominent spontaneous rhythm in the hippocampus that occurs when an animal is moving in its environment. Intracellular recording techniques have shown that LTP induction requires the back-propagation of dendritic action potentials (Magee and Johnston 1997; Markram *et al.*, 1997). Connections in the neocortex are potentiated when the EPSP precedes the back-propagating spike (by 10 ms), but depressed when the spike precedes the EPSP (Markram *et al.*, 1997). It has also been shown that bursts of postsynaptic action potentials are necessary for the induction of LTP *in vitro* (Thomas *et al.*, 1998).

The second method for the induction of LTP is to couple low-frequency stimulation (0.1-1 Hz) with depolarisation of the postsynaptic membrane to around 0mV

delivered through an intracellular recording electrode (Kelso *et al.*, 1986; Wigstrom *et al.*, 1986; Sastry *et al.*, 1986; Gustafsson *et al.*, 1987).

1.5.4 The role of NMDA receptors in LTP

Activation of NMDA receptors appears to be crucial for LTP at most synapses (Collingridge *et al.*, 1983; Bliss and Collingridge, 1993) except for the NMDA-independent LTP in the mossy fibers (Harris and Cotman 1986; Zalutsky and Nicoll, 1990). The role of the NMDA receptor in LTP arises from its unusual properties particularly its voltage-dependent block by extracellular Mg^{2+} and its permeability to Ca^{2+} (Nowak *et al.*, 1984). During a tetanic stimulation there is a strong and sustained depolarisation of cells due to the activation of AMPA receptors. This excitatory postsynaptic potential (EPSP) can be blocked with the AMPA antagonist, CNQX. GABAergic interneurons are also stimulated by a tetanus and the GABA released binds with $GABA_A$ and $GABA_B$ receptors. $GABA_A$ activation increases Cl^- activation and $GABA_B$ activation blocks K^+ channels. Both of these effects lead to hyperpolarisation. This hyperpolarisation consolidates the block of NMDA receptors. However, the release of GABA also leads to the activation of presynaptic $GABA_B$ autoreceptors which inhibits further release of GABA and ultimately leads to the decay of the postsynaptic hyperpolarisation after about 10ms (Davies *et al.*, 1991). Continued AMPA receptor activation causes an influx of sodium into the cell and there is also a subsequent increase in extracellular potassium concentration. In order for the NMDA channel to open, the membrane must be sufficiently depolarized to expell Mg^{2+} from the channel and L-glutamate must also bind to the NMDA receptor to facilitate channel opening. AMPA receptor activation is eventually sufficient to relieve the voltage-dependent Mg^{2+} block of the NMDA receptors and the release of glutamate by even a strong tetanus is sufficient to fully activate NMDA receptors. The colocalisation of NMDA and non-NMDA receptors at individual excitatory synapses enables this activation of NMDA receptors to occur (Bekkers and Stevens, 1989).

With this sequence of events the three main characteristics of NMDA-dependent LTP is demonstrated. The need for a co-operativity threshold is seen when a weak tetanus releases enough glutamate to activate the NMDA receptor but does not provide enough depolarisation to relieve the Mg^{2+} blockade. With a strong tetanus

many fibres can be activated and the activation of NMDA receptors is aided by the spread of depolarisation between neighbouring synapses. This is exploited experimentally where the depolarisation is provided by injecting current into the cell. The release of L-glutamate by presynaptic terminals must allow the NMDA receptors to be activated during tetanus and concurrently the ambient or spontaneous release of L-glutamate must not be able to activate NMDA receptors.
Thus, the NMDA receptor is essential for the triggering of LTP and in accordance with this NMDA receptor antagonists have been shown to block the induction of LTP (Collingridge *et al.*, 1983; Harris *et al.*, 1984; Bashir *et al.*, 1990).
NMDA receptors have a 100-fold greater affinity for glutamate than AMPA receptors (Patneau and Mayer, 1990). However, after a brief glutamate transient they may exhibit similar occupancy because their opening probabilities are principally determined by their binding rates which are similar for both receptors (Kullmann, 1999). However, if the glutamate wave is relatively slow then the NMDA receptors are probably selectively activated by glutamate, because their unbinding and desensitisation rates are slower than those of AMPA receptors. NMDA receptors are also thought to be present extrasynaptically, relatively far from the release sites. This in turn gives rise to the possibility that cross-talk may occur between neighbouring glutamatergic synapses (Kullmann *et al.*, 1996; Kullmann and Asztely, 1998).

LTP in the mossy fibres is independent of NMDA receptor activation. The first evidence for this came from studies which showed that NMDA binding is very low in the stratum lucidum which is the termination zone of mossy fibres (Monaghan and Cotman, 1985). Subsequent studies showed that LTP in this area is independent of NMDA receptors (Harris and Cotman, 1986; Zalutsky and Nicoll, 1990).

1.5.5 The role of Ca^{2+} in LTP

Very early on it was apparent that a postsynaptic rise in Ca^{2+} was required for induction of LTP as an intracellular injection of the Ca^{2+} chelator, EGTA, blocked LTP completely (Lynch *et al.*, 1983). NMDA channels are permeable to Ca^{2+} and it is influx through these channels that allows Ca^{2+} concentration to rise postsynaptically following a tetanus. Enhanced entry of Ca^{2+} through NMDA

receptors has been shown when LTP is triggered by afferent stimulation 0-200 ms before antidromic stimulation (Yuste and Denk, 1995; Magee and Johnston, 1997; Koester and Sakmann, 1998; Schiller *et al.*, 1998). In these cases there is supralinear summation of dendritic spine $[Ca^{2+}]_i$ transients with back-propagated action potential $[Ca^{2+}]_i$ transients leading to a large Ca^{2+} signal in the dendritic spine. If the order of stimulation is reversed then there is a small Ca^{2+} signal generated and LTD ensues. This might be due in part to the Ca^{2+}-dependent inactivation of NMDA receptors. Ca^{2+} can also enter through voltage-gated Ca^{2+} channels. The localisation of NMDA receptors on dendritic spines helps to localise the Ca^{2+} signal and also to restrict the diffusion of Ca^{2+}. Dendritic spines are micron-sized protrusions from neuronal dendrites which form the majority of excitatory synaptic contacts. A greater understanding of the nature of Ca^{2+} dynamics at these spines has been revealed by fluorescent Ca^{2+} indicators, confocal and two-photon imaging (Denk *et al.*, 1996). A recent study has found that Ca^{2+} transients in spines and thin dendrites have large amplitudes (1-2μM) and decay rapidly (time-constant 10-20 ms) (Sabatini *et al.*, 2002). Uptake into the endoplasmic reticulum serves to rapidly remove Ca^{2+} from the cytosol (Majewska *et al.*, 2000; Kovalchuk *et al.*, 2000; Sabatini *et al.*, 2002). However ~5% of Ca^{2+} entering remains unbound and the buffering capacity in the spines is three to fivefold lower than in the main apical dendrites (Helmchen *et al.*, 1996; Maravall *et al.*, 2000). Thus, the relatively large surface-to-volume ratio in the spine aids fast Ca^{2+} signalling. Influx of Ca^{2+} through depolarised NMDA receptors can cause a large rise in Ca^{2+} (>10μM) (Petrozzino *et al.*, 1995) which cannot be achieved by impulse-like Ca^{2+} influx during action potentials, even at high frequencies (Sabatini *et al.*, 2002). The distribution of Ca^{2+} will depend on the localisation and the relative kinetics of Ca^{2+} binding proteins (Markram *et al.*, 1998). It has been hypothesised that a Ca^{2+} sensor with a slow on-rate for binding may be activated by low-frequency NMDA-receptor activation but less so by action potentials (Sabatini *et al.*, 2002). These new studies might be able to resolve the problem of how the same messenger, Ca^{2+}, can cause activation of different enzyme cascades during LTP and LTD induction protocols (Zucker, 1999).

Only a brief Ca^{2+} transient is thought to be necessary for LTP induction. When the transient is limited to 3 s, using a photo-activatable caged Ca^{2+} chelator, LTP can still be induced (Malenka *et al.*, 1992). Recently, a caged Ca^{2+} chelator was used to

elevate postsynaptic $[Ca^{2+}]_i$ to high levels for a few seconds and also modest levels for 1 min. The result was that LTP was induced with the former manipulation and long-term depression (LTD) with the latter (Yang *et al.*, 1999). These studies indicate that the amplitude and duration of $[Ca^{2+}]_i$ is crucial for selecting the enzymatic machinery that is activated and the type of plasticity that is induced. Influx of Ca^{2+} through L-type Ca^{2+} channels has also been implicated in mossy fibre LTP (Kapur *et al.*, 1998), in LTD induction in CA1 pyramidal cells (Coussens *et al.*, 1997; Norris *et al.*, 1998), and in LTP induction in adult CA1 cells (Izumi and Zorumski, 1998). LTD in the cerebellum is thought to involve Ca^{2+}-induced Ca^{2+} release from stores regulated by inositol 1,4,5-trisphosphate (IP_3) (Inoue *et al.*, 1998).

The Ca^{2+} signal can also be reinforced by release of Ca^{2+} from intracellular stores. Ryanodine which inhibits Ca^{2+}-induced Ca^{2+} release and thapsigargin which depletes intracellular Ca^{2+} stores have been shown to reduce the Ca^{2+} transient associated with NMDA receptor activation (Alford and Collingridge, 1992).

It has also been shown that inositol-1,4,5-trisphosphate (IP_3) generated via the activation of mGluRs is involved in releasing Ca^{2+} from intracellular stores and can contribute to the induction of LTP (Fujii *et al.*, 2003). Activation of mGluRs has been shown to induce LTP even when NMDA receptors are blocked (Bortolotto and Collingridge, 1993). This method of induction was shown to be sensitive to thapsigargin thus suggesting that the release of Ca^{2+} from intracellular stores is able to substitute for the influx of Ca^{2+} through NMDA receptors. AMPA receptors lacking the GluR2-subunit are also permeable to Ca^{2+}.

1.5.6 The role of silent synapses and AMPA receptors in LTP

The understanding of the role of AMPA receptors in LTP has been greatly enhanced with the development of the silent synapse theory. Silent synapses are synapses that contain NMDA receptors but no AMPA receptors and are therefore functionally silent during low-frequency, basal transmission. However, AMPA receptors can be recruited into the postsynaptic membrane by LTP induction. Thus, this theory links AMPA receptor trafficking to LTP expression. The first step towards the elucidation of this theory was the observation that evoked monoquantal events can be detected in a postsynaptic neuron which are apparently exclusively mediated by NMDA

receptors (Kullmann and Siegelbaum, 1995). However, after LTP these events are replaced by dual component AMPA and NMDA receptor-mediated signals. Subsequent studies used minimal stimulation to excite small numbers of presynaptic axons and it was shown that NMDA receptor-mediated EPSCs occurred exclusively before LTP and after LTP AMPA-receptor mediated EPSCs emerged (Isaac *et al.*, 1995; Liao *et al.*, 1995; Durand *et al.*, 1996). More recently, paired recording of synaptically coupled pyramidal neurons have definitively shown the existence of silent synapses (Montgomery *et al.*, 2001). LTP is thought to lead to the insertion of AMPA receptors into the postsynaptic density from a cytoplasmic store, or by a translocation from an extrasynaptic location (Malenka and Nicoll, 1999). The postsynaptic density is an electron-dense thickening underneath the postsynaptic membrane at excitatory synapses that contain receptors, structural proteins linked to the actin cytoskeleton and signalling elements such as kinases and phosphatases.

Microscopic techniques have been used to examine the distribution of glutamate receptors in synapses in the rat brain (Petralia and Wenthold 1992; Martin *et al.*, 1993; Molnar *et al.*, 1993; Baude *et al.*, 1995; Nusser *et al.*, 1998; Petralia *et al.*, 1999). Non-synaptic AMPARs have been found in high concentrations on both surfaces and intracellular regions of dendrites and non-synaptic AMPARs seem to outnumber synaptic AMPARs (Shi *et al.*, 1999). The distance between the non-synaptic and synaptic region is a few microns. This is compatible with insertion of AMPARs into the membrane following LTP as these receptors can travel these small distances in a matter of seconds (Malinow, 2003). It has also been found that synapses in the CA1 region are relatively devoid of AMPARs (Petralia *et al.*, 1999; Takumi *et al.*, 1999). Electrophysiological evidence that silent synapses are more prevalent in younger rats has been substantiated by studies that have shown that there is a smaller number of AMPARs present in synapses in the early developmental stage (Durand *et al.*, 1996; Liao and Malinow 1996; Rumpel *et al.*, 1998). The size of dendritic spines has also been related to their function with the discovery that small spines have very few AMPARs (Matsuzaki *et al.*, 2001). In organotypic slices a GFP-tagged GluR1 receptor has been developed which was shown to move towards the dendritic shaft and dendritic spine following high-frequency stimulation (Shi *et al.*, 1999). These receptors were found to be present for up to 50min after the HFS and also their movement was blocked by inhibition of the NMDARs.

Electrophysiologically, AMPA receptors lacking GluR2 exhibit strong rectification. Rectification is measured as the ratio of the response observed at –60mV to that at +40mV. Thus, these AMPARs with rectification can be monitored electrophysiologically and it has been shown that LTP and an overexpression of active CaMKII induce delivery of GluR1-AMPARs into synapses (Hayashi *et al.*, 2000). GluR1 also interacts with PDZ-domain proteins to enable LTP or CaMKII to deliver GluR1-receptors into synapses.

GluR1 has a CaMKII phosphorylation site at Ser831 which is activated during LTP and allows for increased conductance through the channel (Barria *et al.*, 1997; Mammen *et al.*, 1997; Derkach *et al.*, 1999). The PKA phosphorylation of GluR1 has been shown to be necessary but not sufficient for delivery of GluR1-containing receptors to synapses (Ehlers, 2000; Esteban *et al.*, 2003).

In the current model of the AMPA-receptor-anchoring assembly actin filaments form a link between the CaMKII/actinin complex and another complex of two GluR1 binding proteins – 4.1N and SAP97. This scaffolding arrangement serves to bind AMPA receptors tightly to the membrane and it also links AMPA to NMDA receptors as the CaMKII/actinin complex can attach itself to NMDA receptors as well as the GluR1 anchoring protein complex (Lisman and Zhabotinsky, 2001; Lisman *et al.*, 2002). The use of CaMKII as a structural protein as well as an enzymatic protein explains why it is found in such a high concentration in the PSD (~100μM) (Strack *et al.*, 1997; Zhabotinsky, 2000). This structural model of CaMKII can also account for depotentiation as dephosphorylation of CaMKII with phosphatases would reverse the strengthening of the anchoring assembly and contribute to a reduction in potentiation (O'Dell and Kandel, 1994). The phosphorylation state of CaMKII also enables it to be used for memory storage despite the changing nature of receptor trafficking. While AMPA-receptor anchoring may be upregulated or downregulated the phosphorylation state will not be affected by these changes (Sheng, 2001). As CaMKII controls multiple mechanisms of AMPA-mediated transmission there is a certain redundancy in the system and the disruption of one mechanism will not inhibit LTP. This strengthens the expression of LTP and means that mutations which target CaMKII are likely to have a much greater effect than mutations which target just one of the processes described above.

1.6 The locus of expression of LTP

Whether LTP is expressed pre- or postsynaptically has remained a constant source of debate for the last twenty years. One likely reason for such a protracted debate is that LTP uses different mechanisms at different types of synapses. For example, mossy fibre LTP is unique in the hippocampus in that it is NMDA-independent (Nicoll and Malenka, 1995). However, even for NMDA-dependent LTP, it is possible that different synapses use different mechanisms for LTP expression. An overview of the possibilities for the expression of LTP is that it might be due to (1) an increase in the amount of glutamate released presynaptically, (2) an increase in receptor number postsynaptically or a functional change in these receptors, (3) an extrasynaptic change that might lead to increased neurotransmitter availability at receptors and (4) morphological modifications (Bliss and Collingridge, 1993).

An early study measured the concentration of glutamate extracellularly and showed LTP is associated with an increase in glutamate overflow (Dolphin *et al.*, 1982). This gave rise to the idea of a retrograde messenger that has still not been identified. Another early proposal that has recently been confirmed was that glutamate receptors would be inserted into the postsynaptic membrane following LTP (Lynch and Baudry, 1984). Paired-pulse facilitation (PPF) which is an indicator of presynaptic release probability has been used to determine whether LTP expression is pre or postsynaptic. Because LTP of the perforant path is not associated with a change of PPF it was proposed that LTP in this region was postsynaptic (McNaughton, 1982). This was supported by later studies (Manabe *et al.*, 1993; Asztely *et al.*, 1996) but also challenged by others (Schulz *et al.*, 1994). Schultz *et al.* (1994), found that a large initial PPF was associated with a decrease in PPF with LTP, while a smaller initial PPF was associated with an increase in PPF and they suggested that this inverse correlation was due to the recruitment of additional release sites that have low release probability.

Other studies looked at the differences between NMDA and AMPA receptor responses following LTP. A change in the AMPA component of EPSC was taken to be evidence for a postsynaptic modification and a change in both the receptors was taken to be evidence of a presynaptic modification. Some studies found an increase in the AMPA component (Muller *et al.*, 1988; Perkel and Nicoll 1993) and others found the opposite (Bashir *et al.*, 1991).

A decrease in the coefficient of variation and failure rate of synapses was found to indicate a presynaptic expression of LTP (Malinow and Tsien, 1990; Bekkers and Stevens, 1990). However, there is a body of evidence that does not agree with the presynaptic expression of LTP. Occlusion of LTP should be possible if LTP is due to an increase in the probability of transmitter release. Transmitter release can be increased by application of the K^+ channel blocker, 4-AP (Muller and Lynch, 1989). However, when AP-4 is applied and Ca^{2+} is also elevated artificially then there is no further increase in transmission. Experiments which produce a ten-fold change in transmitter release by blocking or activating presynaptic adenosine receptors failed to alter LTP (Asztely *et al.*, 1994).

Antagonists which block glutamate receptors in a use-dependent manner have also been used to study the dynamics of transmitter release. MK-801 was used to block NMDA receptors and its rate of block after repeated stimulation was compared with control synapses and no difference was observed (Manabe and Nicoll, 1994). A similar experiment was carried out with a polyamine compound which blocked a GluR2-lacking AMPA receptor in a use-dependent manner and LTP was found to have no effect on the rate of inhibition (Mainen *et al.*, 1998).

Astrocytic glutamate transporters surround excitatory synapses and are very sensitive to changes in glutamate concentration (Bergles and Jahr, 1997). Following LTP there was no change in the EPSCs of these transporters suggesting that there was no increase in transmitter release (Diamond *et al.*, 1998; Luscher *et al.*, 1998).

FM1-43 is a fluorescent marker that can be taken up by endoplasmic vesicles. Transmitter release can be monitored by measuring the rate at which the marker is lost from the presynaptic membrane. This is called the rate of destaining. Following a HFS of 50 or 200 Hz there was no increase in the rate of destaining in relation to the control which did not receive a HFS. This strongly argues that there is no increase in transmitter release following HFS. However the same study did find an increase in destaining following 200 Hz HFS that induced an LTP that was dependent on NMDA receptors and L-types Ca^{2+} channels (Zakharenko *et al.*, 2001).

In reality there are probably modifications at both the pre and postsynaptic membranes. Induction of LTP has been shown recently to increase the size of synapses measured 2 hours after induction (Ostroff *et al.*, 2002). This increase in synaptic size must lead to a change in the size of the presynaptic active zone and

presumably to an increase in the number of release sites. Thus a simple model for LTP expression would involve the addition of AMPA receptors to the postsynaptic membrane and a growth in the size of the synapse leading to an increase in the number of presynaptic release sites. This simple model of a hybrid pre- and postsynaptic expression of LTP would incorporate the silent synapse theory and the model of Bolshakov *et al.*, (1997).

Different induction protocols seem to require different expression mechanisms. With low-frequency presynaptic stimulation coupled to postsynaptic depolarisation there is no change in the NMDA component (Kullman 1994; Selig *et al.*, 1995; Montgomery *et al.*, 2001) but with high-frequency stimulation there is a change in the NMDA component (Bashir *et al.*, 1991; Xie *et al.*, 1992; Asztely *et al.*, 1992). Thus, much of the controversy surrounding the pre- and postsynaptic debate may be due to different induction protocols using different expression mechanisms.

The persistence of LTP has been shown to be dependent on protein synthesis (Krug *et al.*, 1984; Goelet *et al.*, 1986) and on the intensity of NMDA receptor activation (Malenka, 1991). It has been proposed that there is a critical time during early phase, protein synthesis independent LTP when LTP can be consolidated by plasticity associated proteins (Nguyen *et al.*, 1994). The concept of synaptic tagging was introduced to explain how proteins are targeted to active synapses (Frey and Morris, 1997). Frey and Morris (1997), suggested that plasticity proteins synthesised in the cell body after activation of dendrites are distributed in a random fashion. Rather than these proteins being directed to activated dendrites, they suggested that activated synapses have a "tag" which would enable the synapse to sequester the randomly distributed plasticity proteins. They performed an experiment where a strong tetanus was applied which induced protein synthesis-dependent LTP (also called late-LTP) in one pathway and 60 min later the same tetanus was applied in a second pathway. However, between the two tetani anisomycin was allowed to perfuse through the bath and protein synthesis was therefore inhibited before the second tetanus was applied. In accordance with their hypothesis they found that LTP was produced in both pathways. Following these experiments they also found that a weak stimulus delivered to the second pathway which was unable to induce LTP on its own was capable of inducing LTP when stimulated weakly up to 2 hours prior to the induction of late LTP on the other

pathway (Frey and Morris, 1998). These findings have applications for the synaptic plasticity and memory hypothesis (SPM). An animal that explores its environment will induce LTP that will decay rapidly over time. LTP will only be maintained at synapses that are potentiated shortly before or after the events that lead to stimulation of plasticity-related proteins. This is more likely to occur when the animal is emotionally charged or when it is strongly motivated to learn.

1.7 The role of adrenergic, muscarinic acetylcholine and metabotropic glutamate receptors in LTP

1.7.1 The role of adrenergic receptors in LTP

An early study found that noradrenaline had no effect on synaptic plasticity in Schaffer collateral/commissural-CA1 synapses (Dunwiddie *et al.*, 1982). However, a number of studies since this early work have indicated that adrenergic receptors play an important role in the modulation of LTP in all areas of the hippocampus. Since LTP induction is dependent on the activation of NMDA receptors and the regulation of intracellular Ca^{2+}, it is apparent that activation of α_1-adrenergic receptors which can increase Ca^{2+} via phospholipase C (PLC), might have a facilitatory role in the induction of LTP. The conductance of NMDA receptors has been found to be modulated by α_1-adrenergic receptor activation (Segal *et al.*, 1991). Also, α_2-adrenergic receptors and β-adrenergic receptors which can decrease and increase cAMP levels respectively through G-protein interaction are able to modulate LTP by determining the level of phosphorylation of PKA. The cAMP pathway can also recruit ERK in neurons via PKA (Martin *et al.*, 1997; Impey *et al.*, 1998) and it is well documented that the cAMP pathway can participate in both transient and persistent forms of synaptic plasticity (Huang *et al.*, 1994; Weisskopf *et al.*, 1994; Blitzer *et al.* 1995; Huang *et al.*, 1996; Blitzer *et al.*, 1998; Winder *et al.*, 1998). Therefore the signalling cascades that are activated through adrenergic receptors can regulate the activity and function of downstream molecules after NMDA receptor activation during LTP induction. Application of noradrenaline or a β-adrenergic agonist (isoproterenol) to hippocampal slices has been shown to induce long-lasting potentiation (LLP) in the medial perforant pathway and long-lasting depression (LLD) in the lateral perforant pathway (Dahl and Sarvey, 1990).

Neither of these changes in synaptic efficacy requires electrical stimulation and they are both dependent on NMDA receptor activation (Dahl and Sarvey, 1990). In order to examine the relationship between LLP, LLD and LTP, subsequent experiments were performed where slices were tetanised following LLP and LLD. These experiments found that tetanisation after LLP resulted in additional potentiation of the medial perforant path response and tetanisation after LLD brought the synaptic response back to baseline levels (Pelletier *et al.*, 1994). Application of isoproterenol after LTP induction was found to enhance potentiation in the medial perforant path but it had no effect on the potentiated response evoked by lateral perforant path stimulation (Pelletier *et al.*, 1994). These results prove that the pathway specificity in the dentate gyrus is maintained irrespective of the history of previous potentiation. β-adrenergic receptor antagonists were also found to block the induction of the LLP phenomenon in the dentate gyrus (Dahl and Sarvey, 1989). β-adrenergic receptor antagonists have consistently been shown to reduce the induction of LTP in both medial and lateral perforant path synapses (Bliss *et al.*, 1983; Bramham *et al.*, 1997; Stanton and Sarvey, 1985) and also to reduce the persistence of LTP in the dentate gyrus (Swanson-Park *et al.*, 1999). Other effects of β-adrenergic receptor activation that have been observed in the dentate gyrus are an increase in synapsin phosphorylation and an increase in voltage-dependent Ca^{2+} channel activity (Gray and Johnston, 1987; Parfitt *et al.*, 1992).

In CA1, isoproterenol has been found to lower the threshold for LTP induction (Sah and Bekkers, 1996). Isoproterenol can enable the induction of LTP during a 3 min train of 5 Hz stimulation which would normally produce no change in synaptic response (Thomas *et al.*, 1996; Winder *et al.*, 1999). Winder *et al.*, used knockout mice to demonstrate that β_1-adrenergic receptors were responsible for this effect (Winder *et al.*, 1999). In the absence of isoproterenol the prolonged 5 Hz stimulation is thought to produce a significant activation of phosphatases which counteract the kinase phosphorylation necessary for LTP (Thomas *et al.*, 1996). Also, LTP produced by coupling prolonged low frequency stimulation with isoproterenol was found to be completely dependent on PKA (Thomas *et al.*, 1996). This is of interest because PKA has been shown to activate a gate-like signalling pathway that enables the induction of LTP by inhibiting protein phosphatases (Blitzer *et al.*, 1995; Iyengar, 1996). Isoproterenol has also been shown to

antagonize the effects of the phosphatase, calcineurin, on the NMDA receptor ion channel in cultured hippocampal neurons by activating PKA (Raman *et al.*, 1996). When calcineurin is not opposed by PKA it can inhibit the induction of LTP by activating protein phosphatase 1 through dephosphorylation of inhibitor 1. Therefore, central to the role of β-adrenergic modulation of LTP is the convergence of PKA and calcineurin on inhibitor 1 (Thomas *et al.*, 1996). While isoproterenol can lower the threshold for LTP induction in CA1, β-adrenergic antagonists have not been found to block LTP induction in CA1 (Dunwiddie *et al.*, 1982; Swanson-Park, 1999). Isoproterenol can also increase the width of the time widow of stimulation required to produce associative LTP in CA1, an effect which is dependent on PKA and MAP kinase activation (Lin *et al.*, 2003).

In CA1, β-adrenergic receptor activation seems to have a critical role in the modulation of the early induction phase of LTP (Sah and Bekkers, 1996; Otmakhova and Lisman, 1998; Swanson-Park, 1999). It has been proposed that induction of LTP can be gated by calcium-activated potassium channels that control membrane depolarisation (Sah and Bekkers, 1996). Since β-adrenergic receptors and calcium-activated potassium channels are both located on proximal dendritic spines in the CA1, the β-adrenergic receptors are in an ideal position to regulate LTP induction (Swanson *et al.*, 1987). In CA1, noradrenaline has been shown to have no effect on LTP induced by theta-burst stimulation but to inhibit the induction of LTD by low frequency stimulation (Katsuki *et al.*, 1997). Noradrenaline also facilitated the expression of LTP in response to 10 Hz stimulation by a β-adrenergic dependent mechanism (Katsuki *et al.*, 1997). In contrast to naïve synapses, depotentiation of LTP by low-frequency stimulation in the CA1 can only be fully inhibited by the activation of both α- and β-adrenergic receptors (Katsuki *et al.*, 1997). Consistent with this, the α-adrenergic receptor agonist, St 587, was shown to have a minor facilitatory effect on LTP induction in CA1 (Pussinen and Sirviö, 1998).

Activation of NMDA receptors that does not lead to LTP can prevent LTP induction within a time window of 1 hour (Coan *et al.*, 1989; Izumi *et al.*, 1992a). This NMDA-mediated block of LTP can also be overcome with an α_1-adrenergic agonist (Izumi *et al.*, 1992b). Izumi *et al.* (1992b), found that β-adrenergic receptor activation had no effect on the induction of LTP at potentiated synapses but this

result was not replicated in a subsequent study (Moody *et al.*, 1999). Despite the discrepancies in the literature, α-adrenergic receptors do appear to have a minor role to play in the modulation of LTP in the hippocampus.

In early studies, β-adrenergic agonists were found to modulate the early phase of mossy fibre LTP (Hopkins and Johnston, 1988). This was to be expected considering that there is an extensive noradrenergic projection to the stratum lucidum of the CA3 region where the glutamatergic mossy fibres terminate (Moore and Bloom, 1979; Loy *et al.*, 1980). It was proposed by Hopkins and Johnston that β-adrenergic receptors were mediating their effects postsynaptically via an influx of Ca^{2+} through postsynaptic voltage-gated Ca^{2+} channels. This hypothesis was challenged by Weisskopt *et al.*, (1994) and Huang *et al.*, (1994) with evidence that cAMP induces mossy fibre LTP presynaptically. A further study showed that pairing isoproterenol with a weak train that is normally subthreshold for LTP could produce an early-phase of LTP in mossy fibres and that this LTP occludes paired-pulse facilitation suggesting that β-adrenergic receptors are acting presynaptically (Huang and Kandel, 1996). These results indicating that β-adrenergic receptors are modulating LTP at the presynaptic terminal are consistent with studies showing that β-adrenergic receptors can phosphorylate synapsin I, which is a vesicle-associated protein of the presynaptic terminal (Parfitt *et al.*, 1992). Also, evidence that β-adrenergic receptor activation can enhance evoked and spontaneous transmitter release in hippocampal pyramidal neurons (Chavez-Noriega and Stevens, 1994; Gereau and Conn, 1994) is in agreement with a presynaptic locus for β-adrenergic modulation of mossy fibre LTP.

Recent studies have also shown a requirement for β-adrenergic receptor activation in the induction of some forms of protein-synthesis dependent late-LTP in the dentate gyrus (Straube and Frey, 2003). Straube and Frey (2003), showed that late-LTP induced by two mild stimulation protocols was blocked by propranolol but that a stronger protocol with repeated tetanisation overcomes the dependence of late-LTP on β-adrenergic receptor activation. This indicates that protein synthesis is required for LTP consolidation and that β-adrenergic receptor activation may be used to recruit proteins under certain stimulation protocols but not under others (Straube and Frey, 2003). This recruitment of proteins through diverse sources is in

agreement with the idea of synaptic tagging as porposed by Frey and Morris (1997). Thus, synapses that are tagged can attract proteins which are synthesised by β-adrenergic activation as well as proteins which are synthesised by the actual glutamatergic stimulation.

Overall, the studies to date suggest that the noradrenergic system plays an important role in the modulation of LTP. However, the behavioural significance of this system is still unclear. Blockade of β-adrenergic receptors has been found to impair memory in some studies (Cahill *et al.*, 1994; van Stegeren *et al.*, 1998; Gibbs and Summers, 2000) and to have no effect on memory in others (Pisa and Fibiger, 1983; Wenk *et al.*, 1987; O'Carroll *et al.*, 1999). But noradrenergic activation is also thought to have a crucial role in the emotional reinforcement of memory (Gibbs and Summers, 2000). During exploration there is selective activation of the entorhinal-dentate-CA3 pathway and this pathway is quiescent during behavioually quiet times (Buzsaki and Gage, 1991). This is in accordance with the activation of dentate gyrus and CA3 by the locus coeruleus when an animal is exploring its environment. During quiet periods of slow-wave sleep or consummatory behaviour there are also sporadic bursts in the CA3 that propagate "sharp waves" in CA1 neurons (Buzsaki, 1986), and this may correspond to a flow of information from the CA3 through CA1 to the cortex for consolidation purposes (Buzsaki and Gage, 1991; McClelland *et al.*, 1995). Therefore, there might be a conjunction of adrenergic activity with synaptic activity that helps to consolidate plasticity in the brain.

1.7.2 The role of muscarinic acetylcholine receptors in LTP

There are many studies showing that muscarinic acetylcholine receptors (mAChRs) have an important role in both LTP and LTD. In CA1, perfusion of the general mAChR antagonist, scopolamine, before tetanus was found to suppress LTP. However, application of scopolamine after tetanus did not affect LTP (Hirotsu *et al.*, 1989). Low concentrations of muscarine (1μM), a muscarinic receptor agonist, has been found to facilitate LTP in the dentate gyrus, whereas higher concentrations (10μM) depress EPSPs but have no effect on LTP induction (Burgard and Sarvey, 1990). The M_1 receptor is thought to be responsible for the facilitation of LTP because the M_1 receptor antagonist, pirenzepine, inhibits the facilitatory effect of

muscarine (Burgard and Sarvey, 1990). The medial septal area is the main cholinergic projection to the hippocampus and intraseptal infusion of oxotremorine (an mAChR agonist) has been shown to improve memory in aged rats. Intraseptal infusion of oxotremorine has also been shown to block LTP in the lateral perforant path of aged rats but to have no effect on medial perforant path LTP (Pang *et al.*, 1993). In the same study, generation of LTD in the medial perforant path was enhanced with oxotremorine (Pang *et al.*, 1993). Application of physostygmine, an inhibitor of AChE leads to an enhancement of population spikes that resembles LTP (Ito *et al.*, 1988; Levkovitz and Segal, 1994). In the CA1, the mAChR agonist carbachol can enhance LTP and an mAChR antagonist, atropine, suppresses associative LTP (Blitzer *et al.*, 1990; Sokolov and Kleschevnikov, 1995). During cholinergically induced theta oscillations hippocampal synapses are in a state of heightened plasticity and they can be modified by brief stimulation which would normally be ineffective for eliciting LTP (Huerta and Lisman, 1995). Auerbach and Segal (1996) have shown that a low concentration of carbachol (0.2-0.5μM) applied for 20 min gives rise to an LTP which they call muscarinic LTP (LTP_m) in the CA1. This LTP_m is not accompanied by a change in paired pulse facilitation and it can be blocked by M_2 receptor antagonists methoctramine and AFDX-116. Therefore they concluded that LTP_m is mediated postsyanptically by an M_2 muscarinic receptor. This LTP_m is independent of NMDA receptor activity but it does demonstrate the property of occlusion i.e. carbachol can no longer produce LTP_m after saturation of LTP with repeated suprathreshold tetani (Auerbach and Segal, 1994). LTP_m is also accompanied by a prolonged enhancement of AMPA receptor activation which is in contrast to the transient increase in NMDA receptor activity (Auerbach and Segal, 1996; Markram and Segal, 1990; Markram and Segal, 1992). The slow time-course of this effect suggests that AMPA receptors are being regulated by a second messenger system. LTP_m has also been shown to be dependent on an increase in intracellular Ca^{2+} but not on an increase in Ca^{2+} entry into the cell from the extracellular fluid (Auerbach and Segal, 1994). Higher concentrations of carbachol (2-5μM) gave rise to a fast onset depression of EPSPs (Auerbach and Segal, 1996). This depression of synaptic activity was accompanied by an increase in paired-pulse facilitation indicating that it may be a presynaptic effect. The M_3 antagonist, 4-diphenylacetoxy-*N*-methylpiperidine methiodide (4-DAMP), blocked the depression (Auerbach and Segal, 1996). A subsequent study has also found an enhancement of

LTP with a low concentration of carbachol (0.1μM) which was significantly attenuated by the M_2 antagonist AF-DX (Shimoshige *et al.*, 1997). An agonist of M_1 receptor, MT2, has been shown to facilitate memory consolidation of an inhibitory avoidance task in the rat when applied to the dorsal hippocampus immediately after training and pirenzepine caused retrograde amnesia (Ferriera *et al.*, 2003). With higher concentrations this facilitatory effect was lost due to the fact that higher concentrations of MT2 blocks the M_4 receptor (Ferreira *et al.*, 2003). Overall these results support a role for both M_1 and M_4 in memory consolidation.

Activation of mAChRs has also been shown to block voltage and calcium-gated potassium currents which underlie slow afterhyperpolarisation (I_{AHP}), depolarisation evoked sustained K^+ currents (I_M) and resting leak K^+ currents (I_L) (Cole and Nicoll, 1983; Madison *et al.*, 1987; Dutar and Nicoll, 1988; Muller and Misgeld, 1989). Blockade of these currents are thought to underlie increased reactivity of synapses after ACh application. M_1 receptors have been proposed as the mediators of the block of I_M and I_L while M_2 has been implicated in the block of I_{AHP} (Dutar and Nicoll, 1988; Muller and Misgeld, 1989).

The differential expression of mAChR subtypes in hippocampal pathways is consistent with distinct physiological responses of pathways to cholinergic modulation. In the dentate gyrus M_1 receptors are found in postsynaptic dendrites and spines and M_2, M_3 and M_4 receptors are found in presynaptic axon terminals (Rouse *et al.*, 1999). In the lateral perforant path M_3 receptors are the predominant presynaptic receptor whereas M_2, M_3 and M_4 are equally expressed in the medial perforant path (Rouse *et al.*, 1999).

Relating to the commissural and associational pathways, M_2 has been found to be presynaptically located on the GABAergic neurons of the associational pathway and M_4 receptors are presynaptic on the excitatory associational and commissural pathways (Rouse and Levey, 1997). Both associational and commissural pathways are thought to be important in memory formation as kainic acid lesion studies found that rats with damaged CA3 region had deficits in both working and spatial memory (Sutherland *et al.*, 1983; Jarrard *et al.*, 1984). mAChRs are thought to have a regulatory role in the release of glutamate and GABA and are therefore important in coordinating basal forebrain activity and consolidating memory formation in the hippocampus.

The role of mAChRs in the CA1 area is different to that of mAChRs in the perforant path. Muscarinic receptors increase the excitability of pyramidal cells in the CA1 and prime them to fire action potentials more easily. This priming of synaptic activity has been shown to be mediated by increased activation of NMDA receptors (Markram and Segal, 1990; Segal, 1992; Markram and Segal, 1992). Both M_1 and M_3 are located postsynaptically in the CA1 and carbachol potentiates NMDA receptor current in the CA1 (Marino *et al.*, 1998). The M_1 receptor has also been shown to be colocalised with the NMDA receptor in CA1 pyramidal cells (Marino *et al.*, 1998) and the potentiation of NMDA current by carbachol was shown to be attenuated by a highly specific M_1 toxin (Marino *et al.*, 1998). Therefore, the mAChR modulation of NMDA receptors is likely to be mediated by the M_1 receptor.

It has been shown recently that mAChRs can activate the extracellular signal-regulated kinase (ERK) pathway (Rosenblum *et al.*, 2000; Berkeley *et al.*, 2001; Berkeley and Levey, 2003). ERK activation has been implicated in many aspects of learning and memory including LTP at both the cellular and molecular level (English and Sweatt, 1997; Blum *et al.*, 1999). Carbachol has been shown to induce prolonged activation of ERKI/II in primary cortical neurons and COS-7 cells expressing mAChRs (Rosenblum *et al.*, 2000). Intracellular and extracellular chelators were shown to have no effect on this increase in ERK activity by carbachol but inhibitors of PI_3K blocked the increase (Rosenblum *et al.*, 2000). This group has proposed that the carbachol mediated increase in ERKI/II activity is primarily induced by the $G_{\beta\gamma}$ subunit. In the dendrites and somata of CA1 pyramidal cells carbachol has been shown to cause an atropine-sensitive ERK activation which was found to be mediated by the M_1 receptor (Berkeley *et al.*, 2001). The same study showed that carbachol induced ERK activation was absent in M_1 knockout mice (Berkeley *et al.*, 2001). Carbachol induces ERK staining that is visible in cell bodies and dendrites 15 min after treatment and this staining remains elevated for 2 hours (Berkeley and Levey, 2003). This sustained staining suggests that mAChRs could be responsible for altering dendritic morphology or local changes in neuronal excitability.

1.7.3 The role of metabotropic glutamate receptors in LTP

Since the first study showing mGluR involvement in LTP induction (Reymann and Matthies, 1989) there has been considerable debate surrounding the role of mGluRs in LTP. In this first study AP4, was found to block LTP after 2.5-3 hours. Subsequent studies found that AP3 blocked LTP within 1 hour of tetanus (Behnisch *et al.*, 1991; Izumi *et al.*, 1991). Many studies have reported that MCPG also blocks the induction of LTP (Bashir *et al.*, 1993; Mannahan-Vaughan, 1997; Breakwell *et al.*, 1998; Fitzjohn *et al.*, 1998; Grover, 1998; Wilsch *et al.*, 1998). MCPG has also been shown to block Ca^{2+}-induced LTP (Yang *et al.*, 1998). But some studies have also reported no block of LTP induction with MCPG (Martin and Morris, 1997; Chinestra *et al.*, 1993; Manzoni *et al.*, 1994; Selig *et al.*, 1995a). Some of the discrepancies in the literature are due to the fact that MCPG is a weak, competitive antagonist. It also has antagonistic activity at $mGluR_1$, $mGluR_2$, $mGluR_5$ and $mGluR_8$. MCPG-sensitive mGluR involvement in LTP induction is also dependent upon the previous history of the synapse (Bortolotto *et al.*, 1994).

ACPD, the group I and II agonist, has been shown to facilitate and induce LTP in CA1 (McGuinness *et al.*, 1991b; Bortolotto and Collingridge, 1992, 1993, 1995; Manahan-Vaughan and Reymann, 1995; Breakwell *et al.*, 1996). One group has found that this ACPD induced LTP is dependent on NMDA receptors (Breakwell *et al.*, 1996) while another found that it is NMDA receptor independent (Bortolotto and Collingridge, 1993, 1995).

The role of mGluRs in LTP has been most extensively studied in CA1 synapses where the $mGluR_5$ is the most strongly expressed subtype (Shigemoto *et al.*, 1997). $mGluR_5$ knockout mice were found to have a complete deficit in the NMDA-receptor dependent component of LTP while the AMPA-receptor mediated component of LTP was unaffected (Jia *et al.*, 1998). The studies using $mGluR_1$ knockout mice present a complex picture. In one study there was a deficit in LTP in the CA1 and normal LTP in the CA3 (Aiba *et al.*, 1994) while in another study LTP was unaffected in the CA1 and dentate gyrus and inhibited in the mossy fibre CA3 pathway (Conquet *et al.*, 1994). $mGluR_1$ is expressed at a very low level in CA1 synapses (Shigemoto *et al.*, 1997).

A great advance in the study of mGluRs came with the discovery of LY341495 which blocks group II mGluRs at low nanomolar concentrations but blocks all mGluRs at higher concentrations of ~100μM (Kingston *et al.*, 1998). However this antagonist has been shown to have no effect on LTP in CA1 synapses while MCPG at the same synapses blocks LTP. This leaves the possibility that MCPG is acting at a novel mGluR subtype that has not yet been discovered or MCPG might be acting at some other receptor besides an mGluR (Fitzjohn *et al.*, 1998).

Antagonists of $mGluR_1$ (AIDA and EtCCC) have failed to produce a block of LTP induction (McCaffery *et al.*, 1998). It has been proposed that activation of group II mGluRs might have an inhibitory influence on LTP. In support of this, inhibition of group II mGluRs with MSOPPE has been found to enhance LTP in one study (Behnisch *et al.*, 1998). However, other studies have failed to replicate this either *in vivo* or *in vitro* in the CA1 (Manahan-Vaughan, 1997; Holscher *et al.*, 1997; Breakwell *et al.*, 1998). Antagonists at group III mGluRs in the CA1 have failed to show a block of induction of LTP (Breakwell *et al.*, 1998; Fitzjohn *et al.*, 1998).

The theory of the molecular switch for MCPG-sensitive mGluR receptors was first proposed by Bortolotto *et al.* (1994). The switch is set when MCPG-sensitive mGluRs are activated and once the switch has been set, activation of MCPG-sensitive mGluRs is not necessary for the induction of LTP. If the switch has been set, LTP will not be affected by MCPG. Initially it was thought that the same mGluR was responsible for both switch setting and the induction of LTP. However, these two processes have now been distinguished pharmacologically. LY341495 has been found to block the molecular switch and conversely DHPG can set the molecular switch (Fitzjohn *et al.*, 1998; Bortolotto and Collingridge, 1999). Thus, the mGluR responsible for the switch setting must be one of the known mGluRs. Inhibitors of CaMKII and PKC have also been shown to block the setting of the molecular switch (Bortolotto and Collingridge, 1998; Bortolotto and Collingridge, 1999). This suggests that CaMKII and PKC could be activated by a PLC coupled mGluR via release of Ca^{2+} from intracellular stores.

ACPD has been reported to produce potentiation of the magnitude and duration of LTP (McGuinness *et al.*, 1991; Otani *et al.*, 1993) and also to facilitate subsequent tetanus induced LTP (this is called priming) (Cohen and Abraham, 1996). ACPD is thought to be able to potentiate NMDA-induced depolarisations (Doherty *et al.*, 1997) and this might be able to explain some of its effects mentioned above. DHPG

can also cause a priming effect and application of DHPG can produce a slow onset potentiation (Manahan-Vaughan and Reymann, 1997; Cohen *et al.*, 1998). The time-course of slow-onset potentiation is similar to the time-course of MCPG-sensitive LTP so it has been proposed that ACPD can induce LTP by activating a novel receptor (Bortolotto *et al.*, 1999).

Induction of LTP has been shown to be inhibited by group II agonists ACPD and DCG-IV (Holscher *et al.*, 1997; Breakwell *et al.*, 1998; Behnisch *et al.*, 1998). It has been suggested that the block of LTP induction by MCPG might be due to an agonist action at group II mGluRs (Breakwell *et al.*, 1998) but this is difficult to reconcile with the fact that the group II antagonist LY341495 does not prevent the MCPG block of LTP induction (Fitzjohn *et al.*, 1998).

MCPG has been shown to block the induction of LTP in the dentate gyrus (Richter-Levin *et al.*, 1994; Riedel and Reymann, 1993; Wang *et al.*, 1995; Manahan-Vaughan *et al.*, 1998). Further evidence for the switch mechanism has been obtained in the perforant path where MCPG was found to block LTP only after a low-frequency stimulation (Wang *et al.*, 1995). $mGluR_1$ knockout mice exhibit normal LTP in the lateral and medial perforant path *in vitro* (Conquet *et al.*, 1994) but a reduction has been observed in *in vivo* studies (Bordi *et al.*, 1996). This discrepancy is probably due to an indirect effect on the inhibitory mechanism of the *in vivo* model (Bordi *et al.*, 1997). $mGluR_5$ knockout mice also exhibit normal LTP in the dentate gyrus (Lu *et al.*, 1997).

Similar to CA1 synapses, activation of group II mGluRs in the dentate gyrus by DCG-IV has been shown to block the induction of LTP (Huang *et al.*, 1997). Antagonism of group II receptors with MCCG has been shown to block this effect while having no effect on LTP itself (Bortolotto and Collingridge, 1995). The group I activator DHPG has been shown to induce LTP *in vitro* (O'Leary and O'Connor, 1997) which is in contrast to CA1 (Bortolotto and Collingridge, 1995).

In the mossy fibre pathway where LTP is NMDA receptor independent MCPG has again been shown to block the induction of LTP (Bashir *et al.*, 1993; Fitzjohn *et al.*, 1998). However, this finding has not been replicated in all studies (Hsia *et al.*, 1995). Similar to the CA1, LY341495 was found to have no effect on the induction of LTP in mossy fibre pathway (Fitzjohn *et al.*, 1998). This again suggests that a novel receptor that is sensitive to MCPG might be responsible for the block of LTP. Knockout studies with $mGluR_1$ showed a small deficit in LTP in one study

(Conquet *et al.*, 1994) and no deficit in another study (Hsia *et al.*, 1995). $mGluR_5$ knockout mice showed normal LTP in the mossy fibre pathway (Lu *et al.*, 1997).

mGluR receptors are also involved in LTD in the hippocampus but again there are many discrepancies in the literature. LTD of naïve synapses has been shown to be sensitive to MCPG in the CA1 (Manahan-Vaughan, 1997; Bolshakov and Siegelbaum, 1994; Oliet *et al.*, 1997; Otani and Connor, 1998), to AIDA (Oliet *et al.*, 1997), MSOPPE (Manahan-Vaughan, 1997) and EGLU (Manahan-Vaughan, 1997). Other studies have found that MCPG and LY341495 had no effect on LTD (Oliet *et al.*, 1997; Selig *et al.*, 1995a; Selig *et al.*, 1995b; Fitzjohn *et al.*, 1998). Two distinct forms of LTD have also been found, with one form dependent on mGluRs and the other on NMDA receptors (Oliet *et al.*, 1997). A form of LTD that is dependent on AMPA/kainate and mGluR activation has also been reported (Kemp and Bashir, 1999). Transient application of $mGluR_5$ agonists such as ACPD (Bolshakov and Siegelbaum, 1994; Overstreet *et al.*, 1997), DHPG (Fitzjohn *et al.*, 1998; Cohen *et al.*, 1998; Palmer *et al.*, 1997) and CHPG (Palmer *et al.*, 1997) give rise to a chemically induced LTD. Once established this LTD can be reversed by application of mGluR antagonists MCPG or LY341495 (Fitzjohn *et al.*, 1998; Palmer *et al.*, 1997).

LTD in the perforant path can be blocked by MCPG and MCCG (Huang *et al.*, 1997; O'Mara *et al.*, 1995) and can be primed by the group II agonist DCG-IV (Manahan-Vaughan, 1998). $mGluR_2$ knockout mice show no LTD in the mossy fibre pathway (Yokoi *et al.*, 1996). Thus, it is becoming apparent that group II mGluRs have a critical role in the induction of LTD.

1.8 The role of protein synthesis in LTP

LTP that persists for longer than two hours has been shown by many groups to require gene expression and protein synthesis (Krug *et al.*, 1984; Frey *et al.*, 1988; Otani *et al.*, 1989; Matthies *et al.*, 1990; Frey *et al.*, 1993; Nguyen *et al.*, 1994; Frey *et al.*, 1996; Frey and Morris, 1997). Inhibition of LTP by protein synthesis inhibitors was first discovered *in vivo* (Krug *et al.*, 1984) and was later confirmed *in vitro* (Frey *et al.*, 1988). Thus, LTP can be divided into two phases, an early-LTP (E-LTP) which is unaffected by protein synthesis inhibitors and a late-LTP (L-LTP) which is inhibited by protein synthesis inhibitors (Matthies, 1989). The time frames

of these two phases of LTP are not rigid and they vary with the experimental set-up (i.e. *in vitro* or *in vivo* experiments) and with the type of protein synthesis inhibitors that are used. Early studies demonstrated that injection of protein synthesis inhibitors before HFS produced a significant reduction in LTP after 3 hours (Krug *et al.*, 1984; Otani *et al.*, 1989; Otani *et al.*, 1992). Studies using the mRNA synthesis inhibitor, actinomycin D, have also shown the inhibition of L-LTP after 3 hours (Nguyen *et al.*, 1994). Transient application of actinomycin D before HFS has been shown to leave L-LTP relatively intact suggesting that newly synthesised mRNA might only be required at later time points (Frey *et al.*, 1996). This idea is supported by experiments which show that mRNA for ERK2 and Raf-1 are increased 24 hours after HFS (Thomas *et al.*, 1994). Some studies have however shown effects of protein synthesis inhibitors after about one hour (Stanton and Sarvey, 1984; Osten *et al.*, 1996). In contrast to L-LTP which requires new protein synthesis, E-LTP is dependent on covalent modification of pre-existing proteins at the synapse (Goelet *et al.*, 1986). E-LTP precludes the additional induction of LTP (Frey *et al.*, 1995) and it is thought that synapses that can undergo plastic changes contain receptors or channels termed "fast-acting plasticity processors" (FAPPs) (Frey and Morris, 1998) that can transiently activate previously silent synapses or increase transmitter release (Bliss and Collingridge, 1993; Liao *et al.*, 1995; Isaac *et al.*, 1995). After about two hours these FAPPs are inactivated and new protein synthesis must then produce new processors in order for E-LTP to be converted into L-LTP (Frey and Morris, 1998). New protein synthesis occurs in the cell body of neurons and these proteins are delivered to activated synapses by a mechanism that is not yet understood. Recent evidence also suggests that local protein synthesis in the dendrites might have an important role to play in long-term synaptic plasticity (Aakalu *et al.*, 2001).

Most protein synthesis occurs in the cell body of neurons (Wenzel *et al.*, 1993) and there are four hypotheses as to how the newly synthesised proteins are delivered to the appropriate synapses – the "mail" hypothesis, the "local synthesis hypothesis", the "synaptic tag" hypothesis and the "sensitisation" hypothesis.

The mail hypothesis involves the conference of a specific "synaptic address" to each newly synthesised protein. Considering that CA1 pyramidal cells contain more than 10,000 synapses it would be a cumbersome and inefficient process if each new protein had to be directed to one particular synapse.

The synaptic tag hypothesis obviates the need for elaborate protein trafficking by labelling activated synapses with a molecular "tag". According to this hypothesis, proteins could be distributed diffusely from the cell body and only those synapses with a "tag" would be able to sequester the newly synthesised proteins.

The sensitisation hypothesis proposes that plasticity related proteins synthesised in the cell body are distributed to every synapse. The number of plasticity proteins at the synapse would then determine the threshold for LTP induction, with E-LTP occurring if there are only a few plasticity proteins present at the synapses and L-LTP occurring if there are many proteins present at the synapses (Frey and Morris, 1998).

According to the local protein synthesis hypothesis, dendrites have the translational machinery required to synthesize proteins in response to stimulation. In this scheme there is no difficulty in targeting specific proteins because protein synthesis will only occur in the dendritic area of activated synapses. The first evidence for local protein synthesis came from a study which revealed a preferential localisation of ribosomal clusters at the base of dendritic spines in the dentate gyrus (Steward and Levy, 1982). Since that time most of the components necessary for translation (including rRNAs, translational factors, endoplasmic reticulum (ER) and Golgi apparatus) have been found in dendrites (Tiedge and Brosius, 1996; Gardiol *et al.*, 1999; Pierce *et al.*, 2000). mRNA for proteins that are integral to LTP such as NR1, CaMKIIα and ARC have also been discovered in dendrites (Silva *et al.*, 1992a,b; Holland and Heinemann, 1994; Guzowski *et al.*, 2000). In Schaffer collateral-CA1 synapses, an increase in the concentration of CaMKIIα in dendrites was seen after potentiation and this increase was blocked by anisosmycin (Ouyang *et al.*, 1999). Studies using [^{3}H]leucine to label newly synthesised proteins have also shown that translation is increased in dendrites but not in the cell body following electrical stimulation (Feig and Lipton, 1993). The presence of polyribosomes can be used as a marker to indicate which dendritic spines have undergone protein synthesis-dependent LTP. In a recent study only 12% of dendritic spines were found to contain polyribsomes under control conditions, but this increased to 39% after the induction of LTP (Ostroff *et al.*, 2002). In the same study, the size of the postsynaptic densities on dendritic spines that contained polyribosomes increased after HFS suggesting that local changes in protein synthesis stabilise the growth of the synapse following stimulation.

The increase in protein synthesis that occurs after LTP is also thought to be responsible for an increase in the number of large spines, axo-spinous perforated synapses and perforated synaptic densities with larger apposition zones between pre- and postsynaptic structures (Desmond and Levy, 1988; Geinisman *et al.*, 1991; Geinisman *et al.*, 1993; Buchs and Muller, 1996). Time-lapse imaging has been used to show new spines emerging from the potentiated region of a dendrite hours after tetanus (Engert and Bonhoeffer, 1999). This complements studies which show an increase in presynaptic boutons 1-2 hours after the induction of LTP (Bozdagi *et al.*, 2000). Changes in bouton number were shown to be blocked by protein synthesis inhibitors (Bozdagi *et al.*, 2000).

L-LTP is observed in CA1 mini-slices that have had cell bodies of presynaptic Schaffer collaterals removed (Nayak *et al.*, 1998). This indicates that presynaptic protein synthesis is not required for L-LTP in this area. A later study in cultured CA1 and CA3 cells monitored the number of active presynaptic terminals with the fluorescent dye FM1-43 and found a significant increase in active terminals following plasticity induced by a membrane permeable analogue of cAMP (Ma *et al.*, 1999). Crucially, anisomycin was found to block this effect. Other indirect evidence for presynaptic protein synthesis comes from studies which show an increase in vesicular recycling following potentiation of synapses in models which resembled LTP (Malgaroli *et al.*, 1995; Ryan *et al.*, 1996).

The mechanism used by neurons to initiate translation following stimulation of synapses is still uncertain but one protein that is thought to have an important role in this process is the kinase called target of rapamycin (TOR). Once TOR is activated it can phosphorylate proteins that are normally bound to eukaryotic initiation factor-4E (4E-BPs). Phosphorylated 4E-BPs can then dissociate from initiation factor-4E (eIF-4E) and trigger translation. In relation to the pre- and postsynaptic locus of protein synthesis, TOR, eIF-4E and 4E-BPs have been located both pre- and postsynaptically. Activation of TOR is inhibited by rapamycin (Raught *et al.*, 2001) and recently L-LTP has also been shown to be inhibited by rapamycin (Tang *et al.*, 2002) indicating that the rapamycin sensitive cascade might have a crucial role to play in protein synthesis-dependent LTP.

1.9 The role of protein kinases in LTP

1.9.1 Ca^{2+}/calmodulin-dependent kinase (CaMKII)

CaMKII consists of a family of 28 isoforms that are encoded for by four genes α,β,γ,δ. The α- and β- subunits are the main isoforms in the brain where they form dodecameric holoenzymes. Each isoform has a catalytic domain that contains the ATP and substrate-binding sites, as well as sites for anchoring proteins. If the catalytic domain is truncated by genetic manipulation then the kinase is constitutively active. The full-length form has little catalytic activity under basal conditions due to the fact that the autoinhibitory domain of each subunit inhibits its own catalytic domain. The autoinhibitory domain has a pseudosubstrate region which binds to the catalytic domain at the substrate-binding site. The autoinhibitory domain binds to the catalytic domain and inhibits enzyme activity. This inhibition is relieved when Ca^{2+} /calmodulin binds to a region that overlaps with the pseudosubstrate region. This also causes Thr286 to be exposed on the autoinhibitory domain and allows it to be phosphorylated by a neighbouring subunit (Hanson and Schulman 1992; Ouyang *et al.*, 1997). This phosphorylation leaves the enzyme permanently phosphorylated even if the Ca^{2+} levels fall or Ca^{2+}/calmodulin dissociates from the enzyme. This Ca^{2+}-independent activity is considered to be a biochemical memory trace of the previous Ca^{2+} elevation. Point-mutations that substitute alanine for threonine at position 286 (T286A) have resulted in profound impairments in hippocampal learning (Giese *et al.*, 1998). Interestingly, presynaptic plasticity was not affected by the T286A mutant suggesting that autophosphorylation is critical for postsynaptic but not presynaptic function (Giese *et al.*, 1998). The tetracycline-controlled transactivator system (tTA) has been developed to repress the mutated gene during development and then lift the repression at the appropriate experimental time (Mayford *et al.*, 1996). Results using this technique were also consistent with a role for α-CaMKII in hippocampal learning and LTP.

With a weak stimulus, CaMKII is transiently activated and will be inactivated 0.1-0.2 sec after the fall in Ca^{2+} concentration but if the stimulus is strong, as after a high- frequency stimulation protocol, then autophosphorylation will occur.

Persistent activation is thought to occur because CaMKII molecules in the PSD act as a bistable switch. This switch is turned on when a threshold number of kinase sites are phosphorylated. This state of activation can be maintained for a long time because the kinase acts faster than the PSD phosphatase on Thr286 sites. The dynamics of this activation might account for the all-or-none nature of LTP induction.

The characteristics of the CaMKII molecule have rendered it an attractive model for a molecular switch which was first proposed by Lisman in 1985 (Lisman, 1985). Transcriptional signals derived from the nucleus are unlikely to be a good model for information storage as each synapse can be modified independently. This problem can be resolved with a kinase switch that is transformed into a stable "on" state after phosphorylation. The kinase can remain in the "on" state even after the stimulus has been removed and it can also phosphorylate itself through an autocatalytic reaction. A hyperphosphorylated kinase will also result in the saturation of phosphatases which try to dephosphorylate the kinase and in this way the phosphorylation reaction is likely to proceed at a faster rate than the dephosphorylation reaction. The resulting rapid rephosphorylation of sites is one mechanism whereby the switch can remain stable despite the turnover of individual molecules.

All of these facets of kinase activity can be seen with CaMKII (Zhabotinsky, 2000; Lisman and Zhabotinsky, 2001). One of the most important aspects of CaMKII dynamics is that only protein phosphatase 1 (PP1) is found in the PSD and interestingly even if protein phosphatase 2A (PP2A) is added to the PSD experimentally it is unable to dephosphorylate CaMKII (Strack *et al.*, 1997; Yoshimura *et al.*, 1999). The ability of PP1 to dephosphorylate CaMKII is thought to lie in its immobilisation by scaffolding proteins in the PSD (Watanabe *et al.*, 2001). As yet, it is uncertain which protein causes this immobilisation but possible candidates are spinophilin, neurabin, yotiao and intermediate filaments (Watanabe *et al.*, 2001).

As the concentration of CaMKII in the PSD is about 100μM and that of PP1 is between 1-10μM, it is likely that PP1 is in a saturated state when CaMKII becomes hyperphosphorylated (Strack *et al.*, 1997; Zhabotinsky, 2000). Experimental models have shown that the low basal level of Ca^{2+}/calmodulin is sufficient to expose the Thr286 and cause autophosphorylation (Molloy and Kennedy, 1991). Sustained

phosphorylation of CaMKII is facilitated by the ring structure of the molecule. Initially the six Thr286 sites in the ring of CaMKII are phosphorylated and then one of these sites is dephosphorylated by PP1. However, once this dephosphorylated site binds Ca^{2+}/calmodulin it can again become rephosphorylated by a neighbouring CaMKII which is phosphorylated. This model is only possible with the ring structure of CaMKII and would not occur if the protein had a linear array of subunits.

A low concentration of PP1 in the PSD is a critical requirement for the bistability of the CaMKII switch. If the concentration of PP1 was high then the system would expend a large amount of energy dephosphorylating sites that are constantly becoming phosphorylated, whereas a low concentration of PP1 means that the PSD can be more energy efficient. This hypothesis has been supported by experimental models which have shown that it takes up to 45 hours under basal Ca^{2+} concentration conditions for 10% dephosphorylation of CaMKII to occur after CaMKII catalytic activity is inhibited and even when Ca^{2+} is raised above resting levels the same reaction takes 1.5 hours (Lisman and Zhabotinsky, 2001).

While CaMKII has a turnover rate of about 1 month (Sedman *et al.*, 1986) the characteristics of PP1 saturation enable CaMKII to function as a stable source of information which is resistant to the vicissitudes of protein turnover. This can be demonstrated as follows: when an unphosphorylated enzyme replaces a phosphorylated one it is subject to a low level of phosphatase activity because of the saturation kinetics of PP1 and this ensures that the unphosphorylated enzyme is quickly phosphorylated which returns the system to it original "on" state. This model provides all the characteristics necessary for long-term information storage.

The late-phase of LTP has generally been thought to be dependent on protein synthesis as it is inhibited by protein synthesis inhibitors (Frey *et al.*, 1993). However, stimulation of cAMP was found to bypass the early-phase of LTP and cause potentiation which was dependent on activation of PKA and CREB, and CaMKII inhibitors were found to attenuate the potentiation and the structural changes associated with this stimulation of the cAMP pathway (Bailey *et al.*, 1996; Ma *et al.*, 1999; Makhinson *et al.*, 1999). One possibility is that the stimulation of cAMP inhibits PP1 and this in turn causes the activation of CaMKII (Brown *et al.*, 2000). This hypothesis is supported by studies which have shown that both genetic

and pharmacological inhibition of CaMKII leads to a reduction in early and late-phase LTP (Bortolotto and Collingridge, 1998; Giese *et al.*, 1998).

mRNA for α-CaMKII is found in both dendrites and cell bodies while mRNA for β-CaMKII is found exclusively in cell bodies (Walaas *et al.*, 1988; Burgin *et al.*, 1990). Neuronal depolarisation has been shown to recruit α-CaMKII mRNA into granules that are directed towards dendritic processes (Rook *et al.*, 2000). As dendrites have the machinery necessary for local protein synthesis, CaMKII may well be synthesised locally in the dendrite after the induction of LTP. There is also experimental evidence to support this proposal as there is an actual increase in α-CaMKII as well as α-CaMKII phosphorylation after LTP induction which suggests that there is rapid translation of α-CaMKII mRNA after a stimulus (Ouyang *et al.*, 1999; Giovannini *et al.*, 2001).

The use of CaMKII as a structural protein in the AMPA-receptor anchoring assembly might also explain why the inhibition of protein synthesis causes a reduction in late- phase LTP. As proteins such as actinin, and the GluR1 anchoring proteins, 4.1N and SAP97, are incorporated into the membrane there will be a reduction in the precursor pools of new proteins (Steward and Schuman, 2001) and this pool of precursor proteins must be replenished if AMPA receptors are continually inserted into the membrane. When protein synthesis is inhibited, these pools of proteins will become depleted and CaMKII will no longer have the proteins necessary to form the scaffolding network which strengthens the AMPA-NMDA receptor complex in the PSD. Under these conditions, protein synthesis inhibitors may well reduce late-phase LTP by disrupting CaMKII-mediated AMPA receptor trafficking and anchoring. Despite this reduction in late-phase LTP, CaMKII would still function as a synaptic tag and studies have confirmed this by showing that amnesia induced by the application of protein synthesis inhibitors is reversible over time (Davis and Squire, 1984).

CaMKII is autophosphorylated following the induction of LTP (Fukunaga *et al.*, 1993). Genetic and pharmacological experiments have shown that CaMKII is required for LTP induction and there is almost complete block of LTP when CaMKII function is altered (Otmakhov *et al.*, 1997; Giese *et al.*, 1998). Also, application of CaMKII induces a potentiation that is very similar to LTP (Lledo *et al.*, 1995). CaMKII moves to the PSD after synaptic activation (Shen *et al.*, 2000;

Dosemeci *et al.*, 2001) where it binds to the NMDA receptors. The possibility of CaMKII becoming autophosphorylated is greatly increased when it is bound to the NMDA receptor. This is because there is a much higher concentration of Ca^{2+} in the vicinity of the NMDA receptor and also because CaMKII bound to NMDA receptor can be phosphorylated more easily (Bayer *et al.*, 2001). CaMKII activation may also require full autophosphorylation of multiple nearby CaMKII holoenzymes in the PSD (Lisman and Zhabotinsky, 2001).

The Ca^{2+}/calmodulin-activated adenylate cyclase is also thought to be an important regulator of LTP induction (Chetkovich and Sweatt 1993; Wong *et al.*, 1999). An elevation of cAMP is thought to work together with CaMKII to facilitate potentiation. cAMP by itself does not trigger LTP directly but leads to changes in neural activity that triggers LTP by a normal NMDA process (Ma *et al.*, 1999; Pakhinson *et al.*, 1999; Bozdagi *et al.*, 2000). An increase in cAMP also inhibits PP1 and therefore increases CaMKII autophosphorylation and CREB activation (Brown *et al.*, 2000; Genoux *et al.*, 2002).

Initial evidence suggested that LTP expression depended on CaMKII phosphorylation of AMPA channels and the subsequent increase in conductance through these channels (Barria *et al.*, 1997; Benke *et al.*, 1998). However, more recent evidence suggests that that LTP can still occur even if this process does not occur (Benke *et al.*, 1998; Hayashi *et al.*, 2000; Lee *et al.*, 2000).

Presently, it is thought that CaMKII causes the trafficking of GluR1 into the extrasynaptic membrane and the diffusion of channels to synapses (Chen *et al.*, 2000; Hayashi *et al.*, 2000; Passafaro *et al.*, 2001). Inhibitors of PI3K have been shown to block the insertion of GluR1 into the extrasynaptic membrane (Passafaro *et al.*, 2001). But this effect is reversible which suggests that there is an upstream memory driving this process. PKC was also shown to be involved in this process (Daw *et al.*, 2002).

1.9.2 Protein kinase C (PKC)

PKC was the first kinase to be implicated in LTP (Bar *et al.*, 1984; Akers *et al.*, 1986; Klann *et al.*, 1991). To date, numerous isoforms have been identified and they are divided into three main groups (Nishizuka, 1995; Mellor and Parker, 1998): the Ca^{2+} and diacylglycerol activated conventional PKCs (cPKCα/β1/β2/γ), Ca^{2+}-

independent, diacylglycerol activated, novel PKCs (nPKCδ/ε/η/θ/μ) and the Ca^{2+}-independent, diacylglycerol-independent, atypical PKCs (PKCλ/ζ). All the isoforms have an N-terminal regulatory region of approximately 20-40kDa and a C-terminal catalytic region of approximately 45kDa. A hinge region connects these two domains and it is in this hinge region that PKC can be proteolytically cleaved by calpain or trypsin. After cleavage, PKC is constitutively active because it is not under the influence of the regulatory domain. PKC activators are thought to induce conformational changes by removing a pseudosubstrate region from the kinase core. The persistently active form of PKC is called PKM.

Only a completely phosphorylated PKC can be activated by second messengers (Pears *et al.*, 1992). Binding of diacylglycerol and phosphatidylserine removes the pseudosubstrate region from the catalytic region (Orr and Newton, 1994). This lowers the Ca^{2+} requirements of the enzyme and allows it to be activated.

PKC can be converted from a membrane-associated form to a membrane bound form after prolonged stimulation (Mochly-Rosen *et al.*, 1991; Hyatt *et al.*, 1994; Liao *et al.*, 1994). The different PKC isoforms respond differently to particular combinations of Ca^{2+}, DAG and phopholipids giving rise to localisation of the PKC that is dependent on the different patterns of stimulation (Tanaka and Nishizuka, 1994).

Immunohistochemical studies have been used to describe the localisation of different isoforms of PKC in the brain. PKCβ1 is found mainly in the brain stem while PKCβ2 is found around Golgi complexes and proximal dendrites (Saito, 1994). PKCγ is located mainly in the postsynaptic area in postsynaptic spines as well as dendrites (Saito, 1994). PKCα has a presynaptic localisation (Shearmann *et al.*, 1991) and PKCε has a strict presynaptic localisation (Saito, 1994).

There has been a lot of debate as to whether PKC is involved in the induction or the maintenance of LTP. Some groups have favoured the idea that permanent PKC activation is necessary for LTP (Klann *et al.*, 1991; Sacktor *et al.*, 1993) while other groups have favoured the idea of transient activation of PKC (Otani and Ben-Ari, 1993; Angenstein *et al.*, 1994; Huber *et al.*, 1995). The study of PKC involvement in LTP has been complicated by the fact that under resting basal conditions there is a considerable amount of PKC in a permanently active state in both the membrane

bound and membrane inserted form (Bazzi and Nelsestuen 1988; Staak *et al.*, 1995; Angenstein *et al.*, 1997). There is also a phosphorylation of PKC substrates such as MARCKS, GAP-43 and neurogranin, under basal conditions and this phosphorylation is blocked by PKC inhibitors but not by tetrodotoxin which is a general inhibitor of synaptic transmission. This suggests that basal activity of PKC might not be important for normal synaptic transmission but it could still have a role in increased synaptic activity after stimulation.

The first study to indicate a role for PKC in LTP found that PKC was translocated to the membrane 1 hour after a tetanus but not 1 min after the tetanus (Akers *et al.*, 1986). A subsequent *in vivo* study found a short-lasting redistribution of PKCγ in the dentate gyrus following tetanus (Angenstein *et al.*, 1994). PKC substrates have been shown to be phosphorylated at different time points following tetanus. GAP-43 phosphorylation was found to increase 10-60 min after tetanus while neurogranin phosphorylation only increased after 60 min (Gianotti *et al.*, 1992; Leahy *et al.*, 1993; Ramakers *et al.*, 1995). Different isoenzymes might be responsible for the phosphorylation of different substrates or the balance between kinase and phosphatase activity might differentially regulate the phosphorylation (Hunter, 1995). Anchor proteins like AKAP-79 may be able to facilitate the interactions between kinases, substrates and phosphatases (Faux and Scott, 1996). Studies where PKC inhibitors were added 30 or 90 min after the tetanus showed that substrate phosphorylation was unaffected by inhibitor application at these time points (Colley *et al.*, 1990; Matthies *et al.*, 1991; Huber *et al.*, 1995).

Inhibitors of PKC such as mellitin (Lovinger *et al.*, 1987), polymyxin B (Lovinger *et al.*, 1987; Colley *et al.*, 1990), H-7 (Malinow *et al.*, 1988; Colley *et al.*, 1990), K-252b (Reymann *et al.*, 1990), sphingosine (Malinow *et al.*, 1988) and staurosporine (Denny *et al.*, 1990) have been shown to block the induction of LTP. However, these inhibitors are not very specific blockers and more direct evidence for the involvement of PKC in the induction of LTP came from studies where the PKC inhibitor peptide, PKC_{19-31}, was injected postsynaptically into CA1 synapses (Malinow *et al.*, 1989; Wang and Feng, 1992). This led to the block of LTP induction at these synapses. However, other studies have shown no deficits in LTP induction in the presence of the PKC inhibitors, H-7 and staurosporine (Muller *et al.*, 1988; Muller *et al.*, 1992). Mutant mice lacking the PKCγ isoform have been

shown to have deficits in LTP induction although LTP could be induced after a preceeding low-frequency stimulation protocol (Abeliovich *et al.*, 1993). This study led to the proposal that PKCγ was not integral for LTP induction but was required for regulation of LTP (Abeliovich *et al.*, 1993). Protein kinase Mζ (PKMζ), the constitutively active catalytic fragment of PKCζ, was shown to increase 10 min after LTP induction and this increase lasted for 2 hours (Sacktor *et al.*, 1993; Osten *et al.*, 1996). Application of protein synthesis blockers inhibited both the maintenance of LTP and the increase in PKMζ which indicates that a protein synthesis-dependent mechanism is involved in the persistent phosphorylation of PKC in CA1 LTP (Osten *et al.*, 1996).

Application of PKC activators (phorbol esters) leads to an increase in synaptic transmission through an increase in transmitter release. This reversible enhancement of synaptic response is not however an LTP (Malenka *et al.*, 1986; Muller *et al.*, 1988).

Recently, a Pavolian olfactory learning task has been employed to investigate the role of PKC in learning in *Drosophila*. The induction of the mouse PKMζ (a persistently active PKC, also termed aPKM) transgene in *Drosophila* was found to enhance memory (Drier *et al.*, 2002). Drier *et al.* (2002) found that the improvement was temporally specific with optimum induction 30 min after training. A specific PKMζ inhibitor, chelerythrine, was found to inhibit memory without affecting learning. Another recent study of PKM in the mouse found that it was necessary and sufficient for the persistence and maintenance of LTP over six hours (Ling *et al.*, 2002). The study of Ling *et al.* (2002) introduced recombinant PKM into the postsynaptic neuron to produce a persistent potentiation that occluded subsequent LTP - an effect which was reversed by chelerythrine. The aPKM potentiation was also seen in the presence of NMDA receptor blockers, thus ruling out the possibility that aPKM is causing an enhancement of NMDA currents (Ling *et al.*, 2002). Staurosporine, a general kinase inhibitor, was added at a concentration below that required to block aPKM and was found to block LTP induction but to have no effect on established LTP. However, chelerythrine completely reversed established LTP. This suggests that the machinery required for the initial maintenance of LTP is already present at the site of induction (i.e. it is not transported from some other location in the cell) and that the process is rapidly activated.

Both chelerythrine and a dominant negative inhibitory form of aPKM were found to inhibit the induction of LTP when they were introduced into the postsynaptic neuron (Ling *et al.*, 2002). Therefore, PKC might have a role both in the maintenance and the induction of LTP.

The PKC substrate, GAP-43 (also called B-50), is thought to have a role in synaptic transmission. Overexpression of a mutated form of GAP-43 which is constitutively phosphorylated has been shown to produce an enhancement of LTP in CA1 hippocampal slices (Hulo *et al.*, 2002). The increased LTP was accompanied by an increase in paired-pulse facilitation and an increase in the summation of responses during HFS. These results suggest that GAP-43 may regulate presynaptic properties of synaptic transmission.

Another recent study found that chelerythrine, Ro-31-8220 and Go 6983, all had no effect on the induction of LTP (Bortolotto and Collingridge, 2000). Bortolotto and Collingridge (2000) also investigated the role of PKC in a molecular switch process which is triggered by the synaptic activation of metabotropic receptors and regulates the induction of LTP. It was found that the PKC inhibitors prevented the setting of the molecular switch. The switch was found to be absent in the $mGluR_5$ knockout mouse and so glutamate acting on $mGluR_5$ is thought to be capable of activating the switch. Activation of $mGluR_5$ will lead to PI hydrolysis which will activate PKC and so inhibition of PKC will block the setting of the molecular switch.

GAP-43 is found mainly in presynaptic plasma membranes and not in dendrites. It has been shown to be important in neurite outgrowth, LTP, neuronal signal transduction and neurotransmitter release (Liu and Storm, 1990; Gispen *et al.*, 1991; Strittmatter *et al.*, 1992; Pasinelli *et al.*, 1995). It is often used as a marker of newly formed synapses (Benowitz and Routtenberg, 1997). Phosphorylation of GAP-43 occurs at serine 41 (Coggins and Zwiers, 1989; Spencer *et al.*, 1992), and once phosphoylated, calmodulin cannot bind to the molecule (Alexander *et al.*, 1987). Phosphorylation of GAP-43 modulates clustering and accessibility of the lipid second messenger PI(4,5)P2 and causes changes in the proteins interaction with calmodulin and actin filaments (Laux *et al.*, 2000; Frey *et al.*, 2000). The clustering of PI(4,5)P2 is important for exo- and endocytosis and therefore GAP-43 is also thought to be involved in vesicle recycling (Neve *et al.*, 1998).

RC3/neurogranin is another PKC substrate and it is located mainly in dendritic spines (Represa *et al.*, 1990; Iniguez *et al.*, 1992; Watson *et al.*, 1992, 1994). LTP

induction has been shown to be inhibited by monoclonal antibodies to RC3/neurogranin which disrupt its PKC phosphorylation site (Federov *et al.*, 1995). In contrast to GAP-43 phosphorylation which was seen to increase 10 min after tetanus, RC3/neurogranin phosphorylation levels were found to increase 60 min after tetanus (Leahy *et al.*, 1993). Application of L-glutamate and ACPD has been shown to increase *in situ* phosphorylation of GAP-43 and RC3/neurogranin. This phosphorylation is dependent on mGluRs because the effect was completely abolished by preincubation with the mGluR antagonist, MCPG (Ramakers *et al.*, 1995; 1997).

1.9.3 Protein kinase A (PKA)

cAMP is produced by adenylyl cyclase and the main target of cAMP is the cAMP-dependent protein kinase (PKA). PKA has been shown to be critical for hippocampal LTP (Frey *et al.*, 1993; Huang and Kandel, 1994; Weisskopt *et al.*, 1994; Blitzer *et al.*, 1995; Impey *et al.* 1996; Abel *et al.*, 1997). In CA1, a strong protocol of 4 one second bursts of 100 Hz with 5 min between each burst induces an LTP that is dependent on PKA (Huang and Kandel, 1994). Transgenic mice with reduced hippocampal PKA activity were shown to have deficits in LTP induced by a similar tetra-burst stimulation (Abel *et al.*, 1997).

PKA is a tetrameric enzyme with two catalytic and two regulatory subunits (Reimann *et al.*, 1971). In the mouse hippocampus there are two different catalytic subunits and four different regulatory subunits, so there are 30 different possible isoforms of the enzyme (Cadd and McNight, 1989). Genetically altered mice with deletions of the $RI\beta_1$ catalytic subunit show deficits in LTP and LTD (Qi *et al.*, 1996), while mice with deletions of the RIβ regulatory subunit have normal LTP but impaired LTD (Brandon *et al.*, 1995). These results suggest that different isoforms of PKA can regulate different forms of synaptic plasticity. This was confirmed with studies showing that transgenic mice with an inhibitory form of a regulatory subunit of PKA exhibited a reduction in LTP expression in CA1. This reduction in LTP was seen with a protocol of 4 one second bursts of 100 Hz applied every five minutes but no impairment of LTP was seen with a protocol of 60 pulses applied in a theta-burst pattern (Woo *et al.*, 2000). A PKA inhibitor, Rp-cAMPS, was found to block

LTP induced by both protocols (Woo *et al.*, 2000). These results suggest that different stimulation protocols recruit different isoforms of LTP.

PKA is transiently activated after high-frequency stimulation (Roberson and Sweatt, 1996). Postsynaptic infusion of a cell-permeant PKA inhibitor (Rp-cAMPS) or cell impermeant PKA inhibitor (PKI_{6-22}) resulted in a rapid decay of LTP induced by four trains of 100 Hz repeated every five minutes (Duffy and Nguyen, 2003). However, postsynaptic infusion of PKI_{6-22} was found to produce no impairment of LTP induced by a milder protocol of 100 Hz for 1 sec (Duffy and Nguyen, 2003). This provides further evidence that PKA seems to be predominantly required for LTP induced by multiple stimulation protocols. Postsynaptic infusion of a constitutively active isoform of PKA has been shown to produce an enhanced synaptic response in CA1 pyramidal neurons (Duffy and Nguyen, 2003). Therefore, activation of postsynaptic PKA is necessary for LTP expression and also sufficient for initiating facilitated synaptic responses. During multi-train stimulation there is no change in paired-pulse facilitation suggesting that a postsynaptic mechanism is responsible for LTP. Postsynaptic injection of PKI (a PKA inhibitor) has also been shown to occlude the induction of LTD in CA1 pyramidal cells (Kameyama *et al.*, 1998).

FSK and cAMP analogs have been shown to activate PKA and cause an increase in presynaptic transmitter release (Chavez-Noriega and Stevens, 1992; Carroll *et al.*, 1998). The recruitment of previously silent release sites in presynaptic terminals have been implicated in PKA activation by cAMP analogs such as Sp-cAMPS (Bolshakov *et al.*, 1997; Ma *et al.*, 1999; Bozdagi *et al.*, 2000; Zakharenko *et al.*, 2001). Induction of LTP by repeated tetanisation was associated with a change in paired-pulse facilitation only for the first five minutes following tetanus (Duffy and Nguyen, 2003). This suggests that a postsynaptic mechanism is mainly responsible for LTP and that an increase in the probability of transmitter release only briefly contributes to LTP. Long-lasting LTP has also been shown to be attenuated when apical dendrites are severed from the cell bodies of CA1 pyramidal neurons in slices (Frey *et al.*, 1989). PKA-dependent synthesis of postsynaptic AMPA receptors is also required for long-lasting LTP (Nayak *et al.*, 1998).

GluR1 is a possible target for PKA phosphorylation (Banke *et al.*, 2000; Lee *et al.*, 2000) and PKA can also phosphorylate AMPA and NMDA receptors (Wang *et al.*, 1991; Raman *et al.*, 1996; Leonard and Hell, 1997). PKA can also phosphorylate

the protein phosphatase inhibitor, I-1, and this causes the disinhibition of CaMKII which may lead to the expression of long-lasting LTP at the postsynaptic site (Blitzer *et al.*, 1995, 1998; Brown *et al.*, 2000).

PKA has been shown to be involved in ocular dominance. The shift in ocular dominance after a five-day period of monocular deprivation can be prevented by application of the PKA inhibitor, Rp-8-Cl-cAMPS (Beaver *et al.*, 2001). In hippocampal LTP, activation of NMDA receptors has been shown to cause increased cAMP-induced PKA activity (Roberson and Sweatt, 1996). It is thought that Ca^{2+} stimulation of calmodulin-dependent adenylyl cyclases (AC1 and AC8), is the mechanism for NMDA receptor mediated increase in PKA activity (Wong *et al.*, 1999). This link between PKA and the NMDA receptor is supported by evidence that the PKA catalytic and regulatory (RIIβ) subunits are members of the NMDA receptor complex (Husi *et al.*, 2000). There is also evidence that activation of PKA causes an increase in NMDA receptor currents (Westphal *et al.*, 1999). G-protein coupled receptors, such as mGlu, acetylcholine, dopamine, noradrenaline and serotonin receptors are putative modulators of PKA activity because they can all produce an increase in cAMP and the chief target of cAMP is PKA.

PKA might also influence LTP through a presynaptic mechanism because it has been shown to alter presynaptic release and also to be present in axon terminals (Chavez-Noriega and Stevens, 1994; Trudeau *et al.*, 1996).

In the CA1, CA3 and dentate gyrus, late-phase LTP (L-LTP) requires new protein synthesis and RNA synthesis which is mediated by cAMP (Frey *et al.*, 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Nguyen *et al.*, 1994; Nguyen and Kandel, 1996).

PKA has been implicated in early-phase LTP (E-LTP) in mossy fibre LTP (Huang *et al.*, 1994; Weisskopt *et al.*, 1994) but not in E-LTP in CA1 or dentate gyrus. In all three regions of the hippocampus forskolin application can produce transcription dependent L-LTP (Huang and Kandel, 1994; Hunag *et al.*, 1994; Nguyen *et al.*, 1994). Also, CREB and MAP kinase phosphorylation are increased by forskolin application (Sarvey and Voulalas, 1995; Voulalas and Sarvey, 1995). PKA causes phosphorylation of transcription factors such as CREB which is critical for the establishment of L-LTP (Impey *et al.*, 1996). PKA is also thought to be critical for

an intermediate phase of LTP that begins one hour after induction and is dependent on cAMP, but not on protein synthesis (Winder *et al.*, 1998).

Initially it was thought that early LTP was not affected by PKA (Frey *et al.*, 1993; Huang and Kandel, 1994), but more recent studies have shown that PKA inhibitors can affect E-LTP. Application of the PKA inhibitor, H89, has been shown to suppress E-LTP induced by a single tetanus and postsynaptic infusion of the peptide PKA inhibitor, PKI_{6-22}, also suppresses E-LTP induced by a pairing protocol (Otmakhova *et al.*, 2000). This indicates that the locus of expression of PKA action is postsynaptic. The discrepancies between the studies might be due to saturation of some step in the cAMP pathway during tetanus. This has been confirmed by the finding that application of an adenylyl cyclase inhibitor did not affect E-LTP by itself but could produce a reduction in LTP if the cAMP pathway had already been attenuated by a PKA inhibitor (Otmakhova *et al.*, 2000). In the knockout studies of Wong *et al.* (1999) where Ca^{2+}-dependent cyclase activity in the hippocampus was completely abolished, E-LTP was dramatically reduced. This suggests a role for adenylyl cyclase in E-LTP. Knockouts of the Cβ1 catalytic subunit produce a modest decrease in E-LTP induced by three tetani (Qi *et al.*, 1996). Expression of the R(AB) inhibitory form of regulatory subunit produced a substantial reduction of the E-LTP induced by three tetani (Abel *et al.*, 1997). It is still not clear why inhibitors of the cAMP pathway produce a stronger effect on LTP induced by three tetani as opposed to that induced by a single tetanus (Huang and Kandel, 1994; Abel *et al.*, 1997). Biochemical studies have also shown that PKA is transiently activated at 2 and 10 min after tetanus and that this activation is not present at any other stage after LTP induction (Roberson and Sweatt, 1996).

In the mossy fibre pathway mutant mice lacking Cβ1 catalytic subunit or RIβ regulatory subunit of PKA showed attenuated LTP and confirmed that PKA has a role in LTP induction (Huang *et al.*, 1995; Villacres *et al.*, 1998). The CA3 region also receives extensive noradrenergic innervation and activation of β-adrenergic receptors stimulates cAMP production which in turn influences PKA activity. β-adrenergic activation can convert an STP into an LTP and this conversion can be blocked by both PKA inhibitors and protein synthesis inhibitors (Huang and Kandel, 1996). The same study also found that β-adrenergic antagonists, timolol and propranolol, block early and L-LTP in mossy fibres.

An LTP signalling pathway is characterised by mimicry and occlusion of LTP as typified by CaMKII. PKA however is thought to act as a gating mechanism because inhibitors of PKA block LTP but application of PKA activators do not induce LTP. This gating mechanism operates as follows. After a high-frequency stimulation inhibitor-1 (I-1) is activated and inhibits protein phosphatase 1 (PP1) allowing the expression of LTP. LTP can be blocked by calcineurin which inhibits I-1 and allows PP1 to disrupt LTP. Activation of adenylyl cyclase produces cAMP which activates PKA which in turn phosphorylates I-1 and opens the gate for LTP production by blocking PP1.

Widely spaced trains seem to be very effective in activating calcineurin and producing LTP that is dependent on cAMP. A single-train of HFS does not activate calcineurin and so single-train LTP is independent of the cAMP pathway.

The location of the enzymes involved in the gating process in the postsynaptic density suggests that the teleology of the postsynaptic area has evolved to allow efficient regulation of the different pathways involved. Adenylyl cyclase (Mons *et al.*, 1995), PKA (Carr *et al.*, 1992), calcineurin (Coghlan *et al.*, 1995), and PP1 (Dosemeci and Reese, 1993) have all been found in the postsynaptic density. Also, the two enzymes which regulate I-1, PKA and calcineurin, are bound to each other in the postsynaptic density by AKAP (Coghlan *et al.*, 1995).

1.9.4 Mitogen-activated protein kinase (MAP kinase)

The first evidence for the existence of the mitogen-activated protein kinase (MAP kinase) family came in 1982 when Cooper and Hunter described the tyrosine phosphorylation of cellular proteins by epidermal growth factor (Cooper *et al.*, 1982). MAP kinase was subsequently identified and phosphorylation of the kinase was found to occur at its tyrosine and threonine residues (Ray and Sturgill, 1988a,b). ERK (extracellular signal-regulated kinase) 1 and 2 were the first members of the MAP kinase family to be cloned in 1990 (Boulton *et al.*, 1990). Since then many other members of the MAP kinase family have been cloned – p38/HOG (Hafner *et al.*, 1994; Han *et al.*, 1994), JNK/SAPK (c-jun N-terminal kinase/stress-activated protein kinase) (Kaang *et al.*, 1993; Kyriakis *et al.*, 1994), and ERK 5 (Lee *et al.*, 1995; Zhou *et al.*, 1995). While many scientists use the

words ERK and MAP kinase interchangeably, MAP kinase is more correctly the term for the entire superfamily of kinases including ERK, JNK/SAPK and p38.

Although many of the details surrounding the ERK MAP kinase cascade are uncertain there has been considerable progress in the understanding of the second messengers that can control ERK signalling via the small G-proteins Ras and Rap1. Ras and Rap1 are regulated by specific guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). GEFs promote the attachment of membrane-associated G-proteins to GTP. SOS and C3G are Ras and Rap1 GEFs respectively, and they can be recruited from the cytoplasm to multiprotein membrane complexes by adaptor molecules such as Grb and Crk (Pawson and Scott, 1997). ERK signal specificity can be mediated by growth factor receptors that recruit particular adaptor molecules that associate with either Ras- or Rap-GEFs. After nerve growth factor (NGF) stimulation of PC12 cells, FRS2, a membrane associated molecule can complex with Grb2 and SHP-2 (a tyrosine phosphatase). SHP-2 has been shown to be required for sustained activation of ERK (Hadari *et al.*, 1998). FRS2 also binds to Crk which results in activation of the Rap1 pathway (Meakin *et al.*, 1999). Two novel molecules, rAPS and SH2-B, have been described which bind to neurotrophin receptors and activate ERK via the Ras pathway (Qian *et al.*, 1998). PKC can activate Raf-1 directly and also indirectly via Ras. Recently, a family of phorbol ester-binding GEFs has been discovered that can activate ERK via diacylglycerol through a PKC-independent mechanism (Ebinu *et al.*, 1998; Kawsaki *et al.*, 1998b). PKA inhibits Raf1 but activates the Rap1-B-Raf pathway (Vossler *et al.*, 1997). A cAMP GEF has also been discovered that can activate ERK independently of PKA (de Rooij *et al.*, 1998; Kawasaki *et al.*, 1998a). Both PKA and PKC have been shown to activate ERK2 in the CA1 (Roberson *et al.*, 1999) and receptors which are coupled to PKA (β-adrenergic receptors) and PKC (muscarinic and metabotropic glutamate receptors) have been found to activate ERK. These receptors have also been shown to modulate LTP (Hopkins and Johnston, 1988; Bortolotto and Collingridge, 1993; Huerta and Lisman, 1993) so it has been proposed that PKC and PKA might be mediating their effects on LTP through the activation of ERK. Although there are two isoforms of ERK, ERK1 and ERK2, there is no evidence to date for specific localisation of the isoforms in

different cellular compartments and experiments have shown that MEK1 (the major MEK isoform in neurons) activates ERK1 and ERK2 with similar kinetics (Zheng and Guan, 1993). It is clear however, that ERK1 and ERK2 serve different functions in the brain. This is evident in knockout mice where ERK1 knockouts appear to be neurologically normal but ERK2 knockouts are embryonic lethal (Selcher *et al.*, 2001; Adams and Sweatt, 2002).

The best characterised activation of GEFs is through receptor tyrosine kinases (RTKs). RTKs which are autophosphorylated after ligand binding can provide docking sites for adaptor proteins or intermediate factors. Adaptor proteins or intermediate factors are phosphorylated in turn and can then bind Grb2. The binding of Grb2 results in the formation of Grb2-SOS complexes which can activate ERK (Pawson, 1995; Holgado-Madruga *et al.*, 1996; Raabe *et al.*, 1996; Kouhara *et al.*, 1997). Ca^{2+}/calmodulin activated GEFs have also been discovered in neuronal cells (Orban *et al.*, 1999).

Further complexity has been added to the ERK MAP kinase signalling pathways with recent studies showing that cytoplasmic protein tyrosine kinases such as Src kinases and PYK2 (Lev *et al.*, 1995; Gardner *et al.*, 1993; Cantley *et al.*, 1991), G-protein coupled receptors (GPCRs) including the α-adrenergic, muscarinic, D2 dopaminergic, and A1 adenosine receptors (van Biesen *et al.*, 1996; Della Rocca *et al.*, 1997; Gutkind, 1998), and AMPA and NMDA receptors (Wang *et al.*, 1995; Kyosseva *et al.*, 2001; Chandler *et al.*, 2001) can all activate ERK MAP kinase.

Ras activates Raf and causes it to translocate to the membrane where it phosphorylates ERK MAP kinase kinase by a serine/threonine phosphorylation. Rap-1 activates B-Raf which in turn activates ERK MAP kinase kinase again by a serine/threonine phosphorylation. Despite the complexity of the different cascades they all result in the phosphorylation of ERK MAP kinase kinase which is the sole activator of ERK MAP kinase. This final activation is accomplished through a tyrosine/threonine phosphorylation.

GAPs are responsible for the inhibition of Ras and Rap1. SynGAP is a recently discovered member of this family which links the NMDA receptor to the ERK MAP kinase pathway through PSD95 (Husi *et al.*, 2000). The NMDAR- PSD-95 complex is an electron-dense thickening underneath the postsynaptic membrane at excitatory synapses that contains NMDA receptors, structural proteins linked to the actin cytoskelton and other signalling elements. The NMDA channel occupies one

third of the complex and proteomic analysis revealed the presence of about 75 other proteins in the complex (Husi and Grant, 2001). SynGAP knockout mice show enhanced MAPKK and MAPK phosphorylation but surprisingly exhibit reduced LTP (Komiyama *et al.*, 2002). This has been explained by the fact that PSD-95 knockout mice do not require MAP kinase for LTP and it is possible that there is another pathway downstream of PSD-95 other than the SynGAP-MAPK pathway that is affected in the knockout mice (Komiyama *et al.*, 2002). SynGAP can be inhibited by CaMKII leading to increased activation of ERK (Soderling *et al.*, 2001).

ERK can also be turned off by the dual-specific threonine/tyrosine phosphatase MKP-3 which is the most prominent regulator of ERK dephosphorylation in neurons (Muda *et al.*, 1996a,b). The overall activity of ERK is probably determined by the balance of kinase and phosphatase activity. Interestingly, when ERK is activated it can regulate its own activity by increasing the transcription of MKP-3 phosphatases (Sun *et al.*, 1993; Camps *et al.*, 1998).

The role of ERK in invertebrate neuronal plasticity has been extensively studied in the marine mollusc *Aplysia*. Long-term facilitation (LTF) in *Aplysia* is a model for long-term sensitisation of the gill withdrawal reflex. LTF can be produced by exposing sensory-motor neurons to multiple applications of serotonin and it also causes an increase in ERK activity and ERK nuclear localisation (Martin *et al.*, 1997). When ERK is inactivated by anti-ERK antibodies short-term facilitation is not affected but LTF is attenuated (Martin *et al.*, 1997). ERK activation has also been shown to be critical for an *in vivo* analog of classical conditioning in the invertebrate *Hermissenda* (Crow *et al.*, 1998).

ERK activation was first implicated in vertebrate plasticity in 1996. Following HFS in CA1 an increase in ERK2 activation was discovered (English and Sweatt, 1996). Later studies used the ERK MAPKK inhibitor, PD98059 (Alessi *et al.*, 1995; Dudley *et al.*, 1995), to show that ERK activation is required for stable long-term LTP (English and Sweatt, 1997). Another ERK MAPKK inhibitor, U0126 (Favata *et al.*, 1998), has been shown to block LTP induced by theta-frequency stimulation (Winder *et al.*, 1999; Watabe *et al.*, 2000). Other studies have shown that ERK activation is necessary for both NMDA receptor-independent LTP and NMDA receptor-dependent LTP in the dentate gyrus *in vitro* (Coogan *et al.*, 1999), and also for LTP *in vivo* (McGahon *et al.*, 1999; Davis *et al.*, 2000; Rosenblum *et al.*, 2000)

and LTP at the amygdalar inputs into the insular cortex (Jones *et al.*, 1999). Interestingly, in the CA1, the ERK MAPKK inhibitor, PD98059, was found to block the induction of LTP but to have no effect on the expression of LTP (English and Sweatt, 1997).

LTP is conventionally divided into both an early-phase which lasts up to about 60 mins and a subsequent late-phase which is blocked by inhibitors of protein and RNA synthesis. As ERK has a prominent role in regulating gene expression and inhibitors of ERK MAPKK have been shown to block L-LTP (English and Sweatt, 1996, 1997; Impey *et al.*, 1998; Wu *et al.*, 1999) it is thought that ERK activation is necessary for L-LTP. There is evidence for ERK mediated activation of cell protein synthesis machinery (Lin *et al.*, 1994; Waskiewicz *et al*, 1999). ERK can also act more directly through the modulation of ion channel function and the phosphorylation of synapsin I (Jovanovic *et al.*, 1996).

The importance of ERK in learning mechanisms has been emphasised with data showing that performance in fear-conditioning, aversive taste learning and spatial learning studies is associated with an increase in ERK activity and that inhibition of ERK leads to impaired performance in these learning paradigms (Berman *et al.*, 1998; Atkins *et al.*, 1998; Blum *et al.*, 1999). The fact that the ERK MAPKK inhibitor, SL327, blocks cued fear conditioning in rats has led to the proposal that the deficits seen in amygdala-dependent memory formation in RasGRF mutant mice are due to a disruption of the ERK MAP kinase signalling pathway (Atkins *et al.*, 1998). The muscarinic cholinergic system has also been shown to regulate ERK MAP kinase and consequently LTP in the insular cortex (Jones *et al.*, 1999; Rosenblum *et al.*, 2000). In the hippocampus, activation of the cAMP cascade results in an increase in MAPK expression in CA1 (Martin *et al.*, 1997; Roberson *et al.*, 1999) and β-adrenergic receptor activation causes a PKA-dependent increase in ERK activation (Roberson *et al.*, 1999). This may help to explain the modulation of LTP by β-adrenergic receptors which is inhibited by ERK MAPKK inhibitors (Thomas *et al.*, 1996; Winder *et al.*, 1999). Both muscarinic and metabotropic glutamate receptor mediated increases in ERK MAP kinase activation have been shown to be inhibited by PKC inhibitors (Roberson *et al.*, 1999). As both PKA and PKC can activate ERK MAP kinase it is possible that they might be able to differentially regulate ERK MAP kinase in such a way that either pathway may be

stimulated by a particular stimulus providing a fail safe redundancy in the system (Sweatt, 2001).

ERK can activate several transcription factors including CREB, Elk-1 and c-Myc. Elk-1 phosphorylation in response to novel taste has been shown to be mediated by ERK (Berman *et al.*, 1998). The cAMP response element-binding protein, CREB, is a nuclear protein and it has been implicated in memory in invertebrate and vertebrate plasticity models. CREB is located in the nucleus but CREB mRNA has also been found in isolated dendrites of neuronal cultures generated from embryonic rat hippocampi (Crino *et al.*, 1998). It is not yet known if ERK, Rsk2, or both are needed to translocate to the nucleus to phosphorylate CREB. A further possibility is that CREB is transcribed and phosphorylated locally in the dendrite prior to translocation to the nucleus. Translocation of ERK from the cytosol to the nucleus has been seen in CA1 hippocampal cells (Patterson *et al.*, 2001) and Rsk translocation to the nucleus has also been observed in response to growth factor stimulation in HeLa cells (Chen *et al.*, 1992). CREB is not directly phosphorylated by ERK but via the activation of one of the Rsk family of protein kinases such as Rsk2 (Xing *et al.*, 1996; Impey *et al.*, 1998). Mutations of Rsk2 have been shown to lead to Coffin-Lowry Syndrome in humans which is characterised by mental retardation and cranial dysmorphism (Harum *et al.*, 2001). As protein synthesis inhibitors block L-LTP and CREB is required for the synthesis of proteins that are needed for LTP it is likely that ERK lies upstream of CREB phophorylation in the hippocampus (Adams *et al.*, 2000; Sweatt, 2001). Phosphorylation of CREB occurs at serine 133 and is not exclusively mediated by the Rsk family. PKA, CaMKI and IV have all been shown to phosphorylate CREB at serine 133 (Enslen *et al.*, 1994; Matthews *et al.*, 1994; Sun *et al.*, 1995). CaMKII has a dual role in the regulation of CREB as either a poor activator, or an inhibitor when it phosphorylates sites other than serine 133 (Sun *et al.*, 1994). Once activated, CREB can recruit CREB-binding protein, (CBP), and it is then in a position to activate transcription factors such as *c-fos* and the immediate early gene *zif268* (Goodman, 1990; Herdegen and Leah, 1998). In this way, CREB activation can exert diverse effects on an expanded range of genes. Activation of CREB can also increase the transcription of other proteins besides transcription factors, such as synapsin-1 and the α-subunit of CaMKII (Sauerwald *et al.*, 1990; Olson *et al.*, 1995).

A wealth of evidence has shown that CREB has an important role in neuronal plasticity. Hippocampal long-term memory was examined in transgenic mice with a null mutation of CREB and was found to be impaired in contextual fear conditioning and Morris water maze tasks (Bourtchuladze, *et al.*, 1994; Kogan *et al.*, 1997). Short-term memory was not affected in these studies. Another study blocked CREB activity with an injection of antisense nucleotides into the hippocampus and found that these animals also had deficits in long-term memory when tested with the Morris water maze (Guzowski and McGaugh, 1997). An increase in CREB mediated gene expression has been seen following LTP induction in the hippocampus (Impey *et al.*, 1996) and studies using antibodies have shown that CREB phosphorylation is increased in the perforant path 24 hours after LTP induction by high-frequency stimulation (Schulz *et al.*, 1999).

In biochemical studies activation of ERK following PKA and PKC stimulation was found to target CREB (Impey *et al.*, 1998; Roberson *et al.*, 1999). An interesting caveat to the study by Roberson *et al.* (1999) is the finding that, although PKA can phosphorylate CREB directly, the ERK MAP kinase kinase inhibitor, U0126, can completely block forskolin induced activation of CREB (Roberson *et al.*, 1999). This suggests that PKA must activate CREB via ERK and that ERK is the intermediate through which PKA exerts its influence on gene expression.

The kinetics of CREB activation has been shown to be slow, with CREB phosphorylation increasing over the course of an hour (Wu *et al.*, 2001). This slow rise in CREB phosphorylation might facilitate its role in triggering gene expression in L-LTP.

Recently, ERK MAP kinase has been shown to have an important role in the regulation of the A-type K^+ channel, Kv4.2. This voltage-dependent K^+ channel is localised to subsynaptic compartments of somas and dendrites in area CA1 of the hippocampus (Alonso and Widmer, 1997). The normal function of this channel is to repolarise the membrane and to regulate the excitability of neurons and the magnitude of the excitatory postsynaptic potential (Serodio *et al.*, 1994; Hoffman *et al.*, 1997). Activation of A-type K^+ channels with PKA or PKC causes a depolarising shift in the activation curve of A-type K^+ currents and consequently the number of back-propagating action potentials in dendrites increases (Hoffman and Johnston, 1998). Back-propagating action potentials regulate Ca^{2+} influx through NMDA receptors and are therefore crucial for the induction of LTP. ERK MAP

kinase kinase inhibitors cause a hyperpolarising shift in the K^+ channel activation curve and also inhibit the increase in back-propagating action potentials mediated by activators of PKA or PKC (Adams and Sweatt, 2002). There is also evidence that Kv4.2 is a good substrate for ERK (Adams *et al.*, 2000). When the Kv4.2 channel is phosphorylated by ERK the probability of current passing through the channel decreases and this enables back-propagating action potentials to invade dendrites more easily. When ERK activity is blocked with PD98059 or U0126, the complex spikes that normally result from theta-burst stimulation are also blocked. These complex spikes are responsible for back-propagating action potentials in CA1 dendrites. It is clear from the foregoing evidence that regulation of Kv4.2 via ERK has an important role in neuronal excitability and plasticity.

The nature of the ERK MAPK cascade with three kinases in series allows for enormous signal amplification in an essentially all-or-none manner. This has been demonstrated in *Xenopus* oocytes where the ERK cascade is seen to operate in a digital manner and can change rapidly from a state of low activity to a state of maximal activity (Ferrell and Machleder, 1998). Other studies have shown that ERK can function as a coincidence detector. Neither β-adrenergic receptor agonists nor muscarinic receptor agonists can activate ERK when they are applied separately but if they are both applied ERK can be activated (Watabe *et al.*, 2000). The simultaneous application of β-adrenergic and muscarinic receptor agonists can also trigger activity-dependent hippocampal LTP and this LTP is blocked by ERK MAPKK inhibitors (Watabe *et al.*, 2000). Therefore ERK coordinates the induction of LTP through a three-way coincidence detection – it integrates the two cell-surface signals with synaptic activity to induce LTP. This can be extended to a four-way coincidence detection if we include ERKs regulation of back-propagating action potentials.

Under some conditions, such as temporally spaced stimuli, ERK can be persistently activated and this persistent activation can be used for biochemical information storage (Weng *et al.*, 1999; Wu *et al.*, 2001). One of the proposed mechanisms for persistent ERK activation is that ERK promotes the expression of genes for upstream activators of ERK and thus activation of ERK sets up a positive feedback cycle (Roberson and Sweatt, 2001). Repetitive spaced stimuli have been found to induce more robust forms of memory in most forms of learning, so the evidence to

date indicates that persistent ERK activation following repetitive stimuli might be a critical factor in the maintenance of long lasting memories. ERK activation might also regulate an inhibitory feedback function by promoting the transcription of MAP kinase phosphatases (MKPs). These phosphatases might be capable of consolidating memory by locking the synapase into an aplastic state and protecting it from alteration by subsequent signals.

2 Materials and methods

2.1 Animals

The regulations as issued by the Department of Health and Children regarding the use of animals in scientific research were followed throughout the course of these experiments. All experiments were carried out using male Wistar rats weighing between 40-80g (3-5 week old animals). The animals were supplied by the BioResources Unit, Trinity College Dublin. Every precaution was taken to ensure that the conditions under which the animals were kept remained constant. The animals were housed in cages containing 5-10 rats and food and water was available at all times. The animal house of the BioResources Unit was on a twelve hour light/dark cycle and the temperature of the animal house was maintained between 21-23°C.

2.2 Artificial cerebrospinal fluid (ACSF)

The incubation and experimental solution contained the following (in mM): NaCl, 120; $NaHCO_3$, 26; $NaH_2PO_4.2H_2O$, 1.25; KCl, 2.5; $Mg_2SO_4.7H_2O$, 2; $CaCl_2$, 2; D-glucose, 10. The pH was adjusted to 7.35-7.45 by gassing with 95% O_2/5% CO_2. The saline was prepared each morning before experiments commenced.

2.3 Preparation of slices

The rats were rapidly decapitated using a guillotine without anaesthesia. After decapitation, excess skin and muscle was cut away from the skull using a pair of dissecting scissors. An incision was then made at the base of the skull which facilitated the removal bone from this area of the skull. A second incision was then made along the midline of the skull to enable the remaining right and left hemispheres of the skull to be peeled away using a pair of forceps. At this point the dura was removed using a pair of spring scissors and after this the cerebellum was removed with a scalpel. Another cut was made with the scalpel to separate the area of the brain containing the hippocampus from the forebrain and a spatula was then used to tip the section of brain containing the hippocampus out of the skull and into a bath of cold (<5°C), oxygenated ACSF (95% O_2/5% CO_2). The total time elapsed

from the moment of decapitation to the transfer of the sectioned brain to the bath of cold, oxygenated ACSF was not more than 1.5 min. The brain was incubated in the cold ACSF for ~10 sec and subsequently transferred onto wet filter paper which had been soaked in ACSF. A thin layer of the base of the brain was then removed to enable the brain to be evenly fixed onto a Teflon block with superglue. The stage containing the Teflon block was immediately transferred to a slicing chamber where the brain was completely submerged in cold (<5°C), oxygenated (95% O_2/5% CO_2) ACSF. A Campden Vibroslice with a hard carbon-steel blade (Gillette) was used to cut slices from the brain. The vibroslice was set at a fast (8) vibration speed and a slow (1) advance speed. For the first cut, the top 4mm of the brain was removed and discarded to reveal the hippocampus. Subsequently, several transverse hippocampal slices were cut to a thickness of 350μm and removed to a submersion type chamber (Medical Systems, New York) where they were maintained in oxygenated (95% O_2/5% CO_2) ACSF at room temperature (20-22°C). Slices were allowed to recover in this chamber for at least one hour before they were transferred to the submerged recording chamber of the experimental rig. Again, slices were allowed to recover for at least one hour in the submerged recording chamber of the experimental rig before any recordings were made.

2.4 Experimental set-up

Before slices was transferred to the recording chamber, ACSF was heated to 31-32°C with a stirring heater device (Lauda, Germany) and the solution was gassed for 30 min with a mixture of 95% O_2/5% CO_2. After this, slices could be transferred to the submerged recording chamber where they were superfused with the heated and fully oxygenated ACSF using a gravity feed system at a rate of 6-7ml per min. The volume of the recording chamber was ~0.5ml and slices were prevented from moving in the recording chamber by securing them between two nylon meshes.

The slices were then visualised under illumination with a microscope (World Precision Instruments, Sarasota, FL, USA) at a magnification of X30. Recording and stimulating electrodes were manoeuvred and positioned with two MMN-1 manipulators (Narishige, Japan). The recording chamber, recording and stimulating electrodes and microscope, all rested on an anti-vibration table (AVT 701,

Wentworth Laboratories Ltd., Bedford, UK) and the entire set-up was surrounded by a Faraday cage.

2.5 Electrodes

Bipolar stimulating electrodes were made by twisting two strands of insulated stablohm 650 H-Formvar wire (California Fine Wire Company, CA, USA) together. The two twisted strands of wire were then quickly run through superglue to hold them together. The recording electrodes were low resistance (~1MΩ) glass microelectrodes which were pulled using a Flaming/Brown micropipette puller, P-87 (Sutter Instrument Corp. CA., USA). The recording electrodes were filled with ACSF.

2.6 EPSP recording

Standard electrophysiological techniques were used to record field excitatory postsynaptic potentials (EPSPs) from the medial perforant path of the dentate gyrus. Both stimulating and recording electrodes were placed ~1-2mm apart in the middle third of the molecular layer of the perforant pathway and test EPSPs were evoked at a frequency of 0.033 Hz. An input-output curve (afferent stimulus intensity versus EPSP amplitude) was plotted for each experiment at this test frequency and only slices which had a peak EPSP of >3mV were used in experiments. Stimulation and recording from the medial perforant pathway was verified by the use of paired-pulse stimulation (interstimulus interval 40ms). Paired-pulse depression (~30%) was indicative of the correct location in the medial perforant pathway. The inner (suprapyramidal) blade of the dentate gyrus was used in all studies.

Paired-pulse stimulation was used in all experiments at a test frequency of 0.0167 Hz and in each experiment the amplitude of the test EPSP was adjusted to ~1-1.2mV which was approximately one-third of the maximum EPSP as determined by the I/O curve. The baseline was considered to be stable if no change occurred in the EPSP for 30 min before the application of a drug or the delivery of a high-frequency stimulation protocol.

Stimulation pulses were generated by a Master-8, eight channel, programmable pulse generator (A.M.P.I., Jerusalem, Israel) which was driven by IBM compatible PC computer protocols. The stimulation pulses were fed through a stimulation isolation unit (Iso-flex, A.M.P.I., Jerusalem, Israel) to isolate the electrode from ground. The EPSPs were monitored on an oscilloscope (Gould) while records of EPSPs were generated by pCLAMP6 computer software (Axon instruments Inc., Foster City, CA, USA). EPSPs were amplified by an Axopatch 1D amplifier (Axon instruments Inc., Foster City, CA, USA) and converted from analogue to digital form (DigiData 1200, Axon instruments Inc., Foster City, CA, USA) before being stored on a Gateway 2000, P5-100 personal computer for subsequent off-line data processing. Data was analysed using Microsoft Excel and Prism software.

2.7 High-frequency stimulation protocols

Four different high-frequency stimulation (HFS) protocols were used to elicit four different types of LTP as outlined below.

Conventional LTP: 8 trains of 8 pulses at 200 Hz with a 2 sec intertrain interval (ITI).

100 Hz LTP: 4 trains of 1 sec stimulation at 100 Hz with a 20 sec ITI.

200 Hz LTP: 10 trains of 200 msec stimulation with a 5 sec ITI.

Multiple spaced 200 Hz LTP: the 200 Hz LTP protocol repeated 3 times with a 10 min interval between each tetanus.

The stimulation voltage was increased to give an EPSP of two-thirds of maximum amplitude during all HFS protocols.

2.8 Data analysis and statistics

The two-tailed Student's *t* test was used for statistical comparison. Values are the means ± SEM for *n* slices in each experiment.

2.9 Compounds

All experiments were out in the presence of 50μM picrotoxin to block $GABA_A$ mediated activity. Picrotoxin was dissolved in dimethyl sulphoxide (DMSO) but the final concentration of the solvent was less than 0.05% of the circulating solution and no effect on synaptic transmission was observed at this concentration. Propranolol hydrochloride, scopolamine hydrochloride, MPEP hydrochloride, and emetine dihydrochloride were dissolved in H_2O. LY 341495, Bisindolylmaleimide I, anisomycin, KT 5720, KN-93, KN-62 and PD 98059 were dissolved in DMSO.

Propranolol hydrochloride	Sigma
Scopolamine hydrochloride	Sigma
LY 341495	Tocris Cookson, Bristol, UK
MPEP hydrochloride	Sigma
Bisindolylmaleimide I	Sigma
Emetine dihydorchloride	Sigma
Anisomycin	Tocris Cookson, Bristol, UK
KT 5720	Calbiochem, Luzern, Switzerland
KN-62	Calbiochem, Luzern, Switzerland
KN-93	Calbiochem, Luzern, Switzerland
PD 98059	Calbiochem, Luzern, Switzerland
Picrotoxin	Sigma

3 Results

3.1 Different stimulation protocols induce multiple forms of LTP

LTP was induced with a number of different high-frequency stimulation (HFS) protocols. The first protocol employed was 8 trains of 8 stimuli at 200 Hz with an intertrain interval of 2 sec (Fig. 3.1). After a 20 min baseline LTP was evoked by applying the HFS. EPSP amplitude rose to a peak of 201.5±10.8% at 1 min post-HFS ($p<0.01$, $n=5$). LTP declined for 20 min and then remained at a reasonably constant level for the remainder of the experiment. LTP measured 145.7±9.0% at 120 min ($p<0.01$, $n=5$).

LTP produced by 4 trains of 100 Hz for 1 sec, with a 20 sec intertrain interval was then examined (Fig. 3.2). LTP reached a peak of 152.6±3.7% at 5 min post-HFS ($p<0.01$, $n=5$) and declined quite slowly to 129.3±8.0% at 100 min post-HFS.

A protocol of 10 trains of 200 msec stimulation at 200 Hz with a 5 sec intertrain interval was examined next (Fig.3.3). This produced a large initial LTP of 190.1±13.4% which declined to a level which was not significantly different from 100 Hz LTP after 80 min (153.7±6.7%, $n=5$, $p=0.08$).

Finally a very strong protocol was employed which consisted of the 200 Hz protocol repeated 3 times with 10 min between each tetanus (Fig. 3.4). This protocol produced a strong potentiation that reached a peak of 198.5±5.8% at 8 min after the 3rd 200 Hz ($p<0.01$, $n=5$) and was sustained with little decline throughout the experiment. LTP measured 182.7±8.7% at 120 min after the 3rd 200 Hz.

A comparison between 100 Hz LTP, 200 Hz LTP and multiple spaced 200 Hz LTP is shown in Fig. 3.5. It can be seen that the early-phase of LTP (E-LTP) produced by 200 Hz HFS was not significantly different from E-LTP produced by multiple spaced 200 Hz HFS but it declined rapidly to the level of 100 Hz LTP. The multiple spaced 200 Hz LTP was significantly different from 100 Hz LTP at 5 min ($p<0.05$, $n=5$) and it was significantly different from 200 Hz LTP at 25 min ($p<0.05$, $n=5$). The level of LTP produced is clearly dependent on the frequency of stimulation and on the duration of the high-frequency burst. In addition to this, the 10 min spacing between bursts in the multiple spaced 200 Hz protocol seems to have a particularly

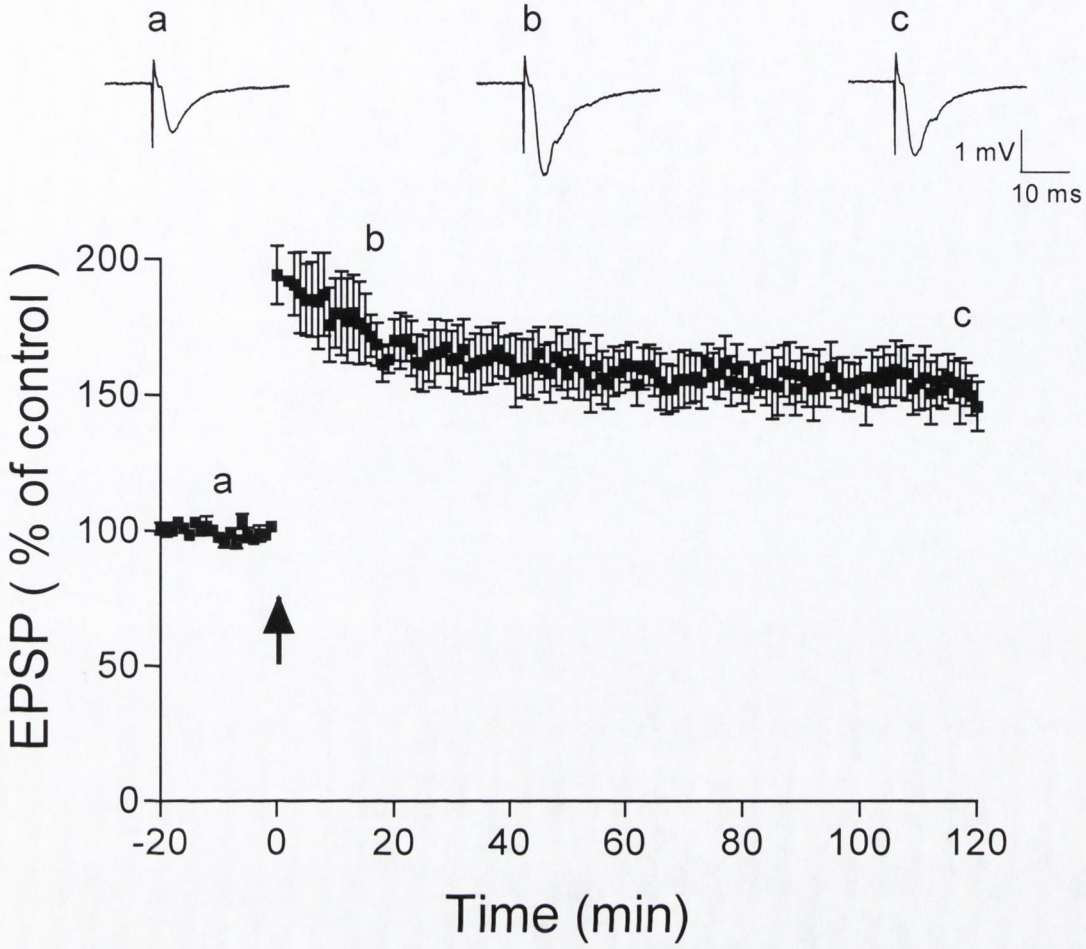

Fig. 3.1 LTP induced by conventional HFS. The arrow indicates the point at which a high-frequency stimulation of 8 trains of 8 stimuli at 200 Hz was applied. LTP reached a peak of 201.5±10.8% (n=5) 1 min after HFS and this value decreased to 145.7±9.0% at 120 min. The traces show sample fEPSPs 5 min before HFS (a), 5 mins after HFS (b) and 120 min after HFS (c).

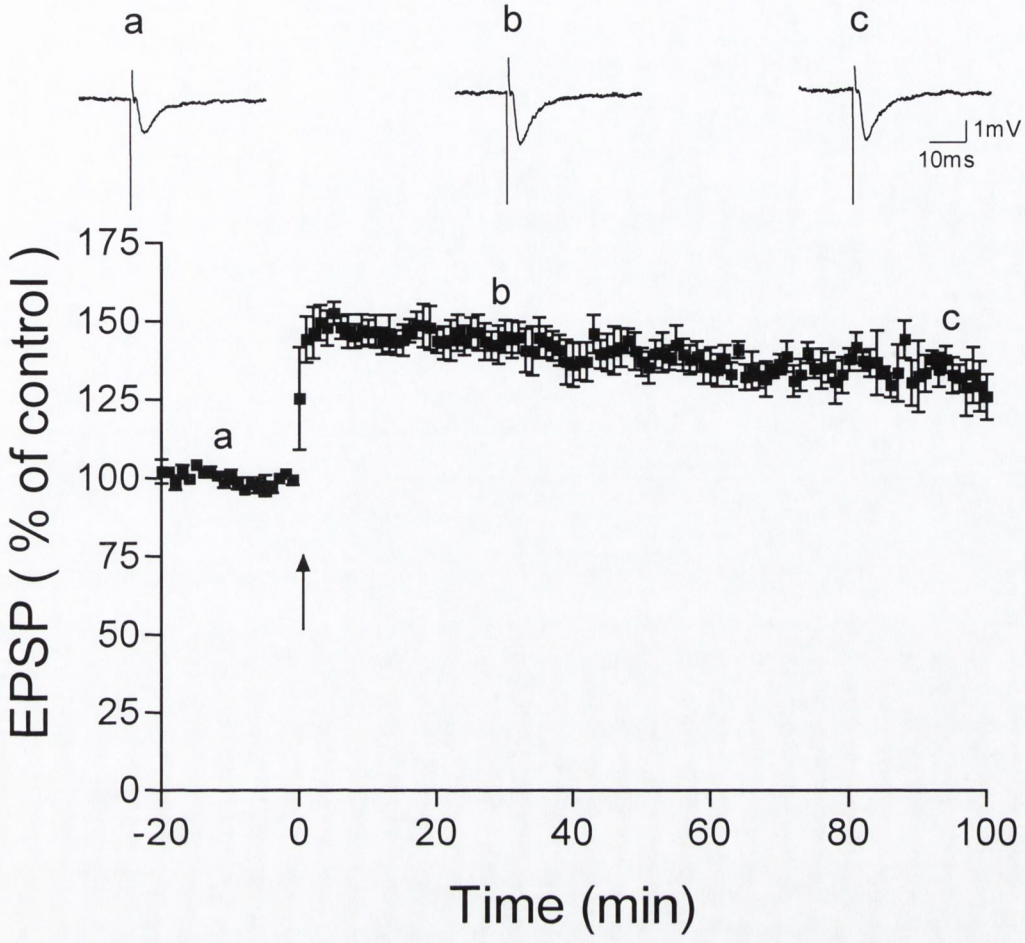

Fig. 3.2 100 Hz LTP. 4 trains of 100 Hz with 20 sec intertrain interval gave a mild LTP with a peak value of 152.6±3.7% at 5 min after the stimulus ($p<0.01$, $n=5$). The traces show fEPSPs prior to HFS (a), 30 min after HFS (b) and 100 min after HFS (c). The arrow indicates the point at which HFS was applied.

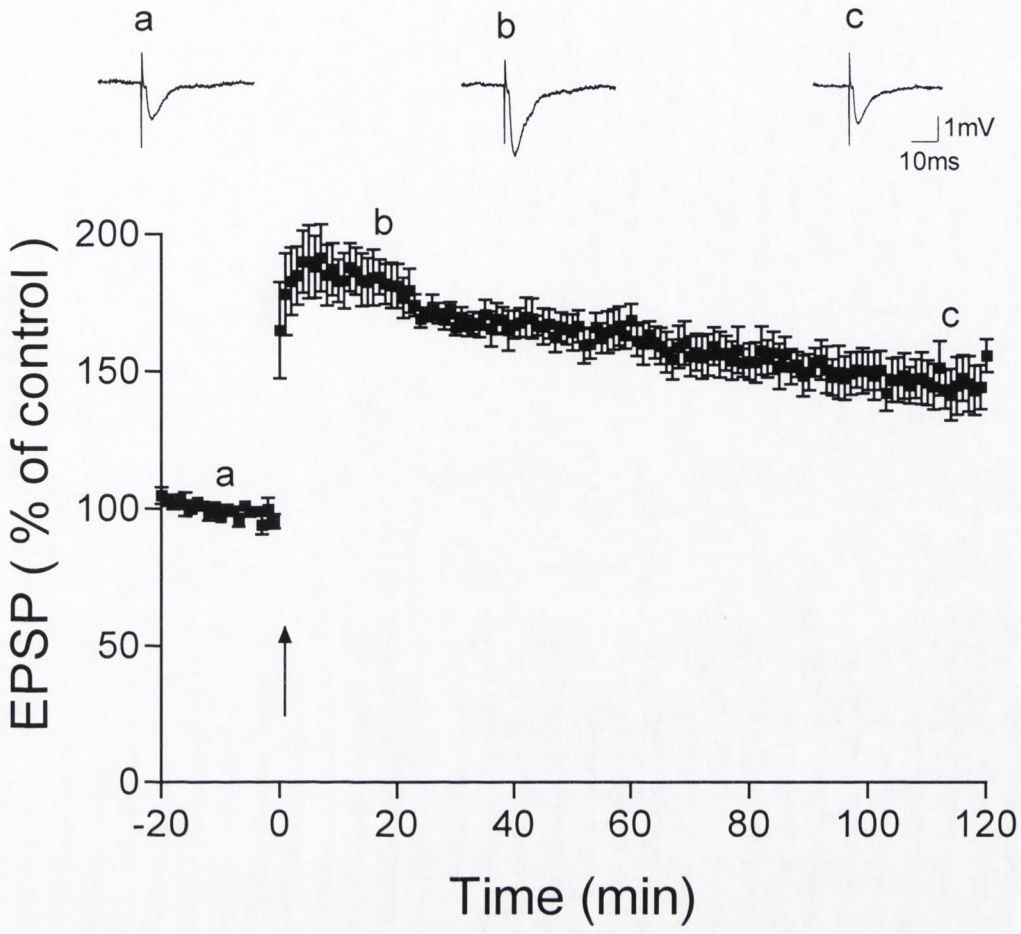

Fig. 3.3 Single 200 Hz LTP. A peak of 190.1±13.4% is reached 5 min after the HFS and LTP decays to a level of 153.7±6.7% 80 min after HFS which is not significantly different from 100 Hz LTP (n=5). The traces show fEPSPs prior to HFS (a), 5 min after HFS (b) and 120 min after HFS (c). The arrow indicates the point at which HFS was applied.

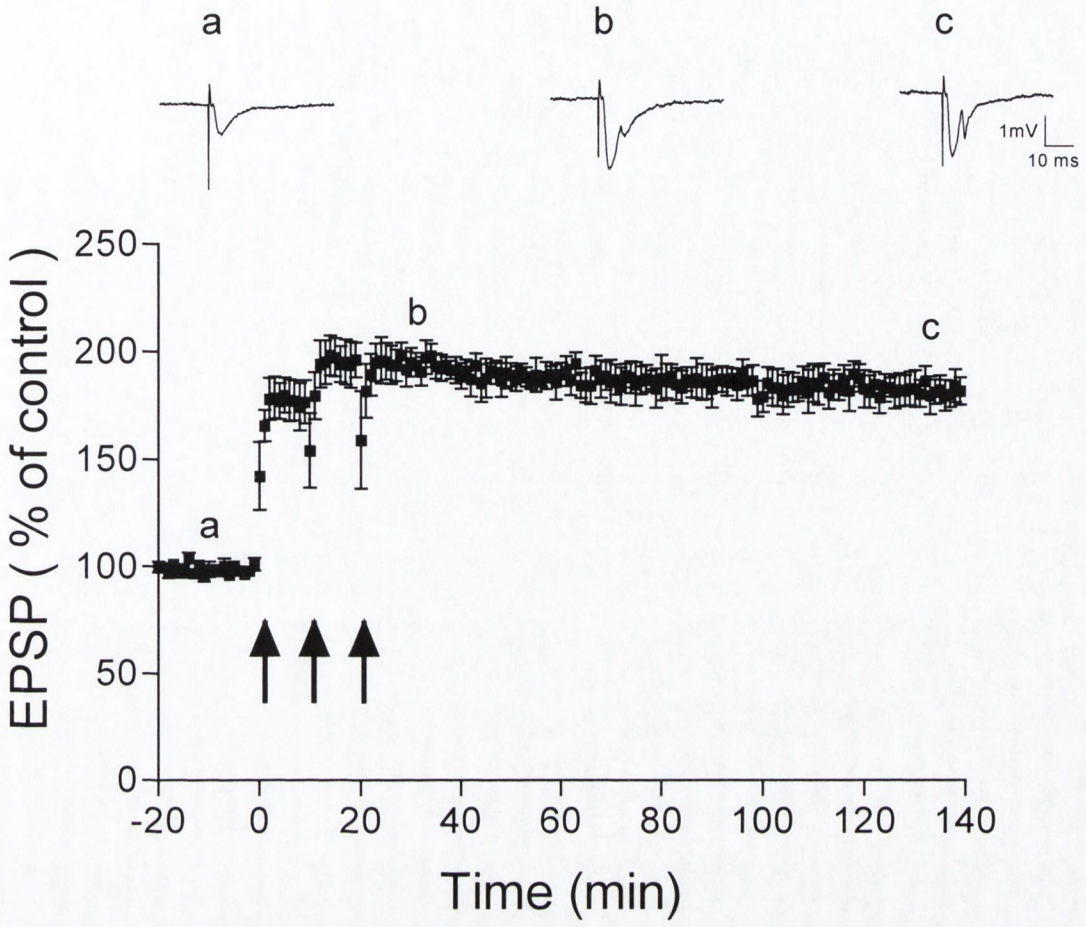

Fig. 3.4 Multiple spaced 200 Hz. LTP. LTP reached a peak of 198.5±5.8% 8 min after the 3rd 200 Hz ($p<0.01$, $n=5$). This high level of LTP was maintained with little decline for the duration for the duration of the experiment. LTP measured 182.7±8.7% 120 min after the 3rd 200 Hz. The traces show fEPSPs prior to HFS (a), 10 min (b) and 100 min (c) after the 3rd HFS. The arrows indicate the times when HFS was applied.

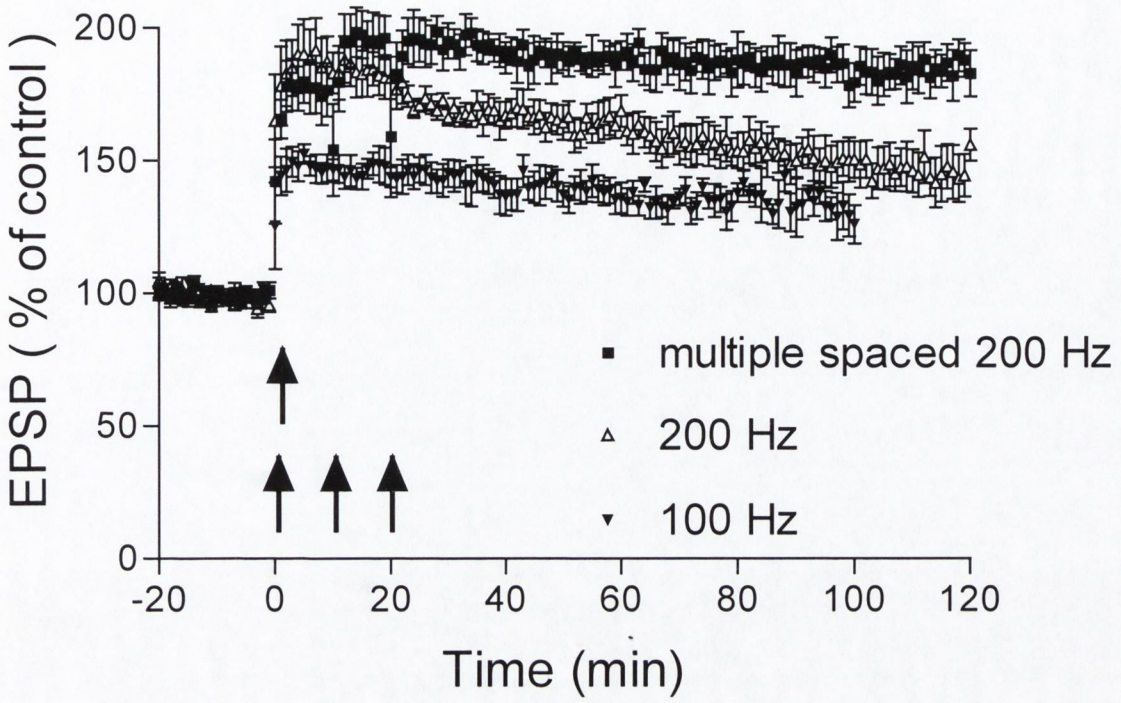

Fig. 3.5 Comparison of 3 HFS protocols. Multiple spaced 200 Hz HFS produced the strongest potentiation which did not decline substantially over the period of 120 min. 200 Hz HFS produced an early phase LTP which was not significantly different from 3 spaced 200 Hz but it declined to a level not significantly different from 100 Hz LTP after 80 min.

strong effect on the efficacy of synaptic transmission between neurons in the dentate gyrus.

3.2 Blockade of β-adrenergic receptors does not significantly inhibit LTP induced by three different protocols

The effect of a block of β-adrenergic receptors on LTP induced by three different HFS protocols was investigated. Propranolol hydrochloride was used to block both β_1- and β_2-adrenergic receptors. This adrenergic antagonist blocks both β_1- and β_2-adrenergic receptors with equal potency and has no effect on α-adrenergic receptors (Whitehurst *et al.*, 1999).

1μM propranolol hydrochloride was applied for 1 hour before the start of the experiment. There was no significant difference between LTP produced by the 100 Hz protocol in control and drug treated slices (Fig 3.6). Peak LTP was 161.4±4.9% in drug treated slices compared with 152.6±3.7% in the control slices. LTP in the drug treated slices was not significantly different from the controls at any point during the experiment ($p>0.1$, $n=2$).

There was no significant inhibition of the single 200 Hz LTP with 1μM propranolol hydrochloride (Fig. 3.7). Peak LTP was 190.1±13.4% and 189.1±17.3% in control and propranolol treated slices respectively. LTP declined in drug treated slices with a similar time course to the control and after 120 min LTP measured 129.3±7.1% in the drug treated slice compared with 155.9±5.9% in the control ($p>0.1$, $n=3$).

In the final experiment the effect of propranolol hydrochloride on multiple spaced 200 Hz HFS was examined (Fig. 3.8). 1μM propranolol hydrochloride was applied to the slice for 1 hour before recording and was present throughout the experiment. Peak LTP was 192.5±10.9% in the drug treated slice and 196.0±10.8% in the control slice. Although there was no significant difference between control and the drug treated slices there was a non-significant trend towards a reduction in LTP in the drug treated slices. At 140 min LTP was 181.7±6.3% and 153.9±10.6% in control and propranolol treated slices respectively ($p>0.1$, $n=4$).

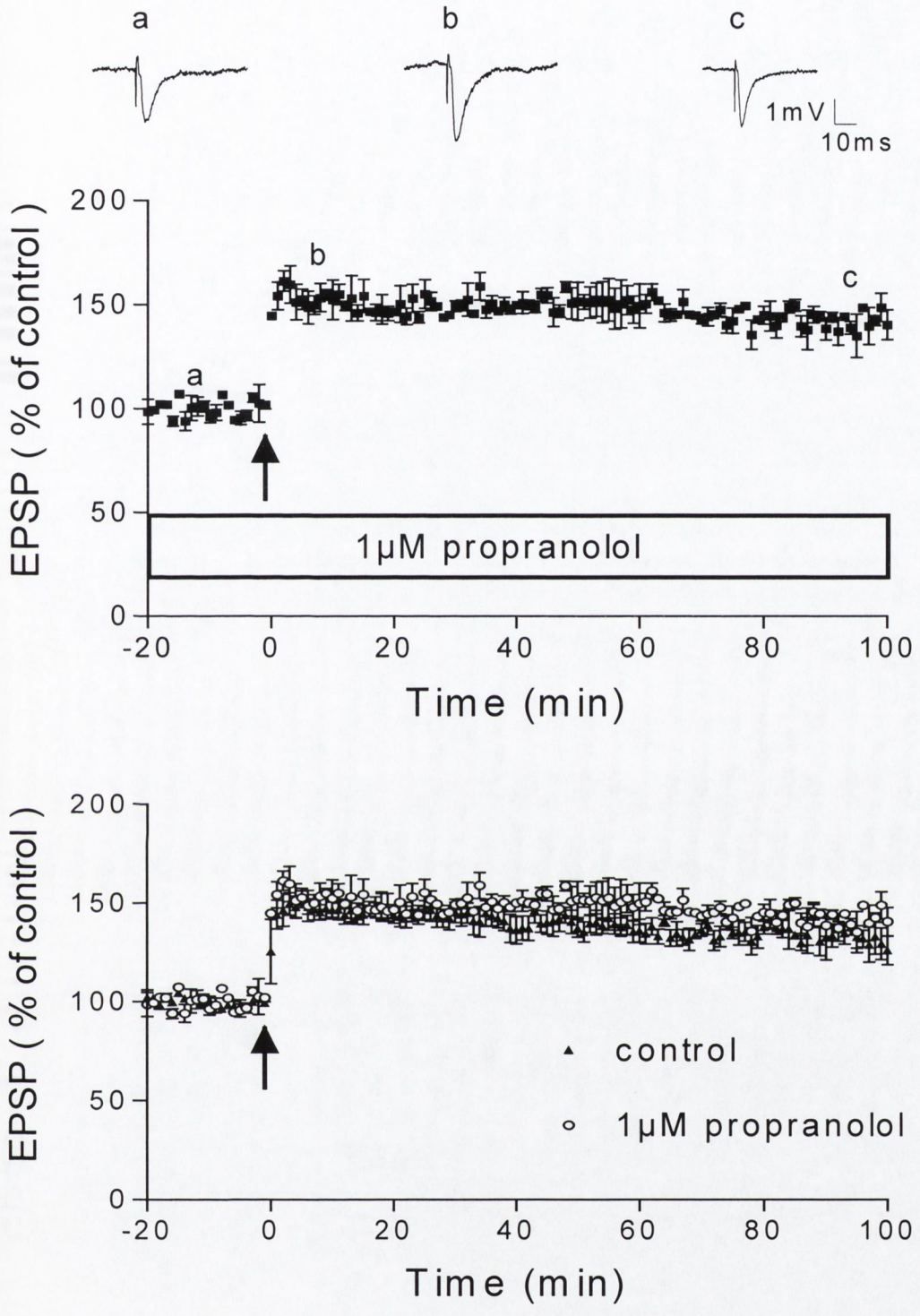

Fig. 3.6 No inhibition of 100 Hz LTP with propranolol. There was no significant difference between the control and slices treated with 1μM propranolol. The top graph shows the LTP produced in the drug treated slices. The lower graph shows a comparison between control and drug treated slices. At 80 min LTP was 137.8±3.5% and 145±4.3% in control and drug treated slices respectively ($p>0.1$, $n=2$). The traces show sample fEPSPs 5 min before HFS (a), 5 mins after HFS (b) and 100 min after HFS (c). The arrow indicates the time when HFS was applied.

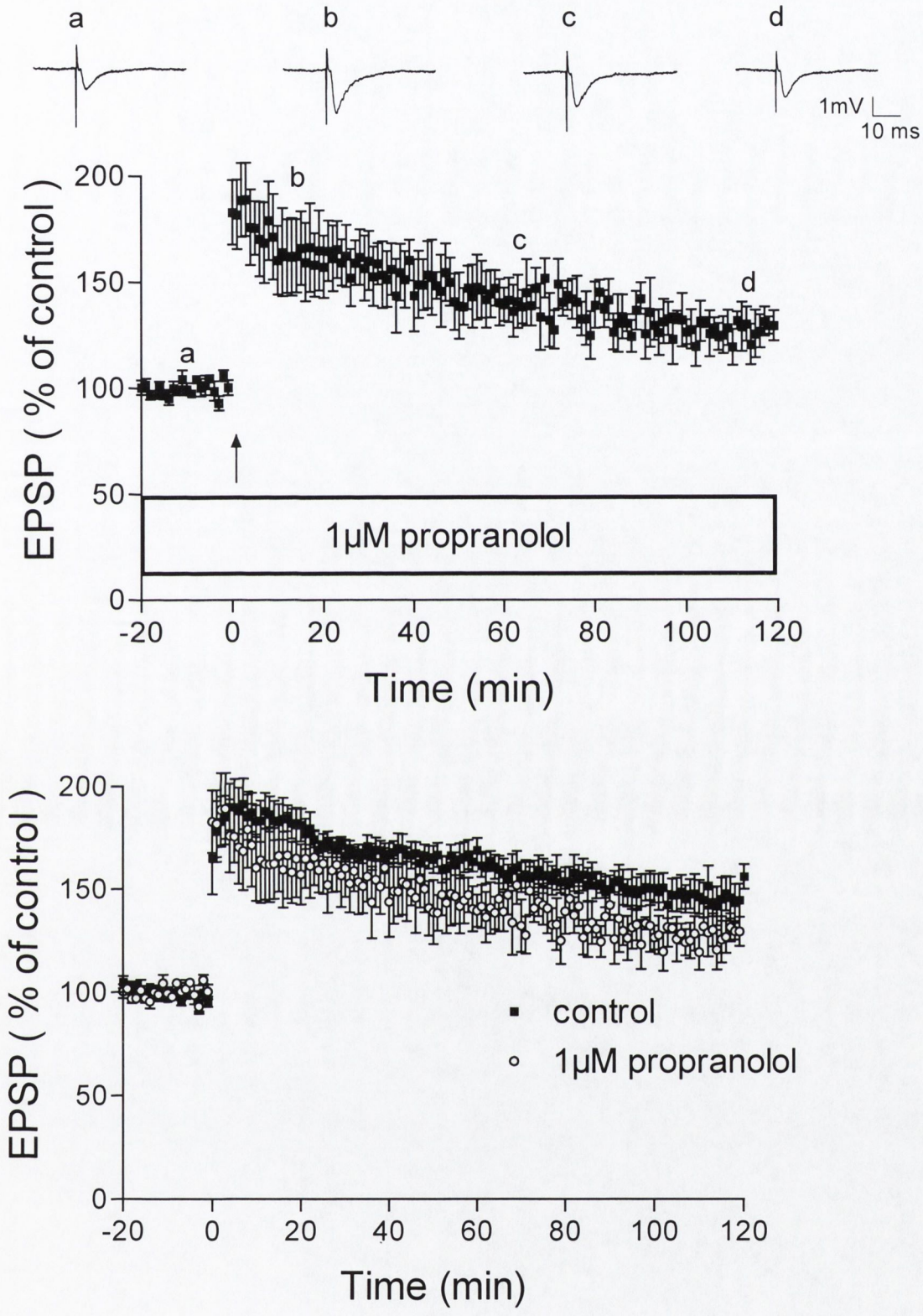

Fig. 3.7 No inhibition of single 200 Hz LTP with propranolol. Slices treated with propranolol hydrochloride showed a non-significant reduction in LTP. At 120 min LTP was 155.9±5.9% and 129.3±7.1% in control and propranolol slices respectively (n=3, p>0.1). The traces show sample fEPSPs 5 min before HFS (a), 5 (b), 60 min (c) and 120 min (d) after HFS. The arrow indicates the time when HFS was applied.

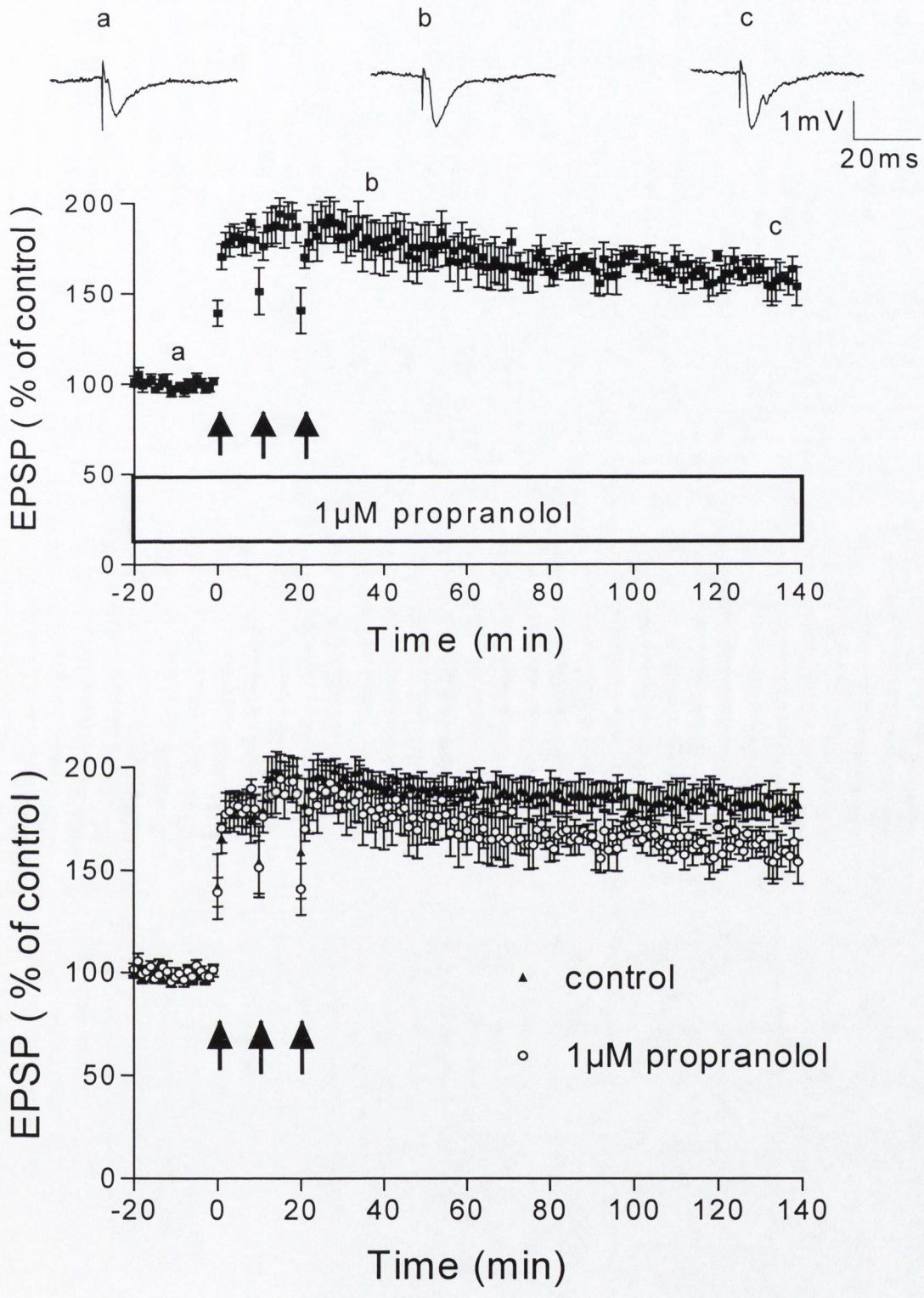

Fig 3.8. Propranolol causes a non-significant decrease in multiple spaced 200 Hz LTP. At 140 min LTP was 181.7±6.3% and 153.9±10.6% in control and propranolol treated slices respectively ($p>0.1$, $n=4$). The top graph shows the effect of propranolol on the multiple spaced 200 Hz protocol and the lower graph shows the comparison between control and drug treated slices. The traces show sample fEPSPs 5 min before HFS (a), 5 mins (b) and 120 min (c) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

These experiments show that activation of β-adrenergic receptors is not essential for the induction of LTP at any of the frequencies studied. Application of propranolol hydrochloride was seen to produce a mild reduction of LTP which was not significant suggesting that β-adrenergic receptors have a modulatory role in LTP induction and maintenance.

3.3 Antagonism of muscarinic acetylcholine receptors significantly reduces the late-phase of multiple spaced 200 Hz LTP

The involvement of muscarinic acetylcholine receptors (mAChRs) in LTP induced by the 3 spaced 200 Hz protocol was investigated by applying a mAChR antagonist to the slices 1 hr before the experiments and allowing the drug to circulate for the duration of the experiment. The potent muscarinic antagonist scopolamine hydrochloride was used to block mAChRs. Scopolamaine-induced amnesia in rats is a commonly used model of memory deficit (Rush, 1988; Park *et al.*, 2000). Scopolamine hydrochloride (100μM) was found to produce a significant decrease in late phase LTP (Fig. 3.9). Early phase LTP was not affected by scopolamine (peak LTP was 196.2±9.2% and 198.5±5.8% in drug treated and control slices respectively). Reduction of LTP only became significant in the late-phase of LTP. LTP measured 152.4±5.6% in scopolamine treated slices and 181.7±6.3% in control slices 2 hrs after the 3rd 200 Hz ($p<0.05$, $n=5$).

These experiments demonstrate that the activation of mAChRs is required for the full expression of late-phase LTP induced by the multiple spaced 200 Hz protocol.

3.4 Antagonism of mGluRs significantly reduces the late-phase of multiple spaced 200 Hz LTP

Since the first report that an mGluR antagonist, MCPG, blocked the induction of LTP (Bashir *et al.*, 1993) there has been considerable controversy over the role of mGluRs in hippocampal synaptic plasticity. Some studies have reported an inhibition of LTP with MCPG (Bashir *et al.*, 1993; Brown *et al.*, 1994; Richter-Levin *et al.*, 1994; Little *et al.*, 1995; Breakwell *et al.*, 1998) and in other studies the

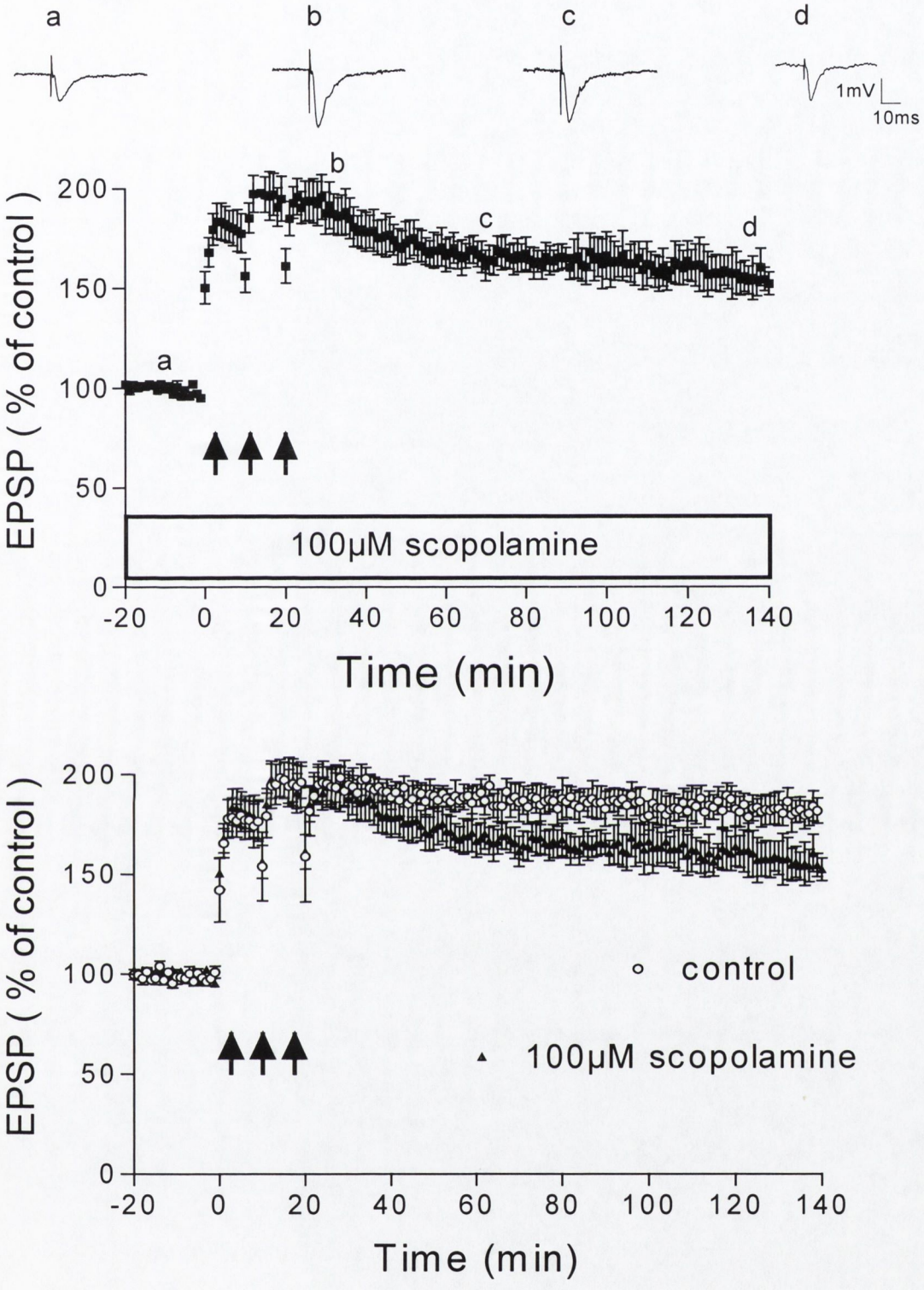

Fig. 3.9 Reduction of the late-phase of multiple spaced 200 Hz LTP with scopolamine. At 120 min after the 3rd 200 Hz LTP measured 152.2±5.6% and 181.7±6.3% in drug treated and control slices respectively ($p<0.05$, $n=5$). Peak LTP was 196.2±9.2% in the drug treated slice as opposed to 198.5±5.8% in control. Thus the scopolamine affected only late phase LTP. The lower graph shows a comparison between drug treated slices and control. The traces show sample fEPSPs 5 min before HFS (a), 5 mins (b) and 120 min after (c) the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

MCPG actions could not be confirmed (Chinestra *et al.*, 1993; Manzoni *et al.*, 1994; Selig *et al.*, 1995; Thomas and O'Dell, 1995; Rush *et al.*, 2001b). Deficits in LTP have been described in mice lacking $mGluR_1$ receptors (Aiba *et al.*, 1994; Conquet *et al.*, 1994) and $mGluR_5$ receptors (Lu *et al.*, 1997) but these studies have also been confusing since deficits in LTP in area CA1 of the $mGlu_1$ receptor knockout were not observed in the study by Conquet's group.

The role of mGluRs in multiple spaced 200 Hz LTP was investigated using the mGluR antagonist LY 341495. LY 341495 has been shown to inhibit group II mGluRs at low nanomolar concentration and group I mGluRs at micromolar concentrations (Fitzjohn *et al.*, 1998; Kingston *et al.*, 1998).

LY 341495 (20μM) was found to reduce the late phase of multiple spaced 200 Hz LTP. In the drug treated slices LTP measured 155.5±8.4% at 120 min compared with 182.7±8.7% in control slices ($n=5$, $p<0.05$) (Fig. 3.10). Neither the induction nor the early-phase of LTP was affected by LY 341495.
In order to dissect out the receptor subtypes that might be involved in this reduction of late phase LTP the same experiment was performed with the $mGluR_5$ antagonist MPEP hydrochloride. There was no significant difference between LTP produced in the presence of 9μM MPEP hydrochloride and control slices. At 60 min, LTP measured 180.2±4.0% and 189.41±5.4% in drug treated slices and control respectively ($n=3$, $p>0.1$) (Fig. 3.11).
These experiments demonstrate that activation of mGluRs are required for the full expression of late-phase LTP induced by multiple spaced 200 Hz protocol. However the receptor subtypes involved are still not known but from the MPEP experiments it is clear that $mGluR_5$ is not required for this LTP.

3.5 The role of protein synthesis in LTP induced by different stimulation protocols

3.5.1 Emetine inhibits the induction of multiple spaced 200 Hz LTP

Previous studies have demonstrated that the expression of LTP requires new protein synthesis (Frey *et al.*, 1991; Huang and Kandel, 1994; Huang *et al.*, 1994). There

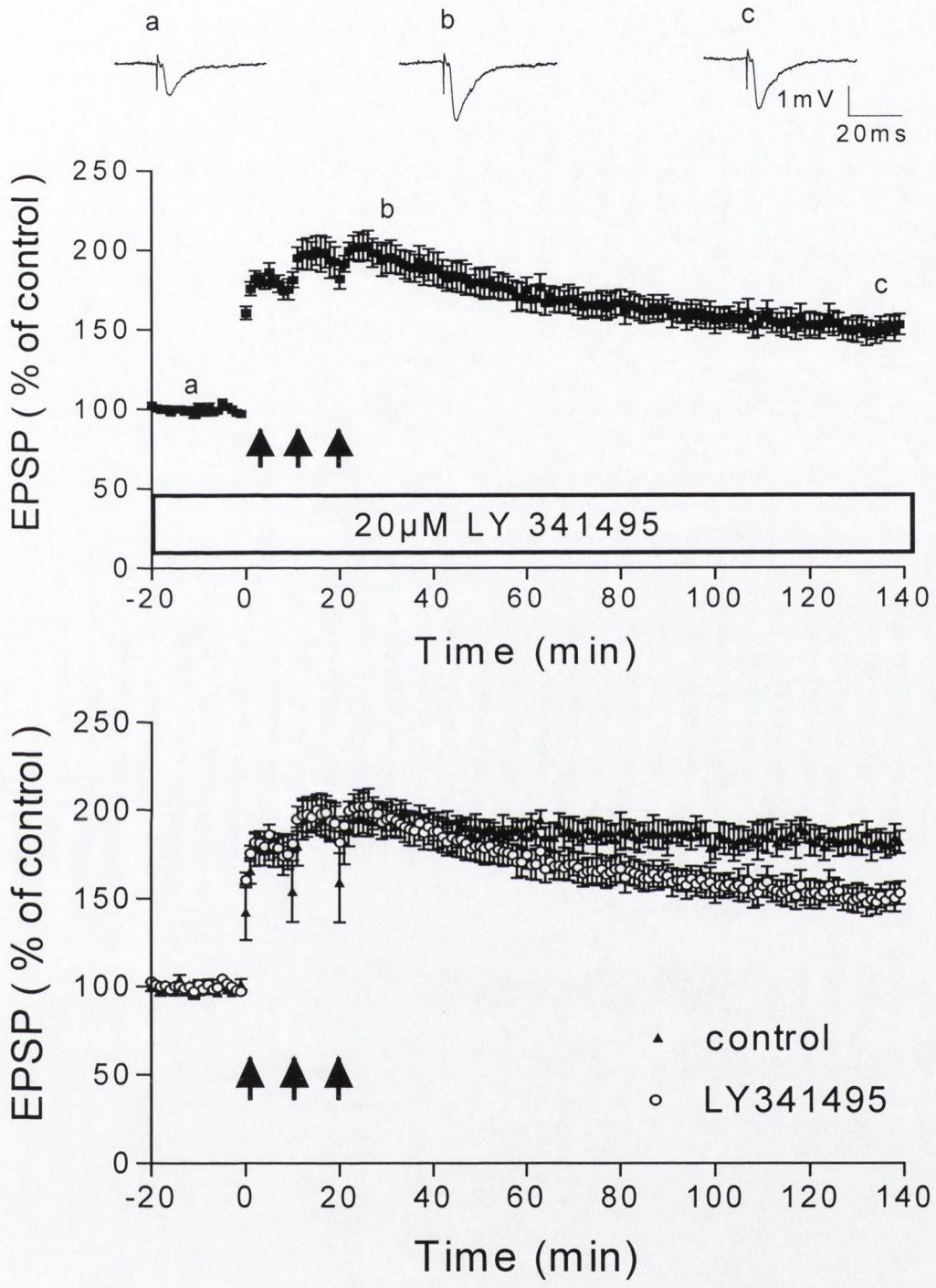

Fig. 3.10 Reduction of the late-phase of multiple spaced 200 Hz LTP with LY 341495. LTP was significantly reduced 100 min after the 3rd 200 Hz ($p<0.05$, $n=5$). Neither LTP induction nor early phase LTP was affected by the drug. The lower graph shows a comparison of control and drug treated slices. The traces show sample fEPSPs 5 min before HFS (a), 5 mins (b) and 120 min (c) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

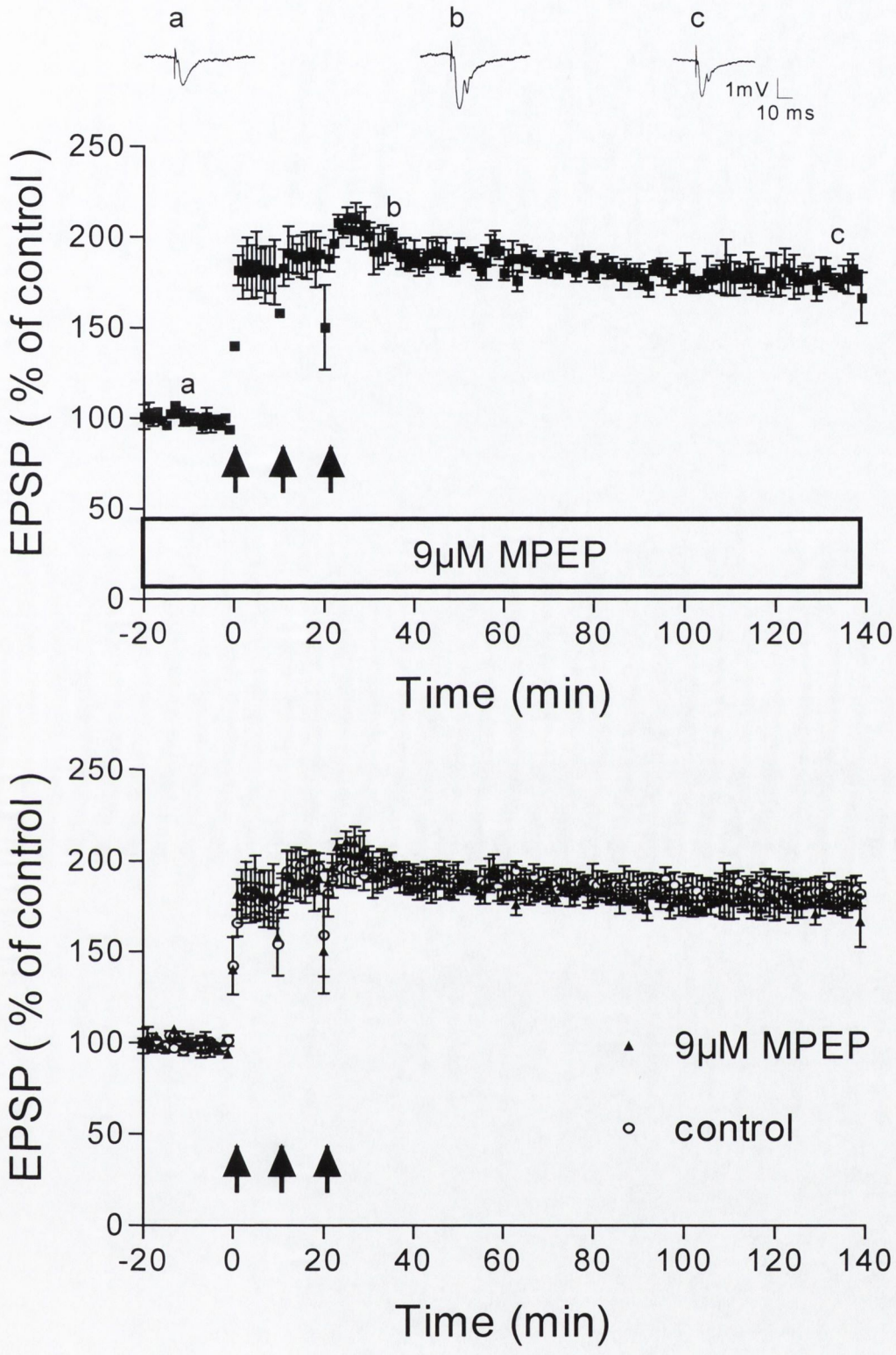

Fig. 3.11 No inhibition of multiple spaced 200 Hz LTP with MPEP. The drug was applied for 1 hr before recording and was present throughout the experiment. There was no significant difference between the drug treated slice and the control at any point during the experiment. The lower graph shows a comparison of control and drug treated slices. The traces show sample fEPSPs 5 min before HFS (a), 5 mins (b) and 120 min (c) after the 3rd 200 Hz in the drug treated slices. The arrows indicate the times when HFS was applied.

are also some *in vivo* studies in the dentate gyrus which have shown that the maintenance of LTP requires new protein synthesis (Krug *et al.*, 1984; Otani *et al.*, 1989; Fazeli *et al.*, 1993). The protein synthesis inhibitor emetine was used in the present experiments to investigate the role of protein synthesis in LTP induced by the multiple spaced 200 Hz protocol. Slices were treated with 20μM emetine for 1 hour before recording and the drug was present for the duration of the experiment. Emetine was found to completely inhibit the induction of LTP. Furthermore, there was a depression of EPSPs following each HFS in the drug treated slices. EPSPs measured 15.0±8.6%, 9.1±4.3% and 1.7±0.03% immediately after the 1st, 2nd and 3rd HFS respectively (n=2) (Fig. 3.12). The EPSPs tended to increase towards baseline levels over time but there was still a significant depression at 50 min with EPSP measuring 80.1±21.2% ($p<0.05$, n=2).

These results demonstrate that protein synthesis is required for the induction of multiple spaced 200 Hz LTP.

3.5.2 Anisomycin inhibits the induction of multiple spaced 200 Hz LTP

The effect of another protein synthesis inhibitor, anisomycin, on multiple spaced 200 Hz LTP was also investigated. Anisomycin is a transcriptional inhibitor and at a concentration of 25μM it has been shown to block at least 85% of incorporation of ^{3}H-leucine into hippocampal slices (Frey *et al.*, 1988). In these experiments slices were treated with 5μM anisomycin for 1 hour before recording and the drug was allowed to circulate in the bath for the duration of the experiment. Similar to the previous results with emetine, anisomycin was found to block the induction of LTP with the multiple spaced 200 Hz protocol. EPSPs measured 108.8±15.4% and 116.6±24.10% at 25 min and 80 min respectively (n=2) (Fig. 3.13).

These results show that protein transcription is required for the induction of multiple spaced 200 Hz LTP.

3.5.3 Anisomycin inhibits the late-phase of multiple spaced 200 Hz LTP

To investigate the effects of protein synthesis on late-phase LTP anisomycin was applied immediately after the 3rd 200 Hz. The requirement of newly synthesized proteins for late LTP is less well studied than the mechanisms of early LTP because

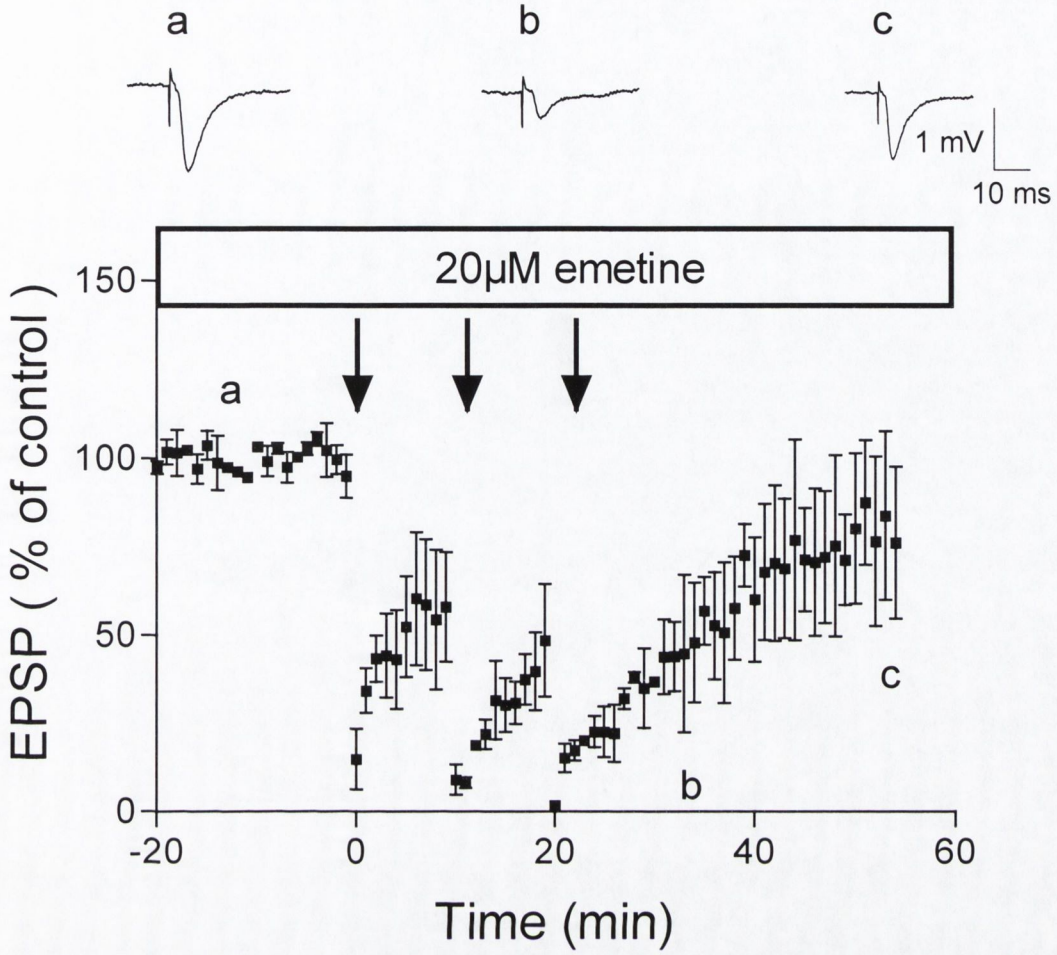

Fig. 3.12 Inhibition of multiple spaced 200 Hz LTP with emetine. The drug was applied for 1 hr before recording and was present throughout the experiment. LTP was completely inhibited in drug treated slices and HFS actually produced a depression of EPSPs (80.1±21.2% at 50 min). The traces show sample fEPSPs 10 min before HFS (a), 5 min (b) and 30 min (c) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

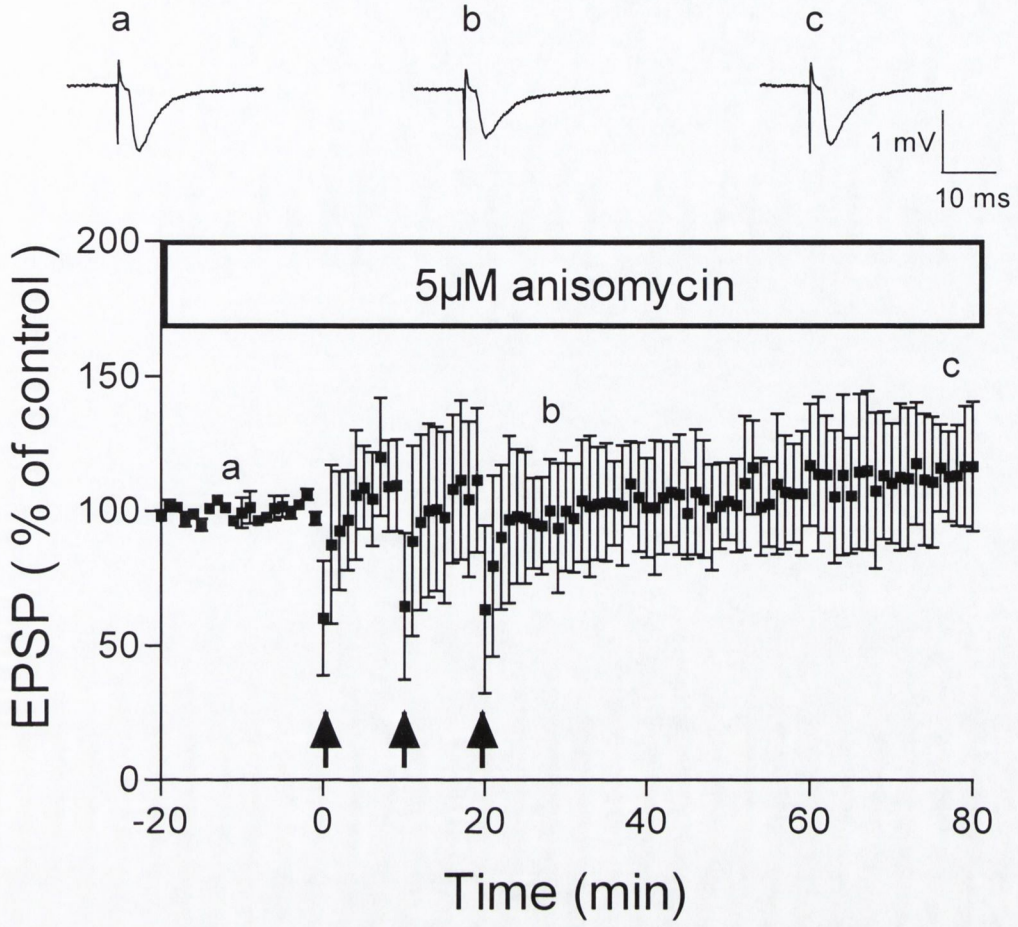

Fig. 3.13 Inhibition of multiple spaced 200 Hz LTP with anisomycin. LTP was completely inhibited in the presence of 5μM anisomycin and EPSPs measured 116.6±24.1% at 80 min (n=2). The traces show sample fEPSPs 10 min before HFS (a), 10 min (b) and 60 min (c) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

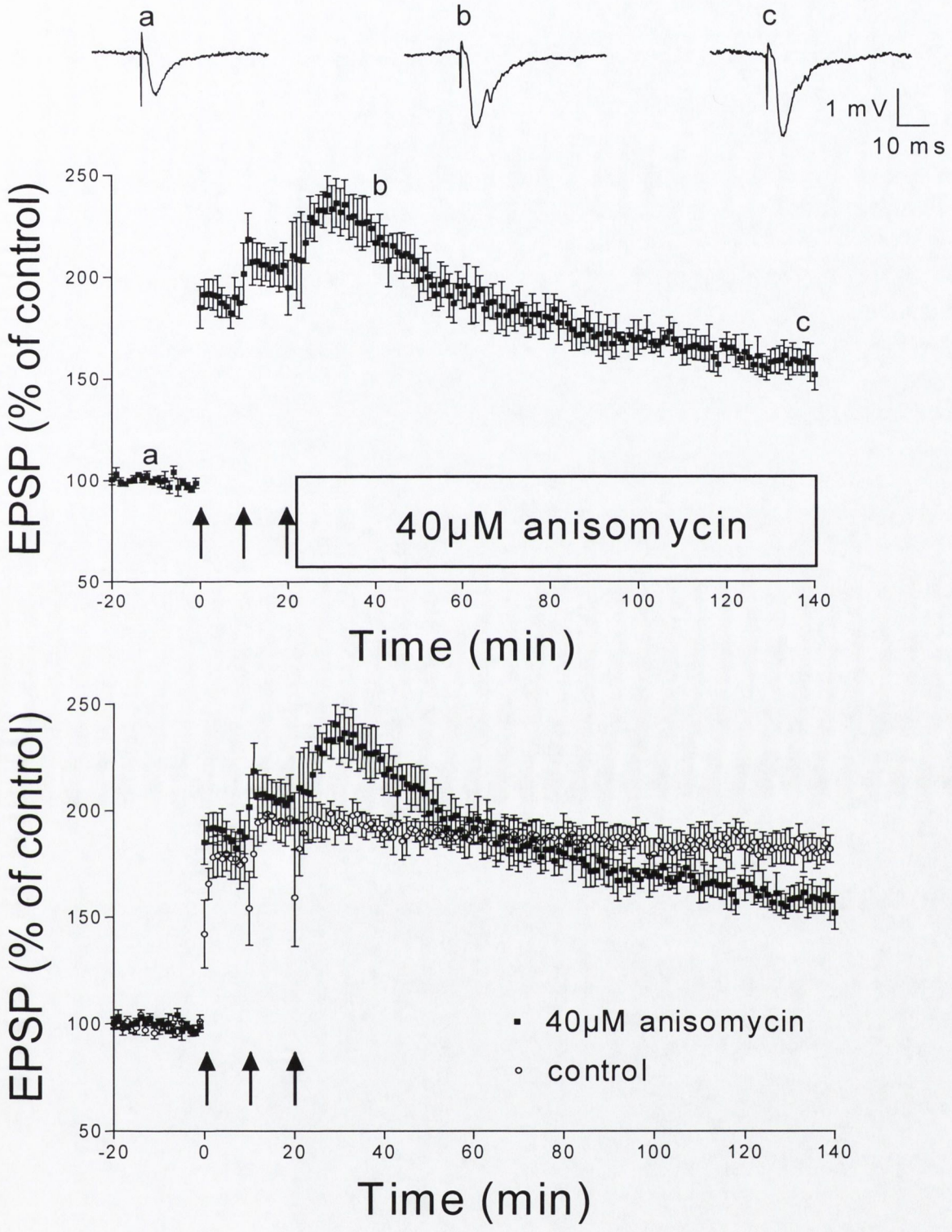

Fig. 3.14 Anisomycin inhibits the late-phase of multiple spaced 200 Hz LTP. LTP rose to 240.2±9.2% 10 min after the 3rd 200 Hz (n=5) and then decreased to 158.2±6.0% at 130 min which was significantly different from the control ($p<0.05$, n=5). The lower graph shows a comparison of the control and drug treated slices. The traces show sample fEPSPs 10 min before HFS (a), 10 min (b) and 120 min (c) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

most LTP experiments continue for less than two hours after tetanisation. Inhibition of late phase LTP was first discovered *in vivo* (Krug *et al.*, 1984) and was subsequently shown *in vitro* (Frey *et al.*, 1988). In the present set of experiments, the application of 40μM anisomycin immediately after the 3rd 200 Hz did not inhibit the early phase of LTP. At 30 min LTP measured 240.2±9.2% in the drug treated slices and LTP began to decline rapidly after this time point (Fig 3.14). At 130 min LTP was significantly reduced below the control value with EPSPs measuring 158.2±6.0% and 182.9±8.5% in drug treated and control slices respectively ($p<0.05$, n=5) (Fig 3.14).

These experiments demonstrate that protein synthesis is required for the late-phase of multiple spaced 200 Hz LTP.

3.5.4 Anisomycin does not inhibit mild LTP induced by 8 trains of 8 pulses at 200 Hz

The effects of anisomycin on LTP induced by 8 trains of 8 pulses at 200 Hz were investigated next. 40μM anisomycin was applied to the slices 1 hour before the recording and was allowed to circulate in the bath for the duration of the experiment. There was no significant difference between control and drug treated slices in either early or late-phase LTP. At 5 min after the HFS, LTP measured 168.7±19.6% and 185.1±13.2% (n=3, $p>0.05$) (Fig. 3.15) in drug treated and control slices respectively. The potentiation remained relatively stable at this level over the course of 2 hours and at 120 min LTP measured 150.0±22.5% and 145.7±9.03% in drug treated and control slices respectively (n=3, $p>0.05$) (Fig. 3.15).

3.6 Antagonism of PKC reduces both the early and late-phases of multiple spaced 200 Hz LTP

There is a considerable body of evidence demonstrating a role for PKC in LTP (Lovinger *et al.*, 1987; Malinow *et al.*, 1988, 1989; Reymann *et al.*, 1988, 1990; Colley *et al*, 1990, Wang and Feng, 1992; Sacktor *et al.*, 1993; Hvalby *et al.*, 1994; Osten *et al.*, 1996). However, the poor specificity of the protein kinase inhibitors

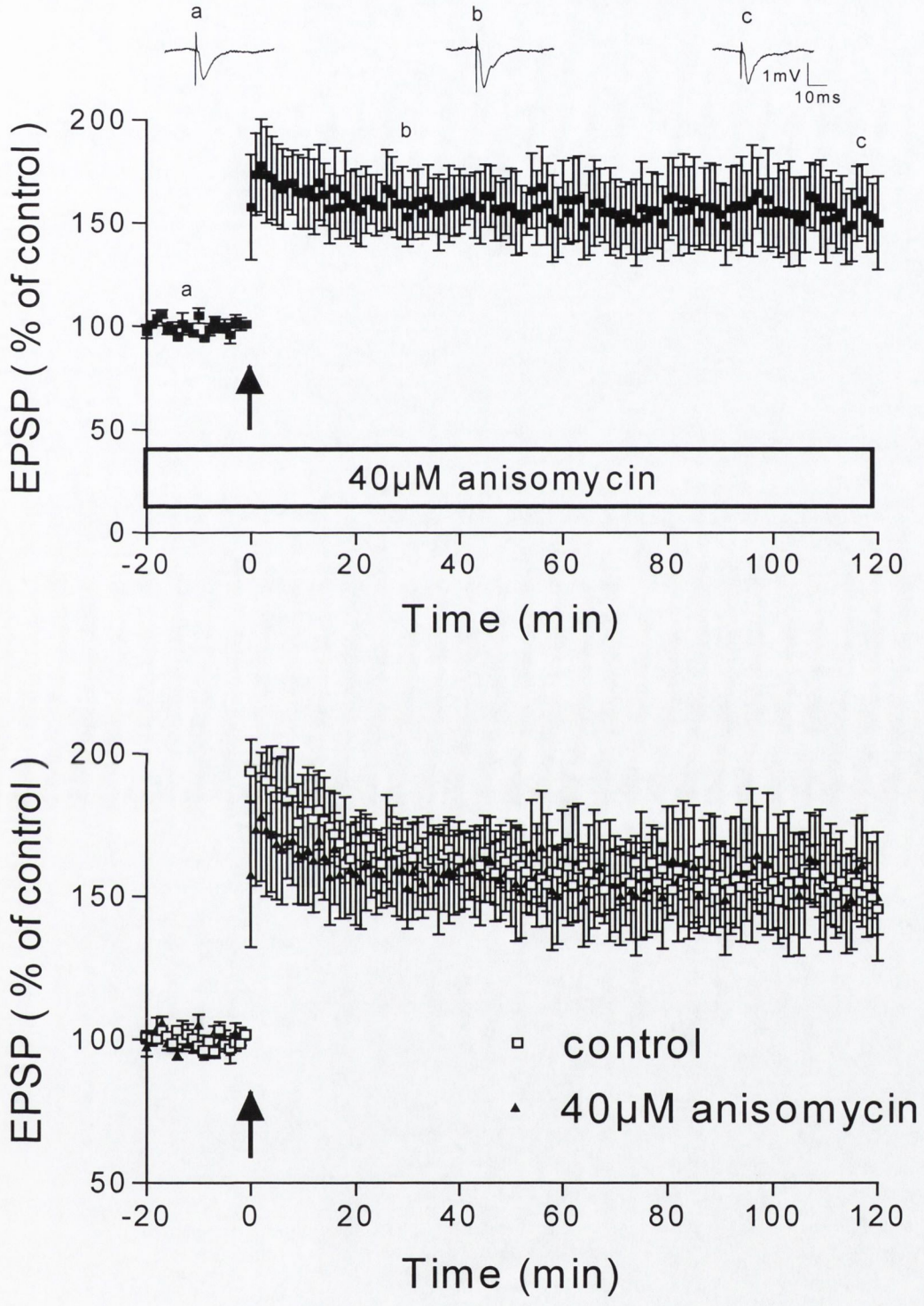

Fig. 3.15 Anisomycin has no effect on conventional LTP induced by 8 trains of 8 pulses at 200 Hz. 40μM anisomycin was applied 1 hr before recording and was present for the duration of the experiment. There was no significant difference between the control and the drug treated slices at any point during the experiment (n=3, $p>0.05$). The traces show sample fEPSPs 10 min before HFS (a), 30 min (b) and 120 min (c) after HFS. The arrow indicates the time when HFS was applied.

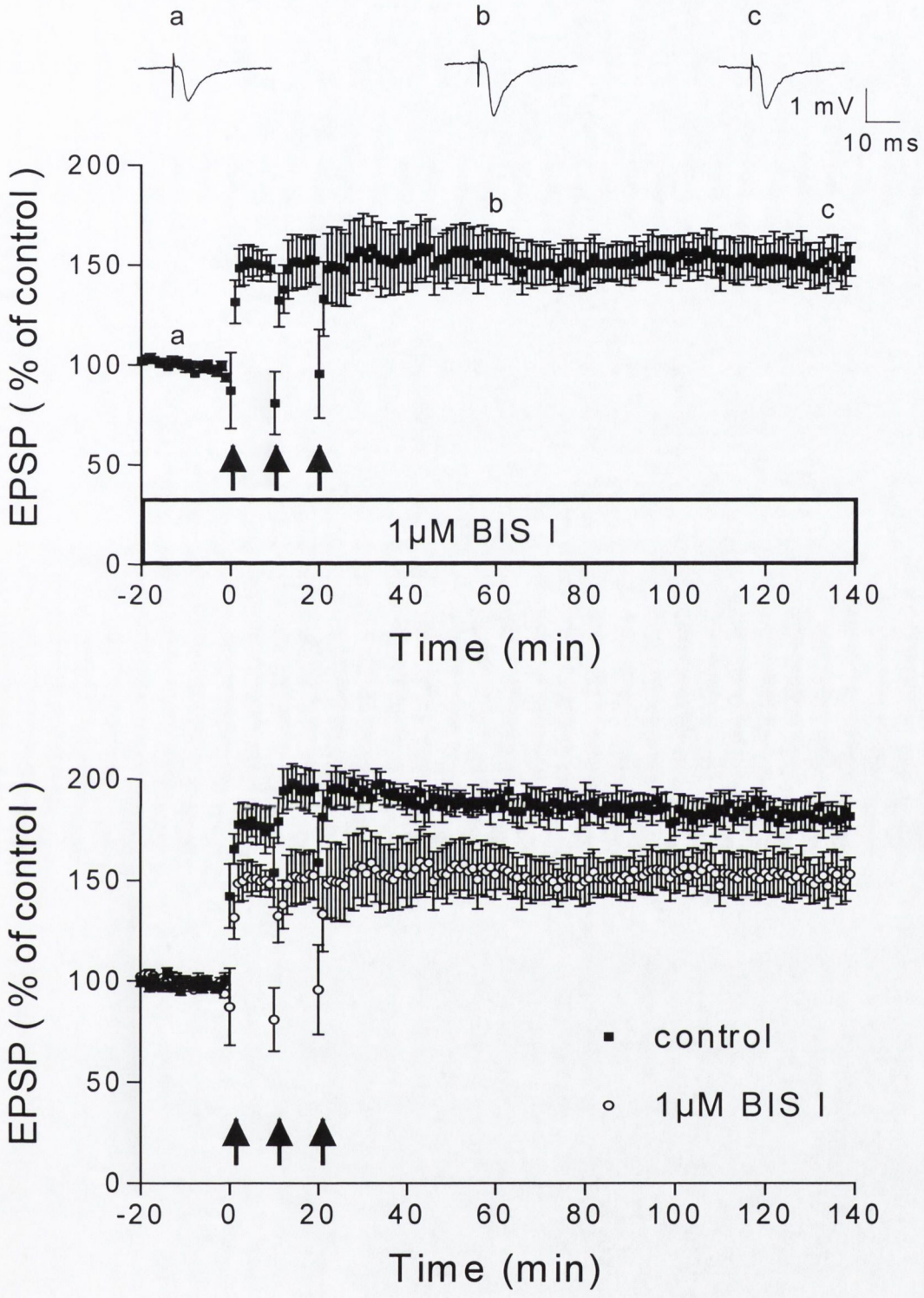

Fig 3.16 Reduction of induction and early and late-phase of multiple spaced 200 Hz LTP with PKC inhibitor BIS I. LTP measured 150.8±8.5% and 179.8±8.1% at 5 min post the 1st HFS in drug treated slices and control respectively ($p<0.05$, n=5). At 140 min LTP measured 152.8±8.3% in the drug treated slices compared with 181.7±6.3% in control ($p<0.05$, n=5). The lower graph shows a comparison of drug treated slices and control. The traces show sample fEPSPs 5 min before HFS (a), 40 min (b) and 120 min (c) after the 3rd 200 Hz in the drug treated slices. The arrows indicate the times when HFS was applied.

used in these studies have led other investigators to challenge some of these results (Muller *et al.*, 1992; Lopez-Molina *et al.*, 1993; Roberson *et al.*, 1996).

In the present study PKC was inhibited with the potent and selective PKC inhibitor Bisindolylmaleimide I (Bis I). This compound has been shown previously to inhibit PKC in enzyme assays with a K_i of 10nM and to inhibit other kinases only at much higher concentrations, for example, PKA with a K_i of 2mM (Nixon *et al.*, 1992). Bis I acts competitively with respect to ATP and in the slice preparation there is a higher cellular ATP concentration than in enzyme assays (Nixon *et al.*, 1992). For this reason, a concentration of 1μM was used in the present experiments which is considerably higher than the K_i value of Bis I for PKC.

The role of PKC in multiple spaced 200 Hz LTP was examined by applying Bis I to the slice 1 hour before recording and leaving it circulate in the bath throughout the duration of the experiment. Induction of LTP was affected by the drug as LTP was significantly reduced 5 min after the 1st 200 Hz HFS. LTP measured 150.8±8.5% at this time point compared with 179.8±8.1% in control (n=5, $p<0.05$) (Fig. 3.16). LTP was stable at this level throughout the experiment and at 140 min it measured 152.8±8.3% compared with 181.7±6.3% in control (n=5, $p<0.05$).

These experiments show that PKC is involved in the induction of LTP. However it does not block LTP completely but allows the expression of LTP at a reduced level over a period of 2 hours.

3.7 The PKA inhibitor, KT 5720, reduces early and late-phases of multiple spaced 200 Hz LTP

LTP induction has been shown to produce a rise in cAMP (Chetkovich and Sweatt, 1996), activation of PKA (Roberson and Sweatt, 1993; Blitzer *et al.*, 1995), and phosphorylation of some PKA substrates (Blitzer *et al.*, 1998), but the role of this pathway is not fully understood. It is generally agreed that late phase LTP is dependent on cAMP and protein synthesis (Frey *et al.*, 1993; Huang and Kandel, 1994; Winder *et al.*, 1998) and that there is also an intermediate phase of LTP that begins approximately one hour after induction and is dependent on cAMP, but not

protein synthesis (Winder *et al.*, 1998). However, there is less consensus regarding the role of PKA in early LTP. Early LTP is defined as the plasticity which occurs 30-60 min after induction, a time at which short-term plasticity processes have decayed. Some studies show that early-phase LTP is not dependent on adenylyl cyclase or PKA (Frey *et al.*, 1993; Huang and Kandel, 1994) and other studies have shown a reduction of early-phase LTP with PKA inhibitors (Otmakhova *et al.*, 2000).

The PKA inhibitor KT 5720 was applied to the slices in order to investigate the role of PKA in multiple spaced 200 Hz LTP. KT 5720 is a potent and selective inhibitor against PKA which blocks the enzymatic activity of the catalytic subunit of PKA (K_i = 56 nM). The drug was applied to the slice for at least 1 hour before recording and it was allowed to circulate in the bath for the duration of the experiment. At 20 min after the 3rd 200 Hz, LTP measured 188.7±5.9% and 156.9±12.6% in control and drug treated slices respectively. Thus there was a significant reduction of LTP below control levels at this time point ($p<0.05$, n=7) (Fig. 3.17). LTP continued to decline in drug treated slices and at 140 min LTP measured 119.8±10.1% compared with 181.7±6.3% in control.

In order to differentiate between a disruption of the induction process and an inhibition of the expression of LTP after HFS, KT 5720 was applied to the slice directly after the 3rd 200 Hz. In these experiments, a significant difference between control and drug treated slices was first observed at 70 min (n=4, $p<0.05$) (Fig 3.18) with EPSPs in slices treated with KT 5720 after the 3rd 200 Hz measuring 149.6±8.7% at this time compared with 189.3±5.2% in control slices. Thus, there was a more gradual reduction of LTP when KT 5720 was applied directly after the 3rd 200 Hz compared with the previous experiments where KT 5720 was present throughout the experiment. However, this significant reduction of LTP with KT 5720 added after the 3rd 200 Hz still fell within the definition of early-phase LTP. LTP continued to decline with a similar time course to the previous experiments and at 140 min LTP had declined to 124.6±9.1% compared to the control value of 181.6±6.3%.

Fig. 3.9 shows a comparison between slices which were treated with KT 5720 throughout the experiment and slices where KT 5720 was added after the 3rd 200

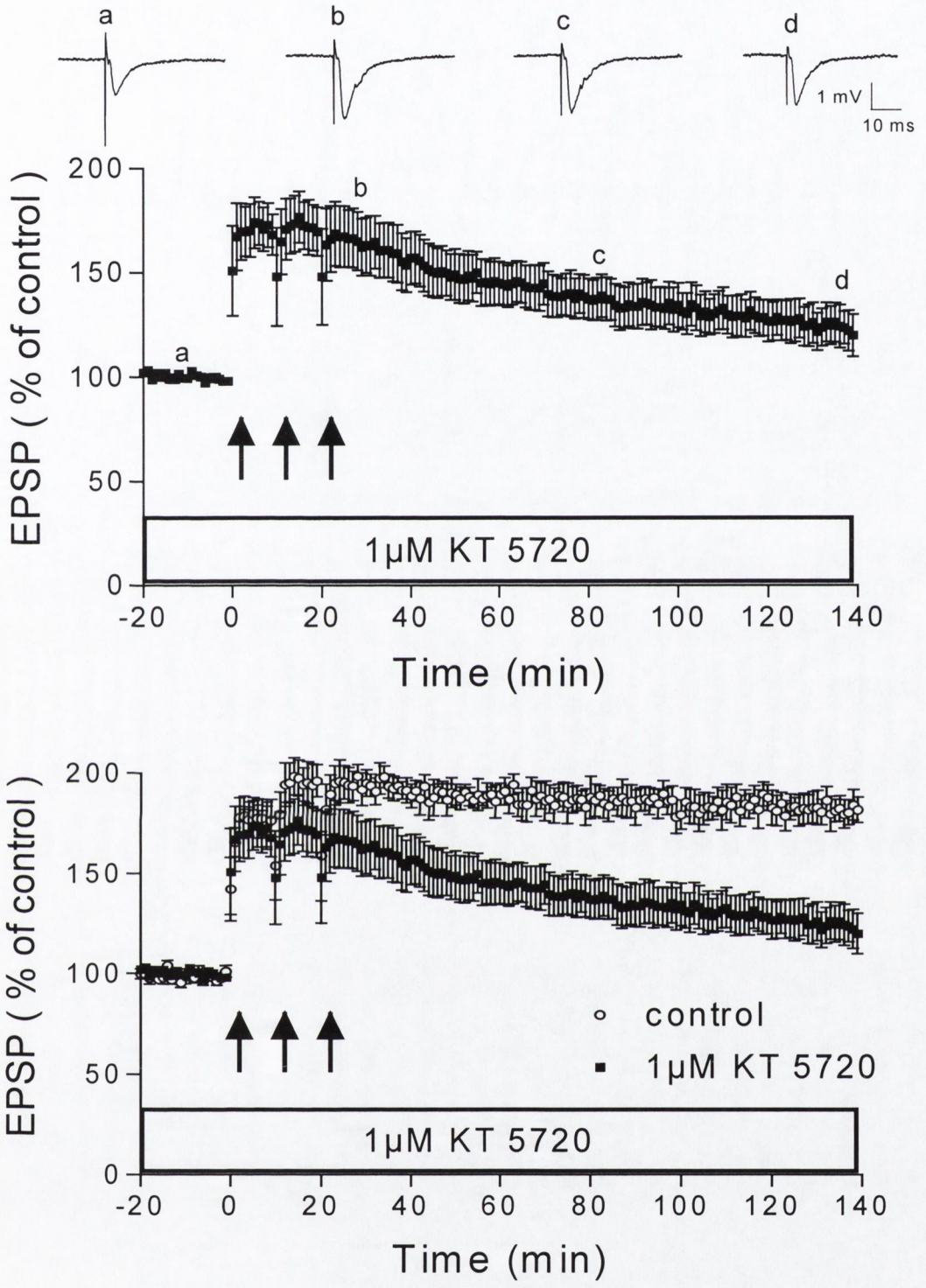

Fig. 3.17 Application of the PKA inhibitor, KT 5720, reduces the early and late-phases of LTP induced by the multiple spaced 200 Hz protocol. 20 min after the 3rd 200 Hz LTP in the drug treated slices were significantly reduced below control levels (EPSPs measured 188.7±5.9% and 156.9±12.6% in control and drug treated slices respectively, $p<0.05$, $n=7$). LTP continued to decline in the presence of the PKA inhibitor and at 140 min EPSPs measured 119.8±10.1% in the drug treated slices compared with 181.7±6.3% in control. The traces show sample fEPSPs 10 min before HFS (a), 5 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

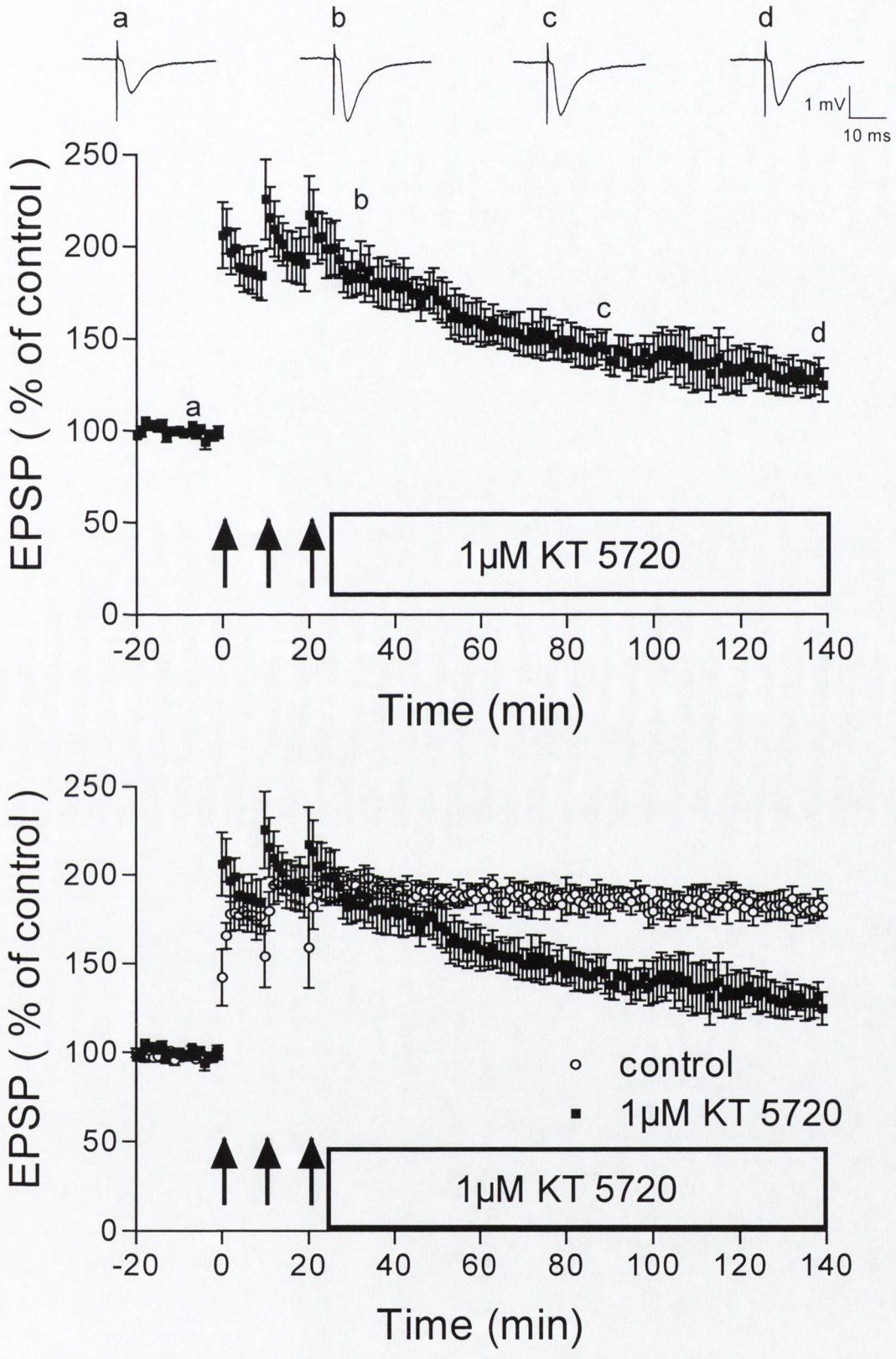

Fig. 3.18 Application of the PKA inhibitor after the 3rd 200 Hz reduces the early and late-phase of multiple spaced 200 Hz LTP. LTP was significantly reduced at 70 min with EPSPs measuring in 149.6±8.7% compared with 189.3±5.2% in control slices (n=4, $p<0.05$). The traces show sample fEPSPs 10 min before HFS (a), 5 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

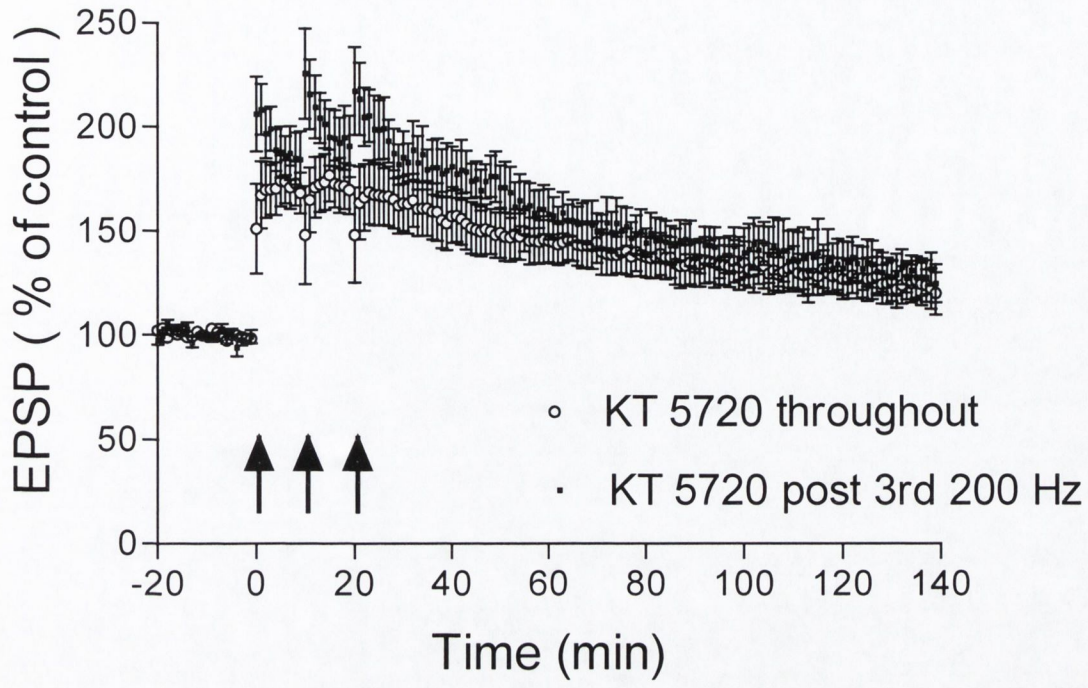

Fig. 3.19 A comparison between slices treated with KT 5720 after the 3rd 200 Hz and slices treated with KT 5720 throughout the experiment. There was no sifificant difference between the two sets of experiments ($p>0.05$).

Hz. There is no significant difference between the two sets of experiments at any time point.

3.8 The role of CaMKII in multiple spaced 200 Hz LTP

3.8.1 The CaMKII inhibitor, KN-62, reduces the early and late-phases of multiple spaced 200 Hz LTP

LTP induction in the CA1 has been shown to be blocked by postsynaptic application of peptide inhibitors of CaMKII (Malinow *et al.*, 1989; Hvalby *et al.*, 1994; Feng 1995). CaMKII is also thought to have an important role in the maintenance of LTP. One hypothesis is that CaMKII activity is maintained constitutively by a positive feedback process involving the autophosphorylation of Thr286 which promotes further autophosphorylation of the kinase (Lisman 1985, Saitoh and Schwartz 1985; Miller and Kennedy 1986; Schworer *et al.*, 1988; Zhabotinsky 2000). Compounding this theory, biochemical work has shown that LTP induction can trigger a long-lasting increase in the autophosphorylated form of CaMKII and its Ca^{2+}-independent activity (Fukunaga *et al.*, 1993, 1995; Barria *et al.*, 1997; Ouyang *et al.*, 1997, 1999; Lee *et al.*, 2000). Also, genetic studies have shown that a mutation of Thr286 prevents LTP induction (Giese *et al.*, 1998).

The potent selective inhibitor of CaMKII, KN-62 (K_i = 900nM) (Ito *et al.*, 1991; Tokumitsu *et al.*, 1990), was used to investigate the role of CaMKII in multiple spaced 200 Hz LTP. Slices were incubated in KN-62 (3μM) for at least an hour before recording and the drug was allowed to circulate in the bath for the duration of the experiment. Peak LTP after the 3rd tetanus was 166.1±11.1% (at 27 min) and 198.5±5.8% (at 28 min) in the drug treated slices and control slices respectively. Thus there was a significant reduction in peak LTP in the presence of the CaMKII inhibitor ($p<0.05$, n=5) (Fig. 3.20). LTP continued to decline in the early and late phases of LTP and at 140 min LTP measured 181.7±6.3% and 135.9±11.1% in control and drug treated slices respectively ($p<0.05$, n=5)(Fig. 3.20).

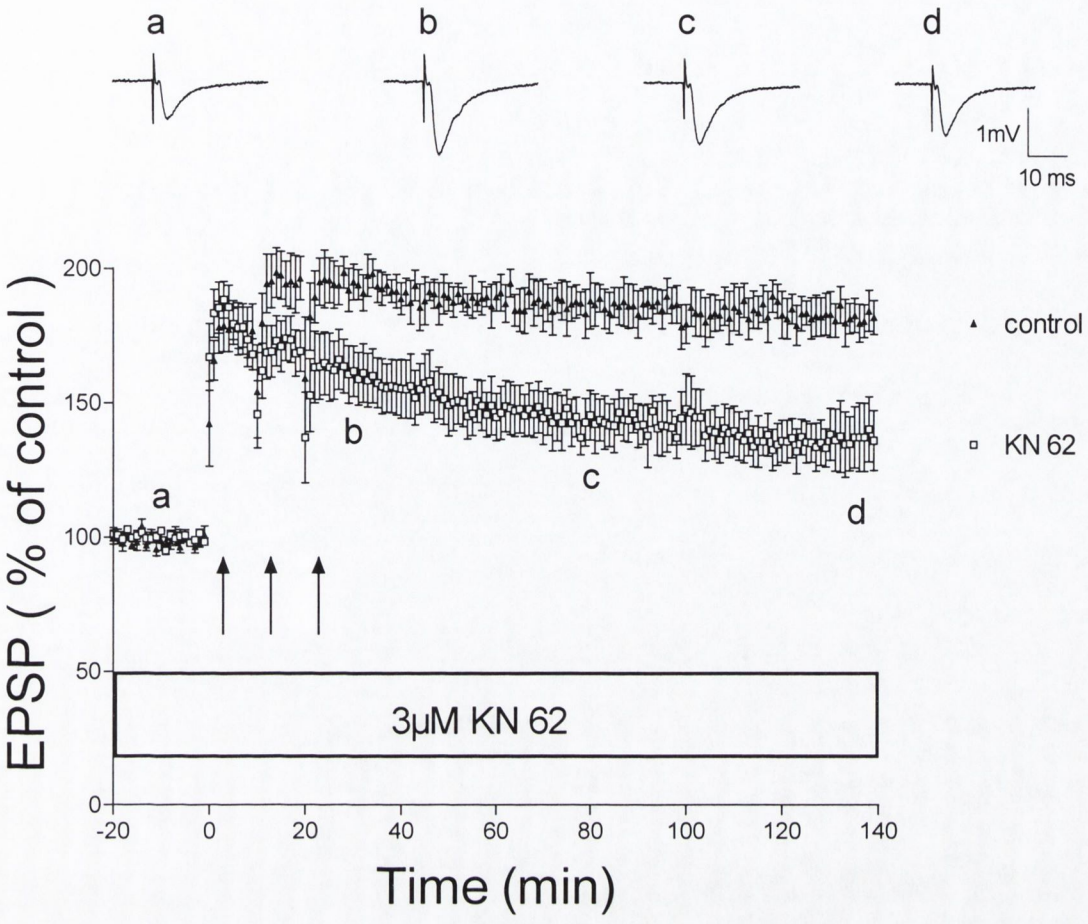

Fig. 3.20 KN-62 reduces both the early and late-phases of LTP induced by the multiple spaced 200 Hz protocol. Peak LTP after the 3rd 200 Hz was 166.1±11.1% in the drug treated slices was compared with 198.5±5.8% in control ($p<0.05$, $n=5$). At 140 min LTP measured 181.7±6.3% and 135.9±11.1% in control and drug treated slices respectively ($p<0.05$, $n=5$). The traces show sample fEPSPs 10 min before HFS (a), 5 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

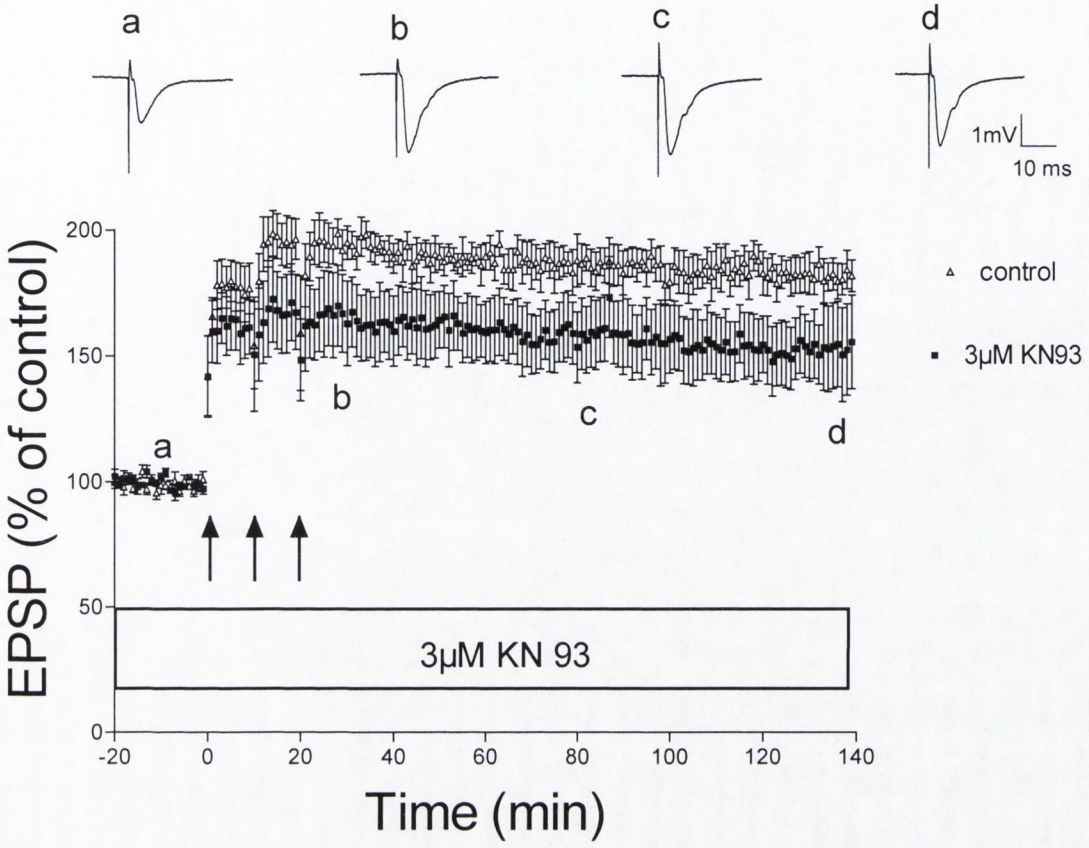

Fig. 3.21 KN-93 does not significantly reduce LTP induced by the multiple spaced 200 Hz protocol. 3μM KN 93 was applied throughout the experiment and peak LTP after the 3rd tetanus measured 169.9±16.1 compared with 198.5±5.8% in control ($p>0.05$, $n=4$). LTP remained stable at this level in the presence of the drug and at 120 min LTP measured 154.5±11.2% and 182.7±8.7% in drug treated slices and control respectively. ($p>0.05$, $n=4$). The traces show sample fEPSPs 10 min before HFS (a), 5 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

3.8.2 The CaMKII inhibitor, KN-93, causes a non-significant reduction in multiple spaced 200 Hz

The same experiment was carried out using an alternative CaMKII inhibitor, KN-93. Again, slices were incubated in the drug for one hour prior to the recording and the drug was allowed to circulate in the bath for the duration of the experiment. Peak LTP was reduced, although not significantly, in the presence of 3μM KN-93, with EPSPs measuring 169.9±16.1 at 28 min in KN-93 treated slices compared with 198.5±5.8% in control slices at the same time point ($p>0.05$, $n=4$)(Fig. 3.21). LTP remained relatively constant at this reduced level over the course of the experiment and at 120 min EPSPs measured 154.5±11.2% in the drug treated slices compared with 182.7±8.7% in control ($p>0.05$, $n=4$).

3.9 The role of MAP kinase in LTP induced by different stimulation protocols

3.9.1 The MAP kinase inhibitor, PD 98059, does not inhibit LTP induced by 8 trains of 8 stimuli at 200 Hz

In the first set of experiments investigating the role of MAP kinase in LTP, the selective MAP kinase inhibitor PD 98059 was found to have no effect on the decremental LTP evoked by a standard 8 trains of 200 Hz for 5 ms. In control slices the mild HFS induced a decremental LTP measuring 201.5±10.8% at peak, and 180.2±12.7% and 161.5±6.7% at 10 min and 1 hr respectively following HFS (Fig 3.22). LTP in slices perfused with 50μM PD 98059 was 152.7±24.9% and 145.9±9.7% at 10 min and 1 hr respectively, values not significantly different from control ($n=2$, $p>0.05$) (Fig. 3.22). Peak LTP in the drug treated slice was 168.4±21% which was not significantly different from peak LTP in control slices ($n=2$, $p>0.05$) (Fig, 3.22). Therefore, the application of PD 98059 did not inhibit the induction of LTP, the early-phase or the late-phase of LTP induced by 8 trains of 200 Hz for 5 ms.

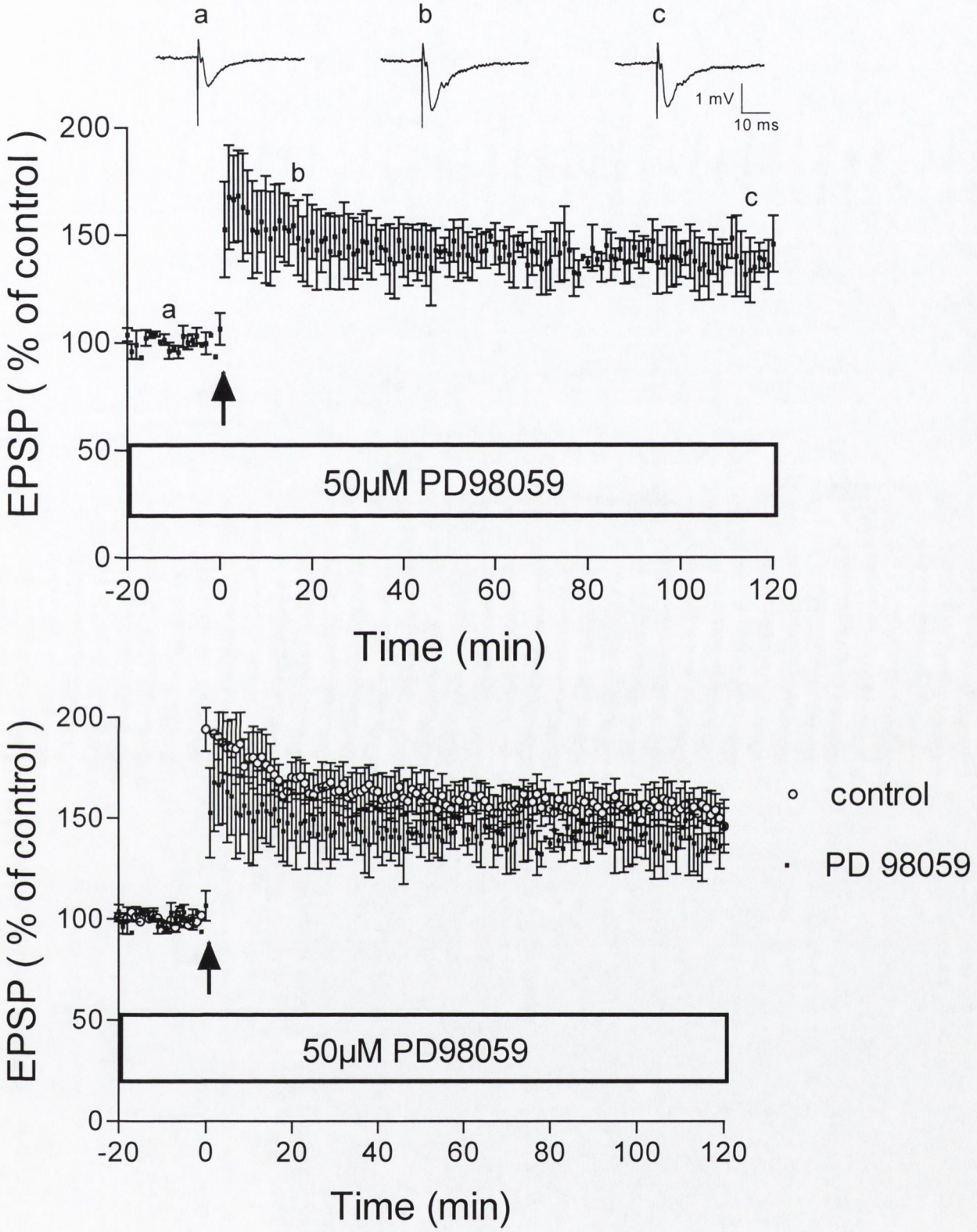

Fig. 3.22 PD 98059 does not inhibit LTP induced by mild stimulation protocol. At 10 and 60 min post HFS LTP measured 152.7±24.9% and 145.9±9.7% values which were not significantly different from control (n=2, $p>0.05$). The lower graph shows a comparison between control and drug treated slices. The traces show sample fEPSPs 10 min before HFS (a), 10 min (b) and 120 min (c) after HFS. The arrows indicates the time when HFS was applied.

3.9.2 PD 98059 inhibits multiple spaced 200 Hz LTP

Multiple spaced 200 Hz HFS induced a very robust LTP that measured 198.5±5.8% at peak, and 189.4±5.2% and 181.7±6.3% at 30 min and 2 hr post the 3rd 200 Hz, levels of LTP that are significantly higher than that induced by a single HFS at 30 min and 2 hr post HFS ($p<0.05$). PD 98059 was used to investigate the role of MAP kinase in multiple spaced 200 Hz LTP. Slices were incubated in PD 98059 for at least one hour before the experiment and the drug was allowed to circulate in the bath for the duration of the experiment. LTP was significantly reduced 5 min after the 1st 200 Hz with LTP measuring 179.8±8.1% and 153.1±1.8 in control and drug treated slices respectively ($p<0.05$, $n=4$) (Fig. 3.23). At 30 min and 90 min after the 3rd 200 Hz, LTP was 139.0±3.0% and 123.6±6.4%, respectively, compared with 189.4±5.2% and 185.5±8.8% in the control slices. Thus, the MAP kinase inhibitor significantly inhibited both the early and late phases of LTP induced by the 3 spaced 200 Hz protocol.

3.9.3 PD 98059 inhibits the expression of multiple spaced 200 Hz LTP

The inhibition of LTP by the MAP kinase inhibitor could have been caused either by a block of the induction or expression of LTP. To investigate this further, PD 98059 was applied at different time points during subsequent experiments in an effort to elucidate the point at which the block of MEK was inhibiting LTP.

Application of PD 98059 immediately after the 3rd HFS resulted in a significant inhibition of LTP expression. LTP was significantly inhibited 10 min after the 3rd 200 Hz with EPSPs at this time point measuring 160.0±9.3% and 195.0±6.7% in drug treated slices and control slices respectively ($p<0.05$, $n=5$)(Fig. 3.24). LTP continued to decrease rapidly in the presence of the drug and EPSPs measured 134.5±12.7% and 131.0±12.0% at 30 min and 90 min post-HFS respectively ($p<0.01$, $n=5$) (Fig. 3.24). Such inhibited levels of LTP expression were not significantly different from those in the previous experiments where PD 98059 was present both prior to, and following, HFS ($p>0.05$) (Fig. 3.25). The inhibition of

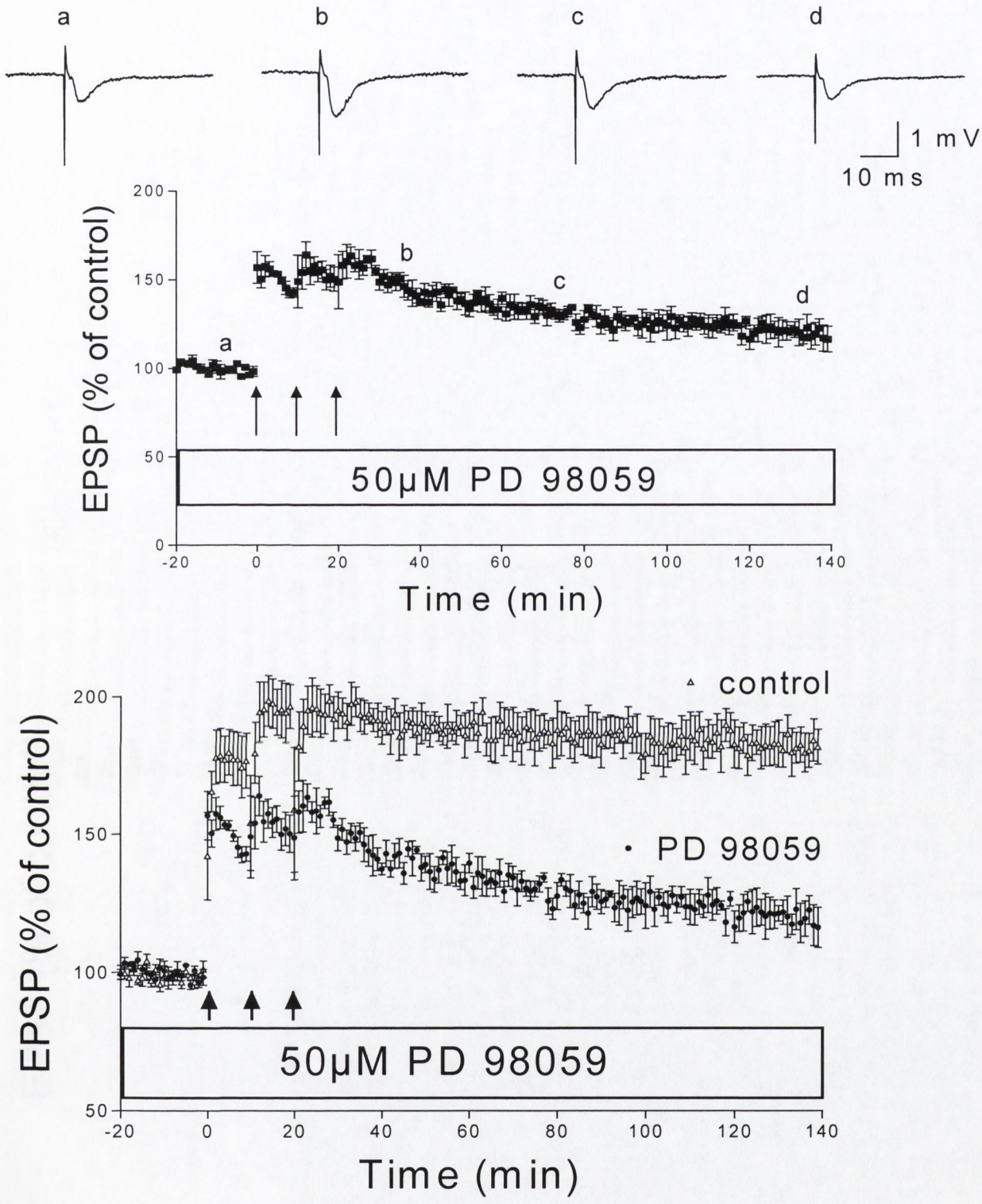

Fig. 3.23 PD 98059 inhibits large amplitude LTP induced by multiple spaced 200 Hz. LTP measured 153.1±1.8% 5 min after the 1st 200 Hz in slices incubated with 50µM PD98059 and this compared with 179.8±8.1% in control slices. LTP continued to decline in the presence of the drug and at 120 min after the 3rd 200 Hz LTP measured 116.3±7.2% and 181.7±6.3% in drug treated slices and control slices respectively. The traces show sample fEPSPs 10 min before HFS (a), 10 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

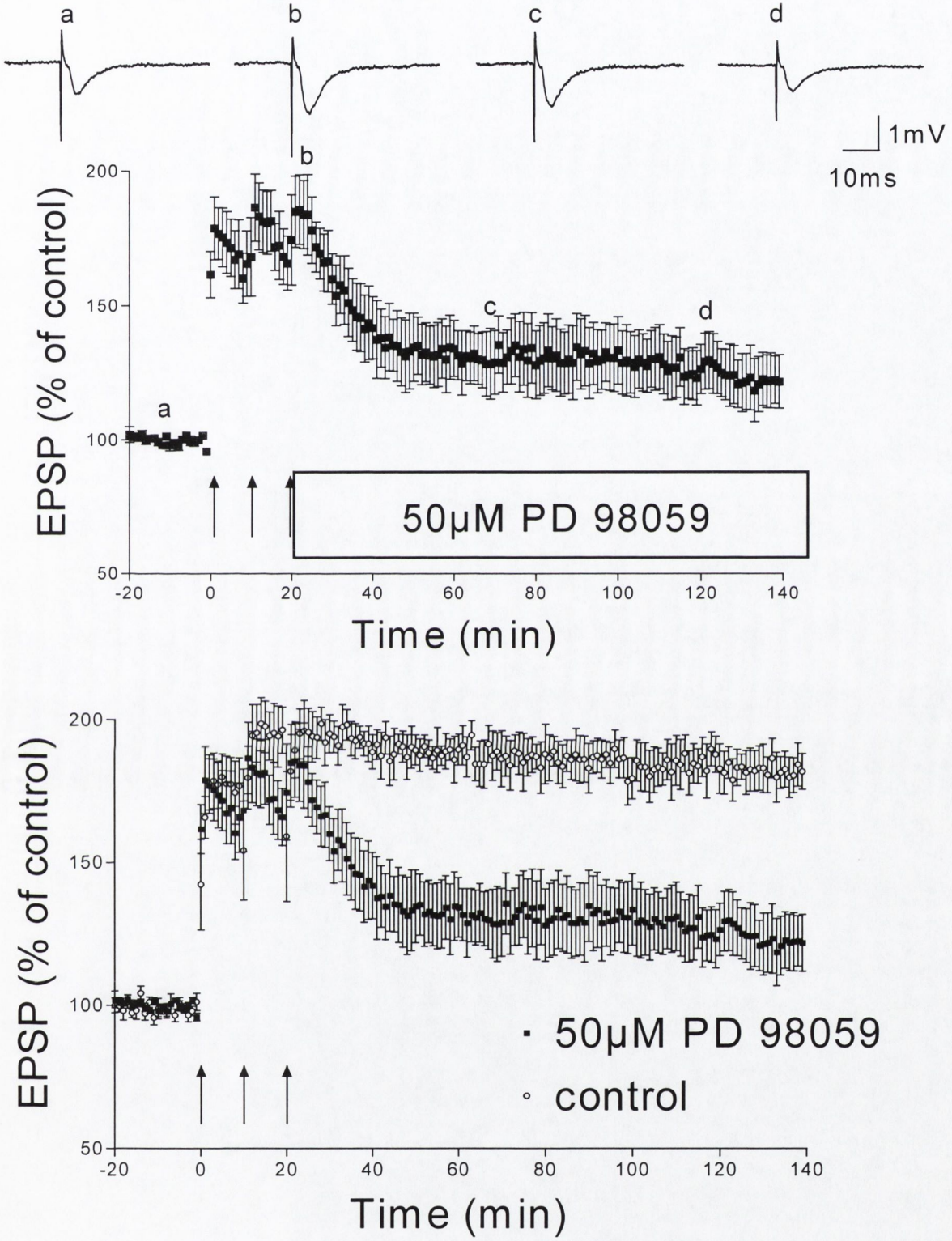

Fig 3.24 PD 98059 inhibits the expression of LTP induced by multiple spaced 200 Hz protocol. The reduction of LTP was significant 10 min after the 3rd 200 Hz ($p<0.05$, n=5). At this time point LTP was 159.97±9.3% and 195.0±6.7% in drug treated and control slices respectively. LTP continued to decline and measured 134.5±12.7% and 131.0±12.0% at 30 min and 90 min post-HFS respectively. The lower graph shows a comparison between the control and drug treated slices. The traces show sample fEPSPs 10 min before HFS (a), 5 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

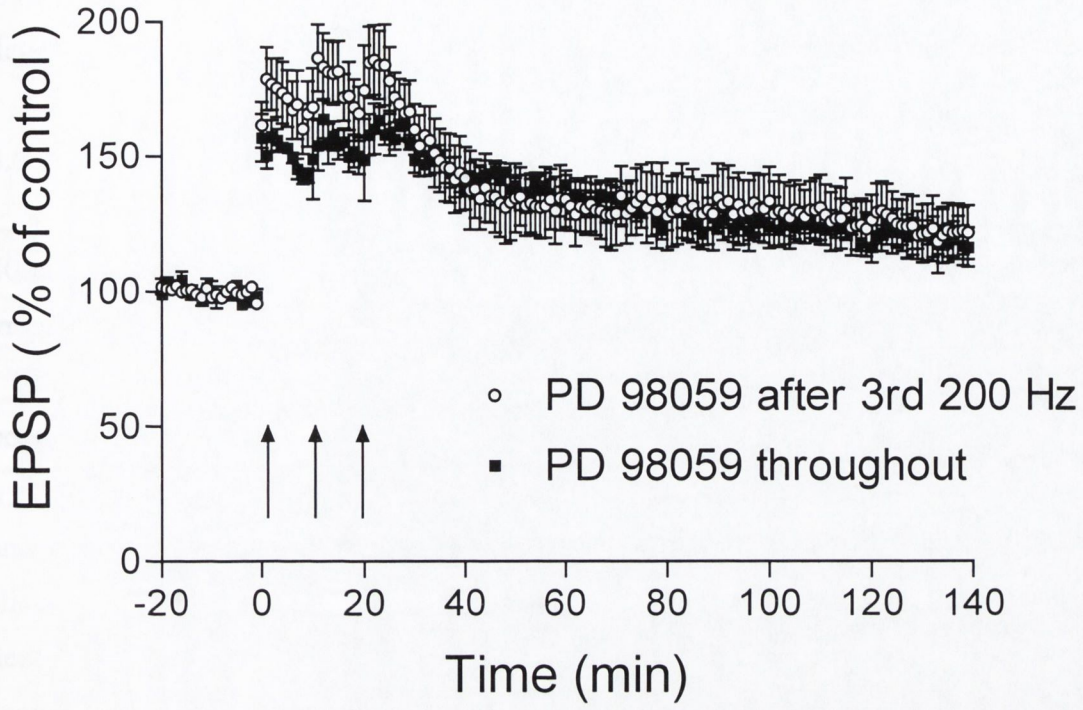

Fig. 3.25 No significant difference between PD 98059 added after the 3rd 200 Hz or when present throughout the experiment. At 30 min after the 3rd 200 Hz EPSPs measured 134.5±12.7% in slices where PD 98059 was added the 3rd 200 Hz and 139.0±3.0% in slices where PD was present throughout the experiment. There was no significant difference between the two graphs at any time point in the experiment.

LTP with application of PD 98059 after the 3rd 200Hz shows that the LTP is still dependent on MAP kinase even after its induction with high-frequency stimulation.

3.9.4 PD 98059 does not block the induction of multiple spaced 200 Hz LTP

50μM PD 98059 was applied for 1 hr prior to HFS, and was washed out immediately following the 3rd 200 Hz. This protocol did not inhibit LTP induction. LTP measured 185.7±4.8% and 184.9±25.0% at 30 min and 90 min respectively post-HFS values which were not significantly different from the controls ($p>0.05$, $n=3$) (Fig. 3.26). LTP was maintained at this high level throughout the experiment and at 120 min EPSPs measured 185.5±22.4% and 182.7±8.7% in drug treated slices and control slices respectively. This demonstrates that MAP kinase is necessary for the expression, but not the induction, of LTP induced by multiple spaced 200 Hz protocol.

3.9.5 Inhibition of the expression of multiple spaced 200 Hz LTP by PD 98059 is reversible

PD 98059 was applied 30 min after the 3rd 200 Hz, perfused for 1 hr and then washed out. LTP fell to 148.7±9.5% and 139.1±7.7%, 30 min and 1 hr respectively after the application of PD 98059 (Fig. 3.27). LTP recovered quite rapidly after washout with EPSPs measuring 164.1±15.3% at 5 min after the onset of washout. This level of LTP at 5 min after the onset of washout was not significantly different from control LTP at the same time point ($p>0.1$, $n=4$). EPSPs continued to rise during the remainder of the washout and at 140 min (which was 30 min after the start of washout) LTP measured 185.0±9.9% compared to 181.7±6.3% in control at the same time point ($p>0.05$, $n=4$). Thus, LTP fully recovers to control levels after the washout of PD 98059. These experiments demonstrate that MAP kinase is persistently active for at least 2 hr after the induction of LTP, and furthermore, that the MEK inhibitor blocked the expression of the signal underlying LTP but did not inhibit the persistent signal itself, i.e. the maintenance of LTP was not inhibited.

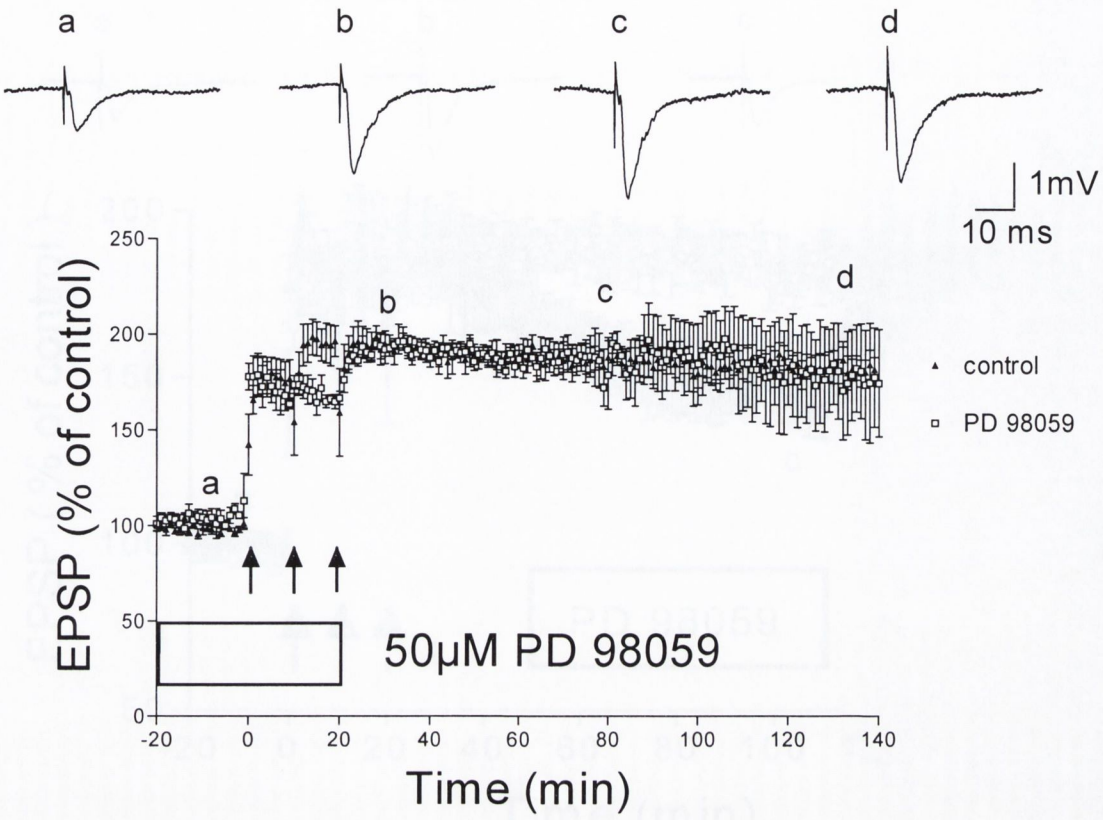

Fig. 3.26 PD 98059 does not block the induction of multiple spaced 200 Hz LTP. LTP measured 185.7±4.8% and 184.9±25.0% at 30 min and 90 min respectively, values not significantly different from the control ($p>0.05$, $n=3$). LTP was maintained at this high level and at 120 min LTP measured 185.5±22.4% and 182.7±8.7% in drug treated slices and control slices respectively. The traces show sample fEPSPs 10 min before HFS (a), 10 min (b), 60 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

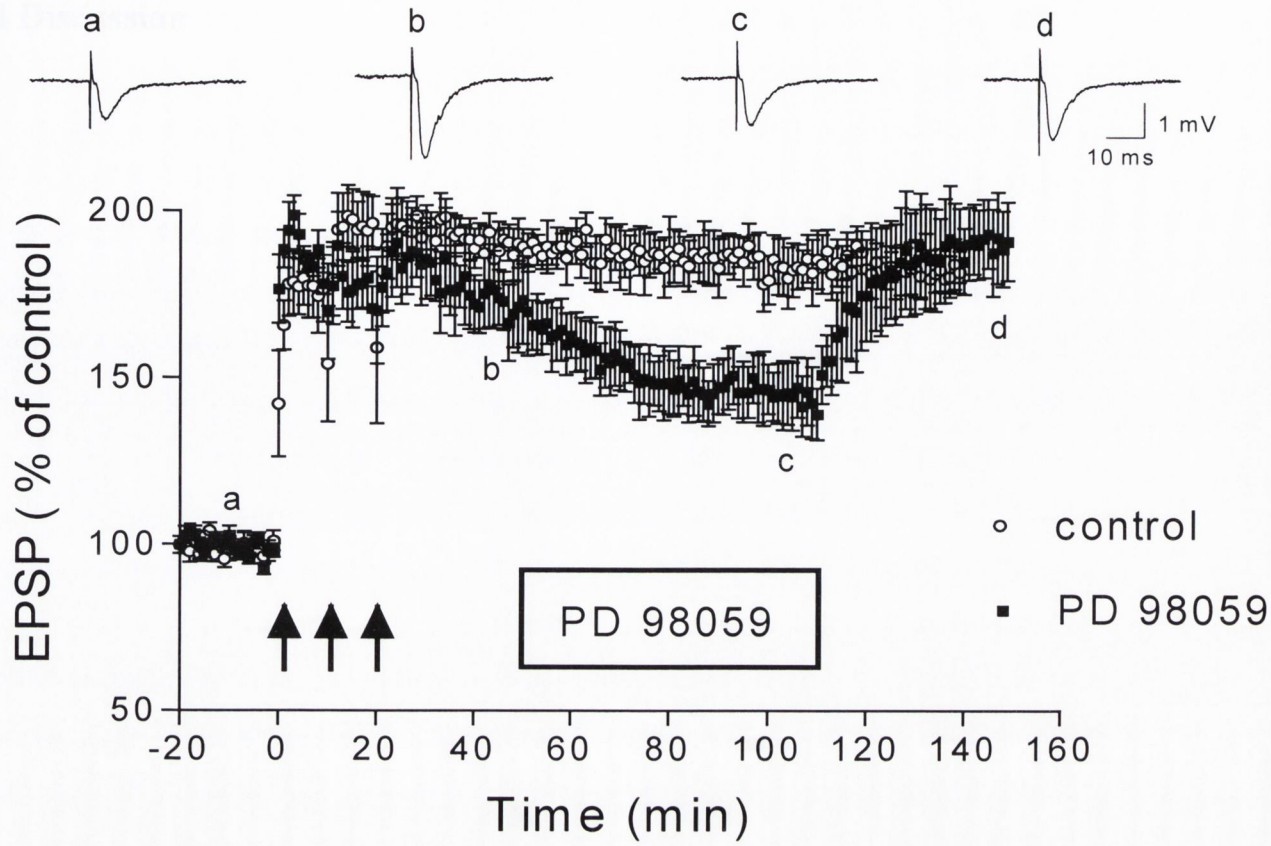

Fig. 3.27 Inhibition of the expression of LTP by MAP kinase inhibitor is reversible. 50μM PD 98059 was added to the bath solution 30 min after the 3rd 200 Hz and circulated for 1 hour before it was washed out. LTP declined very quickly after exposure to PD 98059 and after 10 min it was significantly reduced below control levels (162.7±6.8% and 189.4±5.4% in drug treated and control slices respectively, $p<0.05$, $n=4$). LTP recovered very quickly after the washout of LTP and at 150 min (40 min after the start of washout) EPSPs measured 191.0±11.8%. The traces show sample fEPSPs 10 min before HFS (a), 20 min (b), 80 min (c) and 120 min (d) after the 3rd 200 Hz. The arrows indicate the times when HFS was applied.

4 Discussion

4.1 Different stimulation protocols induce different types of LTP

These three protocols are of particular interest because they elicit three distinct types of LTP with three different time-courses. Both the 100 Hz and the multiple spaced 200 Hz protocols give rise to steady state LTP with little decline. However, the magnitude of the LTP produced by the two protocols is very different, with the 100 Hz protocol producing a sustained LTP at ~130% and the multiple spaced 200 Hz LTP producing an LTP that is maintained at ~200%. The 200 Hz protocol provides a good intermediate between these two protocols as it rises initially to the same level as the multiple spaced LTP and then declines gradually over the course of 80 min to the low amplitude LTP produced by the 100 Hz protocol. These experiments demonstrate that the magnitude of LTP is dependent on the frequency and duration of stimulation applied to the hippocampal slice. This has been found to be the case in all areas of the hippocampus but the magnitude of LTP does not increase indefinitely with increases in stimulation duration and frequency. LTP increases up to a maximum and after this, further stimulation will lead to a reduction in LTP. Therefore, in the hippocampus the degree of LTP induction follows an inverted U-shaped function of the number of stimulus trains (Christie *et al.*, 1995; Abraham and Huggett, 1997). In the present study we have shown an increase in LTP magnitude up to a maximal point with a very high intensity protocol. The possibility of a reduction in LTP through over-stimulation with the strongest protocol was alleviated with a wide spacing of 10 mins between each repetition of the 200 Hz protocol. In a study in the CA1 a protocol of 24 trains of theta-burst stimulation inhibited LTP production (Abraham and Huggett, 1997). Each theta-burst train consisted of 10 bursts of 5 pulses at 100 Hz delivered at 200 ms intervals, with a 30 sec interval between each theta-burst train, so in total the 24 train theta-burst stimulation consisted of 1,200 pulses. By comparison, the multiple spaced 200 Hz protocol used in the present study also consisted of 1,200 pulses but it did not inhibit LTP, conversely, it induced a strong LTP that was sustained at ~200% for 2 hours. The critical difference between the two protocols was that the 24 train theta-burst protocol was sustained for 12 minutes (i.e. 24 trains with 30 sec between each train) whereas the multiple spaced 200 Hz protocol involved 3 bursts of stimulation which lasted for 50 seconds with a time window of 10 minutes between each burst. Therefore, it seems likely that the wide spacing between bursts

of stimulation in the multiple spaced 200 Hz protocol enables synapses to overcome the problem of over-stimulation. Sustained stimulation could possibly lead to a prolonged increase in intracellular Ca^{2+} in the dendrite which would favour LTD or depotentiation of any existing LTP. This was seen in the CA1 where LTP that was generated by the first few trains of theta-burst stimulation was eliminated by the end of a 32 train cycle (Abraham and Huggett, 1997). This suggests that there is a depotentiation process occurring towards the end of the stimulation protocol which lasts for 12 minutes. The selective adenosine 1 (A_1) receptor antagonist, DPCPX, blocked this presumed depotentiation by a mechanism that is not fully understood (Abraham and Huggett, 1997). Activation of the A_1 receptor causes a decrease in cAMP accumulation (Van Calker *et al.*, 1979) and when activated might inhibit the cAMP-dependent cascades that are required for the stabilisation of LTP (Frey *et al.*, 1993; Huang *et al.*, 1994). Activation of adenosine receptors might also be able to reduce NMDA receptor mediated transmission either by an adenosine mediated reduction in presynaptic glutamate release (Dunwiddie, 1985; Greene and Haas, 1991; Mitchell *et al.*, 1993) or by an increase in postsynaptic potassium currents (Greene and Haas, 1985; Gerber *et al.*, 1989). Interestingly, the inhibition of LTP with over-stimulation has been shown to recover after 90 minutes and it has also been shown to affect only LTP as heterosynaptic LTD is not affected by over-stimulation (Fujii *et al.*, 1991; Christie and Abraham, 1992; Huang *et al.*, 1992; Wexler and Stanton, 1993). These results suggest that over-stimulation leads to pathological changes in the synapses arising from the excitotoxic effects of too much afferent stimulation.

In the present study, the ten minute spacing of the multiple 200 Hz protocol probably allows Ca^{2+} concentration in the postsynaptic neuron to rise rapidly and dramatically leading to the predominant activation of kinases that are thought to be responsible for LTP induction. A prolonged and sustained stimulation would lead to a shift in favour of phosphatase activity which would facilitate LTD induction. High-intensity tetanic stimulation has also been shown to induce a form of LTP which is dependent on the activation of voltage-dependent Ca^{2+} channels and can be induced even when NMDA receptors are completely blocked (Grover and Teyler, 1990; Morgan and Teyler, 2001). This has prompted other studies to investigate the role of different Ca^{2+} sources that are recruited by stimulation protocols of varying strength. The endoplasmic reticulum is a large source of Ca^{2+} for various neuronal

signalling processes (Berridge 1998; Mattson *et al.*, 2000). After high-frequency stimulation, Ca^{2+} can be released from the endoplasmic reticulum by both the ryanodine (RyR) and inositol 1,4,5-trisphosphate receptor (IP3R). An increase in cytosolic Ca^{2+} leads to the activation of RyRs and initiates the phenomenon of Ca^{2+}-induced Ca^{2+}-release (CICR) (Zucchi and Ronca-Testoni, 1997; Berridge 1998) while IP3Rs are primarily activated by IP3 which is generated by the activation of metabotropic receptors linked to PLC (Berridge, 1993, 1998; Simpson *et al.*, 1995). Depletion of Ca^{2+} stores with thapsigargin and the block of RyRs with ryanodine have both been shown to inhibit LTP induced by weak conditioning stimuli (Obenaus *et al.*, 1989; Harvey and Collingridge, 1992; Behnisch and Reymann, 1995; Wang *et al.*, 1996). RyRs have been shown to be the most sensitive Ca^{2+} receptor during weak stimulation (Raymond and Redman, 2002). Inhibition of RyRs blocks LTP induced by 1 theta-burst stimulation (TBS) (Raymond and Redman, 2002). Therefore, RyRs might have a prominent role in the induction and expression of the low amplitude LTP induced by the 100 Hz protocol used in this study. Studies in organotypic cultures have shown that Ca^{2+} influx through NMDA receptors in response to single synaptic events can activate RyRs (Emptage *et al.*, 1999). This suggests that Ca^{2+} influx through NMDA receptors might not in itself, be sufficient to induce LTP but a further amplification of the Ca^{2+} signal via RyRs might also be required. LTP induced by mild stimulation protocols has also been shown to be dependent on NMDA receptor activation and independent of voltage-dependent Ca^{2+} channels (VDCCs) (Morgan and Teyler, 2001). This type of LTP has been correlated with short term memory (STM) (<21 hours) in a hippocampal-dependent behavioural task (Woodside *et al.*, 1999; Borroni *et al.*, 2000).

As the number of trains of stimulation increases, LTP has been shown to be more reliant on IP3 receptors (Raymond and Redman, 2002). A stronger stimulation protocol might be required to cause a more prolonged glutamate release which would be able to activate perisynaptically located mGluRs and raise IP3 concentration (Lujan *et al.*, 1996; Scanziani *et al.*, 1997). IP3 receptors can also be activated by an increase in cytosolic Ca^{2+} concentration (Berridge, 1993, 1998) and it is possible that the intermediate 200 Hz protocol is capable of raising the Ca^{2+} concentration sufficiently for the activation of IP3 receptors. A mild stimulation (5 trains of four pulses at 100 Hz separated by 200 ms and repeated six times with an interburst interval of 10 sec) similar to the intermediate 200 Hz protocol used in the

present study has been shown to induce a compound LTP composed of both NMDA-dependent and VDCC-dependent LTP (vdccLTP) (Morgan and Teyler, 2001). This type of stimulation mimics more prolonged complex spike bursting which might occur when an animal is subjected to intensive training (Barnes, 1979). In contrast, inhibitors of IP3 receptors or RyRs have not been found to have any effect on LTP induced by high-frequency or high-intensity stimulation (Morgan and Teyler, 2001; Raymond and Redman, 2002). In the present study, the strongest stimulus induced an LTP that exhibited little decay. This type of LTP might be related to vdccLTP which can only be induced in the CA1 by high-intensity tetanic stimulation (Grover and Teyler, 1990; Morgan and Teyler, 2001). This vdccLTP can also be induced when NMDA receptors are blocked which indicates that a high-intensity stimulation can produce a sufficient magnitude of activity in the hippocampus to by-pass the predominant pathway of LTP induction i.e. the influx of Ca^{2+} through NMDA receptors. In the absence of NMDA receptor activation, cell depolarisation has to rely on AMPA currents which must be able to depolarise the cell to around -10mV for VDCCs to open (Tsein *et al.*, 1988). Field EPSPs of up to 3.5mV have been recorded in CA1 of behaving animals which may be similar to the level of depolarisation required to induce vdccLTP (Buzaki, 1986). This very strong LTP has recently been implicated in long-term memory (LTM) (>7 days) in behaving animals performing a spatial task (Woodside *et al.*, 1999; Borroni *et al.*, 2000). Also, the use of multiple spaced stimulation protocols has been shown to convert an NMDA-dependent LTP into an LTP that requires the activation of both NMDA receptors and VDCCs (Morgan and Teyler, 2001). It is thought that the spaced stimulation enables the threshold for VDCC activation to be achieved by increasing the postsynaptic response to a given presynaptic signal (Morgan and Teyler, 2001). This is particularly interesting in light of the studies which have implicated NMDA-dependent LTP in STM and VDCC-dependent LTP in LTM. A scheme could be envisaged where continued intensive training would convert short-term memories into long term memories by sequential activation of VDCCs mediated by prolonged complex bursting in hippocampal neurons.

The next question to address is the physiological relevance of LTP produced by the multiple spaced 200 Hz protocol. High-frequency EEG waves of up to 200 Hz have been recorded in the hippocampus and other limbic structures (Buzsaki *et al.*, 1992; Ylinen *et al.*, 1995; Chrobak and Buzsaki, 1996). These so called "ripples" are

superimposed on physiological sharp waves which are short, aperiodic field potentials of a large magnitude (1-3mV) (Buzsaki *et al.*, 1983; Buzsaki, 1986; Suzuki and Smith, 1987). Ripples have been observed in both awake and anaesthetised animals and intracellular recordings from pyramidal cells have shown that ripple waves can be as large as 4mV (Ylinen *et al.*, 1995). Therefore, the multiple spaced 200 Hz protocol may well reflect a pattern of neuronal activity that occurs naturally in animals. Synchronised somatic IPSPs interrupted by synchronous discharges of CA1 pyramidal neurons every 5-6 msec are thought to be the currents responsible for ripple activity (Draguhn *et al.*, 1998; Csicsvari *et al.*, 1999). Although ripple activity is fastest in the CA1, it has also been found in the subiculum, parasubiculum and deep layers of the entorhinal cortex indicating that it is a phenomenon that occurs throughout the hippocampal-entorhinal axis. The nature of these high-frequency patterns is not well understood but recent studies have indicated that fast ripples of between 140-200 Hz emerge from the CA1 whereas slower oscillatory patterns of 100-130 Hz are generated in the CA3 and transferred to the CA1 (Csicsvari *et al.*, 1999). Other hippocampal rhythms such as theta (5-10 Hz) and gamma (40-100 Hz) have been observed in the rat during exploration and rapid eye movement (REM). In contrast, ripple activity is associated with immobility, consummatory behaviour and slow-wave sleep (Vanderwolf, 1969; Bland, 1986; Chrobak and Buzaki, 1996). A functional co-operativity between different patterns of activity has previously been proposed to serve as a process for information storage. For example, mnemonic memory is thought to be dependent on encoding in synapses of CA3 neurons during theta-associated behaviour and the consolidation of this memory is thought to rely on the initiation of sharp waves in the CA3 during slow-wave sleep which would cause synaptic connections that were active during the learning state to be spontaneously reactivated (Nadasdy *et al.*, 1999). The repetition of sequences of activity during slow-wave sleep is hypothesized to transfer stored representations in the hippocampus to neocortical networks (Buzsaki, 1989; Wilson and McNaughton, 1994; McClelland *et al.*, 1995; Siapas and Wilson, 1998). Also of interest is the finding that the replay of sequences observed during theta behaviour are predominantly associated with sharp wave bursts (Nadasdy *et al.*, 1999). When a neuronal sequence of activity associated with the theta rhythm in the awake animal is replayed as a sharp wave during slow wave sleep it is also time-compressed

(Nadasdy *et al.*, 1999). The significance of this is that neurons which discharge in a temporally discontinuous manner during theta behaviour are gathered together during the sharp wave on the timescale of the time constant of NMDA receptors (Nadasdy *et al.*, 1999). Thus, the time-compressed nature of the replay allows plasticity to occur in neurons which discharged at frequencies that were too low to activate NMDA receptors but were nevertheless still able to store information for later use. The multiple spaced 200 Hz protocol has very similar properties to the time-compressed replay phenomenon and it is possible that the large amplitude sustained LTP produced by the multiple spaced 200 Hz protocol might be a representation of the consolidation of previously experienced events that occurs during slow-wave sleep in rodents.

While high-frequency oscillatory behaviour can be found throughout the hippocampus, it is also worth noting that the dentate gyrus can be activated by both the entorhinal input (a feed-forward path) and also by the CA3 (a feed-back path). Therefore, high-frequency activity in areas adjacent to the dentate gyrus might be able to influence granule cell firing. Within the dentate gyrus itself, large amplitude (2-4mV) field potentials, termed dentate spikes, have been recorded during behavioural immobility and slow-wave sleep (Bragin *et al.*, 1995). These dentate spikes are associated with a large depolarisation of granule cells and are thought to reflect a combination of synchronous perforant path-mediated EPSPs on dendrites and IPSPs on the perisomatic region of the granule cells mediated by the feed-forward activation of inteneurons (Penttonen *et al.*, 1997). Also, depolarisation and discharge of anatomically identified granule cells has been observed to coincide with hippocampal sharp waves (Ylinen *et al.*, 1995). This depolarisation and discharge of granule cells could result from direct CA3-granule cell projections (Li *et al.*, 1994) or by the direct recruitment of mossy cells by the CA3 collaterals (Soltesz *et al.*, 1993; Buckmaster *et al.*, 1996). Anatomical studies have shown that CA3 pyramidal cells can send axon collaterals to the dentate gyrus where they can directly innervate granule cells (Li *et al.*, 1994). While granule cells are very hyperpolarised and silent during either theta or gamma activity, the firing of action potentials seems to be facilitated during sharp wave related population bursts of the CA3 region (Penttonen *et al.*, 1997). However, the relationship between dentate spikes and sharp waves is complex. During dentate spike mediated feed-forward activation of the granule cells there is a net decrease in the excitability of the CA3-

CA1 axis, but during sharp wave bursts all cell populations in the hippocampus, including granule cells, CA3-CA1, subicular pyramidal cells, presubicular, and layer V neurons in the entorhinal cortex, exhibit an organized transient network discharge (Penttonen *et al.*, 1997).

Another possible scenario for the induction of LTP by HFS protocols is the use of back-propagating action potentials which can serve as a postsynaptic associative signal (Linden, 1999). In this way the back-propagating action potential can be used as a global dendritic associative signal which can potentially reach all of the synapses on a single neuron. Studies have shown that a single back-propagating action potential can induce a small Ca^{2+} influx (Markram *et al.*, 1995) but that when a back-propagating action potential is paired with an EPSP then the Ca^{2+} transient is much larger but only if the action potential is followed by the EPSP and not vice versa (Koester and Sakmann, 1998).

The induction protocols used in the present study show that the level of LTP can be varied simply by changing the frequency of the stimulation and also the number of trains of stimulation. In particular it shows that the spacing of trains is particularly important for the induction of large amplitude LTP and that without this spacing LTP would not be produced due to the over-stimulation of the slice. Also, repeated trains of the multiple spaced 200 Hz protocol did not significantly increase the level of LTP above that produced by the first train but it clearly produced some intrinsic change in the neurons because LTP induced by this protocol was sustained over the course of two hours whereas LTP induced by a single 200 Hz protocol continually declined over the same time-course.

The large amplitude LTP induced by the multiple spaced 200 Hz protocol might be used to record and store an "index" of cortical targets activated during experiential events (Teyler and DiScenna, 1986). Thus, the hippocampus is required to store the information until such time as it is transferred to the cortex and can be subsequently discarded by the hippocampus (Teyler, 1989). The fact that very robust VDCC-dependent LTP does not undergo depotentiation after low-frequency stimulation indicates that this function of the vdccLTP might be related to its ability to encode information which is, in time, passed onto the cortex. An alternative view is that while cortical plasticity is quite malleable, a form of LTP in the hippocampus that is not easily reversed is required in order for an accurate index to be presented to the cortex (Buonomano and Merzenich, 1998).

4.2 Adrenergic receptors

The results show that the β-adrenergic receptor antagonist, propranolol, had no effect on the induction of LTP produced by all three stimulation protocols. Block of the adrenergic receptors did result in a trend towards a non-significant reduction in the expression of LTP produced by both the 200 Hz and the multiple spaced 200 Hz protocol. In both cases there was a greater reduction in the magnitude of LTP towards the end of the experiments. These results suggest that β-adrenergic receptor activation is not essential for LTP produced by mild stimulation protocols and that the role of β-adrenergic receptors increases as the stimulation protocol increases in strength. However, even with the two stronger protocols, β-adrenergic receptor activation still only appears to have a minor modulatory role in LTP.

There have been mixed findings to date regarding the role of β-adrenergic receptors in the induction and expression of LTP in the hippocampus. Some studies have found that β-adrenergic receptors are involved in the induction of LTP in both the medial and lateral perforant path synapses (Bliss *et al.*, 1983; Stanton and Sarvey, 1985; Bramham *et al.*, 1997) while another study reported that noradrenaline depletion resulted in an increase in the population spike amplitude and a depression in the population EPSP (Robinson and Racine, 1985). Robinson and Racine (1985) also found that there were no differences in LTP levels one week after the potentiation tests. Together, these studies present a complicated picture of β-adrenergic receptor activity during the induction and expression of LTP.

As β-adrenergic receptors are positively coupled to G-proteins and cause a rise in cAMP when they are activated, it is surprising that their blockade with propranolol had only a minor effect on LTP expression in the present study. Significantly, dopaminergic receptors which are also positively coupled to G-proteins and have a similar receptor distribution to adrenergic receptors in the dentate gyrus have been shown to have no effect on LTP in this region (Swanson-Park *et al.*, 1999). It is also of interest that both noradrenaline and dopamine can regulate the excitability of granule cells (Whitton, 1997; Yanagihashi and Ishikawa, 1992). The study of Swanson-Park *et al.* (1999) also found regional differences in β-adrenergic receptor regulation of LTP; propranolol had no effect on LTP in CA1 while in contrast to the

present study it reduced both the induction and expression of LTP in the dentate gyrus. It is possible that the placement of electrodes causes only one particular population of catecholamines to be activated in each subregion although this is unlikely as agonists of adrenergic and dopaminergic receptors also caused differential effects on the induction and persistence of LTP (Swanson-Park *et al.*, 1999). Another possibility is that other G-protein coupled receptors such as mGluRs or acetylcholine muscarinic receptors might be upregulated in response to adrenergic blockade and thus, a greater activation of these receptors might compensate for the lack of cAMP formation by adrenergic receptors. Also of interest is the fact that G_i-linked receptors for serotonin, GABA, and glutamate can potentiate increases in cAMP formation after β-adrenergic receptor activation (Andrade, 1993; Bourne and Nicoll, 1993; Gereau and Conn, 1994). G_i βγ subunits are thought to mediate this response by enhancing the activity of the type II adenylyl cyclase which is activated after the stimulation of β-adrenergic receptors (Tang and Gilman, 1991; Federman *et al.*, 1992; Cooper *et al.*, 1995). This finding opens up the possibility that other neurotransmitters might have a modulatory role in β-adrenergic receptor activity and also that neurotransmitters such as acetylcholine, serotonin, and dopamine, might by themselves be able to overtake the role of noradrenaline in instigating the cellular cascades required for LTP production. In support of this, stimulation of cholinergic activity has been found to cause an increase in EPSPs similar to LTP (Auerbach and Segal, 1996). While the activation of β-adrenergic receptors has been shown to increase ERK phosphorylation through the cAMP cascade (Winder *et al.*, 1999), it must be remembered that there are multiple pathways which converge on ERK and that the PKA-mediated phosphorylation of the adaptor molecule, Rap1, which in turn phosphorylates ERK can still occur when adrenergic receptors are inhibited. It has been demonstrated that different neurotransmitters can interact to enhance LTP (Robinson and Racine, 1982; Wang *et al.*, 1999; Watabe *et al.*, 2000) but the ability of different neurotransmitters to compensate for one another in not well understood. The results of this study indicate that there might be a fail-safe cooperative compensation occurring in the dentate gyrus when one receptor is blocked and that mGluRs, muscarinic receptors and NMDA receptors are capable of increasing calcium transients sufficiently in the absence of adrenergic receptors to ensure that

LTP is induced in response to a high- frequency stimulation. Studies in the dentate gyrus and CA1 have also shown that decaying forms of LTP can be prolonged by mGluR activation (Manahan-Vaughan and Reymann, 1996; Raymond *et al.*, 2000). Also of note is a recent study which has shown that mild stimulation protocols induce LTP which can be reduced with propranolol but stronger induction protocols elicit LTP which is unaffected by β-adrenergic receptor inhibition (Straube and Frey, 2003). The study of Straube and Frey (2003) was carried out in freely moving rats and the duration of the experiments were of a much longer time scale (24 hours) than in the present study. Despite these differences, a significant reduction in LTP induced by the mild stimulation protocol was only seen after four hours and with the strongest protocol there was no significant difference between control and propranolol treated animals. It is difficult to compare the findings of Straube and Frey (2003) to those of the present study because of the differences between the *in vivo* and *in vitro* experimental set-ups but it is of interest that over a two hour period both studies found a negligible effect of propranolol on LTP. Further studies employing inhibition of more that one G-protein coupled receptor might be of use in determining whether or not there is compensatory mechanism operating between these receptors.

4.3 Muscarinic acetylcholine receptors

The results from the present experiments show that inhibition of muscarinic acetylcholine receptors (mAChRs) with scopolamine hydrochloride had no effect on the induction of LTP with the multiple spaced 200 Hz protocol but it did cause a significant reduction in late-phase LTP. Previous studies in the dentate gyrus *in vivo* have found that cholinergic denervation with AF64A attenuated the magnitude of LTP induced by a weak tetanus in a concentration dependent manner but that LTP induced by a stronger tetanus was unaffected by the cholinergic toxin (Abe *et al.*, 1994). The attenuation of LTP induced by a weak tetanus was found to be mediated by the M_1 receptor as it was blocked by pirenzepine and was not affected by either the M_3 antagonist, 4-DAMP, or the M_2 antagonist, AF-DX116. The *in vitro* results from the present study suggest that activation of muscarinic receptors is required for the expression of late-phase LTP. This is in contrast to CA1 where the same

concentration of scopolamine as was used in this study (100μM) was found to suppress both the early and late phases of LTP (Hirotsu *et al.*, 1989). One of the reasons for the differences between the different hippocampal subregions could be related to the differentential localisation of muscarinic receptors within the hippocampus. M_1 receptors which are positively coupled to adenylyl cyclase are most abundant in the somata and dendrites of pyramidal neurons and granule cells (Dawson *et al.*, 1989; Joyce *et al.*, 1989; Levey *et al.*, 1995). The M_2 are predominantly expressed in nonpyramidal neurons and in discrete bands of fibres and puncta surrounding pyramidal neurons and other layers while M_3 receptors are found in pyramidal neurons, the neuropil in stratum lacunosum-moleculare and the outer third of the molecular layer of the dentate gyrus (Levey *et al.*, 1995). This regional and laminar distribution of mAChRs indicates that different hippocampal subregions have different roles in cholinergic modulation of excitatory hippocampal circuits during the induction and expression of LTP induced by high-frequency stimulation.

Another possible explanation for the reduction in late-phase LTP mediated by the muscarinic antagonist, scopolamine, is that the full complement of proteins and RNA necessary for late-phase LTP might not be synthesised when mAChRs are inhibited. Similar to the CA1 and CA3 regions, late-phase LTP in the dentate gyrus requires the participation of PKA for the generation of new proteins and RNA (Frey *et al.*, 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Huang *et al.*, 1994; Nguyen and Kandel, 1996). As stimulation of mAChRs has been shown to increase the activation of ERK (Rosenblum *et al.*, 2000) and also to increase the concentration of Ca^{2+} in neurons (Felder, 1995) it is likely that activation of mAChRs receptors can ultimately contribute to an increase in gene expression and RNA synthesis via the ERK cascade. M_1, M_3 and M_5 receptors all activate PLC and consequently lead to production of Ca^{2+} and DAG which can both activate PKC. Activated PKC can in turn activate the Ras/Raf-1 wing of the ERK pathway and cause activation of CREB which can bring about a change in gene expression and the synthesis of new proteins. Consistent with this, ERKI/II has been shown to be activated by glutamate via Ca^{2+}-dependent mechanisms (Xia *et al.*, 1996) and application of the acetylcholine esterase inhibitor, physostigmine, has been shown to increase endogenous ERKI/II activity in hippocampal tissue while the muscarinic antagonist, atropine, decreases ERKI/II activity (Rosenblum *et al.*, 2000).

However, investigation of pathways downstream of mAChRs has revealed a complicated picture. In COS-7 cells expressing mAChRs, carbachol was found to increase ERK activation by a PKC-independent mechanism. However, Ca^{2+} chelators and PI_3K inhibitors attenuated ERK activation while inhibitors of Src tyrosine kinase completely blocked ERK activation (Rosenblum *et al.*, 2000). In relation to the present study it is likely that scopolamine application prevented the full activation of ERK and as it has been reported that ERKI/II signalling is necessary for Ca^{2+}-stimulated transcription in hippocampal neurons (Impey *et al.*, 1998) it is reasonable to suppose that the decline in late-phase LTP in the presence of scopolamine was the result of an attenuation of ERK mediated protein synthesis. ERK is the only member of the MAP kinase family that is known to translocate to the nucleus and after it has moved to the nucleus it can upregulate gene expression (Impey *et al.*, 1998). CREB has been shown to be a downstream target of ERK (Roberson *et al.*, 1999) and ERK mediates a phosphorylation of CREB at Ser^{133} via RSK2 (Roberson *et al.*, 1999; Impey *et al.*, 1998). CREB is a transcription factor and once it is activated it can lead to protein synthesis.

In the dentate gyrus the proteins necessary for late-phase LTP are thought to be synthesised postsynaptically because blockade of protein synthesis in the dentate gyrus inhibits late-phase LTP, whereas an inhibition of protein synthesis in the entorhinal cortex where the cell bodies of presynaptic perforant path axons are found has no effect on late-phase LTP (Otani and Abraham, 1989). Furthermore, in the transverse hippocampal slice preparation, the cell bodies of the presynaptic perforant axons which are situated in the entorhinal cortex are absent and studies with this preparation have shown that protein synthesis inhibitors attenuate late-phase LTP (Nguyen and Kandel, 1996).

The effects of stimulation of mAChRs are dependent on the dissociated α and $\beta\gamma$ subunits of the G-protein to which they are coupled. G_α subunit activation is Ca^{2+}- and PKC-dependent (Hawes *et al.*, 1995) while activation of ERKI/II in neurons is thought to be primarily induced by $G_{\beta\gamma}$. The $G_{\beta\gamma}$ subunit activates the Src tyrosine kinase and the PI_3K which in turn activate the ERK cascade via the small G-protein Ras. Clearly, activation of mAChRs can activate ERK through a diverse array of pathways and this diversity might enable cross-talk between the different signal transduction cascades to occur.

The results of the present study indicate that the reduction of late-phase LTP may be due to an attenuation of ERKI/II activation. However, as other receptors are also capable of activating ERK when mAChRs are inhibited there is not an inhibition of LTP, only a reduction in late-phase LTP. Overall, it can be concluded that mAChRs do not have a role in the induction of LTP in the dentate gyrus but that they do have an important regulatory role in the expression of late-phase LTP.

4.4 Metabotropic glutamate receptors

The present study investigated the role of metabotropic glutamate receptors (mGluRs) in multiple spaced 200 Hz LTP using the mGluR antagonists LY341495 and MPEP hydrochloride. LY341495 is the first compound that has been developed that can block all known mGluRs. It can inhibit group II mGluRs at low nanomolar concentrations and group I and III mGluRs at micromolar concentrations (Kingston *et al.*, 1998). In the present study LY341495 was found to have no effect on the induction of LTP by the strong multiple spaced 200 Hz protocol but it did produce a significant reduction of late phase LTP. A previous study in the CA1 has shown that LY341495 has no effect on either the induction or expression of LTP induced by 100 Hz for 1 sec (Fitzjohn *et al.*, 1998). However, in the study of Fitzjohn *et al.*, LY341495 was either applied for 20 min prior to tetanus or directly after the tetanus for 20 min. In both cases LY341495 had no effect on LTP. In the present study, LY341495 was present throughout the experiment and so the difference between the two studies might be due to an inhibition of mGluR activation during late-phase LTP which would only occur if LY341495 was present throughout the experiment. The concentration of LY341495 used in the present study (20μM) is sufficient to block all mGluRs and so it is interesting to compare its effect on LTP with that of the commonly used weak competitive antagonist, MCPG, which inhibits $mGluR_1$, $mGluR_2$, $mGluR_5$ and $mGluR_8$. The finding in this study that LTP induction is not affected by LY341495 is consistent with studies which have shown that LTP induction is not affected by (*S*)-MCPG (Chinestra *et al.*, 1993; Bortolotto *et al.*, 1994; Manzoni *et al.*, 1994; Selig *et al.*, 1995; Thomas and O'Dell, 1995; Wang *et al.*, 1995; Martin and Morris, 1997).

Although the CA1 has been the main focus of attention for the study of mGluR activity in LTP, two of the studies which failed to show a block of LTP induction

with MCPG were in the dentate gyrus (Wang *et al.*, 1995; Martin and Morris, 1997). Opposing the above are studies which have shown that group I mGluR antagonists can inhibit the induction of LTP (Bashir *et al.*, 1993; Sergueeva *et al.*, 1993; Aiba *et al.*, 1994; O'Connor *et al.*, 1994; Richter-Levin *et al.*, 1994; Little *et al.*, 1995; Manahan-Vaughan, 1997; Breakwell *et al.*, 1998). Another study tried to resolve these discrepancies by proposing that the role of mGluRs in LTP is dependent on the type and strength of tetanisation applied (Wilsch *et al.*, 1998). This group noted that studies which have employed strong tetanisation protocols such as theta-burst stimulation or multiple 100 Hz for 1 sec have found that MCPG does not block LTP induction (Manzoni *et al.*, 1994; Selig *et al.*, 1995a; Thomas and O'Dell, 1995) while studies using a protocol of 100 Hz for less than 1 sec produced LTP which was inhibited by MCPG. The hypothesis of Wilsch *et al.* (1998) is that the commonly used protocol of 100 Hz for 1 sec (Bashir *et al.*, 1993; Chinestra *et al.*, 1993; Bortolotto *et al.*, 1994; Thomas and O'Dell, 1995) marks a critical threshold where the dependence of LTP on mGluR activation is determined by unknown experimental conditions. Considered in the light of the present experiments, this hypothesis might be valid for the induction of LTP but it does not account for the reduction in late-phase LTP seen in the present study. Wilsch *et al.* (1998) found that MCPG had no effect on their strong multiple 100 Hz protocol but again the antagonist was only present for 10 min prior to the tetanus and was washed out immediately after the tetanus. Therefore, it is possible that application of the antagonist for the duration of the experiment might have produced a reduction in late-phase LTP similar to that seen in the present study.

The induction of LTP in the CA1 and the dentate gyrus requires the elevation of intracellular Ca^{2+} concentration in the postsynaptic neuron (Lynch *et al.*, 1983; Malenka *et al.*, 1988). After tetanisation, the main source of this rise in postsynaptic Ca^{2+} is influx through NMDA receptors (Collingridge *et al.*, 1983; Malenka, 1994), while Ca^{2+} influx through VDCCs and the release of Ca^{2+} from IP_3 and ryanodine sensitive intracellular Ca^{2+} stores (ICSs) can also augment the Ca^{2+} concentration (Thastrup *et al.*, 1990; Berridge, 1993; Frenguelli *et al.*, 1996). Strong tetanisation protocols have been shown to elicit LTP with a VDCC-dependent component (Grover and Teyler, 1990) while a weaker protocol will produce LTP with a smaller VDCC component (Grover and Teyler, 1994). The strong protocol used in the present study probably recruits Ca^{2+} influx through VDCCs and this mechanism

might help to ensure that LTP induction is not attenuated even when all mGluRs are blocked with LY341495. With weaker stimulation protocols that do not recruit VDCCs the induction of LTP is more dependent on mGluR-triggered Ca^{2+} release from IP_3-sensitive ICSs. Therefore, the induction of LTP with weaker protocols is more likely to be inhibited by mGluR antagonists. In contrast, the influx of Ca^{2+} through both NMDA and VDCCs during strong protocols is sufficient for the LTP threshold to be reached and under these conditions Ca^{2+} release from ICSs would be functionally redundant. This scheme of events is supported by evidence that the Ca^{2+}-ATPase inhibitor, thapsigargin, has no effect on the induction of LTP by multiple 100 Hz tetanus but can impair LTP generation with a single 100 Hz protocol (Behnisch and Reymann, 1995).

While the results of this study are consistent with the scheme of LTP induction presented above, the next question to address is the reduction of late-phase LTP in LY341495 treated slices. One possibility is that activation of group I mGluRs facilitates local protein synthesis in dendrites and dendritic spines. Studies have shown that there is virtually a full complement of protein synthesis machinery present in the dendrites of many different neuronal types (Tiedge and Brosius, 1996; Torre and Steward, 1996; Gardiol *et al.*, 1999). More importantly, mRNAs encoding for proteins which are thought to have an integral role in LTP such as αCaMKII, GAP43, tyrosine kinase B, CREB, activity-related cytoskeletal protein and several glutamate receptor subunits have been found near dendritic spines (Steward, 1997; Tongiorgi *et al.*, 1997; Gao, 1998). Taken together with studies which have shown that the persistence of LTP is affected by inhibitors of group I mGluRs and inhibitors of translation but not by inhibitors of transcription (Raymond *et al.*, 2000) the evidence to date suggests that the activation of mGluRs can facilitate the synthesis of new proteins from pre-existing mRNA. This transcription-independent protein synthesis has been demonstrated in both the CA1 and the dentate gyrus (Otani *et al.*, 1989; Raymond *et al.*, 2000) indicating that local protein synthesis in dendrites may be a general feature of hippocampal plasticity. Raymond *et al.*, found a decrease in late-phase LTP in the presence of the group I antagonist, AIDA, and while the magnitude of LTP induced in their study is not as large as in the present one, there is a striking similarity in the time-course of LTP decline in both studies, with a reduction in LTP below control levels becoming apparent one hour after tetanus in both cases (Raymond *et al.*, 2000 (Fig. 4B),

present study Fig. 3.10). This suggests that the mGluR-dependent persistence of late-phase LTP might be mediated by the same mechanisms in both studies.

The results of the present study are also consistent with previous findings in the CA3 synapses which have shown that activation of mGluRs is use-dependent and that as the strength of high-frequency stimulation is increased there is a greater activation of mGluRs (Scanziani *et al.*, 1997). This is probably due to a greater increase in glutamate concentration in the synaptic cleft after stronger stimulation and as group I mGluRs are located peripherally on the postsynaptic density (Lujan *et al.*, 1996) it is likely that they are only fully activated after stronger stimulation protocols or when they are pharmacologically primed by DHPG.

A possible mechanism by which mGluRs could contribute to the persistence of late-phase LTP is through the activation of PKC. Activation of group I mGluRs leads to the production of IP_3 and DAG (Birse *et al.*, 1993). IP_3 binds to IP_3R on ICSs and causes the release of Ca^{2+} which together with DAG can activate PKC. PKC in turn can phosphorylate translation initiation factors, such as the eIF4E subunit of the mRNA binding complex eIF4F (Sonenberg, 1996) and lead ultimately to an increase in protein synthesis which can maintain the persistence of late-phase LTP.

While LTP has commonly been divided into two phases – an early LTP dependent on post-translational modifications and a late LTP dependent on transcription and translation – the recent evidence suggesting that mGluR activation can cause local protein synthesis supports the idea that there is also an intermediate phase that is protein synthesis-dependent but transcription-independent (Otani *et al.*, 1989; Raymond *et al.*, 2000). In the present study the decline of LTP which is seen 1 hour after tetanus in LY341495 treated slices might signify the transition from early-phase LTP to an intermediate phase of LTP. One example of a contributory factor in the decline of LTP might be a decrease in AMPA receptor phosphorylation by αCaMKII in the presence of LY341495. If the synthesis of αCaMKII in dendrites is inhibited by the mGluR antagonist then its expression will decline and LTP might be attenuated as a consequence of this fall in αCaMKII activity. Another implication of the present study is that persistent mGluR activation might be necessary for maintenance of late-phase LTP. A commonly used protocol in experiments employing mGluR antagonists is to apply the drug for 10-20 min before the tetanus and to wash the drug out shortly after the tetanus. It is possible that results obtained in this study differ from previous findings because the activation of mGluRs is

inhibited throughout the experiment whereas it is possible that mGluRs can be reactivated after the washout of an antagonist once LTP has been successfully induced by a tetanus. It is reasonable to assume that the persistent activation of mGluRs leading to the production of DAG and Ca^{2+} which in turn can trigger many internal cascades through the phosphorylation of PKC and CaMKII will have an influence on the persistence of late-phase LTP. It is also interesting to note that group I receptor activation has recently been shown to increase the phosphorylation of CREB, Elk-1, and ERK in the rat dorsal striatum (Choe and Wang, 2001). Similar studies have not yet been carried out in the hippocampus but the results of the present study indicate that the inhibition of mGluRs might well be able to attenuate the phophosphorylation of CREB, Elk-1 and ERK. As activation of these proteins can lead to the synthesis of new proteins, an inhibition of their activation would contribute to an attenuation of late-phase LTP similar to that seen in the present study. In addition to this, in the dentate gyrus, ERK signalling can regulate both Elk-1 and CREB by targeting the serum response element (SRE) and the Ca^{2+} and cAMP response elements (CaCRE) (Davis *et al.*, 2000). A possible scheme of events in the hippocampus might be that activation of mGluRs triggers Ca^{2+}-dependent signalling cascades, such as CaMKII and MAP kinases, which in turn cause CREB and Elk-1 phosphorylation.

In an effort to determine the receptor subtype involved in the reduction of late-phase LTP the specific noncompetitive antagonist of $mGluR_5$, MPEP hydrochloride (Gasparini *et al.*, 1999; Spooren *et al.*, 2001), was applied to the slice. There was no significant difference between control LTP and LTP induced in the presence of MPEP hydrochloride which rules out the possibility that LY341495 is mediating its reduction of late-phase LTP via an $mGluR_5$. This result is somewhat surprising as $mGluR_5$ has been shown to have a role in the potentiation of NMDA component of LTP (Lu *et al.*, 1997) and $mGluR_5$ knockout mice were shown to have impaired LTP in the same study (Lu *et al.*, 1997). Furthermore, a recent study has found that MPEP blocks *in vivo* LTP in the CA1 and that intracerebroventricular injection of MPEP results in a marked impairment of spatial learning in rats (Balschun and Wetzel, 2002). Again, there is not unanimous agreement in the literature as one study has shown that the $mGluR_5$ antagonist, LY344545, had no effect on LTP in the CA1 (Doherty *et al.*, 2000). Clearly, there is a need for further studies to be

carried out using selective mGluR antagonists in order to determine the role of the different receptor subtypes in LTP.

To date most studies have concentrated on the role of mGluRs in the induction of LTP and there has been less emphasis on the role of mGluRs in the persistence of LTP. The present study demonstrates that mGluR activation is integral for the persistence of late-phase LTP but the cascades which are triggered by mGluR activation still need to be elucidated.

4.5 Protein synthesis

The results from the present study demonstrate the effects of two protein synthesis inhibitors, anisomycin and emetine, on LTP induced by two different high-frequency stimulation protocols. Both of these drugs are inhibitors of translation with anisomycin blocking peptide bond formation while emetine blocks ribosome translation along mRNA (Vazquez, 1974). When the slices were incubated in emetine for 1 hour prior to recording and the drug was present throughout the experiment there was a complete block of LTP induction with the multiple spaced 200 Hz protocol. Surprisingly, there was a marked depression in the EPSPs immediately after each tetanus and these depressed EPSPs tended to recover during the 10 min intertrain interval but they were still significantly depressed at the onset of the next 200 Hz stimulation. After the final 200 Hz stimulation, EPSPs were almost completely obliterated but they recovered to ~80% of baseline levels after 30 min. A block of the induction of multiple spaced 200 Hz LTP was also seen in slices incubated in anisomycin but in these experiments the depression of EPSPs immediately after each tetanus was not as large as that seen with emetine (~60%) and EPSPs returned to baseline levels within the 10 min intertrain interval. After the 3rd 200 Hz stimulation anisomycin-treated slices remained stable at baseline levels and exhibited no potentiation. These results indicate that, in contrast to milder stimulation protocols, the induction of large amplitude multiple spaced 200 Hz LTP requires protein synthesis.

To date, LTP in all three regions of the hippocampus has consistently been divided into two phases – an early-phase (E-LTP) which lasts 1-3 hours and is independent of protein and RNA synthesis and a longer lasting late-phase (L-LTP) which is dependent on new RNA and protein synthesis and is thought to be mediated by

cAMP (Frey *et al.*, 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Huang *et al.*, 1994; Nguyen *et al.*, 1994; Nguyen and Kandel, 1996). Interestingly, there are striking parallels between the division of LTP into an early and a late phase and the distinctions between short and long-term memory which have been elucidated by cognitive psychological studies. Short-term memory lasts for minutes to hours while long-term memory can persist for weeks or longer (Polster *et al.*, 1991) and the induction of these long-term memories can be blocked by the transient application of protein synthesis and RNA inhibitors while short-term memories are unaffected by the same inhibitors (Davis and Squire, 1984; Castellucci *et al.*, 1989; Crow and Forrester, 1990; Tully *et al.*, 1994). The switch from short-term to long-term memory requires the induction of new proteins for both implicit and explicit forms of memory storage but once long-term memory has been induced new protein synthesis is no longer required for the persistence of the memory (Davis and Squire, 1984). The switch from short-term to long-term memory has also been defined on a molecular level for the gill- and siphon-withdrawal reflexes in the marine snail, *Aplysia*. Again, short-term facilitation of synaptic transmission in *Aplysia* has been found to be increased by modification of pre-existing proteins which is mediated by both PKA and PKC (Castellucci *et al.*, 1980; Montarolo *et al.*, 1986; Ghirardi *et al.*, 1992; Byrne *et al.*, 1993) and long-term facilitation has been found to result from new protein synthesis which is mediated by the activation of CREB-1 and the disinhibition of CREB-2 (Montarolo *et al.*, 1986; Dash *et al.*, 1990; Alberini *et al.*, 1994; Bartsch *et al.*, 1995). Therefore, it can be seen that there seems to be an evolutionary conservation across species of the basic mechanisms which are required for the expression and maintenance of learning and memory. However, the results from the present experiments are in contrast to previous experiments in the dentate gyrus which have reported that protein synthesis inhibitors attenuate L-LTP but have no effect on E-LTP (Krug *et al.*, 1984; Frey *et al.*, 1988; Otani *et al.*, 1989; Nguyen and Kandel, 1996). The difference between the results of the present set of experiments and previous experiments in the dentate gyrus is most likely related to the strong nature of the multiple spaced 200 Hz protocol. It is possible that, in contrast to mild decremental LTP, large amplitude non-decremental LTP induced by the multiple spaced 200 Hz protocol requires protein synthesis for both induction and L-LTP. It is also possible that inhibitors of protein synthesis cause a decrease in the levels of a housekeeping

protein such as a calcium binding protein which normally regulates the levels of Ca^{2+} influx after a tetanus. In the absence of this protein, a strong stimulation protocol would produce a large postsynaptic increase in Ca^{2+} which would no longer be buffered by the calcium binding protein and this unregulated rise in cytoplasmic Ca^{2+} would be toxic to neurons and inhibit the induction of LTP (Choi, 1988; Namgung *et al.*, 1995). Consistent with this, a previous *in vivo* study in the dentate gyrus found that when mice were pretreated with cycloheximide for 4 hours before LTP, there was a sudden inhibition of the entire response after the tetanus in half of the animals examined (Namgung *et al.*, 1995). Calbindin-D_{28k} is one calcium binding protein which normally buffers the concentration of Ca^{2+} after a tetanus and this protein is depleted by 50% 2-3 hours after treatment with cycloheximide (Theofan and Norman, 1986).

The difference between the induction of new protein synthesis by different stimulation protocols was highlighted by the finding that a conventional mild amplitude LTP induced by 8 trains of 8 pulses at 200 Hz with a 2 sec intertrain interval was not affected by anisomycin. Neither the induction nor the maintenance of LTP was affected by the protein synthesis inhibitor despite the fact that it was used at a much higher concentration than that which blocked the induction of multiple spaced 200 Hz LTP (40μM as opposed to 5μM). Therefore, these two stimulation protocols appear to represent opposite ends of the spectrum as far as the dependence of LTP on protein synthesis is concerned, with mild amplitude LTP being completely independent of protein synthesis over a two hour period and large amplitude LTP being completely dependent on protein synthesis for the induction of LTP. Interestingly, in a study by Nguyen and Kandel (1996), emetine was found to have no effect on a decremental LTP induced by 3 trains of 100 Hz/1sec with 1 min intertrain interval, whereas it brought a non-decremental LTP induced by 10 trains of the same protocol back to baseline levels 120 min after the tetanus (Fig 3, Nguyen and Kandel, 1996). It is possible that the 10 trains of 100 Hz/1sec protocol represents an LTP that lies between a decremental LTP which is protein synthesis independent and a non-decremental LTP which is protein synthesis-dependent. Overall, the results of the present experiments, taken together with those of Nguyen and Kandel (1996), indicate that the dependence of LTP on protein synthesis is critically modulated by the strength and intensity of the stimulation protocol.

In relation to the concentration of protein synthesis inhibitors used, emetine has previously been shown to block protein synthesis in whole cell hippocampal slices at concentrations similar to that used in the present experiments (Stanton and Sarvey, 1984) and anisomycin at a concentration of 20μM causes an 85% reduction in leucine incorporation into hippocampal proteins (Frey *et al.*, 1988). Anisomycin acts very rapidly as an injection of the drug into the hippocampus immediately after tetanus has been found to cause an 89% inhibition of protein synthesis within 15 min (Otani *et al.*, 1989).

In the present study it was found that application of anisomycin directly after the 3rd 200 Hz of the multiple spaced 200 Hz protocol did not cause any reduction in LTP over the first ~30 min. After ~30 min, LTP began to decline below control levels and LTP in the drug treated slice continued to decline until it was significantly lower than control levels at 110 min post-tetanus. These results extend the previous findings which demonstrated that slices incubated in a protein synthesis inhibitor exhibited a block of induction of multiple spaced 200 Hz LTP. Once LTP has been successfully induced by the multiple spaced 200 Hz protocol it appears that inhibition of protein synthesis is not detrimental to E-LTP but it does affect more persistent L-LTP as LTP is significantly reduced below control levels ~2 hours after the tetanus.

Previous *in vivo* studies in the dentate gyrus have found that an injection of anisomycin directly after tetanus causes a reduction in LTP while an injection of anisomycin 15 min after tetanus does not have any effect on LTP (Otani *et al.*, 1989). These findings from *in vivo* experiments indicate that the proteins required for the maintenance of LTP are synthesised within the first 15 minutes after the induction of LTP by high-frequency stimulation. In relation to behavioural studies, protein synthesis is thought to be necessary for the formation of long-term memory and it is interesting to note that anisomycin produces an amnestic effect when it inhibits >80% of protein synthesis but these effects are only seen after 2-4 hours (Flood *et al.*, 1973; Greksch *et al.*, 1980; Mizumori *et al.*, 1985; Staubli *et al.*, 1985).

Unlike the late-phase of LTP which has been shown to require new RNA and protein synthesis in all three regions of the hippocampus (Frey *et al.*, 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Huang *et al.*, 1994; Nguyen *et al.*, 1994; Nguyen and Kandel, 1996), there have been conflicting reports regarding the

dependence of early-phase LTP on protein synthesis in the dentate gyrus, CA3 and CA1. Some studies have found that E-LTP in the CA3 (Huang and Kandel, 1996; Nguyen and Kandel, 1996) and CA1 (Krug *et al.*, 1984; Frey *et al.*, 1988; Huang *et al.*, 1996; Mochida *et al.*, 2001) is independent of protein synthesis. Opposing this view are other studies which have reported that the early maintenance of LTP in CA1 (Stanton and Sarvey, 1984; Deadwyler *et al.*, 1987; Clayton, 2000; Scharf *et al.*, 2002), the dentate gyrus (Otani and Abraham, 1989; Otani *et al.*, 1989 Nguyen *et al.*, 1994) and CA3 (Barea-Rodriguez *et al.*, 2000; Calixto *et al.*, 2003) is protein synthesis-dependent. Even within studies there have been conflicting results as in the study of Stanton and Sarvey (1984) which found that emetine, cycloheximide and puromycin blocked LTP in CA1 while, surprisingly, anisomycin was unable to block LTP.

Although it is impossible to confirm whether or not protein synthesis is occurring in the pre- or postsynaptic cells of the dentate gyrus using bath application of protein synthesis inhibitors, the evidence to date suggests that it is occurring in the postsynaptic granule cells. Inhibition of protein synthesis in the entorhinal cortex where the cell bodies of the presynaptic perforant path axons are located has no effect on L-LTP indicating that presynaptic cells are not involved in the protein synthesis which maintains L-LTP (Otani and Abraham, 1989). Furthermore, high-frequency stimulation which induces LTP leads to the production of a wide range of mRNAs and proteins as well as immediate-early genes in dentate granule cells (Cole *et al.*, 1989; Hughes and Dragunow, 1995). A quantitative increase in protein synthesis has also been reported after high-frequency stimulation in *in vivo* studies in the dentate gyrus (Fazeli *et al.*, 1993). Other studies in CA1 in which dendrites were severed from CA1 pyramidal cell bodies reported that only a transient early-phase LTP remained under these conditions (Frey *et al.*, 1989). Taken together, this evidence suggests that the protein synthesis which is induced by high-frequency stimulation is occurring at a postsynaptic site in the dentate gyrus. However, in mossy fibre LTP there is evidence for both presynaptic and postsynaptic cells contributing to the induction and maintenance of LTP which can be influenced by protein synthesis inhibitors. A retrograde signalling cascade involving EphB receptor-ephrin ligand interaction which links an influx of Ca^{2+} in the postsynaptic cell with an increase in presynaptic transmitter release has been discovered in this

region and similar to the effect of emetine, inhibition of these receptors produces an early decline of LTP (Contractor *et al.*, 2002; Calixto *et al.*, 2003).

While the traditional view of protein synthesis is that it occurs in the cell body of the neuron, recent studies have found that local protein synthesis can also occur in the postsynaptic cell in response to a train of stimulation (Aakalu *et al.*, 2001; Job and Eberwine, 2001; Smith *et al.*, 2001; Steward and Schuman, 2001; Steward and Worley, 2002). Local protein synthesis has also been implicated in the conversion of silent synapses into functionally active synapses as the expression and insertion of AMPA receptors can be induced in dendritic spines by high-frequency stimulation (Kacharmina *et al.*, 2000; Malinow and Malenka, 2002). The expression of other proteins such as cellular adhesion molecules (cadherins and proteins of cytoskeleton) and CaMKII are also induced locally by high-frequency stimulation (Kim and Lisman, 1999; Krucker *et al.*, 2000; Chicurel *et al.*, 1993). The finding that CaMKII can be induced locally is particularly interesting as this molecule is thought to be critical for the induction and maintenance of NMDA receptor-dependent LTP (Finn and Browning, 1980; Mody *et al.*, 1984; Ouyang *et al.*, 1999; Krucker *et al.*, 2000) and αCaMKII has been shown to have an important role in learning and memory (Silva *et al.*, 1992a; Silva *et al.*, 1992b). It is possible that the translation of pre-existing CaMKII mRNAs in the postsynaptic cells in the dentate gyrus are required for the induction of multiple spaced 200 Hz LTP and incubation of slices in either emetine or anisomycin prior to the multiple spaced 200 Hz protocol would thus inhibit the induction of large amplitude LTP by preventing the local protein synthesis of CaMKII. Also, it has been demonstrated that new proteins are synthesised about 15-45 min after high-frequency stimulation, which is consistent with the finding from the present study that anisomycin applied after the final tetanus did not cause a reduction in the early-phase LTP (Lynch *et al.*, 1994; Osten *et al.*, 1996; Lanahan and Worley, 1998; Ouyang *et al.*, 1999).

The most important finding from the present study is that the mechanisms which underlie the induction and maintenance of multiple spaced 200 Hz LTP are markedly different from those which underlie LTP induced by a more temporally compressed stimulation. In particular, the large temporal spacing of the multiple spaced 200 Hz protocol appears to have a critical effect on the induction of LTP. At a behavioural level it has been known for a long time that multiple training trials which are spaced far apart are generally more effective at promoting robust learning

and memory than trials which are compressed together. This phenomenon is know as the trial spacing effect and while it has been extensively studied in behaviour models, very few studies have tried to replicate its effects at the cellular electrophysiological level using spaced stimulation in hippocampal experiments *in vivo* or *in vitro* (Ebbinghaus, 1885; Hintzman, 1974; Lattal, 1999; Rescorla, 1988; Wu *et al.*, 2001a). In the fruit fly, *Drosophila melanogaster*, a temporally spaced protocol has been found to produce enhanced long-term memory for conditioned odour avoidance as compared with a more temporally massed protocol but this improvement was eliminated with inhibitors of protein synthesis (Tully *et al.*, 1994). Other studies have shown that the induction of an inhibitory CREB transgene reduces the development of long-term memory after spaced training while the induction of an activator isoform of CREB enables the development of long-term memory even with a massed stimulation protocol (Yin *et al.*, 1994). Together these studies suggest that a temporally spaced protocol is more effective than a massed protocol at activating CREB-modulated protein synthesis.

In the mouse model, knockouts of the α- and δ-isoforms of CREB have been shown to have impaired long-term memory but this reduced long-term memory can be rescued by spaced training (Kogan *et al.*, 1997). Again, this provides evidence that the temporal nature of behavioural training is vitally important for initiating long-term memory via mechanisms that are dependent on protein synthesis. To date only one study has examined the role of protein synthesis in the trial spacing effect in both behavioural long-term memory and LTP in rodents (Scharf *et al.*, 2001). Scharf *et al.* (2001) found that LTP induced by a temporally spaced tetraburst protocol of 100 Hz with a 5 min intertrain interval was larger than the LTP induced by a temporally massed tetraburst protocol of 100 Hz with a 20 sec intertrain interval. In addition to this they also found that the maintenance of LTP induced by a temporally spaced protocol was impaired much more rapidly by anisomycin than the LTP induced by the temporally massed protocol. These electrophysiological experiments were coupled with behavioural experiments which showed that temporally spaced training improved long-term memory for contextual fear conditioning to a greater extent than temporally massed training and the improvement in long-term memory in both cases was impaired with anisomycin (Scharf *et al.*, 2001). Although there is no direct evidence that L-LTP contributes to long-term memory, the similarities between the responses of both the behavioural

and the electrophysiological models to distinct temporal patterns of training and stimulation suggest that both processes may be closely related. The fact that enhanced memory and LTP are both dependent on protein synthesis also strengthens this hypothesis.

Another factor which is thought to be important for the expression of L-LTP is the phosphorylation of CREB which can be mediated by both PKA and MAP kinase (Impey *et al.*, 1998; Roberson *et al.*, 1999). In mice which express an inhibitory subunit of PKA, L-LTP induced by spaced stimulation has been shown to be impaired whereas L-LTP induced by a massed theta-burst protocol is unaffected (Abel *et al.*, 1997; Woo *et al.*, 2000a,b). In addition to this, contextual fear conditioning has been shown to be impaired after spaced, but not after massed, training in PKA mutant mice (Woo *et al.*, 2000b). Spaced stimulation has also been shown to persistently activate MAP kinase and to initiate the protrusion of new dendritic filopodia in cultured hippocampal neurons (Wu *et al.*, 2001a). All of this evidence overwhelmingly points to the fact that different stimulation protocols can recruit different intracellular cascades depending on the strength and the temporal nature of the stimulation protocol employed.

4.6 PKC

The results with BIS-1 show that LTP induction is significantly reduced but not inhibited in the presence of the PKC inhibitor. LTP also remains stable at this reduced level (~150%) for the duration of the experiment. These results suggest that PKC activation is required for the full expression of LTP induced by the multiple spaced 200 Hz protocol but that even in the presence of the PKC inhibitor this strong protocol can still induce a mild LTP which is independent of PKC activity.

A variety of other extracellularly applied PKC inhibitors such as mellitin, polymyxin B, H7, sphinosine, K-252a, staurosporine, and calphostin C have been shown to inhibit LTP induction in the hippocampus (Lovinger *et al.*, 1987; Malinow *et al.*, 1988; Colley *et al.*, 1990; Denny *et al.*, 1990). Other studies have found similar results with the intracellular application of peptide inhibitors $PKC_{19\text{-}31}$ and $PKC_{19\text{-}36}$ (Wang and Feng, 1992; Malinow *et al.*, 1989). The reliability of these early studies has however been questioned because of the lack of specificity of the inhibitors used and more recent studies have found that more potent inhibitors of

PKC such as chelerythrine, Ro-31-8220 and Gö 6983, have no effect on the induction of LTP (Bortolotto and Collingridge, 2000).

In the present study, slices were treated with BIS-1 (K_i=10nM) for the duration of the experiment. BIS-1 is a specific inhibitor of PKCα, βI, βII, γ, δ, and ε, so it is unlikely that the reduction in LTP induction is due to non-specific actions of the inhibitor. Regional and methodological differences might account for the differences between the present study and that of Collingridge and Bortolotto (2000). In contrast to the present study, the Collingridge and Bortolotto (2000) study was carried out in the CA1 and a moderate stimulation protocol of 100 Hz for 1 sec was used. It is possible that the large magnitude LTP induced with the multiple spaced 200 Hz protocol is more dependent on PKC activation than an LTP induced by a milder protocol. As a stronger stimulation protocol will produce a greater increase in postsynaptic Ca^{2+} and is also capable of supplementing the post-tetanic rise in Ca^{2+} by recruiting VDCCs (Grover and Teyler, 1990; Morgan and Teyler, 2001) the increased Ca^{2+} concentration after the multiple spaced 200 Hz protocol is likely to cause a greater activation of PKC, which would contribute to the greater magnitude of LTP. Previous studies have also demonstrated that PKC is involved in the induction of the NMDA receptor component of LTP in the dentate gyrus (O'Connor *et al.*, 1995) and that PKC subspecies can be stimulated 15 sec after tetanic stimulation (Sacktor *et al.*, 1993). A postsynaptic rise in Ca^{2+} can cause the translocation of PKCα/β/γ from the cytosol to the membrane (Angenstein and Staak, 1997) and when these PKC isozymes bind DAG they can become completely activated (Huang and Huang, 1993). The DAG necessary for PKC activation can be provided by stimulation of group I mGluRs. Stimulation of group I mGluRs which are coupled to PLC produce DAG and IP_3 and so if mGluRs are involved in the triggering of PKC activation, inhibition of mGluRs should produce similar effects on LTP as inhibition of PKC. The previous results in this study with the mGluR antagonist, LY341495, show that inhibition of mGluRs significantly reduced late-phase LTP but had no effect on LTP induction. Taken together, these results suggest that the activation of PKC might be mediated by different receptors during the time-course of the experiment, with late-phase activation being dependent on mGluRs and early-phase activation mediated by another as yet undetermined receptor or group of receptors. PKC seems to have a widespread role in synaptic plasticity as there is also strong evidence for the involvement of PKC in synaptic depression

with studies showing that PKC inhibitors can block the induction of mGluR-LTD in both CA1 and the dentate gyrus (Oliet *et al.*, 1997; Wang *et al.*, 1998).

Interestingly, the mGluR antagonist, MCPG, has been shown to block the translocation of PKCγ into the cytosol which normally occurs after the induction of LTP (Angenstein and Staak, 1997). This suggests that mGluRs might also have a modulatory role in activating PKC during LTP induction. In conjunction with this, the induction of LTP both pharmacologically, with the mGluR agonist, ACPD, and electrically, by high-frequency stimulation, has been shown to be inhibited with the PKC inhibitor $PKC_{19\text{-}31}$ (O'Connor *et al.*, 1995). Taken together with the present results showing that the induction of multiple spaced 200 Hz LTP is significantly attenuated by BIS-1, it is likely that PKC activation is mediating a phosporylation of the NMDA receptor or a site downstream of the NMDA receptor. Supporting this view are previous studies which have found that PKC inhibitors can inhibit a reversible potentiation of NMDA receptors in the CA1 (Aniksztejn *et al.*, 1992). Furthermore, phorbol esters which directly activate PKC have been found to enhance the NMDA-mediated response in dorsal spinal horn neurons and *Xenopus* oocytes (Gerber *et al.*, 1989; Kelso *et al.*, 1992) while PKC injection into terminal neurons potentiated NMDA-mediated currents and this potentiation was abolished with a peptide inhibitor (Chen and Huang, 1992).

In contrast, some studies have found that transient activation of PKC with a phorbol ester can substitute for weak synaptic activity in priming synapses to suppress LTP (Stanton, 1995) and PKC phosphorylation of mGluRs can also desensitise these receptors under certain conditions (De Blasi *et al.*, 2001). However, the role of PKC mediated phosphorylation of mGluRs appears to be quite complex as the phosphorylation of $mGluR_5$ at Thr840 is not involved in the desensitisation of the receptor but is responsible for the generation of Ca^{2+} oscillations in response to agonists in $mGluR_{5a}$-receptor expressing cells and similarly stimulation of the phosphorylated $mGluR_{1a}$ induces a single peak Ca^{2+} response (Kawabata *et al.*, 1996). Further evidence which suggests that PKC may have an important role in regulating the increase in Ca^{2+} during LTP induction has come from studies in cultured astrocytes which have shown that mGluR mediated Ca^{2+} oscillations in these cells are inhibited by PKC inhibitors (Nakahara *et al.*, 1997). An interaction between PKC and mGluR has also been implicated in the potentiation of $mGluR_5$ by NMDA receptors. NMDA receptors can potentiate group I mGluR-mediated

responses in hippocampal slices (Luthi *et al.*, 1994; Alagarsamy *et al.*, 1999) and this has been found to be dependent on the activation of calcineurin and the dephosphorylation of the PKC phosphorylation sites that participate in $mGluR_5$ desensitisation (Alagarsamy *et al.*, 1999). Recent studies have also shown that calmodulin can inhibit $mGluR_5$ receptor signalling by binding directly to the receptor at the PKC phosphorylation sites (Minakami *et al.*, 1997).

Overall, PKC seems to have a dynamic role in the regulation of second messenger signalling and the integration of the post-tetanic rise in Ca^{2+} with downstream effector molecules. The results of the present study confirm its importance in the induction and maintenance of LTP induced by a strong multiple spaced 200 Hz protocol.

The stability of the mild LTP induced in the presence of BIS-1 suggests that PKC is persistently activated by the multiple spaced 200 Hz protocol under control conditions. Persistent PKC activation may reflect the fact that PKC substrates are phosphorylated at different time points with GAP-43 phosphorylation seen to increase 10-60 min after tetanus while neurogranin phosphosphorylation increases only after 60 min (Gianotti *et al.*, 1992; Leahy *et al.*, 1993; Ramakers *et al.*, 1995; Oestreicher *et al.*, 1997). In particular, the interaction between phosphorylated GAP-43 and $PI(4,5)P_2$ can influence neurite outgrowth and growth cone steering (Laux *et al.*, 2000). GAP-43 has also been implicated in vesicular recycling (Neve *et al.*, 1998) and consistent with this, noradrenaline release from synaptosomes has been found to be dependent on phosphorylation of GAP-43 (Robinson, 1991). Although the elimination of wild-type GAP-43 does not affect LTP in CA1, the over-expression of a mutated GAP-43 which is constitutively active did result in enhanced LTP and increased paired-pulse facilitation (Hulo *et al.*, 2002). Behavioural studies have also shown that there is enhanced learning in the same mouse model that over-expresses the persistently active GAP-43 (Routtenburg *et al.*, 2000). Overall, the evidence suggests that the inhibition of GAP-43 in BIS-1 treated slices may well contribute to the attenuation of the induction and expression of multiple spaced 200 Hz of LTP.

RC3/neurogranin is a specific substrate for PKCγ which is found postsynaptically (Ramakers *et al.*, 1999). A model has been proposed whereby RC3/neurogranin can regulate calmodulin availability in dendritic spines and calmodulin in turn can regulate the ability of RC3/neurogranin to amplify the mobilisation of Ca^{2+} in

response to mGluR stimulation (Gerendasy and Sutcliffe, 1997). RC3/neurogranin can release CaM in response to influxes of Ca^{2+} and it is the relationship between phosphorylated and unphosphorylated RC3/neurogranin which determines the level of activation of CaMKII (Krucker *et al.*, 2002). Thus, PKC can also influence the induction and expression of LTP by regulating CaMKII activity through the phosphorylation of RC3/neurogranin. Inhibition of PKC such as occurs in the present study might cause a reduction in LTP induction and expression by disrupting this PKC-RC3/neurogranin-CaMKII pathway.

4.7 PKA

The results with the cell-permeable inhibitor of PKA, KT 5720, show that PKA activation is required for both the induction and the maintenance of multiple spaced 200 Hz LTP. As KT 5720 has no preferential affinity for blocking particular isoforms of PKA at the concentration used in the present study (1μM) (Kase *et al.*, 1987) this reduction of both LTP induction and maintenance cannot be attributed to any one isoform but indicates a general dependence of multiple spaced 200 Hz LTP on PKA activation.

When KT 5720 was applied before the tetanus there was an obvious but non-significant reduction in the magnitude of the EPSPs after each of the three 200 Hz trains and the ensuing LTP continued to decline rapidly with the reduction becoming significant 20 min after the 3rd 200 Hz. While there was a large reduction in LTP when KT 5720 was present throughout the experiment, LTP was not completely inhibited and EPSPs were still mildly potentiated at the end of the experiment (~125% after 2 hours). This finding is consistent with a number of recent studies which have re-examined the role of PKA in early-phase LTP and LTP induction.

Until recently it was thought that PKA did not have a role in early-phase LTP or LTP induction and both pharmacological and genetic studies reported that inhibition of the cAMP pathway did not affect early-phase LTP or LTP induction (Qi *et al.*, 1996; Abel *et al.*, 1997; Winder *et al.*, 1998). Late-phase LTP, by contrast, has been shown to be dependent on PKA activation (Frey *et al.*, 1993; Huang and Kandel, 1994). The present findings however are in agreement with more recent studies which have shown that PKA and adenylyl cyclase activation are required for both

early and late-phase LTP (Otmakhova *et al.*, 2000; Wong *et al.*, 1999; Woo *et al.*, 2003; Duffy and Nguyen, 2003).

To further investigate whether or not persistent PKA activation is required for the expression of multiple spaced 200 Hz LTP, KT 5720 was also applied immediately after the final 200 Hz stimulation. Under these conditions LTP again declined rapidly and the level of LTP for the final hour of the experiment was virtually identical to that of the previous experiment with KT 5720 present during HFS (Fig. 3.19). This result suggests that PKA activation is not only required for the induction of multiple spaced 200 Hz LTP but that persistent PKA activation is also necessary for the full expression of late-phase LTP. Previous studies in the hippocampus have not investigated the effects of PKA inhibitors applied after tetanus but biochemical studies in the CA1 have found that PKA activity is only transiently activated between 2 and 10 min and decays by 45 min after LTP-inducing HFS (Roberson and Sweatt, 1996). It is unlikely that the application of the PKA inhibitor immediately after the 3rd 200 Hz stimulation could have produced an inhibition of PKA activation within the time window of 2 to 10 mins, therefore one possibility is that the onset of PKA activation occurs at a later time point in the dentate gyrus. Another possibility is that persistent PKA activation is required for the expression of the large amplitude LTP induced by the multiple spaced 200 Hz protocol. Consistent with this, recent studies have shown that the temporal spacing of multiple trains of HFS critically modulates the PKA-dependence of LTP in CA1 and dentate gyrus (Woo *et al.*, 2003; Woo *et al.*, 2000; Nguyen and Kandel, 1996). LTP induced by two compressed protocols (four trains of 100 Hz/1sec spaced 20 sec or 3 sec apart) was normally expressed in R(AB) transgenic mice which have reduced PKA activity but LTP was impaired in R(AB) mice if a more temporally spaced tetraburst stimulation was used (four trains of 100 Hz/1sec spaced 5 min apart) (Woo *et al.*, 2003). Extrapolating from these results, a likely scenario for the present study is that the longer spacing of 10 min between each 200 Hz train induces a large amplitude LTP with an even greater dependence on PKA activation. Using R(AB) mice it is not possible to determine whether or not the LTP induced by compressed protocols represents a PKA-independent form of LTP or an LTP that requires the activation of particular isoforms of PKA that are still expressed in the hippocampus of R(AB) mice. This problem has been circumvented by the use of KT 5720 which blocks all isoforms of PKA with equal potency. Studies have shown

that the LTP induced by compressed protocols in R(AB) mice are also unaffected by KT 5720 which demonstrates that LTP does not require PKA activation under these experimental conditions (Woo *et al.*, 2003).

In contrast, KT 5720 has been shown to impair LTP induced by 4 trains of 100 Hz spaced 5 min apart in wild-type mice (Woo *et al.*, 2003). The reduction of LTP induced by temporally spaced stimulation with KT 5720 displays a similar time-course in the present study to that of Woo *et al.* (2003) indicating that the inhibition of PKA might be affecting a common PKA-dependent pathway in both the dentate gyrus and the CA1.

These studies with stronger stimulation protocols complement earlier studies which have shown that milder single 100 Hz trains are less effective at inducing PKA-dependent LTP (Huang and Kandel, 1994; Abel *et al.*, 1997). The complicated interplay between stimulation protocols and PKA activation is demonstrated by the fact that R(AB) mice have impaired LTP, with the temporally spaced protocol (four 100 Hz/1sec trains applied every 5 min) and normal LTP with a protocol of 60 pulses applied in a theta-burst pattern, while Rp-cAMPS can block the expression of LTP induced by both of these protocols (Woo *et al.*, 2000). The explanation for this is that the theta-burst stimulation protocol must be capable of recruiting isoforms of PKA containing regulatory subunits other than the mutant RIα subunit while the tetraburst protocol recruits the RIα subunit which is deficient in R(AB) mice. One possibility is that theta-burst stimulation might activate tetramers of PKA-containing RII subunits whose subcellular localisation is regulated by interactions with A-kinase anchoring proteins (Scott *et al.*, 1990; Carr *et al.*, 1991). This combination of pharmacological and genetic experiments demonstrates that different stimulation protocols are capable of recruiting different isoforms of PKA for the induction and expression of LTP.

Overall, the evidence to date suggests that the amount of stimulation and the spacing of stimulation can both critically modulate the PKA-dependence of LTP. This is in contrast to protein synthesis which has recently been shown to be required for LTP induced by both compressed and spaced tetanic stimulation (Scharf *et al.*, 2002). Different pathways have previously been shown to be differentially activated depending on the frequency and intensity of synaptic stimulation (Cavus and Teyler, 1996).

Both PKA and adenylyl cyclase can be activated when Ca^{2+} influxes through NMDA receptors during HFS (Chetkovich *et al.*, 1991; Chetkovich and Sweatt, 1993; Roberson and Sweatt, 1996) and this influx of Ca^{2+} through NMDA receptors is critical for LTP induced by 100 Hz trains in the CA1 (Collingridge *et al.*, 1983). However, the possibility that temporally spaced stimulation might alter the NMDA-dependence of LTP was ruled out by the finding that both temporally spaced and temporally compressed protocols are both equally dependent on NMDA receptor activation (Woo *et al.*, 2003).

A contributory factor in the greater PKA-dependence of LTP induced by temporally spaced stimulation may be the reduced activation of inhibitory synaptic pathways with this protocol in comparison with more compressed protocols. The enhanced synaptic excitation due to decreased activation of inhibitory pathways may have a greater PKA-dependence.

PKA has also been shown to have an important role in ERK and CREB pathways. Both PKA and receptors coupled to PKA such as β-adrenergic receptors have been found to activate ERKII (Roberson *et al.*, 1999) which can lead to new protein synthesis. There is added complexity in the PKA-ERK cascade as PKA inhibits the Ras-Raf1 wing of the ERK pathway but activates the Rap1-B-Raf pathway (Vossler *et al.*, 1997). The sustained large amplitude LTP induced multiple spaced 200 Hz protocol may reflect an enhanced activation of ERK through PKA stimulation which would contribute to the synthesis of new proteins that sustain late-phase LTP. The transcription factor CREB can also be directly phosphorylated by PKA at serine 133 (Enslen *et al.*, 1994) and phosphorylated CREB can activate the transcription factor *c-fos* and the immediate early gene *zif268* (Goodman, 1990; Hergen and Leah, 1998). Thus, downstream of PKA activation there is a diverse range of cascades which can contribute to the expression and maintenance of large amplitude LTP induced by multiple spaced 200 Hz protocol. Confirming this is the finding that, while PKA can activate CREB directly, the forskolin induced activation of CREB is inhibited by the ERK inhibitor U0126 (Roberson *et al.*, 1999). This indicates that PKA can influence CREB-mediated transcriptional activation both directly and indirectly.

Late-phase LTP which is dependent on transcription can be induced in all three hippocampal regions with forskolin application (Huang and Kandel, 1994; Huang *et al.*, 1994; Nguyen *et al.*, 1994) and CREB and ERK are also increased in the dentate

gyrus after acute forskolin application (Sarvey and Voulalas, 1995; Voulalas and Sarvey, 1995). Therefore, the application of forskolin which activates PKA can prepare the neuron with the molecular machinery necessary for new protein synthesis which supports late-phase LTP.

Other targets for PKA-mediated phosphorylation are GluR1 and AMPA receptors (Banke *et al.*, 2000; Lee *et al.*, 2000; Wang *et al.*, 1991). The phosphorylation of GluR1 is thought to be particularly important for the bidirectional regulation of plasticity (Banke *et al.*, 2000; Lee *et al.*, 2000). NMDA receptor mediated currents can also be increased after PKA phosphorylation (Raman *et al.*, 1996; Leonard and Hell, 1997) and in addition to this, PKA has been shown to be linked to NMDA receptors by the synaptic scaffolding protein yotiao (Westphal *et al.*, 1999). The PKA-mediated phosphorylation of inhibitor-1 (I-1) is an important gating mechanism for the regulation of LTP induction and expression. Phosphorylated I-1 suppresses the activity of protein phosphatase-1 (PP-1) and consequently protects CaMKII from inhibition by PP-1, thus ensuring the successful induction and expression of LTP (Blitzer *et al.*, 1995, 1998; Brown *et al.*, 2000). More recently, CREB phosphorylation has been shown to be inhibited when fusion proteins containing PKI and nuclear localisation signals are delivered into the nuclear compartment of neurons (Matsushita *et al.*, 2001). This inhibition of CREB phosphorylation caused a block of late-phase LTP and indicates that PKA-mediated phosphorylation of CREB might be a critical target for the expression of late-phase LTP (Impey *et al.*, 1996; Matsushita *et al.*, 2001).

The results of the present study complement previous studies investigating the relationship between LTP induced by different stimulation protocols. This study also extends previous findings in so far as it demonstrates that the full expression of large amplitude LTP induced by multiple spaced 200 Hz requires PKA activation both during and after the high-frequency stimulation.

More recent studies have addressed the question of the cellular locus of PKA activation which is necessary for the expression of LTP. In one study injection of cell-impermeant inhibitors of PKA or adenylyl cyclase were found to have no effect on early-phase LTP but these experiments did not continue beyond 40 min post-tetanus so it was impossible to determine whether or not PKA was required for late-phase LTP (Otmakhova *et al.*, 2000). Postsynaptic injection of Sp-cAMPS which activates PKA produces mild synaptic facilitation while postsynaptic injection of

Rp-cAMPS which inhibits PKA results in an impairment of late-phase LTP in the Schaffer collateral pathway (Blitzer *et al.*, 1995). While these studies point to a postsynaptic location for PKA activation, the possibility of a presynaptic locus of activation cannot be ruled out because both of these drugs are cell-permeable. A recent study however has employed a cell-impermeant PKA inhibitor (PKI_{6-22}) and found that postsynaptic injection of this inhibitor causes a significant reduction of LTP induced by four 100 Hz trains at 5 min intervals within 1.5 hours (Duffy and Nguyen, 2003). Postsynaptic injection of PKI_{6-22} did not however have any effect on LTP induced by a single 100 Hz train (Duffy and Nguyen, 2003). This is consistent with the body of evidence which indicates that PKA is differentially activated by different stimulation protocols (Frey *et al.*, 1993; Matthies and Reymann, 1993; Huang and Kandel, 1994; Blitzer *et al.*, 1995; Impey *et al*, 1996; Abel *et al.*, 1997; Woo *et al.*, 2000; Woo *et al.*, 2003). Furthermore, a postsynaptic injection of the constitutively active PKA catalytic subunit, C_{α}, caused a long-lasting synaptic facilitation in CA1 pyramidal neurons (Duffy and Nguyen, 2003). Confirming this hypothesis of a postsynaptic locus of PKA activation are studies which have shown that the PKA-dependent synthesis of postsynaptic AMPA receptors is required to support the induction of LTP by multiple trains of high-frequency stimulation (Nayak *et al.*, 1998).

4.8 CaMKII

The results from the present set of experiments have shown that the induction of multiple spaced 200 Hz LTP is significantly reduced in the presence of the specific CaMKII inhibitor KN-62. After the slices were incubated in 3μM KN-62, LTP was ~30% below control immediately after the 3rd 200 Hz and it declined to a level that was ~45% below control by 140 min. Another set of experiments was then carried using an alternative CaMKII inhibitor, KN-93. Again, peak LTP after induction was reduced (by ~28%) but in this case the reduction was not significant. The decline of LTP in the presence of KN-93 was not as great as that seen with KN-62 and at 140 min post-tetanus, LTP in KN-93 treated slices was ~28% below the control level. Previous studies have found that KN-62 blocks the induction of LTP in CA1 (Ito *et al.*, 1991; Bortolotto and Collingridge, 1998) while LTP in mossy fibre CA3 synapses was unaffected by KN-62 (Ito *et al.*, 1991). There has only been one

previous study investigating the effects of KN-93 on LTP in the hippocampus and that study found that KN-93 blocked the induction of LTP in CA1 (Kasahara *et al.*, 2001). It is difficult however to draw comparisons between the present study and that of Kasahara *et al.* (2001) as they used a very high concentration of KN-93 (50μM) which blocks both CaMKIV and CaMKII whereas the much lower concentration of KN-93 (3μM) used in the present study selectively blocks only CaMKII. KN-93 has a K_i value of 370nM for CaMKII (Fan *et al.*, 1997; Fan *et al.*, 1999) so the concentration of 3μM used in the present experiment should be sufficient to block CaMKII. KN-62 has a K_i value of 900nM for CaMKII and it competes with Ca^{2+}/CaM which might also enable it to inhibit other calmodulin-sensitive enzymes (Fan *et al.*, 1999; Kidaka and Yokokura, 1996). The possible inhibition of other calmodulin-sensitive enzymes besides CaMKII with KN-62 might account for its greater reduction of LTP induction and expression in comparison to KN-93.

The most likely explanation for the difference between the effect of the CaMKII inhibitors on LTP induction in the present experiments and that of previous studies is the nature of the stimulation protocol employed. Mild stimulation protocols of 100 Hz for 1 sec (Bortolotto and Collingridge, 1998) or 2 trains of 100 Hz for 1 sec with 20 sec intertrain intervals (Kasahara *et al.*, 2001) were used in the previous studies while the present study employed a stronger intensity protocol of multiple 200 Hz trains with a 10 min spacing between each train. It is possible that both the strong intensity and the spaced nature of this protocol elicits a large amplitude LTP which is only partially dependent on CaMKII activation for its induction and maintenance. The large post-tetanic rise in Ca^{2+} concentration after such a protocol may well activate a greater number of kinase cascades than milder stimulation protocols and under these conditions the inhibition of CaMKII could be compensated for by other kinases and receptors such as PKA, PKC, CaMKIV, mGluRs, mAChRs and adrenergic receptors.

Previous studies have consistently shown a block of LTP induction with peptide inhibitors of CaMKII but again these studies used either much milder stimulation protocols than the one used in the present study or else a pairing protocol where low-frequency stimulation is coupled with depolarisation of the postsynaptic membrane (Malinow *et al.*, 1989; Wang and Kelly, 1995; Otmakhov *et al.*, 1997; Hvalby *et al.*, 1994). The results with the pairing protocol rule out the possibility

that CaMKII inhibitors block LTP induction by interfering with the postsynaptic depolarisation rather than the core processes downstream of NMDA receptors (Otmakhov *et al.*, 1997). Inhibitors of calmodulin have also been found to block both CaMKII activation and LTP induction (Malenka *et al.*, 1989; Liu *et al.*, 1999). From the foregoing it is evident that there is a wealth of evidence derived from a pharmacological approach implicating a role for CaMKII in LTP induction. The results of the present study suggest that the dependence of LTP on CaMKII activation is correlated with the strength of the high-frequency stimulation protocol and that the induction of large magnitude LTP induced by multiple spaced 200 Hz is only partially dependent on CaMKII activation. While the induction of multiple spaced 200 Hz LTP is not completely inhibited in the presence of either KN-62 or KN-93, it should still be noted that there is a large decrease in peak LTP and in late-phase LTP in the presence of both drugs.

One problem with the pharmacological approach is that CaMKII inhibitors can probably inhibit CaMKI and CaMKIV to some extent (Lisman *et al.*, 2002). The roles of these CaMK subtypes are still not well known but CaMKIV has been shown to activate CREB and it is thought to regulate gene expression in a neuronal activity-dependent manner (Bito *et al.*, 1996). In addition to this, CaMKIV-deficient mice exhibit reduced CREB phosphorylation and also reduced potentiation at a later stage of CA1 LTP (Ho *et al.*, 2000; Riber *et al.*, 2000). Therefore, if CaMKIV is inhibited by KN-62 and KN-93 in the present experiments, it might contribute to a reduction in late-phase LTP through an inhibition of CREB-dependent protein synthesis. In contrast to the long lasting activation of CaMKII which is seen after LTP induction (Fukunaga *et al.*, 1993; Fukunaga *et al.*, 1995), CaMKIV shows a more transient activation after high-frequency stimulation (Fukunaga *et al.*, 1992; Kasahara *et al.*, 1999; Kasahara *et al.*, 2001). One possible explanation for this difference in time scale between the two subtypes is that a large increase in Ca^{2+} concentration will activate CaMKIV and its dephosphorylating enzyme, PP2B, simultaneously which would cause CaMKIV to be rapidly dephosphorylated (Kasahara *et al.*, 1999). In contrast, CaMKII cannot be deposphorylated by this phosphatase (Goto *et al.*, 1985).

The role of CaMKII in LTP has been confirmed with genetic studies using single amino acid modifications of the Thr286 phosphorylation site of CaMKII. When Thr286 is replaced with alanine CaMKII can exhibit Ca^{2+}-dependent activity and it

is unable to become persistently activated. In agreement with previous pharmacological experiments, LTP was almost completely blocked in this mouse model (Giese *et al.*, 1998). Again, these studies suggest that KN-93 and KN-62 are not producing their effects through an inhibition of the generation of dendritic spikes or an inhibition of the summation of synaptic inputs required for LTP induction but that the disruption of CaMKII affects a critical cascade that is necessary for LTP production.

From the present set of experiments it is not possible to distinguish between a pre- or postsynaptic locus of activity for CaMKII but there is evidence that CaMKII can mediate its effects postsynaptically by enhancing AMPA receptor currents (Malenka and Nicoll, 1999). The postsynaptic injection of CaMKII or the expression of a constitutively active CaMKII causes potentiation which occludes with tetanus-induced LTP (Petit *et al.*, 1994; Lledo *et al.*, 1995; Shirke and Malinow, 1997) and this potentiation is thought to be mediated by direct phosphorylation of GluR1 leading to an increase in conductance through these channels as well as the insertion of new GluR1 subunits into the synapse (Benke *et al.*, 1998; Derkach *et al.*, 1999; Hayashi *et al.*, 2000). Similar to LTP, the potentiation induced by injection of CaMKII causes an increase in quantal size as measured by the amplitude of spontaneous EPSCs and a decrease in the probability of failure rate which is the probability that a presynaptic action potential will fail to produce a postsynaptic response (Lledo *et al.*, 1995). The decrease in failure rate is due to the fact that, before LTP, vesicles are released at silent synapses which contain NMDA receptors but no AMPA receptors and are therefore functionally silent during baseline activity, whereas after LTP, these previously silent synapses become active by the insertion of AMPA receptors into the membrane (Liao *et al.*, 1995).

An important mechanism for CaMKII-mediated potentiation is its binding to the NMDA receptor in the PSD. Once it binds to the NR2B subunit it remains active even after the dissociation of Ca^{2+}/calmodulin (Bayer *et al.*, 2001). This activation is transient and lasts for seconds to minutes but is likely to have important consequences as it cannot be reversed by a phosphatase and it causes "trapping" of the kinase whereby a secondary autophosphorylation or the CaM-binding domain is prevented. This transient activation also facilitates further autophosphorylation around the ring of the CaMKII molecule. Overall, the binding of CaMKII to

NMDAR facilitates further autophosphorylation of the kinase which will strengthen the binding of CaMKII to NMDA receptors.

Another way in which CaMKII contributes to LTP is via the phosphorylation of AMPA receptor subunit GluR1 at serine 831. After phosphorylation, the conductance through these channels increases by about 50% (Barria *et al.*., 1997; Mammen *et al.*, 1997; Derkach *et al.*, 1999). Both an NMDA receptor antagonist and the same CaMKII antagonist used in the present study, KN-62, have been shown to block this phosphorylation of GluR1. Interestingly, the time-course of phosphorylation of GluR1 suggests that CaMKII is persistently activated as phosphorylation is increased both 30 min and 1 hour after LTP induction (Lee *et al.*, 2000).

In addition to the phosphorylation of GluR1, CaMKII also has a role in AMPA receptor insertion into the membrane and the capture of AMPA receptors at synaptic sites. While these mechanisms are still under investigation experiments have already demonstrated the transformation of a silent synapse into a functionally active synapse after the induction of LTP and the insertion of new receptors into membranes (Hayashi *et al.*, 2000).

After LTP, AMPA receptors have been shown to move from dendrites to spines and to insert themselves into the membrane by a process of exocytosis (Lledo *et al.*, 1998; Shi *et al.*, 1999). Studies have used the lipophilic dye FM1-43 to demonstrate that inhibitors of CaMKII block this exocytosis while constitutively active CaMKII stimulates the process (Maletic-Savatic *et al.*, 1998). The mechanisms of AMPA receptor anchoring into the membrane are thought to be dependent on translocation of CaMKII into the PSD and its subsequent binding to NMDA receptors (Liao *et al.*, 2001; Lisman and Zhabotinsky, 2001).

Whether or not CaMKII is involved in the maintenance of LTP after high-frequency stimulation has not yet been fully resolved. In the present study the CaMKII inhibitors were present throughout the experiment so it was not possible to determine whether or not the decline of LTP after tetanus was due to disruption of the induction process or to inhibition of the persistent activation of CaMKII. In the presence of KN-62, LTP declined by ~30% over a 2 hr period after the final 200 Hz stimulation whereas the control LTP declined by only ~20% over the same time period. However, in the presence of KN-93, LTP exhibited a much smaller decline of ~15% over a 2 hour period after the final 200 Hz stimulation. It is possible that a

higher concentration of KN-93 is required in order to produce the same magnitude of decline as that seen in KN-62 treated slices. Another possibility is that KN-62 might be inhibiting other CaMKs such as CaMKI and CaMKIV to a greater extent than KN-93. Experiments with both CaMKII inhibitors added after the final tetanus are required to further elucidate the role of CaMKII in the maintenance of multiple spaced 200 Hz LTP. Most studies to date have found that the postsynaptic application of a CaMKII inhibitor do not affect the maintenance of LTP (Fukunaga *et al.*, 1995; Malinow *et al.*, 1989; but see Feng, 1995). A mutation of Thr286 prevents the persistent activation of CaMKII and causes an inhibition of the induction of LTP but again a distinction cannot be made between short-term and long-term persistent activation with this model. The autophosphorylation of CaMKII might extend the activation of the kinase to a few minutes which would be long enough to trigger persistent downstream mechanisms which are the actual source of memory storage.

However, this scheme is difficult to reconcile with recent studies which have shown that GluR1 can be phosphorylated for up to 1 hour by CaMKII after LTP induction (Lee *et al.*, 2000). Other studies have shown that depotentiation causes a decrease in the phosphorylation level of Thr286 (Huang *et al.*, 2001). These elaborate mechanisms suggest that persistent CaMKII phosphorylation has an important role to play in the maintenance of LTP. While the application of CaMKII inhibitors after the induction of LTP have not as yet confirmed this, one possible explanation for their lack of effect on LTP maintenance could be that there is not enough phosphatase activity to dephosphorylate CaMKII during the time-course of these experiments. This proposal has been confirmed recently with the finding that a 15 minute application of the general kinase inhibitor H7 reduced the phosphorylation of several kinase proteins but not CaMKII in the PSD (Gardoni *et al.*, 2001). Theoretical studies have also estimated that it would take more than a day for all CaMKII to become dephosphorylated (Lisman and Zhabotinsky, 2001). In order to fully elucidate the role of CaMKII in the maintenance of LTP future experiments will probably have to be carried out over much longer time-courses to enable PP1 to dephosphorylate CaMKII.

Overall, the results from the present experiments show that while CaMKII has a role in the induction of LTP, its inhibition cannot completely block induction by the multiple spaced 200 Hz protocol. This finding extends the work of previous studies

which have shown that inhibitors of CaMKII completely block the induction of LTP by milder stimulation protocols and suggests that the activation of a large number of cascades by the stronger spaced protocol may result in a certain amount of redundancy in the processes that underlie induction and consequently induction can be rescued by cascades which are unaffected by the CaMKII inhibitors.

4.9 MAP kinase

The results from the present experiments show that the dependence of LTP on ERK MAP kinase (referred to here as MAP kinase) activation is related to the intensity and the temporal nature of the stimulus protocol used to elicit LTP. In all experiments PD98059 (a specific inhibitor of MAP kinase kinase or MEK) was used at a concentration of 50μM which has previously been shown to inhibit MAP kinase activation (Alessi *et al.*, 1995; Pang *et al.*, 1995; English and Sweatt, 1997). The application of PD98059 had no effect on the mild amplitude, decremental LTP induced by a conventional protocol of 8 trains of 200 Hz with a 2 sec intertrain interval. In contrast, the application of PD98059 inhibited the large amplitude non-decremental LTP induced by the multiple spaced 200 Hz protocol. In these experiments LTP declined rapidly after the 3rd 200 Hz train and after 2 hours EPSPs were not significantly different from baseline levels. In order to distinguish between an interference of MAP kinase activation during the induction or the expression of multiple spaced 200 Hz LTP, PD98059 was applied immediately after the 3rd 200 Hz or else during the baseline and then washed out immediately after the 3rd 200 Hz. When PD98059 was applied immediately after the 3rd 200 Hz there was a rapid decline in LTP, similar in time-course to that seen when PD98059 was applied throughout the experiment. Indeed, a comparison of these two sets of experiments revealed no significant difference between them at any point during the recording (Fig. 3.25). These results indicate that in both cases PD98059 is primarily exerting its influence by interfering with the activation of MAP kinase after the induction process. This was confirmed by experiments where PD98059 was present during the baseline and the three spaced 200 Hz trains of stimulation but was washed out immediately after the final tetanus. Under these conditions, LTP was successfully induced and was not significantly different from control LTP at any point during the 2 hour experiment.

Overall, these results indicate that in the dentate gyrus the activation of MAP kinase is determined by the type of protocol used to elicit LTP and that large amplitude LTP has a greater dependence on MAP kinase activation than mild amplitude LTP. In addition to this, the results presented here further elucidate the nature of MAP kinase activation in multiple spaced 200 Hz LTP by demonstrating that MAP kinase activation is not required for the induction of this non-decremental LTP but that it is necessary for its expression. The variable activation of MAP kinase depending on the stimulation protocol used to induce LTP may help to explain some of the differences between studies using inhibitors of MAP kinase. A number of previous studies have shown that HFS-induced LTP is not affected by MEK inhibitors, indicating that MAP kinase activation is not essential for LTP (Opazo *et al.*, 2003; Winder *et al.*, 1999; Watabe *et al.*, 2000; Kasahara *et al.*, 2001) while other studies have shown that hippocampal LTP is inhibited by MEK inhibitors (English and Sweatt, 1997; Atkins *et al.*, 1998; Coogan *et al.*, 1999; Winder *et al.*, 1999; Watabe *et al.*, 2000; Giovanni *et al.*, 2001). It is interesting to note that previous studies have also found differences between the MAP kinase-dependence of LTP induced by HFS protocols and brief theta frequency stimulation protocols (Winder *et al.*, 1999; Watabe *et al.*, 2000). In these studies, MEK inhibitors had no effect on LTP induced by 100 Hz stimulation whereas LTP induced by a 30 sec stimulation of 5 Hz was rapidly inhibited in the presence of the same inhibitors (Winder *et al.*, 1999; Watabe *et al.*, 2000). These results indicate that the activation of MAP kinase is critically dependent on the type of stimulation paradigm used (i.e. high-frequency stimulation protocols or protocols modelled on the theta frequency) as well as the duration or temporal spacing of a particular paradigm. Furthermore, a number of these previous studies concluded that MAP kinase was involved in the induction of LTP based on experiments in which the MAP kinase inhibitor was present both prior to and following high-frequency stimulation (English and Sweatt, 1997; Atkins *et al.*, 1998; Winder *et al.*, 1998; Coogan *et al.*, 1999; Liu *et al.*, 1999; Kanterewicz *et al.*, 2000; Watabe *et al.*, 2000; Giovanni *et al.*, 2001). Such a protocol cannot however distinguish between the induction and expression of LTP. In the present experiments in which PD98059 was present prior to high-frequency stimulation but was washed out immediately after the final tetanus, LTP was not inhibited, clearly demonstrating that the MAP kinase cascade is not involved in the induction of multiple spaced 200 Hz LTP.

Recent studies in cultured pyramidal neurons and dentate gyrus explants have revealed very similar results to the present findings in relation to the effects of temporal spacing of stimulation on the activation of MAP kinase (Wu *et al.*, 2001a). A single stimulation of the cultured neurons by 3 min depolarisation was found to produce the same initial peak MAP kinase phosphorylation as 4 trains of 3 min depolarisation with a 10 min intertrain interval but the spaced stimulation generated a prolonged phosphorylation of MAP kinase, whereas the single stimulation generated a phosphorylation of MAP kinase which returned to baseline levels within 30 min (Wu *et al.*, 2001a). Similar to the single stimulation, a massed stimulation of 12 min depolarisation only produced a transient activation of MAP kinase while a prolonged 42 min stimulation was not as effective as the spaced stimulation at producing a sustained increase in MAP kinase activation (Wu *et al.*, 2001a). Furthermore, the application of PD98059 immediately after the spaced stimulation reduced MAP kinase phosphorylation very rapidly during the first 20 min post-stimulation and phosphorylation returned to baseline levels at 50 min post-stimulus. This time-course of decline of phosphorylated MAP kinase is very similar to the decline in LTP seen in the present study when PD98059 was added after the final tetanus (Fig. 3.24). Together, these results suggest that spaced stimulation has a common mode of action in both pyramidal cell cultures and hippocampal slices. In addition to this, the fall in MAP kinase phosphorylation with PD98059 in cultured neurons strongly indicates that the reduction in LTP seen with the same inhibitor is also due to the inhibition of persistent MAP kinase activation.

Pharmacological investigation of the initial peak in MAP kinase phosphorylation revealed that it was dependent on L-type voltage-dependent Ca^{2+} channels (VDCCs), CaM kinase, Ras and MEK pathways and independent of PKA and PKC cascades (Wu *et al.*, 2001a). The dependence of the initial peak on VDCCs is particularly relevant to the present study as strong 200 Hz-LTP has previously been shown to be dependent on VDCCs (Grover and Teyler, 1990; Cavus and Teyler, 1996; Morgan and Teyler, 2001). It is therefore likely that the multiple spaced 200 Hz protocol used in the present study elicits an LTP with a strong VDCC component and the large influx of Ca^{2+} through these channels is possibly one of the pathways used to rapidly phosphorylate MAP kinase. The hypothesis that CaMK can regulate the initial peak in MAP kinase phosphorylation is consistent with previous studies which have shown that the CaM kinase kinase (CaMKII)/CaMKIV

cascade can strongly activate MAP kinase (Enslen *et al.*, 1996). However, more recent studies have indicated that both of these kinases have very distinct roles in the temporal regulation of synaptic transmission after a depolarisation of cultured neurons or a tetanus delivered to a hippocampal slice (Deisseroth *et al.*, 1996; Hardingham *et al.*, 1999; Ho *et al.*, 2000; Riber *et al.*, 2000; Wu *et al.*, 2001b).

Both CaMK and MAP kinase can activate CREB which is a key regulator of gene expression but CaMK can rapidly activate CREB independently of MAP kinase during the first 10 min post-stimulus while it can also initiate a slower cascade which activates CREB at 60 min post-stimulus using MAP kinase as an intermediate (Wu *et al.*, 2001b). There is also an intermediate phase at 30 min post-stimulus where both the MAP kinase-independent and MAP kinase-dependent pathways contribute equally to the activation of CREB (Wu *et al.*, 2001b). The fast activation of CREB is thought to be mediated by the CaMKIV cascade (Bito *et al.*, 1996) while the later onset dual CaMK-MAP kinase activation of CREB is thought to be due to the translocation of MAP kinsase to the nucleus where it mediates the phosphorylation of CREB indirectly through Rsk2 (Finkbeiner *et al.*, 1997; Martin *et al.*, 1997; Impey *et al.*, 1998). Sustained Ca^{2+} influx after prolonged stimulation has also been shown to inactivate a PP-1 mediated dephosphorylation of CREB which is promoted by calcineurin (Bito *et al.*, 1996; Liu and Graybiel, 1996).

The delayed nature of MAP kinase mediated phosphorylation of CREB is accounted for by a number of factors including the slow kinetics of phosphorylated MAP kinase formation, the large number of intermediates that lie upstream of phosphorylated MAP kinase and the necessity of recruiting Rsk2 for the phosphorylation of CREB.

As some studies have argued that CREB phosphorylation is mediated predominantly by MAP kinase (Impey *et al.*, 1998) while others have shown that in CaMKIV mutant mice there is a decrease in CREB phosphorylation at both early and late time points (Ho *et al.*, 2000; Riber *et al.*, 2000), the most likely solution to these conflicts lies in a cross-talk between CaMK and MAP kinase. One possibility is that CaMKII can regulate MAP kinase signalling by phosphorylating SynGAP which would result in the disinhibition of Ras (Chen *et al.*, 1998; Kim *et al.*, 1998). Another possibility is that while CaMKIV normally stimulates MAP kinase (Enslen *et al.*, 1996) this activation of MAP kinase is lost when CaMK inhibitors are applied to slices or cultured neurons. Consistent with this hypothesis is the finding that both

CaMKIV and MAP kinase 1 and 2 are present in the nucleus and cytoplasm of pyramidal cells (Deisseroth *et al.*, 1996; Deisseroth *et al.*, 1998; Martin *et al.*, 1997).

Another feature of the differential regulation of CREB phosphorylation is that milder stimulation protocols which lead to a modest increase in postsynaptic Ca^{2+} only lead to an activation of the CaMK pathway whereas stronger stimulation protocols which cause a greater rise in postsynaptic Ca^{2+} concentration recruit both pathways and cause a more pronounced increase in CREB phosphorylation (Wu *et al.*, 2001b). This is consistent with the findings from the present study which clearly show that PD98059 had no effect on the LTP elicited by a mild stimulation protocol whereas it attenuated the LTP produced by strong multiple trains of high frequency stimulation. In this way the differential regulation of the MAP kinase and CaM kinase signalling pathways may be useful for conferring information about the strength of the stimulus to the nucleus. Regarding the induction of LTP, the selective activation of the CaMK cascade for the first 10 min after a stimulation suggests that the induction process is primarily concerned with the activation of this cascade and not the activation of the MAP kinase cascade which comes online after the immediate early-phase of LTP (i.e. >10 min post-stimulation). This hypothesis is also consistent with the present results which show that PD98059 has no effect on the induction of LTP whereas it attenuates LTP when applied immediately after the multiple spaced 200 Hz protocol.

Another possible use for the employment of signalling pathways with fast and slow kinetics is in the computation of the distance between the source of the stimulus and the nucleus. A short delay between the arrival of the two signalling pathways would indicate that the stimulus originated from a nearby synapse whereas a longer delay between the arrival of the cascades would indicate that the origin of the stimulus was further away.

While the results clearly show that persistent MAP kinase activation is required for the expression of LTP (Fig. 3.24), the next question to address is the mechanism or mechanisms which underlie this persistent activation of MAP kinase. One possibility is that MEK might be constitutively activated after synaptic stimulation. This however, is unlikely as PD98059 directly inhibits the activation of MEK as opposed to its ability to phosphorylate substrates (Favata *et al.*, 1998; Dudley *et al.*, 1995). The autophosphorylation of the kinase is a more plausible explanation for the

persistent activity of MAP kinase especially when considered in the light of previous studies which have shown that MAP kinase can be autophosphorylated after the induction of LTP in a similar fashion to the autophosphorylation of CaMKII (Murphy *et al.*, 1994; Lisman *et al.*, 2002).

Other kinases such as phosphatidylinositol 3-kinase (PI3-kinase) and PKC have recently been shown to be persistently activated after the induction of LTP (Sanna *et al.*, 2002; Ling *et al.*, 2002). PI3-kinase is a Ras effector and pharmacological inhibition of this kinase has been shown to disrupt LTP (Kelly and Lynch, 2000; Lin *et al.*, 2001; Raymond *et al.*, 2002; Sanna *et al.*, 2002). Furthermore, activation of MAP kinase 2 by stimulation of NMDA receptors has been shown to be completely dependent on PI-3 kinase activity in cultured neurons (Chandler *et al.*, 2001; Perkinton *et al.*, 2002) and the PI-3 inhibitor, LY294002, has also been shown to inhibit established LTP but not the induction of LTP in the CA1 (Sanna *et al.*, 2002). A VDCC-dependent LTP induced by a strong stimulation protocol was also found to be reduced by a PI3-kinase inhibitor when it was applied after the stimulus (Sanna *et al.*, 2002). Thus, the effects of PI3-kinase inhibitors on LTP are very similar to those of PD98059, indicating that PI3-kinase which lies upstream of MEK may be an important form of biochemical memory which contributes to the persistent activation of MAP kinase.

Earlier studies have shown that the inhibition of most forms of PKC during or immediately after a stimulation protocol prevents the induction of LTP whereas the same inhibitors do not have any effect on LTP once it has been successfully established (Denny *et al.*, 1990). However, more recent studies have shown that the activity of the autonomously active, independent catalytic domain of the atypical PKC isosyme, PKMζ, is increased during the maintenance of LTP (Ling *et al.*, 2002). Similar to inhibitors of MAP kinase, two inhibitors of PKMζ, chelerythrine and myristoylated ζ-pseudosubstrate peptide, were both found to inhibit the expression of LTP when applied after LTP induction (Ling *et al.*, 2002). However, in contrast to MAPK, PKMζ was also shown to be involved in the induction of LTP as application of recombinant PKMζ directly induced LTP (Ling *et al.*, 2002).

It should also be noted that persistent PKMζ activity is achieved by the proteolytic cleavage of the catalytic domain of PKC and not by autophosphorylation. There are a number of distinctions between these two processes which confer them with different roles in the regulation of potentiation. For example, proteolytic cleavage is

irreversible whereas autophosphorylation can be reversed by phosphatases. It is possible that different induction protocols can selectively recruit one or other of these mechanisms and the activation of proteolysis might result in the development of a non-decremental LTP which cannot undergo depotentiation.

While CaMKII has consistently been shown to be involved in the induction of LTP (Malinow *et al.*, 1989; Lisman *et al.*, 2002), its role in the expression of LTP is less certain. CaMKII can be autophosphorylated and subsequently become independent of Ca^{2+} (Lisman *et al.*, 1985). As discussed in more detail in a previous section, this property of autophosphorylation has led to the proposal that CaMKII is a "molecular switch" which can store long-term synaptic information (Lisman, 1985; Lisman *et al.*, 2002). Although CaMKII activity has been shown to persist for at least 1 hour after the induction of LTP, the application of CaMKII inhibitors after the induction of LTP has no effect on its subsequent expression (Malinow *et al.*, 1989; Otmakov *et al.*, 1997; Lisman *et al.*, 2002). One hypothesis that has been put forward as an explanation for this is that the phosphatase activity in the postsynaptic density might not be sufficient to dephosphorylate CaMKII (Otmakov *et al.*, 1997; Lisman *et al.*, 2002). The reverse of this might be true for MAP kinase, i.e. the concentration of MAP kinase phosphatases might be at a high enough level to cause the dephosphorylation of MAP kinase.

The further elucidate the nature of the persistent activation of MAP kinase, PD98059 was added to the slice 30 min after the induction of LTP and was washed out after 1 hour. Established LTP was again rapidly reduced but the washout of PD98059 resulted in a reappearance of the maintenance of LTP. This experiment demonstrated that the effect of PD98059 was reversible and also that MAP kinase activation was required for the expression but not the maintenance of LTP induced by the multiple spaced 200 Hz protocol. The persistent signal underlying LTP can be maintained even during an interruption of its expression by PD98059 but once the inhibitor is removed LTP is restored because the inhibitor is only capable of blocking the expression of the signal, it is unable to block the underlying maintenance of the signal.

This distinction between the maintenance and the expression of LTP was first proposed by Malinow *et al.* (1988) who found that the wide-spectrum kinase inhibitor, H-7, could reduce LTP in a reversible manner. They concluded that the mechanisms underlying the maintenance of LTP were not affected by the kinase

inhibitor despite the fact that LTP expression was inhibited by H-7 (Malinow *et al.*, 1988). The results from the present study extend those of Malinow *et al.*, by demonstrating the reversibility of LTP with a specific inhibitor of MEK rather than a wide spectrum kinase inhibitor. The results also suggest that the pharmacological profile of LTP changes after the induction of LTP as PD98059 has no effect on LTP when it is washed out after the induction protocol whereas it rapidly attenuates LTP when applied after the final tetanus. It is interesting to note that the inhibition of both a VDCC-dependent and an NMDA-dependent LTP with the PI-3 kinase, LY294002, was also found to be reversible. As PI-3 kinase is a Ras effector and Ras in turn can activate MAP kinase it is possible that PI-3 kinase can act as a persistent upstream activator of MAP kinase. The fact that LTP recovers after the washout of PD98059 also suggests that the persistent signal does not require the continuous activation of a feedback loop involving MAP kinase or another downstream effector and this further strengthens the idea that an upstream activator of MAP kinase such as PI-3 kinase may be responsible for the persistent signal.

The activation of MAP kinase by multiple spaced stimuli has also been shown to have an important role in the development of new dendritic filopodia. Both electrical and chemical stimulation facilitate the growth of mature spines and new filopodial processes on the dendrite (Maletic-Savatic *et al.*, 1999; Engert and Bonhoeffer, 1999; Toni *et al.*, 1999; Wu *et al.*, 2002a) and these morphological changes are inhibited when dendrites are exposed to MEK inhibitors (Wu *et al.*, 2002a). It has also been discovered that once the new structures are formed, acute application of a MEK inhibitor does not cause them to collapse. This indicates that they are not dependent on ongoing MAP kinase activity for their stability (Wu *et al.*, 2002a). This dependence of the structural morphology on MAP kinase for the expression but not the maintenance of filopodial extensions strongly correlates with the dependence of LTP on MAP kinase for the expression but not the maintenance of enhanced potentiation. Taken together, the evidence suggests that spaced stimulation may give rise to non-decremental LTP through the MAP kinase-dependent formation of new spines which become independent of MAP kinase activity once they are fully formed. MAP kinase activation might also have a role in the regulation of activity-dependent clustering of synapses and the bifurcation of existing spines (Edwards, 1995; Kavalali *et al.*, 1999; Toni *et al.*, 1999). MAP kinase could mediate its effects through an effector such as eIF4e which would

promote local protein synthesis from globally distributed mRNAs (Raught and Gingras, 1999; Schuman, 1999). Another possibility is that after temporally spaced stimulation the bifurcation of existing spines and the development of new filopodia facilitate an interaction with a nuclear derived structural protein which aids the enhanced synaptic transmission after the spaced stimulation.

Overall, the evidence from the present study taken together with previous studies indicates that MAP kinase has a number of important characteristics which suggest that it is a good candidate for storing information and regulating the enhanced potentiation which results from spaced synaptic stimulation.

5 References

AAKALU, G., SMITH, W.B., NGUYEN, N., JIANG, C. & SCHUMAN, E.M. (2001). Dynamic visualization of local protein synthesis in hippocampal neurons. *Neuron* **30**, 489-502.

ABE, E., MURAI, S., MASUDA, Y., SAITO, H. & ITOH, T. (1993). Alpha-sialyl cholesterol reverses AF64A-induced deficit in passive avoidance response and depletion of hippocampal acetylcholine in mice. *British Journal of Pharmacology* **108**, 387-392.

ABE, T., SUGIHARA, H., NAWA, H., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1992). Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/Ca2+ signal transduction. *Journal of Biological Chemistry* **267**, 13361-13368.

ABEL, T., NGUYEN, P.V., BARAD, M., DEUEL, T.A., KANDEL, E.R. & BOURTCHOULADZE, R. (1997). Genetic demonstration of a role for PKA in the late phase of LTP and in hippocampus-based long-term memory. *Cell* **88**, 615-626.

ABELIOVICH, A., CHEN, C., GODA, Y., SILVA, A.J., STEVENS, C.F. & TONEGAWA, S. (1993). Modified hippocampal long-term potentiation in PKC gamma-mutant mice. *Cell* **75**, 1253-1262.

ABRAHAM, W.C. & HUGGETT, A. (1997). Induction and reversal of long-term potentiation by repeated high-frequency stimulation in rat hippocampal slices. *Hippocampus* **7**, 137-145.

ADAMS, J.P., ANDERSON, A.E., VARGA, A.W., DINELEY, K.T., COOK, R.G., PFAFFINGER, P.J. & SWEATT, J.D. (2000). The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. *Journal of Neurochemistry* **75**, 2277-2287.

ADAMS, J.P., ROBERSON, E.D., ENGLISH, J.D., SELCHER, J.C. & SWEATT, J.D. (2000). MAPK regulation of gene expression in the central nervous system.

Acta Neurobiol Exp (Wars) **60**, 377-394.

ADAMS, J.P. & SWEATT, J.D. (2002). Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annual Review of Pharmacology and Toxicology* **42**, 135-163.

AHLQUIST, R.P. (1948). A study of the adrenotropic receptors. *American Journal of Physiology* **153**, 586-600.

AIBA, A., CHEN, C., HERRUP, K., ROSENMUND, C., STEVENS, C.F. & TONEGAWA, S. (1994). Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. *Cell* **79**, 365-375.

AKERS, R.F., LOVINGER, D.M., COLLEY, P.A., LINDEN, D.J. & ROUTTENBERG, A. (1986). Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. *Science* **231**, 587-589.

ALAGARSAMY, S., MARINO, M.J., ROUSE, S.T., GEREAU, R.W.4., HEINEMANN, S.F. & CONN, P.J. (1999). Activation of NMDA receptors reverses desensitization of mGluR5 in native and recombinant systems. *Nature Neuroscience* **2**, 234-240.

ALBERINI, C.M., GHIRARDI, M., METZ, R. & KANDEL, E.R. (1994). C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. *Cell* **76**, 1099-1114.

ALESSI, D.R., CUENDA, A., COHEN, P., DUDLEY, D.T. & SALTIEL, A.R. (1995). PD 098059 is a specific inhibitor of the activation of mitogen-activated protein kinase kinase in vitro and in vivo. *Journal of Biological Chemistry* **270**, 2789-2794.

ALEXANDER, K.A., CIMLER, B.M., MEIER, K.E. & STORM, D.R. (1987). Regulation of calmodulin binding to P-57. A neurospecific calmodulin binding protein. *Journal of Biological Chemistry* **262**, 6108-6113.

ALFORD, S. & COLLINGRIDGE, G.L. (1992). In: Excitatory Amino Acids and Second Messenger Systems (Eds Teichberg, Turski, V.I.). *Springer, Berlin* 43-53.

ALONSO, G. & WIDMER, H. (1997). Clustering of KV4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study. *Neuroscience* **77**, 617-621.

ANDERSEN, P., SUNDBERG, S.H., SVEEN, O. & WIGSTROM, H. (1977). Specific long-lasting potentiation of synaptic transmission in hippocampal slices. *Nature* **266**, 736-737.

ANDRADE, R. (1993). Enhancement of beta-adrenergic responses by Gi-linked receptors in rat hippocampus. *Neuron* **10**, 83-88.

ANDREASEN, M., LAMBERT, J.D. & JENSEN, M.S. (1989). Effects of new non-N-methyl-D-aspartate antagonists on synaptic transmission in the in vitro rat hippocampus. *Journal of Physiology* **414**, 317-336.

ANGENSTEIN, F., HIRSCHFELDER, M. & STAAK, S. (1997). Activation of metabotropic glutamate receptors increases endogenous protein kinase C substrate phosphorylation in adult hippocampal slices. *Brain Research* **745**, 46-54.

ANGENSTEIN, F., RIEDEL, G., REYMANN, K.G. & STAAK, S. (1994). Hippocampal long-term potentiation in vivo induces translocation of protein kinase C gamma. *NeuroReport* **5**, 381-384.

ANGENSTEIN, F. & STAAK, S. (1997). Receptor-mediated activation of protein kinase C in hippocampal long-term potentiation: facts, problems and implications. *Progress in Neuropsychopharmacology and Biological Psychiatry* **21**, 427-454.

ANIKSZTEJN, L., OTANI, S. & BEN-ARI, Y. (1992). Quisqualate Metabotropic Receptors Modulate NMDA Currents and Facilitate Induction of Long-Term

Potentiation Through Protein Kinase C. *European Journal of Neuroscience* **4**, 500-505.

ASZTELY, F., WIGSTROM, H. & GUSTAFSSON, B. (1992). The relative contribution of NMDA receptor channels in the expression of long-term potentiation in the hippocampal CA1 region. *European Journal of Physiology* **4**, 681-690.

ASZTELY, F., XIAO, M.Y. & GUSTAFSSON, B. (1996). Long-term potentiation and paired-pulse facilitation in the hippocampal CA1 region. *NeuroReport* **7**, 1609-1612.

ASZTELY, F., XIAO, M.Y., WIGSTROM, H. & GUSTAFSSON, B. (1994). Effect of adenosine-induced changes in presynaptic release probability on long-term potentiation in the hippocampal CA1region. *Journal of Neuroscience* **14**, 6707-6714.

ATKINS, C.M., SELCHER, J.C., PETRAITIS, J.J., TRZASKOS, J.M. & SWEATT, J.D. (1998). The MAPK cascade is required for mammalian associative learning. *Nature Neuroscience* **1**, 602-609.

AUERBACH, J.M. & SEGAL, M. (1996). Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. *Journal of Physiology* **492**, 479-493.

AUERBACH, J.M. & SEGAL, M. (1994). A novel cholinergic induction of long-term potentiation in rat hippocampus. *Journal of Neurophysiology* **72**, 2034-2040.

BAHN, S., VOLK, B. & WISDEN, W. (1994). Kainate receptor gene expression in the developing rat brain. *Journal of Neuroscience* **14**, 5525-5547.

BAILEY, C.H., BARTSCH, D. & KANDEL, E.R. (1996). Toward a molecular definition of long-term memory storage. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 13445-13452.

BALSCHUN, D. & WETZEL, W. (2002). Inhibition of mGluR5 blocks hippocampal LTP in vivo and spatial learning in rats. *Pharmacology Biochemistry and Behavior* **73**, 375-380.

BANKE, T.G., BOWIE, D., LEE, H., HUGANIR, R.L., SCHOUSBOE, A. & TRAYNELIS, S.F. (2000). Control of GluR1 AMPA receptor function by cAMP-dependent protein kinase. *Journal of Neuroscience* **20**, 89-102.

BAR, P.R., WIEGANT, F., LOPES DA SILVA, F.H. & GISPEN, W.H. (1984). Tetanic stimulation affects the metabolism of phosphoinositides in hippocampal slices. *Brain Research* **321**, 381-385.

BAREA-RODRIGUEZ, E.J., RIVERA, D.T., JAFFE, D.B. & MARTINEZ, J.L.Jr. (2000). Protein synthesis inhibition blocks the induction of mossy fiber long-term potentiation in vivo. *Journal of Neuroscience* **20**, 8528-8532.

BARNES, C.A. (1979). Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *Psychological Review* **93**, 74-104.

BARRIA, A., MULLER, D., DERKACH, V., GRIFFITH, L.C. & SODERLING, T.R. (1997). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* **276**, 2042-2045.

BARTSCH, D., GHIRARDI, M., SKEHEL, P.A., KARL, K.A., HERDER, S.P., CHEN, M., BAILEY, C.H. & KANDEL, E.R. (1995). Aplysia CREB2 represses long-term facilitation: relief of repression converts transient facilitation into long-term functional and structural change. *Cell* **83**, 979-992.

BASHIR, Z.I., ALFORD, S., DAVIES, S.N., RANDAL, l.A.D. & COLLINGRIDGE, G.L. (1991). Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. *Nature* **349**, 156-158.

BASHIR, Z.I., BORTOLOTTO, Z.A., DAVIES, C.H., BERRETTA, N., IRVING, A.J., SEAL, A.J., HENLEY, J.M., JANE, D.E., WATKINS, J.C. &

COLLINGRIDGE, G.L. (1993). Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* **363**, 347-350.

BASHIR, Z.I., TAM, B. & COLLINGRIDGE, G.L. (1990). Activation of the glycine site in the NMDA receptor is necessary for the induction of LTP. *Neuroscience Letters* **108**, 261-266.

BAUDE, A., NUSSER, Z., MOLNAR, E., MCILHINNEY, R.A. & SOMOGYI, P. (1995). High-resolution immunogold localization of AMPA type glutamate receptor subunits at synaptic and non-synaptic sites in rat hippocampus. *Neuroscience* **69**, 1031-1055.

BAUDE, A., NUSSER, Z., ROBERTS, J.D., MULVIHILL, E., MCILHINNEY, R.A. & SOMOGYI, P. (1993). The metabotropic glutamate receptor (mGluR1 alpha) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. *Neuron* **11**, 771-787.

BAYER, K.U., DE KONINCK, P., LEONARD, A.S., HELL, J.W. & SCHULMAN, H. (2001). Interaction with the NMDA receptor locks CaMKII in an active conformation. *Nature* **411**, 801-805.

BAZZI, M.D. & NELSESTUEN, G.L. (1988). Properties of membrane-inserted protein kinase C. *Biochemistry* **27**, 7589-7593.

BEAVER, C.J., JI, Q., FISCHER, Q.S. & DAW, N.W. (2001). Cyclic AMP-dependent protein kinase mediates ocular dominance shifts in cat visual cortex. *Nature Neuroscience* **4**, 159-163.

BEHNISCH, T., FJODOROW, K. & REYMANN, K.G. (1991). L-2-amino-3-phosphonopropionate blocks late synaptic long-term potentiation. *NeuroReport* **2**, 386-388.

BEHNISCH, T. & REYMANN, K.G. (1995). Thapsigargin blocks long-term potentiation induced by weak, but not strong tetanisation in rat hippocampal CA1 neurons. *Neuroscience Letters* **192**, 185-188.

BEHNISCH, T., WILSCH, V.W., BALSCHUN, D. & REYMANN, K.G. (1998). The role of group II metabotropic glutamate receptors in hippocampal CA1 long-term potentiation in vitro. *European Journal of Pharmacology* **356**, 159-165.

BEKKERS, J.M. & STEVENS, C.F. (1989). NMDA and non-NMDA receptors are co-localized at individual excitatory synapses in cultured rat hippocampus. *Nature* **341**, 230-233.

BEKKERS, J.M. & STEVENS, C.F. (1990). Presynaptic mechanism for long-term potentiation in the hippocampus. *Nature* **346**, 724-729.

BELENIKIN, M.S., COSTANTINO, G., PALYULIN, V.A., PELLICCIARI, R. & ZEFIROV, N.S. (2003). Molecular modeling of the ligand-binding domains of the NR3A and NR3B subunits of the NMDA receptor. *Dokl Biochem Biophys.* **389**, 83-89.

BENKE, T.A., LUTHI, A., ISAAC, J.T. & COLLINGRIDGE, G.L. (1998). Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature* **393**, 793-797.

BENOWITZ, L.I. & ROUTTENBERG, A. (1997). GAP-43: an intrinsic determinant of neuronal development and plasticity. *Trends in Neuroscience* **20**, 84-91.

BERGLES, D.E., DOZE, V.A., MADISON, D.V. & SMITH, S.J. (1996). Excitatory actions of norepinephrine on multiple classes of hippocampal CA1 interneurons. *Journal of Neuroscience* **16**, 572-585.

BERGLES, D.E. & JAHR, C.E. (1997). Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**, 1297-1308.

BERKELEY, J.L., GOMEZA, J., WESS, J., HAMILTON, S.E., NATHANSON, N.M. & LEVEY, A.I. (2001). M1 muscarinic acetylcholine receptors activate extracellular signal-regulated kinase in CA1 pyramidal neurons in mouse hippocampal slices. *Molecular and Cellular Neuroscience* **18**, 512-524.

BERKELEY, J.L. & LEVEY, A.I. (2003). Cell-specific extracellular signal-regulated kinase activation by multiple G protein-coupled receptor families in hippocampus. *Molecular Pharmacology* **63**, 128-135.

BERMAN, D.E., HAZVI, S., ROSENBLUM, K., SEGER, R. & DUDAI, Y. (1998). Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. *Journal of Neuroscience* **18**, 10037-10044.

BERRIDGE, M.J. (1993). Inositol trisphosphate and calcium signalling. *Nature* **361**, 315-325.

BERRIDGE, M.J. (1998). Neuronal calcium signaling. *Neuron* **21**, 13-26.

BETTLER, B., BOULTER, J., HERMANS-BORGMEYER, I., O'SHEA-GREENFIELD, A., DENERIS, E.S., MOLL, C., BORGMEYER, U., HOLLMANN, M. & HEINEMANN, S. (1990). Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. *Neuron* **5**, 583-595.

BETTLER, B. & MULLE, C. (1995). Review: neurotransmitter receptors. II. AMPA and kainate receptors. *Neuropharmacology* **34**, 123-139.

BIRSE, E.F., EATON, S.A., JANE, D.E., JONES, P.L., PORTER, R.H., POOK, P.C., SUNTER, D.C., UDVARHELYI, P.M., WHARTON, B., ROBERTS, P.J. & ET AL. (1993). Phenylglycine derivatives as new pharmacological tools for investigating the role of metabotropic glutamate receptors in the central nervous system. *Neuroscience* **52**, 481-488.

BITO, H., DEISSEROTH, K. & TSIEN, R.W. (1996). CREB phosphorylation and dephosphorylation: a Ca(2+)- and stimulus duration-dependent switch for hippocampal gene expression. *Cell* **87**, 1203-1214.

BLACKSTONE, C.D., MOSS, S.J., MARTIN, L.J., LEVEY, A.I., PRICE, D.L. & HUGANIR, R.L. (1992). Biochemical characterization and localization of a

non-N-methyl-D-aspartate glutamate receptor in rat brain. *Journal of Neurochemistry* **58**, 1118-1126.

BLAKE, J.F., BROWN, M.W. & COLLINGRIDGE, G.L. (1988). CNQX blocks acidic amino acid induced depolarizations and synaptic components mediated by non-NMDA receptors in rat hippocampal slices. *Neuoscience Letters* **89**, 182-186.

BLAND, B.H. (1986). The physiology and pharmacology of hippocampal formation theta rhythms. *Progress in Neurobiology* **26**, 1-54.

BLEAKMAN, R., SCHOEPP, D.D., BALLYK, B., BUFTON, H., SHARPE, E.F., THOMAS, K., ORNSTEIN, P.L. & KAMBOJ, R.K. (1996). Pharmacological discrimination of GluR5 and GluR6 kainate receptor subtypes by (3S,4aR,6R,8aR)-6-[2-(1(2)H-tetrazole-5-yl)ethyl]decahyd roisdoquinoline-3 carboxylic-acid. *Molecular Pharmacology* **49**, 581-585.

BLISS, T.V. & COLLINGRIDGE, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31-39.

BLISS, T.V. & GARDNER-MEDWIN, A.R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**, 357-374.

BLISS, T.V., GODDARD, G.V. & RIIVES, M. (1983). Reduction of long-term potentiation in the dentate gyrus of the rat following selective depletion of monoamines. *Journal of Physiology* 475-491.

BLISS, T.V. & LOMO, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *Journal of Physiology* **232**, 331-356.

BLITZER, R.D., CONNOR, J.H., BROWN, G.P., WONG, T., SHENOLIKAR, S., IYENGAR, R. & LANDAU, E.M. (1998). Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. *Science* **280**, 1940-1942.

BLITZER, R.D., GIL, O. & LANDAU, E.M. (1990). Cholinergic stimulation enhances long-term potentiation in the CA1 region of rat hippocampus. *Neuroscience Letters* **119**, 207-210.

BLITZER, R.D., WONG, T., NOURANIFAR, R., IYENGAR, R. & LANDAU, E.M. (1995). Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. *Neuron* **15**, 1403-1414.

BLUM, S., MOORE, A.N., ADAMS, F. & DASH, P.K. (1999). A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. *Journal of Neuroscience* **19**, 3535-3544.

BOLSHAKOV, V.Y., GOLAN, H., KANDEL, E.R. & SIEGELBAUM, S.A. (1997). Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron* **19**, 635-651.

BOLSHAKOV, V.Y. & SIEGELBAUM, S.A. (1994). Postsynaptic induction and presynaptic expression of hippocampal long-term depression. *Science* **264**, 1148-1152.

BORDI, F., REGGIANI, A. & CONQUET, F. (1997). Regulation of synaptic plasticity by mGluR1 studied in vivo in mGluR1 mutant mice. *Brain Research* **761**, 121-126.

BORRONI, A.M., FICHTENHOLTZ, H., WOODSIDE, B.L. & TEYLER, T.J. (2000). Role of voltage-dependent calcium channel long-term potentiation (LTP) and NMDA LTP in spatial memory. *Journal of Neuroscience* **20**, 9272-9276.

BORTOLOTTO, Z.A., BASHIR, Z.I., DAVIES, C.H. & COLLINGRIDGE, G.L. (1994). A molecular switch activated by metabotropic glutamate receptors regulates induction of long-term potentiation. *Nature* **368**, 740-743.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (1992). Activation of glutamate

metabotropic receptors induces long-term potentiation. *European Journal of Pharmacology* **214**, 297-298.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (1993). Characterisation of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus. *Neuropharmacology* **32**, 1-9.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (1999). Evidence that a novel metabotropic glutamate receptor mediates the induction of long-term potentiation at CA1 synapses in the hippocampus. *Biochemical Society Transactions* **27**, 170-174.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (1998). Involvement of calcium/calmodulin-dependent protein kinases in the setting of a molecular switch involved in hippocampal LTP. *Neuropharmacology* **37**, 535-544.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (1995). On the mechanism of long-term potentiation induced by (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) in rat hippocampal slices. *Neuropharmacology* **34**, 1003-1014.

BORTOLOTTO, Z.A. & COLLINGRIDGE, G.L. (2000). A role for protein kinase C in a form of metaplasticity that regulates the induction of long-term potentiation at CA1 synapses of the adult rat hippocampus. *European Journal of Neuroscience* **12**, 4055-4062.

BOULTON, T.G., YANCOPOULOS, G.D., GREGORY, J.S., SLAUGHTER, C., MOOMAW, C., HSU, J. & COBB, M.H. (1990). An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. *Science* **249**, 64-67.

BOURNE, H.R. & NICOLL, R. (1993). Molecular machines integrate coincident synaptic signals. *Cell* **72**, 65-75.

BOURTCHULADZE, R., FRENGUELLI, B., BLENDY, J., CIOFFI, D., SCHUTZ, G. & SILVA, A.J. (1994). Deficient long-term memory in mice with a targeted

mutation of the cAMP-responsive element-binding protein. *Cell* **79**, 59-68.

BOZDAGI, O., SHAN, W., TANAKA, H., BENSON, D.L. & HUNTLEY, G.W. (2000). Increasing numbers of synaptic puncta during late-phase LTP: N-cadherin is synthesized, recruited to synaptic sites, and required for potentiation. *Neuron* **28**, 245-259.

BRADLEY, S.R., LEVEY, A.I., HERSCH, S.M. & CONN, P.J. (1996). Immunocytochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. *Journal of Neuroscience* **16**, 2044-2056.

BRAGIN, A., JANDO, G., NADASDY, Z., VAN LANDEGHEM, M. & BUZSAKI, G. (1995). Dentate EEG spikes and associated interneuronal population bursts in the hippocampal hilar region of the rat. *Journal of Neurophysiology* **73**, 1691-1705.

BRAMHAM, C.R., BACHER-SVENDSEN, K. & SARVEY, J.M. (1997). LTP in the lateral perforant path is beta-adrenergic receptor-dependent. *NeuroReport* **8**, 719-724.

BRANDON, E.P., ZHUO, M., HUANG, Y.Y., QI, M., GERHOLD, K.A., BURTON, K.A., KANDEL, E.R., MCKNIGHT, G.S. & IDZERDA, R.L. (1995). Hippocampal long-term depression and depotentiation are defective in mice carrying a targeted disruption of the gene encoding the RI beta subunit of cAMP-dependent protein kinase. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 8851-8855.

BREAKWELL, N.A., ROWAN, M.J. & ANWYL, R. (1998). (+)-MCPG blocks induction of LTP in CA1 of rat hippocampus via agonist action at an mGluR group II receptor. *Journal of Neurophysiology* **79**, 1270-1276.

BREAKWELL, N.A., ROWAN, M.J. & ANWYL, R. (1996). Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the

submerged rat hippocampal slice. *Journal of Neurophysiology* **76**, 3126-3135.

BROWN, E.M., GAMBA, G., RICCARDI, D., LOMBARDI, M., BUTTERS, R., KIFOR, O., SUN, A., HEDIGER, M.A., LYTTON, J. & HEBERT, S.C. (1993). Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. *Nature* **366**, 575-580.

BROWN, G.P., BLITZER, R.D., CONNOR, J.H., WONG, T., SHENOLIKAR, S., IYENGAR, R. & LANDAU, E.M. (2000). Long-term potentiation induced by theta frequency stimulation is regulated by a protein phosphatase-1-operated gate. *Journal of Neuroscience* **20**, 7880-7887.

BROWN, R.E., RABE, H. & REYMANN, K.G. (1994). (RS)-alpha-methyl-4-carboxyphenylglycine (MCPG) does not block theta burst-induced long-term potentiation in area CA1 of rat hippocampal slices. *Neuroscience Letters* **170**, 17-21.

BROWN, S. & SCHAFER, E.A. (1888). An investigation into the functions of the occipital and temporal lobes of the monkey's brain. *Philosophical Transactions of the Royal Society of London (Biology)* **179**, 303-327.

BUCHS, P.A. & MULLER, D. (1996). Induction of long-term potentiation is associated with major ultrastructural changes of activated synapses. *Proceedings of the National Academy of Sciences of the United States of America* **23**, 8040-8045.

BUCKMASTER, P.S., WENZEL, H.J., KUNKEL, D.D. & SCHWARTZKROIN, P.A. (1996). Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *Journal of Comparative Neurology* **366**, 271-292.

BUONOMANO, D.V. & MERZENICH, M.M. (1998). Cortical plasticity: from synapses to maps. *Annual Review of Neuroscience* **21**, 149-186.

BURGARD, E.C. & SARVEY, J.M. (1990). Muscarinic receptor activation facilitates the induction of long-term potentiation (LTP) in the rat dentate gyrus.

Neuroscience Letters **116**, 34-39.

BURGIN, K.E., WAXHAM, M.N., RICKLING, S., WESTGATE, S.A., MOBLEY, W.C. & KELLY, P.T. (1990). In situ hybridization histochemistry of Ca2+/calmodulin-dependent protein kinase in developing rat brain. *Journal of Neuroscience* **10**, 1788-1798.

BURNASHEV, N., SCHOEPFER, R., MONYER, H., RUPPERSBERG, J.P., GUNTHER, W., SEEBURG, P.H. & SAKMANN, B. (1992). Control by asparagine residues of calcium permeability and magnesium blockade in the NMDA receptor. *Science* **257**, 1415-1419.

BUZAKI, G. & GAGE, F.H. (1991). Long-term potentiation: does it happen in the normal brain? When and how? *In Memory Mechanisms; A Tribute to G.V. Goddard (Eds Abraham, W.C., Corballis, M.C. and White, K.G.) Lawrence Erlbaum, Hillsdale* 79-102.

BUZSAKI, G. (1986). Hippocampal sharp waves: their origin and significance. *Brain Research* **398**, 242-252.

BUZSAKI, G. (1989). Two-stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience* **31**, 551-570.

BUZSAKI, G., HORVATH, Z., URIOSTE, R., HETKE, J. & WISE, K. (1992). High-frequency network oscillation in the hippocampus. *Science* **256**, 1025-1027.

BUZSAKI, G., LEUNG, L.W. & VANDERWOLF, C.H. (1983). Cellular bases of hippocampal EEG in the behaving rat. *Brain Research* **287**, 139-171.

BYLUND, D.B., EIKENBERG, D.C., HIEBLE, J.P., LANGER, S.Z., LEFKOWITZ, R.J., MINNEMAN, K.P., MOLINOFF, P.B., RUFFOLO, R.R.J. & TRENDELENBURG, U. (1994). International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacological Review* **46**, 121-136.

BYMASTER, F.P., HEATH, I., HENDRIX, J.C. & SHANNON, H.E. (1993).

Comparative behavioral and neurochemical activities of cholinergic antagonists in rats. *Journal of Pharmacology and Experimental Therapeutics* **267**, 16-24.

BYRNE, J.H., ZWARTJES, R., HOMAYOUNI, R., CRITZ, S.D. & ESKIN, A. (1993). Roles of second messenger pathways in neuronal plasticity and in learning and memory. Insights gained from Aplysia. *Advances in Second Messenger Phosphoprotein Research* **27**, 47-108.

CADD, G. & MCKNIGHT, G.S. (1989). Distinct patterns of cAMP-dependent protein kinase gene expression in mouse brain. *Neuron* **3**, 71-79.

CAHILL, L., PRINS, B., WEBER, M. & MCGAUGH, J.L. (1994). Beta-adrenergic activation and memory for emotional events. *Nature* **371**, 702-704.

CAJAL, S.R. (1911). Hstologie du systeme Nerveux de l'Homme et des Vertebres. *(Paris: Maloine)* **11**, 993

CALIXTO, E., THIELS, E., KLANN, E. & BARRIONUEVO, G. (2003). Early maintenance of hippocampal mossy fiber--long-term potentiation depends on protein and RNA synthesis and presynaptic granule cell integrity. *Journal of Neuroscience* **23**, 4842-4849.

CALLAHAN, M.J., KINSORA, J.J., HARBAUGH, R.E., REEDER, T.M. & DAVIS, R.E. (1993). Continuous ICV infusion of scopolamine impairs sustained attention of rhesus monkeys. *Neurobiology of Aging* **14**, 147-151.

CAMPS, M., NICHOLS, A., GILLIERON, C., ANTONSSON, B., MUDA, M., CHABERT, C., BOSCHERT, U. & ARKINSTALL, S. (1998). Catalytic activation of the phosphatase MKP-3 by ERK2 mitogen-activated protein kinase. *Science* **280**, 1262-1265.

CANNON, W.B. & URIDIL, J.E. (1921). Studies on the conditions of activity in endocrine glands. VIII. Some effects on the denervated heart of stimulaing the nerves of the liver. *American Journal of Physiology* **58**, 353-354.

CANTLEY, L.C., AUGER, K.R., CARPENTER, C., DUCKWORTH, B., GRAZIANI, A., KAPELLER, R. & SOLTOFF, S. (1991). Oncogenes and signal transduction. *Cell* **64**, 281-302.

CARR, D.W., STOFKO-HAHN, R.E., FRASER, I.D., BISHOP, S.M., ACOTT, T.S., BRENNAN, R.G. & SCOTT, J.D. (1991). Interaction of the regulatory subunit (RII) of cAMP-dependent protein kinase with RII-anchoring proteins occurs through an amphipathic helix binding motif. *Journal of Biological Chemistry* **266**, 14188-14192.

CARR, D.W., STOFKO-HAHN, R.E., FRASER, I.D., CONE, R.D. & SCOTT, J.D. (1992). Localization of the cAMP-dependent protein kinase to the postsynaptic densities by A-kinase anchoring proteins. Characterization of AKAP 79. *Journal of Biological Chemistry* **267**, 16816-16823.

CARROLL, R.C., NICOLL, R.A. & MALENKA, R.C. (1998). Effects of PKA and PKC on miniature excitatory postsynaptic currents in CA1 pyramidal cells. *Journal of Neurophysiology* **80**, 2797-2800.

CASTELLUCCI, V.F., BLUMENFELD, H., GOELET, P. & KANDEL, E.R. (1989). Inhibitor of protein synthesis blocks long-term behavioral sensitization in the isolated gill-withdrawal reflex of Aplysia. *Journal of Neurobiology* **20**, 1-9.

CASTELLUCCI, V.F., KANDEL, E.R., SCHWARTZ, J.H., WILSON, F.D., NAIRN, A.C. & GREENGARD, P. (1980). Intracellular injection of t he catalytic subunit of cyclic AMP-dependent protein kinase simulates facilitation of transmitter release underlying behavioral sensitization in Aplysia. *Proceedings of the National Academy of Sciences of the United States of America* **77**, 7492-7496.

CASTILLO, P.E., MALENKA, R.C. & NICOLL, R.A. (1997). Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature* **388**, 182-186.

CAULFIELD, M.P. & BIRDSALL, N.J. (1998). International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacological Reviews* **50**, 279-290.

CAVUS, I. & TEYLER, T. (1996). Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. *Journal of Neurophysiology* **76**, 3038-3047.

CHANDLER, L.J., SUTTON, G., DORAIRAJ, N.R. & NORWOOD, D. (2001). N-methyl D-aspartate receptor-mediated bidirectional control of extracellular signal-regulated kinase activity in cortical neuronal cultures. *Journal of Biological Chemistry* **276**, 2627-2636.

CHANG, W., CHEN, T.H., PRATT, S. & SHOBACK, D. (2000). Amino acids in the second and third intracellular loops of the parathyroid Ca2+-sensing receptor mediate efficient coupling to phospholipase C. *Journal of Biological Chemistry* **275**, 19955-19963.

CHATTERTON, J.E., AWOBULUYI, M., PREMKUMAR, L.S., TAKAHASHI, H., TALANTOVA, M., SHIN, Y., CUI, J., TU, S., SEVARINO, K.A., NAKANISHI, N., TONG, G., LIPTON, S.A. & ZHANG D. (2002). Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature* **415**, 793-798.

CHAVEZ-NORIEGA, L.E. & STEVENS, C.F. (1994). Increased transmitter release at excitatory synapses produced by direct activation of adenylate cyclase in rat hippocampal slices. *Journal of Neuroscience* **14**, 310-317.

CHEN, H.J., ROJAS-SOTO, M., OGUNI, A. & KENNEDY, M.B. (1998). A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron* **20**, 895-904.

CHEN, L., CHETKOVICH, D.M., PETRALIA, R.S., SWEENEY, N.T., KAWASAKI, Y., WENTHOLD, R.J., BREDT, D.S. & NICOLL, R.A. (2000). Stargazin

regulates synaptic targeting of AMPA receptors by two distinct mechanisms. *Nature* **408**, 936-943.

CHEN, L. & HUANG, L.Y. (1992). Protein kinase C reduces Mg2+ block of NMDA-receptor channels as a mechanism of modulation. *Nature* **356**, 521-523.

CHEN, R.H., SARNECKI, C. & BLENIS, J. (1992). Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Molecular Cell Biology* **12**, 915-927.

CHETKOVICH, D.M., GRAY, R., JOHNSTON, D. & SWEATT, J.D. (1991). N-methyl-D-aspartate receptor activation increases cAMP levels and voltage-gated Ca2+ channel activity in area CA1 of hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 6467-6471.

CHETKOVICH, D.M. & SWEATT, J.D. (1993). NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. *Journal of Neurochemistry* **61**, 1933-1942.

CHICUREL, M.E., TERRIAN, D.M. & POTTER, H. (1993). mRNA at the synapse: analysis of a synaptosomal preparation enriched in hippocampal dendritic spines. *Journal of Neuroscience* **13**, 4054-4063.

CHINESTRA, P., ANIKSZTEJN, L., DIABIRA, D. & BEN-ARI, Y. (1993). (RS)-alpha-methyl-4-carboxyphenylglycine neither prevents induction of LTP nor antagonizes metabotropic glutamate receptors in CA1 hippocampal neurons. *Journal of Neurophysiology* **70**, 2684-2689.

CHOE, E.S. & WANG, J.Q. (2001). Group I metabotropic glutamate receptor activation increases phosphorylation of cAMP response element-binding protein, Elk-1, and extracellular signal-regulated kinases in rat dorsal striatum. *Molecular Brain Research* **94** , 75-84.

CHOI, D.W. (1988). Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends in Neuroscience* **11**, 465-469.

CHRISTIE, B.R. & ABRAHAM, W.C. (1992). NMDA-dependent heterosynaptic long-term depression in the dentate gyrus of anaesthetized rats. *Synapse* **10**, 1-6.

CHRISTIE, B.R., STELLWAGEN, D. & ABRAHAM, W.C. (1995). Reduction of the threshold for long-term potentiation by prior theta-frequency synaptic activity. *Hippocampus* **5**, 52-59.

CHROBAK, J.J. & BUZSAKI, G. (1996). High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *Journal of Neuroscience* **9**,

CLAIBORNE, B.J., AMARAL, D.G. & COWAN, W.M. (1986). A light and electron microscopic anlysis of the mossy fibers of the at dentate gyrus. *Journal of Comparative Neurology* **246**, 435-458.

CLAPHAM, D.E. & NEER, E.J. (1997). G protein beta gamma subunits. *Annual Review of Pharmacological Toxicology* **37**, 167-203.

CLARKE, V.R., BALLYK, B.A., HOO, K.H., MANDELZYS, A., PELLIZZARI, A., BATH, C.P., THOMAS, J., SHARPE, E.F., DAVIES, C.H., ORNSTEIN, P.L., SCHOEPP, D.D., KAMBOJ, R.K., COLLINGRIDGE, G.L., LODGE, D. & BLEAKMAN, D. (1997). A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. *Nature* **389**, 599-603.

CLAYTON, D.F. (2000). The genomic action potential. *Neurobiology of Learning and Memory* **74**, 185-216.

COAN, E.J. & COLLINGRIDGE, G.L. (1987). Effects of phencyclidine, SKF 10,047 and related psychotomimetic agents on N-methyl-D-aspartate receptor mediated synaptic responses in rat hippocampal slices. *British Journal of Pharmacology* **91**, 547-556.

COAN, E.J., IRVING, A.J. & COLLINGRIDGE, G.L. (1989). Low-frequency activation of the NMDA receptor system can prevent the induction of LTP. *Neuroscience Letters* **105**, 205-210.

COGGINS, P.J. & ZWIERS, H. (1989). Evidence for a single protein kinase C-mediated phosphorylation site in rat brain protein B-50. *Journal of Neurochemistry* **53**, 1895-1901.

COGHLAN, V.M., PERRINO, B.A., HOWARD, M., LANGEBERG, L.K., HICKS, J.B., GALLATIN, W.M. & SCOTT, J.D. (1995). Association of protein kinase A and protein phosphatase 2B with a common anchoring protein. *Science* **267**, 108-111.

COHEN, A.S. & ABRAHAM, W.C. (1996). Facilitation of long-term potentiation by prior activation of metabotropic glutamate receptors. *Journal of Neurophysiology* **76**, 953-962.

COHEN, A.S., RAYMOND, C.R. & ABRAHAM, W.C. (1998). Priming of long-term potentiation induced by activation of metabotropic glutamate receptors coupled to phospholipase C. *Hippocampus* **8**, 160-170.

COLE, A.E. & NICOLL, R.A. (1983). Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. *Science* **221**, 1299-1301.

COLE, A.J., SAFFEN, D.W., BARABAN, J.M. & WORLEY, P.F. (1989). Rapid increase of an immediate early gene messenger RNA in hippocampal neurons by synaptic NMDA receptor activation. *Nature* **340**, 474-476.

COLLEY, P.A., SHEU, F.S. & ROUTTENBERG, A. (1990). Inhibition of protein kinase C blocks two components of LTP persistence, leaving initial potentiation intact. *Journal of Neuroscience* **10**, 3353-3360.

COLLINGRIDGE, G.L., HERRON, C.E. & LESTER, R.A. (1988b). Frequency-dependent N-methyl-D-aspartate receptor-mediated synaptic transmission in rat hippocampus. *Journal of Physiology* **399**, 301-312.

COLLINGRIDGE, G.L., HERRON, C.E. & LESTER, R.A. (1988a). Synaptic activation of N-methyl-D-aspartate receptors in the Schaffer collateral-commissural pathway of rat hippocampus. *Journal of Physiology* **399**, 283-300.

COLLINGRIDGE, G.L., KEHL, S.J. & MCLENNAN, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *Journal of Physiology* **334**, 33-46.

CONN, P.J. & PIN, J.P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annual Review of Pharmacological Toxicology* **37**, 205-237.

CONQUET, F., BASHIR, Z.I., DAVIES, C.H., DANIEL, H., FERRAGUTI, F., BORDI, F., FRANZ-BACON, K., REGGIANI, A., MATARESE, V., CONDE, F. & ET AL. (1994). Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. *Nature* **372**, 237-243.

CONTRACTOR, A., ROGERS, C., MARON, C., HENKEMEYER, M., SWANSON, G.T. & HEINEMANN, S.F. (2002). Trans-synaptic Eph receptor-ephrin signaling in hippocampal mossy fiber LTP. *Science* **296**, 1864-1869.

COOGAN, A.N., O'LEARY, D.M. & O'CONNOR, J.J. (1999). P42/44 MAP kinase inhibitor PD98059 attenuates multiple forms of synaptic plasticity in rat dentate gyrus in vitro. *Journal of Neurophysiology* **81**, 103-110.

COOPER, D.M., MONS, N. & KARPEN, J.W. (1995). Adenylyl cyclases and the interaction between calcium and cAMP signalling. *Nature* **374**, 421-424.

COOPER, J.A., BOWEN-POPE, D.F., RAINES, E., ROSS, R. & HUNTER, T. (1982). Similar effects of platelet-derived growth factor and epidermal growth factor on the phosphorylation of tyrosine in cellular proteins. *Cell* **31**, 263-273.

COSSART, R., ESCLAPEZ, M., HIRSCH, J.C., BERNARD, C. & BEN-ARI, Y. (1998). GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. *Nature Neuroscience* **1**, 470-478.

COSTANTINO, G. & PELLICCIARI, R. (1996). Homology modeling of metabotropic glutamate receptors. (mGluRs) structural motifs affecting binding modes and pharmacological profile of mGluR1 agonists and competitive antagonists.

Journal of Medical Chemistry **39**, 3998-4006.

COTECCHIA, S., KOBILKA, B.K., DANIEL, K.W., NOLAN, R.D., LAPETINA, E.Y., CARON, M.G., LEFKOWITZ, R.J. & REGAN, J.W. (1990). Multiple second messenger pathways of alpha-adrenergic receptor subtypes expressed in eukaryotic cells. *Journal of Biological Chemistry* **265**, 63-69.

COUSSENS, C.M., KERR, D.S. & ABRAHAM, W.C. (1997). Glucocorticoid receptor activation lowers the threshold for NMDA-receptor-dependent homosynaptic long-term depression in the hippocampus through activation of voltage-dependent calcium channels. *Journal of Neurophysiology* **78**, 1-9.

CRINO, P., KHODAKHAH, K., BECKER, K., GINSBERG, S., HEMBY, S. & EBERWINE, J. (1998). Presence and phosphorylation of transcription factors in developing dendrites. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 2313-2318.

CROW, T. & FORRESTER, J. (1990). Inhibition of protein synthesis blocks long-term enhancement of generator potentials produced by one-trial in vivo conditioning in Hermissenda. *Proceedings of the Naionational Academy of Sciences of the United States of America* **87**, 4490-4494.

CROW, T., XUE-BIAN, J.J., SIDDIQI, V., KANG, Y. & NEARY, J.T. (1998). Phosphorylation of mitogen-activated protein kinase by one-trial and multi-trial classical conditioning. *Journal of Neurochemistry* **18**, 3480-3487.

CSICSVARI, J., HIRASE, H., CZURKO, A., MAMIYA, A. & BUZSAKI, G. (1999). Fast network oscillations in the hippocampal CA1 region of the behaving rat. *Journal of Neuroscience* **19**, RC20

CULL-CANDY, S.G., HOWE, J.R. & OGDEN, D.C. (1988). Noise and single channels activated by excitatory amino acids in rat cerebellar granule neurones. *Journal of Physiology* **400**, 189-222.

DAHL, D. & SARVEY, J.M. (1990). Beta-adrenergic agonist-induced long-lasting

synaptic modifications in hippocampal dentate gyrus require activation of NMDA receptors, but not electrical activation of afferents. *Brain Research* **526**, 347-350.

DAHL, D. & SARVEY, J.M. (1989). Norepinephrine induces pathway-specific long-lasting potentiation and depression in the hippocampal dentate gyrus. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 4776-4780.

DAS, S., SASAKI, Y.F., ROTHE, T., PREMKUMAR, L.S., TAKASU, M., CRANDALL, J.E., DIKKES, P., CONNER, D.A., RAYUDU, P.V., CHEUNG, W., CHEN, H.S., LIPTON, S.A. & NAKANISHI, N. (1998). Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature* **393**, 377-381.

DASH, P.K., HOCHNER, B. & KANDEL, E.R. (1990). Injection of the cAMP-responsive element into the nucleus of Aplysia sensory neurons blocks long-term facilitation. *Nature* **345**, 718-721.

DAVIES, C.H., STARKEY, S.J., POZZA, M.F. & COLLINGRIDGE, G.L. (1991). GABA autoreceptors regulate the induction of LTP. *Nature* **349**, 609-611.

DAVIES, J., EVANS, R.H., FRANCIS, A.A. & WATKINS, J.C. (1979). Excitatory amino acid receptors and synaptic excitation in the mammalian central nervous system. *Journal of Physiology (Paris)* **75**, 641-654.

DAVIS, H.P. & SQUIRE, L.R. (1984). Protein synthesis and memory: a review. *Psychological Bulletin* **96**, 518-559.

DAVIS, S., VANHOUTTE, P., PAGES, C., CABOCHE, J. & LAROCHE, S. (2000). The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus in vivo. *Journal of Neuroscience* **20**, 4563-4572.

DAW, M.I., BORTOLOTTO, Z.A., SAULLE, E., ZAMAN, S., COLLINGRIDGE,

G.L. & ISAAC, J.T. (2002). Phosphatidylinositol 3 kinase regulates synapse specificity of hippocampal long-term depression. *Nature Neuroscience* **5**, 835-836.

DAWSON, V.L., GAGE, F.H., HUNT, M.A. & WAMSLEY, J.K. (1989). Normalization of subtype-specific muscarinic receptor binding in the denervated hippocampus by septodiagonal band grafts. *Experimental Neurology* **106**, 115-124.

DAY, H.E., CAMPEAU, S., WATSON, S.J.J. & AKIL, H. (1997). Distribution of alpha 1a-, alpha 1b- and alpha 1d-adrenergic receptor mRNA in the rat brain and spinal cord. *Journal of Chemical Neuroanatomy* **13**, 115-139.

DE BLASI, A., CONN, P.J., PIN, J. & NICOLETTI, F. (2001). Molecular determinants of metabotropic glutamate receptor signaling. *Trends in Pharmacological Science* **22**, 114-120.

DE ROOIJ, J., ZWARTKRUIS, F.J., VERHEIJEN, M.H., COOL, R.H., NIJMAN, S.M., WITTINGHOFER, A. & BOS, J.L. (1998). Epac is a Rap1 guanine-nucleotide-exchange factor directly activated by cyclic AMP. *Nature* **396**, 474-477.

DEADWYLER, S.A., DUNWIDDIE, T. & LYNCH, G. (1987). A critical level of protein synthesis is required for long-term potentiation. *Synapse* **1**, 90-95.

DEISSEROTH, K., BITO, H. & TSIEN, R.W. (1996). Signaling from synapse to nucleus: postsynaptic CREB phosphorylation during multiple forms of hippocampal synaptic plasticity. *Neuron* **16**, 89-101.

DEISSEROTH, K., HEIST, E.K. & TSIEN, R.W. (1998). Translocation of calmodulin to the nucleus supports CREB phosphorylation in hippocampal neurons. *Nature* **392**, 198-202.

DELLA ROCCA, G.J., VAN BIESEN, T., DAAKA, Y., LUTTRELL, D.K., LUTTRELL, L.M. & LEFKOWITZ, R.J. (1997). Ras-dependent mitogen-

activated protein kinase activation by G protein-coupled receptors. Convergence of Gi- and Gq-mediated pathways on calcium/calmodulin, Pyk2, and Src kinase. *Journal of Biological Chemistry* **272**, 19125-19132.

DENK, W., YUSTE, R., SVOBODA, K. & TANK, D.W. (1996). Imaging calcium dynamics in dendritic spines. *Current Opinions in Neurobiology* **6**, 372-378.

DENNY, J.B., POLAN-CURTAIN, J., RODRIGUEZ, S., WAYNER, M.J. & ARMSTRONG, D.L. (1990). Evidence that protein kinase M does not maintain long-term potentiation. *Brain Research* **534**, 201-208.

DERKACH, V., BARRIA, A. & SODERLING, T.R. (1999). Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 3269-3274.

DESMOND NL, L.WB. (1988). Synaptic interface surface area increases with long-term potentiation in the hippocampal dentate gyrus. *Brain Research* **453**, 308-314.

DEVRIES. S.H. & SCHWARTZ, E.A. (1999). Kainate receptors mediate synaptic transmission between cones and 'Off' bipolar cells in a mammalian retina. *Nature* **397**, 157-160.

DIAMOND, J.S., BERGLES, D.E. & JAHR, C.E. (1998). Glutamate release monitored with astrocyte transporter currents during LTP. *Neuron* **21**, 425-433.

DIXON, R.A., KOBILKA, B.K., STRADER, D.J., BENOVIC, J.L., DOHLMAN, H.G., FRIELLE, T., BOLANOWSKI, M.A., BENNETT, C.D., RANDS, E., DIEHL, R.E. & ET AL. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature* **321**, 75-79.

DOHERTY, A.J., PALMER, M.J., BORTOLOTTO, Z.A., HARGREAVES, A., KINGSTON, A.E., ORNSTEIN, P.L., SCHOEPP, D.D., LODGE, D. & COLLINGRIDGE, G.L. (2000). A novel, competitive mGlu(5) receptor

antagonist (LY344545) blocks DHPG-induced potentiation of NMDA responses but not the induction of LTP in rat hippocampal slices. *British Journal of Pharmacology* **131**, 239-244.

DOHERTY, A.J., PALMER, M.J., HENLEY, J.M., COLLINGRIDGE, G.L. & JANE, D.E. (1997). (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) activates mGlu5, but no mGlu1, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. *Neuropharmacology* **36**, 265-267.

DOLPHIN, A.C., ERRINGTON, M.L. & BLISS, T.V. (1982). Long-term potentiation of the perforant path in vivo is associated with increased glutamate release. *Nature* **297**, 496-498.

DONEVAN, S.D., BEG, A., GUNTHER, J.M. & TWYMAN, R.E. (1998). The methylglutamate, SYM 2081, is a potent and highly selective agonist at kainate receptors. *Journal of Pharmacology and Experimental Therapeutics* **285**, 539-545.

DOSEMECI, A. & REESE, T.S. (1993). Inhibition of endogenous phosphatase in a postsynaptic density fraction allows extensive phosphorylation of the major postsynaptic density protein. *Journal of Neurochemistry* **61**, 550-555.

DOSEMECI, A., TAO-CHENG, J.H., VINADE, L., WINTERS, C.A., POZZO-MILLER, L. & REESE, T.S. (2001). Glutamate-induced transient modification of the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 10428-10432.

DRAGUHN, A., TRAUB, R.D., SCHMITZ, D. & JEFFERYS, J.G. (1998). Electrical coupling underlies high-frequency oscillations in the hippocampus in vitro. *Nature* **394**, 189-192.

DRIER, E.A., TELLO, M.K., COWAN, M., WU, P., BLACE, N., SACKTOR, T.C. & YIN, J.C. (2002). Memory enhancement and formation by atypical PKM activity in Drosophila melanogaster. *Nature Neuroscience* **5**, 316-324.

DUDLEY, D.T., PANG, L., DECKER, S.J., BRIDGES, A.J. & SALTIEL, A.R. (1995). A synthetic inhibitor of the mitogen-activated protein kinase cascade. . *Proceedings of the National Academy of Sciences of the United States of America* **92**, 7686-7689.

DUFFY, S. & MACVICAR, B.A. (1995). Adrenergic calcium signaling in astrocyte networks within the hippocampal slice. *Journal of Neuroscience* **15**, 5535-5550.

DUFFY, S.N. & NGUYEN, P.V. (2003). Postsynaptic application of a peptide inhibitor of cAMP-dependent protein kinase blocks expression of long-lasting synaptic potentiation in hippocampal neurons. *Journal of Neuroscience* **23**, 1142-1150.

DUNWIDDIE, T.V. (1985). The physiological role of adenosine in the central nervous system. *International Review of Neurobiology* **27**, 63-139.

DUNWIDDIE, T.V., ROBERSON, N.L. & WORTH, T. (1982). Modulation of long-term potentiation: effects of adrenergic and neuroleptic drugs. *Pharmacology Biochemistry and Behavior* **17**, 1257-1264.

DURAND, G.M., KOVALCHUK, Y. & KONNERTH, A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* **381**, 71-75.

DUTAR, P. & NICOLL, R.A. (1988). Classification of muscarinic responses in hippocampus in terms of receptor subtypes and second-messenger systems: electrophysiological studies in vitro. *Journal of Neuroscience* **8**, 4214-4224.

DUVOISIN, R.M., ZHANG, C. & RAMONELL, K. (1995). A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. *Journal of Neurscience* **15**, 3075-3083.

EATON, S.A., JANE, D.E., JONES, P.L., PORTER, R.H., POOK, P.C., SUNTER, D.C., UDVARHELYI, P.M., ROBERTS, P.J., SALT, T.E. & WATKINS, J.C. (1993). Competitive antagonism at metabotropic glutamate receptors by (S)-4-

carboxyphenylglycine and (RS)-alpha-methyl-4-carboxyphenylglycine. *European Journal of Pharmacology* **244**, 195-197.

EBBINGHAUS, H. (1885). Uber das Gedachtnis. *New York: Dover*

EBINU, J.O., BOTTORFF, D.A., CHAN, E.Y., STANG, S.L., DUNN, R.J. & STONE, J.C. (1998). RasGRP, a Ras guanyl nucleotide- releasing protein with calcium- and diacylglycerol-binding motifs. *Science* **280**, 1082-1086.

EDWARDS, F.A. (1995). Anatomy and electrophysiology of fast central synapses lead to a structural model for long-term potentiation. *Physiological Reviews* **75**, 759-787.

EHLERS, M.D. (2000). Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. *Neuron* **28**, 511-525.

EMPTAGE, N., BLISS, T.V. & FINE, A. (1999). Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron* **22**, 115-124.

ENGERT, F. & BONHOEFFER, T. (1999). Dendritic spine changes associated with hippocampal long-term synaptic plasticity. *Nature* **399**, 66-70.

ENGERT, F. & BONHOEFFER, T. (1997). Synapse specificity of long-term potentiation breaks down at short distances. *Nature* **388**, 279-284.

ENGLISH, J.D. & SWEATT, J.D. (1996). Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *Journal of Bioilogical Chemistry* **271**, 24329-24332.

ENGLISH, J.D. & SWEATT, J.D. (1997). A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *Journal of Biological Chemistry* **272**, 19103-19106.

ENSLEN, H., SUN, P., BRICKEY, D., SODERLING, S.H., KLAMO, E. &

SODERLING, T.R. (1994). Characterization of Ca2+/calmodulin-dependent protein kinase IV. Role in transcriptional regulation. *Journal Biology Chemistry* **269**, 15520-15527.

ENSLEN, H., TOKUMITSU, H., STORK, P.J., DAVIS, R.J. & SODERLING, T.R. (1996). Regulation of mitogen-activated protein kinases by a calcium/calmodulin-dependent protein kinase cascade. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 10803-10808.

ESTEBAN, J.A., SHI, S.H., WILSON, C., NURIYA, M., HUGANIR, R.L. & MALINOW, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nature Neuroscience* **6**, 136-143.

FAN, G.H., WANG, L.Z., QIU, H.C., MA, L. & PEI, G. (1999). Inhibition of calcium/calmodulin-dependent protein kinase II in rat hippocampus attenuates morphine tolerance and dependence. *Molecular Pharmacology* **56**, 39-45.

FAN, G.H., ZHANG, W.B., YAO, C.P. & PEI, G. (1997). Modulation by calcium/calmodulin-dependent protein kinase II of functional response of delta opioid receptor in neuroblastoma x glioma hybrid (NG108-15) cells. *Neuropharmacology* **36**, 1763-1769.

FAUX, M.C., SCOTT, J.D., 1996 AUG & 21(8):312-5. (1996). More on target with protein phosphorylation: conferring specificity by location. *Trends in Biochemical Sciences* **21**, 312-315.

FAVATA, M.F., HORIUCHI, K.Y., MANOS, E.J., DAULERIO, A.J., STRADLEY, D.A., FEESER, W.S., VAN DYK, D.E., PITTS, W.J., EARL, R.A., HOBBS, F., COPELAND, R.A., MAGOLDA, R.L., SCHERLE, P.A. & TRZASKOS, J.M. (1998). Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *Journal of Biological Chemistry* **273**, 18623-18632.

FAZELI, M.S., CORBET, J., DUNN, M.J., DOLPHIN, A.C. & BLISS, T.V. (1993). Changes in protein synthesis accompanying long-term potentiation in the dentate gyrus in vivo. *Journal of Neuroscience* **13**, 1346-1353.

FEDERMAN, A.D., CONKLIN, B.R., SCHRADER, K.A., REED, R.R. & BOURNE, H.R. (1992). Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature* **356** , 159-161.

FEDOROV, N.B., PASINELLI, P., OESTREICHER, A.B., DEGRAAN, P.N. & REYMANN, K.G. (1995). Antibodies to postsynaptic PKC substrate neurogranin prevent long-term potentiation in hippocampal CA1 neurons. *European Journal of Neurosience* **7**, 819-822.

FEIG, S. & LIPTON, P. (1993). Pairing the cholinergic agonist carbachol with patterned Schaffer collateral stimulation initiates protein synthesis in hippocampal CA1 pyramidal cell dendrites via a muscarinic, NMDA-dependent mechanism. *Journal of Neuroscience* **13**, 1010-1021.

FELDER, C.C. (1995). Muscarinic acetylcholine receptors: signal transduction through multiple effectors. *FASEB Journal* **9**, 619-625.

FENG, T.P. (1995). The involvement of PKC and multifunctional CaM kinase II of the postsynaptic neuron in induction and maintenance of long-term potentiation. *Progress in Brain Research* **105**, 55-63.

FERGUSON, S.S., DOWNEY, W.E.3., COLAPIETRO, A.M., BARAK, L.S., MENARD, L. & CARON, M.G. (1996). Role of beta-arrestin in mediating agonist-promoted G protein-coupled receptor internalization. *Science* **271**, 363-366.

FERREIRA, A.R., FURSTENAU, L., BLANCO, C., KORNISIUK, E., SANCHEZ, G., DAROIT, D., CASTRO E SILVA, M., CERVENANSKY, C., JERUSALINSKY, D. & QUILLFELDT, J.A. (2003). Role of hippocampal M1 and M4 muscarinic receptor subtypes in memory consolidation in the rat.

Pharmacology Biochemistry Behavior **74**, 411-415.

FERRELL, J.E.J. & MACHLEDER, E.M. (1998). The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes. *Science* **280**, 895-898.

FINKBEINER, S., TAVAZOIE, S.F., MALORATSKY, A., JACOBS, K.M., HARRIS, K.M. & GREENBERG, M.E. (1997). CREB: a major mediator of neuronal neurotrophin responses. *Neuron* **19**, 1031-1047.

FINN, R.C. & BROWNING, M. (1980). Trifluoperazine inhibits hippocampal long-term potentiation and the phosphorylation of a 40,000 dalton protein. *Neuroscience Letters* **19**, 103-108.

FITZJOHN, S.M., BORTOLOTTO, Z.A., PALMER, M.J., DOHERTY, A.J., ORNSTEIN, P.L., SCHOEPP, D.D., KINGSTON, A.E., LODGE, D. & COLLINGRIDGE, G.L. (1998). The potent mGlu receptor antagonist LY341495 identifies roles for both cloned and novel mGlu receptors in hippocampal synaptic plasticity. *Neuropharmacology* **37**, 1445-1458.

FLOOD, J.F., ROSENZWEIG, M.R., BENNETT, E.L. & ORME, A.E. (1973). The influence of duration of protein synthesis inhibition on memory. *Physiol Behav.* **10**, 555-562.

FORD, A.P., WILLIAMS, T.J., BLUE, D.R. & CLARKE, D.E. (1994). Alpha 1-adrenoceptor classification: sharpening Occam's razor. *Trends in Pharmacological Science* **15**, 167-170.

FORSYTHE, I.D. & WESTBROOK, G.L. (1988). Slow excitatory postsynaptic currents mediated by N-methyl-D-aspartate receptors on cultured mouse central neurones. *Journal of Physiology* **396**, 515-533.

FREEDMAN, N.J. & LEFKOWITZ, R.J. (1996). Desensitization of G protein-coupled receptors. *Recent Progress in Hormone Research* **51**, 319-353.

FRENGUELLI, B.G. & MALINOW, R. (1996). Fluctuations in intracellular calcium

responses to action potentials in single en passage presynaptic boutons of layer V neurons in neocortical slices. *Learning and Memory* **3**, 150-159.

FRERKING, M., MALENKA, R.C. & NICOLL, R.A. (1998). Synaptic activation of kainate receptors on hippocampal interneurons. *Nature Neuroscience* **1**, 479-486.

FREY, D., LAUX, T., XU, L., SCHNEIDER, C. & CARONI, P. (2000). Shared and unique roles of CAP23 and GAP43 in actin regulation, neurite outgrowth, and anatomical plasticity. *Journal of Cell Biology* **149**, 1443-1454.

FREY, U., FREY, S., SCHOLLMEIER, F. & KRUG, M. (1996). Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. *Journal of Physiology* **490**, 703-711.

FREY, U., HUANG, Y.Y. & KANDEL, E.R. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* **260**, 1661-1664.

FREY, U., KRUG, M., BRODEMANN, R., REYMANN, K. & MATTHIES, H. (1989). Long-term potentiation induced in dendrites separated from rat's CA1 pyramidal somata does not establish a late phase. *Neuroscience Letters* **97**, 135-139.

FREY, U., KRUG, M., REYMANN, K.G. & MATTHIES, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. *Brain Research* **452**, 57-65.

FREY, U. & MORRIS, R.G. (1997). Synaptic tagging and long-term potentiation. *Nature* **385**, 533-536.

FREY, U. & MORRIS, R.G. (1998). Weak before strong: dissociating synaptic tagging and plasticity-factor accounts of late-LTP. *Neuropharmacology* **37**, 545-552.

FREY, U., SCHOLLMEIER, K., REYMANN, K.G. & SEIDENBECHER, T. (1995). Asymptotic hippocampal long-term potentiation in rats does not preclude

additional potentiation at later phases. *Neuroscience* **67**, 799-807.

FUJII, S., MIKOSHIBA, K., KURODA, Y., AHMED, T.M. & KATO, H. (2003). Cooperativity between activation of metabotropic glutamate receptors and NMDA receptors in the induction of LTP in hippocampal CA1 neurons. *Neuroscience Research* **46**, 509-521.

FUJII, S., SAITO, K., MIYAKAWA, H., ITO, K. & KATO, H. (1991). Reversal of long-term potentiation (depotentiation) induced by tetanus stimulation of the input to CA1 neurons of guinea pig hippocampal slices. *Brain Resarch* **555**, 112-122.

FUKUNAGA, K., MULLER, D. & MIYAMOTO, E. (1995). Increased phosphorylation of Ca2+/calmodulin-dependent protein kinase II and its endogenous substrates in the induction of long-term potentiation. *Journal of Biological Chemistry* **270**, 6119-6124.

FUKUNAGA, K., SODERLING, T.R. & MIYAMOTO, E. (1992). Activation of Ca2+/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. *Journal of Biological* **267**, 22527-22533.

FUKUNAGA, K., STOPPINI, L., MIYAMOTO, E. & MULLER, D. (1993). Long-term potentiation is associated with an increased activity of Ca2+/calmodulin-dependent protein kinase II. *Journal of Biological Chemistry* **268**, 7863-7867.

FURUYA, S., OHMORI, H., SHIGEMOTO, T. & SUGIYAMA, H. (1989). Intracellular calcium mobilization triggered by a glutamate receptor in rat cultured hippocampal cells. *Journal of Physiology* **414**, 539-548.

GALLAGHER, J.P. & HASUO, H. (1989). Excitatory amino acid-receptor-mediated EPSPs in rat dorsolateral septal nucleus neurones in vitro. *Journal of Physiology* **418**, 353-365.

GAO, F.B. (1998). Messenger RNAs in dendrites: localization, stability, and implications for neuronal function. *Bioessays* **20**, 70-78.

GARDIOL, A., RACCA, C. & TRILLER, A. (1999). Dendritic and postsynaptic protein synthetic machinery. *Journal of Neuroscience* **19**, 168-179.

GARDNER, A.M., VAILLANCOURT, R.R. & JOHNSON, G.L. (1993). Activation of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase by G protein and tyrosine kinase oncoproteins. *Journal of Biological Chemistry* **268**, 17896-17901.

GARDONI, F., BELLONE, C., CATTABENI, F. & DI LUCA, M. (2001). Protein kinase C activation modulates alpha-calmodulin kinase II binding to NR2A subunit of N-methyl-D-aspartate receptor complex. *Journal of Biological Chemistry* **276**, 7609-7613.(Abstract)

GASPARINI, F., LINGENHOHL, K., STOEHR, N., FLOR, P.J., HEINRICH, M., VRANESIC, I., BIOLLAZ, M., ALLGEIER, H., HECKENDORN, R., URWYLER, S., VARNEY, M.A., JOHNSON, E.C., HESS S.D., RAO, S.P., SACAAN, A.I., SANTORI, E.M., VELICELEBI, G. & KUHN, R. (1999). 2-Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systemically active mGlu5 receptor antagonist. *Neuropharmacology* **38**, 1493-1503.

GEINISMAN, Y., DE TOLEDO-MORRELL, L., MORRELL, F., HELLER, R.E., ROSSI, M. & PARSHALL, R.F. (1993). Structural synaptic correlate of long-term potentiation: formation of axospinous synapses with multiple, completely partitioned transmission zones. *Hippocampus* **3**, 435-445.

GEINISMAN, Y., DETOLEDO-MORRELL, L. & MORRELL, F. (1991). Induction of long-term potentiation is associated with an increase in the number of axospinous synapses with segmented postsynaptic densities. *Brain Research* **566**, 77-88.

GENOUX, D., HADITSCH, U., KNOBLOCH, M., MICHALON, A., STORM, D. & MANSUY, I.M. (2002). Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature* **418**, 970-975.

GERBER, U., GREENE, R.W., HAAS, H.L. & STEVENS, D.R. (1989). Characterization of inhibition mediated by adenosine in the hippocampus of the rat in vitro. *Journal of Physiology* **417**, 567-578.

GEREAU, R.W.4. & CONN, P.J. (1994). Presynaptic enhancement of excitatory synaptic transmission by beta-adrenergic receptor activation. *Journal of Neurophysiology* **72**, 1438-1442.

GEREAU, R.W.4. & CONN, P.J. (1995). Roles of specific metabotropic glutamate receptor subtypes in regulation of hippocampal CA1 pyramidal cell excitability. *Journal of Neurophysiology* **74**, 122-129.

GERENDASY, D.D. & SUTCLIFFE, J.G. (1997). RC3/neurogranin, a postsynaptic calpacitin for setting the response threshold to calcium influxes. *Molecular Neurobiology* **15**, 131-163.

GHIRARDI, M., BRAHA, O., HOCHNER, B., MONTAROLO, P.G., KANDEL, E.R. & DALE, N. (1992). Roles of PKA and PKC in facilitation of evoked and spontaneous transmitter release at depressed and nondepressed synapses in Aplysia sensory neurons. *Neuron* **9**, 479-489.

GIANOTTI, C., NUNZI, M.G., GISPEN, W.H. & CORRADETTI, R. (1992). Phosphorylation of the presynaptic protein B-50 (GAP-43) is increased during electrically induced long-term potentiation. *Neuron* **8**, 843-848.

GIBBS, M.E. & SUMMERS, R.J. (2000). Separate roles for beta2- and beta3-adrenoceptors in memory consolidation. *Neuroscience* **95**, 913-922.

GIESE, K.P., FEDOROV, N.B., FILIPKOWSKI, R.K. & SILVA, A.J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* **279** , 870-873.

GIOVANNINI, M.G., BLITZER, R.D., WONG, T., ASOMA, K., TSOKAS, P., MORRISON, J.H., IYENGAR, R. & LANDAU, E.M. (2001). Mitogen-activated protein kinase regulates early phosphorylation and delayed expression

of Ca2+/calmodulin-dependent protein kinase II in long-term potentiation. *Journal of Neuroscience* **21**, 7053-7062.

GISPEN, W.H., NIELANDER, H.B., DE GRAAN, P.N., OESTREICHER, A.B., SCHRAMA, L.H. & SCHOTMAN, P. (1991). Role of the growth-associated protein B-50/GAP-43 in neuronal plasticity. *Molecular Neurobiology* **5**, 61-85.

GOELET, P., CASTELLUCCI, V.F., SCHACHER, S. & KANDEL, E.R. (1986). The long and the short of long-term memory--a molecular framework. *Nature* **322**, 419-422.

GOMEZA, J., JOLY, C., KUHN, R., KNOPFEL, T., BOCKAERT, J. & PIN, J.P. (1996). The second intracellular loop of metabotropic glutamate receptor 1 cooperates with the other intracellular domains to control coupling to G-proteins. *Journal of Biological Chemistry* **271**, 2199-2205.

GOOD, P.F., HUNTLEY, G.W., ROGERS, S.W., HEINEMANN, S.F. & MORRISON, J.H. (1993). Organization and quantitative analysis of kainate receptor subunit GluR5-7 immunoreactivity in monkey hippocampus. *Brain Research* **624**, 347-353.

GOODMAN, O.B.Jr., KRUPNICK, J.G., SANTINI, F., GUREVICH, V.V., PENN, R.B., GAGNON, A.W., KEEN, J.H. & BENOVIC, J.L. (1996). Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature* **383**, 447-450.

GOODMAN, R.H. (1990). Regulation of neuropeptide gene expression. *Annual Review of Neuroscience* **13**, 111-127.

GOTO, S., YAMAMOTO, H., FUKUNAGA, K., IWASA, T., MATSUKADO, Y. & MIYAMOTO, E. (1985). Dephosphorylation of microtubule-associated protein 2, tau factor, and tubulin by calcineurin. *Journal of Neurochemistry* **45**, 276-283.

GRAY, R. & JOHNSTON, D. (1987). Noradrenaline and beta-adrenoceptor agonists

increase activity of voltage-dependent calcium channels in hippocampal neurons. *Nature* **327**, 620-622.

GRECKSCH, G., OTT, T. & MATTHIES, H. (1980). The effect of intrahippocampally applied anisomycin on the retention of brightness discrimination in rats. *Behavioral Neural Biology* **29**, 291-288.

GREENE, R.W. & HAAS, H.L. (1985). Adenosine actions on CA1 pyramidal neurones in rat hippocampal slices. *Journal of Physiology* **366**, 119-127.

GREENE, R.W. & HAAS, H.L. (1991). The electrophysiology of adenosine in the mammalian central nervous system. *Progress in Neurobiology* **36**, 329-341.

GROVER, L.M. (1998). Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. *Journal of Neurophysiology* **79**, 1167-1182.

GROVER, L.M. & TEYLER, T.J. (1994). Activation of NMDA receptors in hippocampal area CA1 by low and high frequency orthodromic stimulation and their contribution to induction of long-term potentiation. *Synapse* **16**, 66-75.

GROVER, L.M. & TEYLER, T.J. (1990). Two components of long-term potentiation induced by different patterns of afferent activation. *Nature* **347**, 477-479.

GUSTAFSSON, B., WIGSTROM, H., ABRAHAM, W.C. & HUANG, Y.Y. (1987). Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *Journal of Neuroscience* **7**, 774-780.

GUTKIND, J.S. (1998). The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. *Journal of Biological Chemistry* **273**, 1839-1842.

GUZOWSKI, J.F., LYFORD, G.L., STEVENSON, G.D., HOUSTON, F.P., MCGAUGH, J.L., WORLEY, P.F. & BARNES, C.A. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the

maintenance of long-term potentiation and the consolidation of long-term memory. *Journal of Neuroscience* **20**, 3993-4001.

GUZOWSKI, J.F. & MCGAUGH, J.L. (1997). Antisense oligodeoxynucleotide-mediated disruption of hippocampal cAMP response element binding protein levels impairs consolidation of memory for water maze training. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 2693-2698.

HADARI, Y.R., KOUHARA, H., LAX, I. & SCHLESSINGER, J. (1998). Binding of Shp2 tyrosine phosphatase to FRS2 is essential for fibroblast growth factor-induced PC12 cell differentiation. *Molecular Cell Biology* **18**, 3966-3973.

HAFNER, S., ADLER, H.S., MISCHAK, H., JANOSCH, P., HEIDECKER, G., WOLFMAN, A., PIPPIG, S., LOHSE, M., UEFFING, M. & KOLCH, W. (1994). Mechanism of inhibition of Raf-1 by protein kinase A. *Molecular Cell Biology* **14**, 6696-6703.

HAMPSON, D.R., HUANG, X.P., OBERDORFER, M.D., GOH, J.W., AUYEUNG, A. & WENTHOLD, R.J. (1992). Localization of AMPA receptors in the hippocampus and cerebellum of the rat using an anti-receptor monoclonal antibody. *Neuroscience* **50**, 11-22.

HAN, G. & HAMPSON, D.R. (1999). Ligand binding to the amino-terminal domain of the mGluR4 subtype of metabotropic glutamate receptor. *Journal of Biological Chemistry* **274**, 10008-10013.

HAN, J., LEE, J.D., BIBBS, L. & ULEVITCH, R.J. (1994). A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **265**, 808-811.

HANSON, P.I. & SCHULMAN, H. (1992). Inhibitory autophosphorylation of multifunctional Ca2+/calmodulin-dependent protein kinase analyzed by site-directed mutagenesis. *Journal of Biological Chemistry* **267**, 17216-17224.

HARDINGHAM, G.E., CHAWLA, S., CRUZALEGUI, F.H. & BADING, H. (1999).

Control of recruitment and transcription-activating function of CBP determines gene regulation by NMDA receptors and L-type calcium channels. *Neuron* **22**, 789-798.

HARRIS, E.W. & COTMAN, C.W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. *Neuroscience Letters* **70**, 132-137.

HARRIS, E.W., GANONG, A.H. & COTMAN, C.W. (1984). Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. *Brain Research* **323**, 132-137.

HARUM, K.H., ALEMI, L. & JOHNSTON, M.V. (2001). Cognitive impairment in Coffin-Lowry syndrome correlates with reduced RSK2 activation. *Neurology* **56**, 207-214.

HARVEY, J. & COLLINGRIDGE, G.L. (1992). Thapsigargin blocks the induction of long-term potentiation in rat hippocampal slices. *Neuroscience Letters* **139**, 197-200.

HAWES, B.E., VAN BIESEN, T., KOCH, W.J., LUTTRELL, L.M. & LEFKOWITZ, R.J. (1995). Distinct pathways of Gi- and Gq-mediated mitogen-activated protein kinase activation. *Journal of Biological Chemistry* **270**, 17148-17153.

HAYASHI, Y., MOMIYAMA, A., TAKAHASHI, T., OHISHI, H., OGAWA-MEGURO, R., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1993). Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature* **366**, 687-690.

HAYASHI, Y., SEKIYAMA, N., NAKANISHI, S., JANE, D.E., SUNTER, D.C., BIRSE, E.F., UDVARHELYI, P.M. & WATKINS, J.C. (1994). Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. *Journal of Neuroscience* **14**, 3370-3377.

HAYASHI, Y., SHI, S.H., ESTEBAN, J.A., PICCINI, A., PONCER, J.C. & MALINOW, R. (2000). Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. *Science* **287**, 2262-2267.

HEBB, D.O. (1949). The organisation of the brain: A neuropsychological theory. *Wiley, New York*

HELMCHEN, F., IMOTO, K. & SAKMANN, B. (1996). Ca2+ buffering and action potential-evoked Ca2+ signaling in dendrites of pyramidal neurons. *Biophysical Journal* **70**, 1069-1081.

HERB, A., BURNASHEV, N., WERNER, P., SAKMANN, B., WISDEN, W. & SEEBURG, P.H. (1992). The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron* **8**, 775-785.

HERDEGEN, T. & LEAH, J.D. (1998). Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. *Brain Research Reviews* **3**,

HESTRIN, S., NICOLL, R.A., PERKEL, D.J. & SAH, P. (1990). Analysis of excitatory synaptic action in pyramidal cells using whole-cell recording from rat hippocampal slices. *Journal of Physiology* **422**, 203-225.

HIDAKA, H. & YOKOKURA, H. (1996). Molecular and cellular pharmacology of a calcium/calmodulin-dependent protein kinase II (CaM kinase II) inhibitor, KN-62, and proposal of CaM kinase phosphorylation cascades. *Advances in Pharmacology* **36**, 193-219.

HILLE, B. (1994). Modulation of ion-channel function by G-protein-coupled receptors. *Trends in Neurosience* **17**, 531-536.

HINTZMAN, D.L. (1974). Theoretical implications of the spacing effect. *In: Theories in Cognitive Psychology: the Loyola Symposium, edited by Solso R.L. Hillsdale,*

Erlbaum. 77-99.

HIRASAWA, A., SHIBATA, K., HORIE, K., TAKEI, Y., OBIKA, K., TANAKA. T., MURAMOTO, N., TAKAGAKI, K., YANO, J. & TSUJIMOTO, G. (1995). Cloning, functional expression and tissue distribution of human alpha 1c-adrenoceptor splice variants. *FEBS Letters* **363** , 256-260.

HIROTSU, I., HORI, N., KATSUDA, N. & ISHIHARA, T. (1989). Effect of anticholinergic drug on long-term potentiation in rat hippocampal slices. *Brain Research* **482**, 194-197.

HO, N., LIAUW, J.A., BLAESER, F., WEI, F., HANISSIAN, S., MUGLIA, L.M., WOZNIAK, D.F., NARDI, A., ARVIN, K.L., HOLTZMAN, D.M., LINDEN, D.J., ZHUO, M., MUGLIA, L.J. & CHATILA, T.A. (2000). Impaired synaptic plasticity and cAMP response element-binding protein activation in Ca2+/calmodulin-dependent protein kinase type IV/Gr-deficient mice. *Journal of Neuroscience* **20**, 6459-6472.

HOFFMAN, D.A. & JOHNSTON, D. (1998). Downregulation of transient K+ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC. *Journal of Neuroscience* **18**, 3521-3528.

HOFFMAN, D.A., MAGEE, J.C., COLBERT, C.M. & JOHNSTON, D. (1997). K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. *Nature* **387**, 869-875.

HOLGADO-MADRUGA, M., EMLET, D.R., MOSCATELLO, D.K., GODWIN, A.K. & WONG, A.J. (1996). A Grb2-associated docking protein in EGF- and insulin-receptor signalling. *Nature* **379**, 560-564.

HOLLMANN, M. & HEINEMANN, S. (1994). Cloned glutamate receptors. *Annual Review of Neuroscience* **17**, 31-108.

HOLSCHER, C., ANWYL, R. & ROWAN, M.J. (1997). Activation of group-II metabotropic glutamate receptors blocks induction of long-term potentiation

and depotentiation in area CA1 of the rat in vivo. *European Journal of Pharmacology* **322**, 155-163.

HONORE, T., DAVIES, S.N., DREJER, J., FLETCHER, E.J., JACOBSEN, P., LODGE, D. & NIELSEN, F.E. (1988). Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. *Science* **241**, 701-703.

HOPKINS, W.F. & JOHNSTON, D. (1988). Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *Journal of Neurophysiology* **59**, 667-687.

HOUAMED, K.M., KUIJPER, J.L., GILBERT, T.L., HALDEMAN, B.A., O'HARA, P.J., MULVIHILL. E.R., ALMERS, W. & HAGEN, F.S. (1991). Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science* **252**, 1318-1321.

HSIA, A.Y., SALIN, P.A., CASTILLO, P.E., AIBA, A., ABELIOVICH, A., TONEGAWA, S. & NICOLL, R.A. (1995). Evidence against a role for metabotropic glutamate receptors in mossy fiber LTP: the use of mutant mice and pharmacological antagonists. *Neuropharmacology* **34**, 1567-1572.

HU, J., HAUACHE, O. & SPIEGEL, A.M. (2000). Human Ca2+ receptor cysteine-rich domain. Analysis of function of mutant and chimeric receptors. *Journal of Biological Chemistry* **275**, 16382-16389.

HUANG, C.C., LIANG, Y.C. & HSU, K.S. (2001). Characterization of the mechanism underlying the reversal of long term potentiation by low frequency stimulation at hippocampal CA1 synapses. *Journal of Biological Chemistry* **276**, 48108-48117.

HUANG, K.P. & HUANG, F.L. (1993). How is protein kinase C activated in CNS. *Neurochemistry International* **22**, 417-433.

HUANG, L.Q., ROWAN, M.J. & ANWYL, R. (1997). mGluR II agonist inhibition of LTP induction, and mGluR II antagonist inhibition of LTD induction, in the

dentate gyrus in vitro. *NeuroReport* **8**, 687-693.

HUANG, Y.Y., COLINO, A., SELIG, D.K. & MALENKA, R.C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. *Science* **255**, 730-733.

HUANG, Y.Y. & KANDEL, E.R. (1996). Modulation of both the early and the late phase of mossy fiber LTP by the activation of beta-adrenergic receptors. *Neuron* **16**, 611-617.

HUANG, Y.Y. & KANDEL, E.R. (1994). Recruitment of long-lasting protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learning and Memory* **1**, 74-82.

HUANG, Y.Y., KANDEL, E.R., VARSHAVSKY, L., BRANDON, E.P., QI, M., IDZERDA, R.L., MCKNIGHT, G.S. & BOURTCHOULADZE, R. (1995). A genetic test of the effects of mutations in PKA on mossy fiber LTP and its relation to spatial and contextual learning. *Cell* **83**, 1211-1222.

HUANG, Y.Y., LI, X.C. & KANDEL, E.R. (1994). cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. *Cell* **79**, 69-79.

HUBER, K.M., MAUK, M.D., THOMPSON, C. & KELLY, P.T. (1995). A critical period of protein kinase activity after tetanic stimulation is required for the induction of long-term potentiation. *Learning and Memory* **2**, 81-100.

HUERTA, P.T. & LISMAN, J.E. (1995). Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 in vitro. *Neuron* **15**, 1053-1063.

HUERTA, P.T. & LISMAN, J.E. (1993). Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. *Nature* **364**, 723-725.

HUGHES, P. & DRAGUNOW, M. (1995). Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacological Reviews* **47**, 133-178.

HULO, S., ALBERI, S., LAUX, T., MULLER, D. & CARONI, P. (2002). A point mutant of GAP-43 induces enhanced short-term and long-term hippocampal plasticity. *European Journal of Neuroscience* **15**, 1976-1982.

HUNTER, T. (1995). Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signalling. *Cell* **80**, 225-236.

HURLEY, J.H. (1999). Structure, mechanism, and regulation of mammalian adenylyl cyclase. *Journal of Biological Chemistry* **274**, 7599-7602.

HUSI, H. & GRANT, S.G. (2001). Isolation of 2000-kDa complexes of N-methyl-D-aspartate receptor and postsynaptic density 95 from mouse brain. *Journal of Neurochemistry* **77**, 281-291.

HUSI, H., WARD, M.A., CHOUDHARY, J.S., BLACKSTOCK, W.P. & GRANT, S.G. (2000). Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neuroscience* **3**, 661-669.

HVALBY, O., HEMMINGS, H.C.J., PAULSEN, O., CZERNIK, A.J., NAIRN, A.C., GODFRAIND, J.M., JENSEN, V., RAASTAD, M., STORM, J.F., ANDERSEN, P. & ET AL. (1994). Specificity of protein kinase inhibitor peptides and induction of long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 4761-4765.

HYATT, S.L., LIAO, L., ADEREM, A., NAIRN, A.C. & JAKEN, S. (1994). Correlation between protein kinase C binding proteins and substrates in REF52 cells. *Cell Growth and Differentiation* **5**, 495-502.

IANNAZZO, L. & MAJEWSKI, H. (2000). M(2)/M(4)-muscarinic receptors mediate automodulation of acetylcholine outflow from mouse cortex. *Neuroscience Letters* **287**, 129-132.

IKEDA, K., NAGASAWA, M., MORI, H., ARAKI, K., SAKIMURA, K., WATANABE, M., INOUE, Y. & MISHINA, M. (1992). Cloning and expression of the epsilon 4 subunit of the NMDA receptor channel. *FEBS* **313**, 34-38.

IMPEY, S., MARK, M., VILLACRES, E.C., POSER, S., CHAVKIN, C. & STORM, D.R. (1996). Induction of CRE-mediated gene expression by stimuli that generate long-lasting LTP in area CA1 of the hippocampus. *Neuron* **16**, 973-982.

IMPEY, S., OBRIETAN, K., WONG, S.T., POSER, S., YANO, S., WAYMAN, G., DELOULME, J.C., CHAN, G. & STORM, D.R. (1998). Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron* **21**, 869-883.

INIGUEZ, M.A., RODRIGUEZ-PENA, A., IBARROLA, N., MORREALE DE ESCOBAR, G. & BERNAL, J. (1992). Adult rat brain is sensitive to thyroid hormone. Regulation of RC3/neurogranin mRNA. *Journal of Clinical Investigation* **90**, 554-558.

INOUE, T., KATO, K., KOHDA, K. & MIKOSHIBA, K. (1998). Type 1 inositol 1,4,5-trisphosphate receptor is required for induction of long-term depression in cerebellar Purkinje neurons. *Journal of Neuroscience* **18**, 5366-573.

IRVING, A.J., SCHOFIELD, J.G., WATKINS, J.C., SUNTER, D.C. & COLLINGRIDGE, G.L. (1990). 1S,3R-ACPD stimulates and L-AP3 blocks Ca2+ mobilization in rat cerebellar neurons. *European Journal of Pharmacology* **186**, 363-365.

ISA, T.I.S.I.M.T.K.O.S. (1996). Distribution of neurones expressing inwardly rectifying and Ca(2+)-permeable AMPA receptors in rat hippocampal slices. *Journal of Physiology* **491**, 719-733.

ISAAC, J.T., NICOLL, R.A. & MALENKA, R.C. (1995). Evidence for silent synapses:

implications for the expression of LTP. *Neuron* **15**, 427-34.

ISAACSON, J.S. & NICOLL, R.A. (1991). Aniracetam reduces glutamate receptor desensitization and slows the decay of fast excitatory synaptic currents in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **88** , 10936-10940.

ISHIDA, M., SAITOH, T., SHIMAMOTO, K., OHFUNE, Y. & SHINOZAKI, H. (1993). A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord. *British Journal of Pharmacology* **109**, 1169-1177.

ITO, I., HIDAKA, H. & SUGIYAMA, H. (1991). Effects of KN-62, a specific inhibitor of calcium/calmodulin-dependent protein kinase II, on long-term potentiation in the rat hippocampus. *Neuroscience Letters* **121**, 119-121.

ITO, I., KOHDA, A., TANABE, S., HIROSE, E., HAYASHI, M., MITSUNAGA, S. & SUGIYAMA, H. (1992). 3,5-Dihydroxyphenyl-glycine: a potent agonist of metabotropic glutamate receptors. *NeuroReport* **3**, 1013-1016.

ITO, T., MIURA, Y. & KADOKAWA, T. (1988). Effects of physostigmine and scopolamine on long-term potentiation of hippocampal population spikes in rats. *Canadian Journal of Physiology and Pharmacology* **66**, 1010-1016.

IYENGAR, R. (1996). Gating by cyclic AMP: expanded role for an old signaling pathway. *Science* **271**, 461-463.

IZUMI, Y., CLIFFORD, D.B. & ZORUMSKI, C.F. (1991). 2-Amino-3-phosphonopropionate blocks the induction and maintenance of long-term potentiation in rat hippocampal slices. *Neuroscience Letters* **122**, 187-190.

IZUMI, Y., CLIFFORD, D.B. & ZORUMSKI, C.F. (1992a). Low concentrations of N-methyl-D-aspartate inhibit the induction of long-term potentiation in rat hippocampal slices. *Neuroscience Letters* **137**, 245-248.

IZUMI, Y., CLIFFORD, D.B. & ZORUMSKI, C.F. (1992b). Norepinephrine reverses N-methyl-D-aspartate-mediated inhibition of long-term potentiation in rat hippocampal slices. *Neuroscience Letters* **142**, 163-166.

IZUMI, Y. & ZORUMSKI, C.F. (1998). LTP in CA1 of the adult rat hippocampus and voltage-activated calcium channels. *NeuroReport* **9**, 3689-3691.

JAHR, CE. & STEVENS, C.F. (1993). Calcium permeability of the N-methyl-D-aspartate receptor channel in hippocampal neurons in culture. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11573-11577.

JANE, D.E., JONES, P.L., POOK, P.C., SALT, T.E., SUNTER, D.C. & WATKINS, J.C. (1993). Stereospecific antagonism by (+)-alpha-methyl-4-carboxyphenylglycine (MCPG) of (1S,3R)-ACPD-induced effects in neonatal rat motoneurones and rat thalamic neurones. *Neuropharmacology* **32**, 725-727.

JANE, D.E., PITTAWAY, K., SUNTER, D.C., THOMAS, N.K. & WATKINS, J.C. (1995). New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal rat spinal cord. *Neuropharmacology* **34**, 851-856.

JARRARD, L.E., OKAICHI, H., STEWARD, O. & GOLDSCHMIDT, R.B. (1984). On the role of hippocampal connections in the performance of place and cue tasks: comparisons with damage to hippocampus. *Behavioural Neurosience* **98**, 946-954.

JIA, Z., LU, Y., HENDERSON, J., TAVERNA, F., ROMANO, C., ABRAMOW-NEWERLY, W., WOJTOWICZ, J.M. & RODER, J. (1998). Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. *Learning and Memory* **5**, 331-343.

JOB, C. & EBERWINE, J. (2001). Identification of sites for exponential translation in living dendrites. *Proceedings of the National Academy of Sciences of the*

United States of America **98**, 13037-13042.

JOHNSON, J.W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325**, 529-531.

JONAS, P. & SAKMANN, B. (1992). Glutamate receptor channels in isolated patches from CA1 and CA3 pyramidal cells of rat hippocampal slices. *Journal of Physiology* **455**, 143-171.

JONES, M.W., FRENCH, P.J., BLISS, T.V. & ROSENBLUM, K. (1999). Molecular mechanisms of long-term potentiation in the insular cortex in vivo. *Journal of Neuroscience* **19**, RC36

JOVANOVIC, J.N., BENFENATI, F., SIOW, Y.L., SIHRA, T.S., SANGHERA, J.S., PELECH, S.L., GREENGARD, P. & CZERNIK, A.J. (1996). Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 3679-3683.

JOYCE, J.N., GIBBS, R.B., COTMAN, C.W. & MARSHALL, J.F. (1989). Regulation of muscarinic receptors in hippocampus following cholinergic denervation and reinnervation by septal and striatal transplants. *Journal of Neuroscience* **9**, 2776-2791.

JOYCE, J.N., GIBBS, R.B., COTMAN, C.W. & MARSHALL, J.F. (1989). Regulation of muscarinic receptors in hippocampus following cholinergic denervation and reinnervation by septal and striatal transplants. *Journal of Neuroscience* **9**, 2776-2791.

KAANG, B.K., KANDEL, E.R. & GRANT, S.G. (1993). Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. *Neuron* **10**, 427-435.

KACHARMINA, J.E., JOB, C., CRINO, P. & EBERWINE, J. (2000). Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated

neuronal dendrites. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 11545-11550.

KAMEYAMA, K., LEE, H.K., BEAR, M.F. & HUGANIR, R.L. (1998). Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. *Neuron* **21**, 1163-1175.

KANTEREWICZ, B.I., URBAN, N.N., MCMAHON, D.B., NORMAN, E.D., GIFFEN, L.J., FAVATA, M.F., SCHERLE, P.A., TRZSKOS, J.M., BARRIONUEVO, G. & KLANN, E. (2000). The extracellular signal-regulated kinase cascade is required for NMDA receptor-independent LTP in area CA1 but not area CA3 of the hippocampus. *Journal of Neuroscience* **20**, 3057-3066.

KAPUR, A., YECKEL, M.F., GRAY, R. & JOHNSTON, D. (1998). L-Type calcium channels are required for one form of hippocampal mossy fiber LTP. *Journal of Neurophysiology* **79**, 2181-2190.

KASAHARA, J., FUKUNAGA, K. & MIYAMOTO, E. (2001). Activation of calcium/calmodulin-dependent protein kinase IV in long term potentiation in the rat hippocampal CA1 region. *Journal of Biological Chemistry* **276**, 24044-24050.

KASAHARA, J., FUKUNAGA, K. & MIYAMOTO, E. (1999). Differential effects of a calcineurin inhibitor on glutamate-induced phosphorylation of Ca2+/calmodulin-dependent protein kinases in cultured rat hippocampal neurons. *Journal of Biological Chemistry* **274**, 9061-9067.

KASE, H., IWAHASHI, K., NAKANISHI, S., MATSUDA, Y., YAMADA, K., TAKAHASHI, M., MURAKATA, C., SATO, A. & KANEKO, M. (1987). K-252 compounds, novel and potent inhibitors of protein kinase C and cyclic nucleotide-dependent protein kinases. *Biochemistry and Biophysical Research Communications* **142**, 436-440.

KATSUKI, H., IZUMI, Y. & ZORUMSKI, C.F. (1997). Noradrenergic regulation of

synaptic plasticity in the hippocampal CA1 region. *Journal of Neurophysiology* **77**, 3013-3020.

KAUPMANN, K., HUGGEL, K., HEID, J., FLOR, P.J., BISCHOFF, S., MICKEL, S.J., MCMASTER, G., ANGST, C., BITTIGER, H., FROESTL, W. & BETTLER, B. (1997). Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386**, 239-246.

KAVALALI, E.T., KLINGAUF, J. & TSIEN, R.W. (1999). Activity-dependent regulation of synaptic clustering in a hippocampal culture system. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 12893-12900.

KAWABATA, S., TSUTSUMI, R., KOHARA, A., YAMAGUCHI, T., NAKANISHI, S. & OKADA, M. (1996). Control of calcium oscillations by phosphorylation of metabotropic glutamate receptors. *Nature* **383**, 89-92.

KAWASAKI, H., SPRINGETT, G.M., MOCHIZUKI, N., TOKI, S., NAKAYA, M., MATSUDA, M., HOUSMAN, D.E. & GRAYBIEL, A.M. (1998a). A family of cAMP-binding proteins that directly activate Rap1. *Science* **282**, 2275-2279.

KAWASAKI, H., SPRINGETT, G.M., TOKI, S., CANALES, J.J., HARLAN, P., BLUMENSTIEL, J.P., CHEN, E.J., BANY, I.A., MOCHIZUKI, N., ASHBACHER, A., MATSUDA, M., HOUSMAN, D.E. & GRAYBIEL, A.M. (1998b). A Rap guanine nucleotide exchange factor enriched highly in the basal ganglia. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 13278-13283.

KEINANEN, K., WISDEN, W., SOMMER, B., WERNER, P., HERB, A., VERDOORN, T.A., SAKMANN, B. & SEEBURG, P.H. (1990). A family of AMPA-selective glutamate receptors. *Science* **249**, 556-560.

KELLY, A. & LYNCH, M.A. (2000). Long-term potentiation in dentate gyrus of the rat is inhibited by the phosphoinositide 3-kinase inhibitor, wortmannin.

Neuropharmacology **39** , 643-651.

KELSO, S.R., GANONG, A.H. & BROWN, T.H. (1986). Hebbian synapses in hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 5326-5330.

KELSO, S.R., NELSON, T.E. & LEONARD, J.P. (1992). Protein kinase C-mediated enhancement of NMDA currents by metabotropic glutamate receptors in Xenopus oocytes. *Journal of Physiology* **449**, 705-718.

KEMP, M., ROBERTS, P., POOK, P., JANE, D., JONES, A., JONES, P., SUNTER, D., UDVARHELYI, P. & WATKINS, J. (1994). Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors. *European Journal of Pharmacology* **266**, 187-192.

KEMP, N. & BASHIR, Z.I. (1999). Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. *Neuropharmacology* **38**, 495-504.

KERNER, J.A., STANDAERT, D.G., PENNEY, J.B.Jr., YOUNG, A.B. & LANDWEHRMEYER. G.B. (1997). Expression of group one metabotropic glutamate receptor subunit mRNAs in neurochemically identified neurons in the rat neostriatum, neocortex, and hippocampus. *Molecular Brain Research* **48**, 259-269.

KIDD, F.L. & ISAAC, J.T. (1999). Developmental and activity-dependent regulation of kainate receptors at thalamocortical synapses. *Nature* **400**, 569-573.

KIM, C.H. & LISMAN, J.E. (1999). A role of actin filament in synaptic transmission and long-term potentiation. *Journal of Neuroscience* **19**, 4314-4324.

KIM, J.H., LIAO, D., LAU, L.F. & HUGANIR, R.L. (1998). SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron* **20**, 683-691.

KINGSTON, A.E., ORNSTEIN, P.L., WRIGHT, R.A., JOHNSON, B.G., MAYNE, N.G., BURNETT, J.P., BELAGAJE, R., WU, S. & SCHOEPP, D.D. (1998). LY341495 is a nanomolar potent and selective antagonist of group II metabotropic glutamate receptors. *Neuropharmacology* **37**, 1-12.

KJELSBERG, M.A., COTECCHIA, S., OSTROWSKI, J., CARON, M.G. & LEFKOWITZ, R.J. (1992). Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. *Journal of Biological Chemistry* **26**, 1430-1433.

KLANN, E., CHEN, S.J. & SWEATT, J.D. (1991). Persistent protein kinase activation in the maintenance phase of long-term potentiation. *Journal of Biological Chemistry* **266**, 24253-24256.

KLECKNER, N.W. & DINGLEDINE, R. (1988). Requirement for glycine in activation of NMDA-receptors expressed in Xenopus oocytes. *Science* **241**, 835-837.

KLUKOWSKI, G. & HARLEY, C.W. (1994). Locus coeruleus activation induces perforant path-evoked population spike potentiation in the dentate gyrus of awake rat. *Experimental Brain Research* **102**, 165-170.

KOBILKA, B.K., KOBILKA, T.S., DANIEL, K., REGAN, J.W., CARON, M.G. & LEFKOWITZ, R.J. (1988). Chimeric alpha 2-,beta 2-adrenergic receptors: delineation of domains involved in effector coupling and ligand binding specificity. *Science* **240**, 1310-1316.

KOESTER, H.J. & SAKMANN, B. (1998). Calcium dynamics in single spines during coincident pre- and postsynaptic activity depend on relative timing of back-propagating action potentials and subthreshold excitatory postsynaptic potentials. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 9596

KOGAN, J.H., FRANKLAND, P.W., BLENDY, J.A., COBLENTZ, J., MAROWITZ,

Z., SCHUTZ, G. & SILVA, A.J. (1997). Spaced training induces normal long-term memory in CREB mutant mice. *Current Biology* **7**, 1-11.

KOH, D.S., GEIGER, J.R., JONAS, P. & SAKMANN, B. (1995). Ca(2+)-permeable AMPA and NMDA receptor channels in basket cells of rat hippocampal dentate gyrus. *Journal of Physiology* **485**, 383-402.

KOMIYAMA, N.H., WATABE, A.M., CARLISLE, H.J., PORTER, K., CHARLESWORTH, P., MONTI, J., STRATHDEE, D.J., O'CARROLL, C.M., MARTIN, S.J., MORRIS, R.G., O'DELL, T.J. & GRANT, S.G. (2002). SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with postsynaptic density 95 and NMDA receptor. *Journal of Neuroscience* **22**, 9721-9732.

KONORSKI, J. (1948). Conditioned Reflexes and Neuron Organization. *London: Cambridge University Press*

KOUHARA, H., HADARI, Y.R., SPIVAK-KROIZMAN, T., SCHILLING, J., BAR-SAGI, D., LAX, I. & SCHLESSINGER, J. (1997). A lipid-anchored Grb2-binding protein that links FGF-receptor activation to the Ras/MAPK signaling pathway. *Cell* **89**, 693-702.

KOVALCHUK, Y., EILERS, J., LISMAN, J. & KONNERTH, A. (2000). NMDA receptor-mediated subthreshold Ca(2+) signals in spines of hippocampal neurons. *Journal of Neuroscience* **20** , 1791-1799.

KRUCKER, T., SIGGINS, G.R. & HALPAIN, S. (2000). Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 6856-6861.

KRUCKER, T., SIGGINS, G.R., MCNAMARA, R.K., LINDSLEY, K.A., DAO, A., ALLISON, D.W., DE LECEA, L., LOVENBERG, T.W., SUTCLIFFE, J.G. & GERENDASY, D.D. (2002). Targeted disruption of RC3 reveals a calmodulin-

based mechanism for regulating metaplasticity in the hippocampus. *Journal of Neuroscience* **22**, 5525-5535.

KRUG, M., LOSSNER, B. & OTT, T. (1984). Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Research Bulletin* **13**, 39-42.

KULLMANN, D.M. (1994). Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. *Neuron* **12**, 1111-1120.

KULLMANN, D.M. (1999). Synaptic and extrasynaptic roles of glutamate in the mammalian hippocampus. *Acta Physiologica Scandinavica* **16**, 79-83.

KULLMANN, D.M. & ASZTELY, F. (1998). Extrasynaptic glutamate spillover in the hippocampus: evidence and implications. *Trends in Neuroscience* **21**, 8-14.

KULLMANN, D.M., ERDEMLI, G. & ASZTELY, F. (1996). LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. *Neuron* **17**, 461-474.

KULLMANN, D.M., MIN, M.Y., ASZTELY, F. & RUSAKOV, D.A. (1999). Extracellular glutamate diffusion determines the occupancy of glutamate receptors at CA1 synapses in the hippocampus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **354**, 395-402.

KULLMANN, D.M. & SIEGELBAUM, S.A. (1995). The site of expression of NMDA receptor-dependent LTP: new fuel for an old fire. *Neuron* **15**, 997-1002.

KUNER, T., WOLLMUTH, L.P., KARLIN, A., SEEBURG, P.H. & SAKMANN, B. (1996). Structure of the NMDA receptor channel M2 segment inferred from the accessibility of substituted cysteines. *Neuron* **17**, 343-352.

KUNISHIMA, N., SHIMADA, Y., TSUJI, Y., SATO, T., YAMAMOTO, M., KUMASAKA, T., NAKANISHI, S., JINGAMI, H. & MORIKAWA, K. (2000).

Structural basis of glutamate recognition by a dimeric metabotropic glutamate receptor. *Nature* **407**, 971-977.

KUTSUWADA, T., KASHIWABUCHI, N., MORI, H., SAKIMURA, K., KUSHIYA, E., ARAKI, K., MEGURO, H., MASAKI, H., KUMANISHI, T., ARAKAWA, M. & ET AL. (1992). Molecular diversity of the NMDA receptor channel. *Nature* **358**, 36-41.

KYOSSEVA, S.V., OWENS, S.M., ELBEIN, A.D. & KARSON, C.N. (2001). Differential and region-specific activation of mitogen-activated protein kinases following chronic administration of phencyclidine in rat brain. *Neuropsychopharmacology* **24**, 267-277.

KYRIAKIS, J.M., BANERJEE, P., NIKOLAKAKI, E., DAI, T., RUBIE, E.A., AHMAD, M.F., AVRUCH, J. & WOODGETT, J.R. (1994). The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* **369**, 156-160.

LANAHAN, A. & WORLEY, P. (1998). Immediate-early genes and synaptic function. *Neurobiology of Learning and Memory* **70**, 37-43.

LANGLEY, J.N. (1901). Observations on the phsiological action of extracts of the supra-renal bodies. *Journal of Physiology* **256**, 2010-2013.

LANGLEY, J.N. (1900). The sympathetic and other related systems of nerves. *In E.A. Schafer (Ed.), Text-book of Physiology. London: Pentland.* **Vol 2**, 616-696.

LARSON, J., WONG, D. & LYNCH, G. (1986). Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. *Brain Research* **368** , 347-350.

LATTAL, K.M. (1999). Trial and intertrial durations in Pavlovian conditioning: issues of learning and performance. *Journal of Experimental Psychology and Animal Behavioral Processes* **25**, 433-450.

LAUBE, B., KUHSE, J. & BETZ, H. (1998). Evidence for a tetrameric structure of

recombinant NMDA receptors. *Journal of Neuroscience* **18**, 2954-2961.

LAUX, T., FUKAMI, K., THELEN, M., GOLUB, T., FREY, D. & CARONI, P. (2000). GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. *Journal of Cell Biology* **149**, 1455-1472.

LEAHY, J.C., LUO, Y., KENT, C.S., MEIRI, K.F. & VALLANO, M.L. (1993). Demonstration of presynaptic protein kinase C activation following long-term potentiation in rat hippocampal slices. *Neuroscience* **52**, 563-574.

LEE, H.K., BARBAROSIE, M., KAMEYAMA, K., BEAR, M.F. & HUGANIR, R.L. (2000). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **405**, 955-959.

LEE, J.D., ULEVITCH, R.J. & HAN, J. (1995). Primary structure of BMK1: a new mammalian map kinase. *Biochemical and Biophysical Research Communications* **213**, 715-724.

LEONARD, A.S. & HELL, J.W. (1997). Cyclic AMP-dependent protein kinase and protein kinase C phosphorylate N-methyl-D-aspartate receptors at different sites. *Journal of Biological Chemistry* **272**, 12107-12115.

LESTER, R.A., CLEMENTS, J.D., WESTBROOK, G.L. & JAHR, C.E. (1990). Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature* **346**, 565-567.

LEV, S., MORENO, H., MARTINEZ, R., CANOLL, P., PELES, E., MUSACCHIO, J.M., PLOWMAN, G.D., RUDY, B. & SCHLESSINGER, J. (1995). Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. *Nature* **376**, 737-745.

LEVEY, A.I., EDMUNDS, S.M., KOLIATSOS, V., WILEY, R.G. & HEILMAN, C.J. (1995). Expression of m1-m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. *Journal of*

Neuroscience **15**, 4077-4092.

LEVEY, A.I., KITT, C.A., SIMONDS, W.F., PRICE, D.L. & BRANN, M.R. (1991). Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. *Journal of Neuroscience* **11**, 3218-3226.

LEVKOVITZ, Y. & SEGAL, M. (1994). Acetylcholine mediates the effects of fenfluramine on dentate granule cell excitability in the rat. *European Journal of Pharmacology* **264**, 279-284.

LEVY, W.B. & STEWARD, O. (1979). Synapses as associative memory elements in the hippocampal formation. *Brain Research* **175**, 233-245.

LEWANDOWSKY, M. (1898). Ueber eine Wirkung des Nebennieren-extractes auf das Auge. *Zentralbl. Physiol.* **12**, 599-600.

LEWIS, F.T. (1923). The significance of the term *hippocampus.* *Journal of Comparative Neurology* **35**, 213-230.

LI, H. & ROGAWSKI, M.A. (1998). GluR5 kainate receptor mediated synaptic transmission in rat basolateral amygdala in vitro. *Neuropharmacology* **37**, 1279-1286.

LI, P., WILDING, T.J., KIM, S.J., CALEJESAN, A.A., HUETTNER, J.E. & ZHUO, M. (1999). Kainate-receptor-mediated sensory synaptic transmission in mammalian spinal cord. *Nature* **397**, 161-164.

LI, X.G., SOMOGYI, P., YLINEN, A. & BUZSAKI, G. (1994). The hippocampal CA3 network: an in vivo intracellular labeling study. *Journal of Comparative Neurology* **339**, 181-208.

LIAO, D., HESSLER, N.A. & MALINOW, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* **375**, 400-404.

LIAO, D. & MALINOW, R. (1996). Deficiency in induction but not expression of LTP in hippocampal slices from young rats. *Learning and Memory* **3**, 138-149.

LIAO, D., SCANNEVIN, R.H. & HUGANIR, R. (2001). Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *Journal of Neuroscience* **21** , 6008-6017.

LIAO, L., RAMSAY, K. & JAKEN, S. (1994). Protein kinase C isozymes in progressively transformed rat embryo fibroblasts. *Cell Growth and Differentiation* **5**, 1185-1194.

LIGGETT, S.B., OSTROWSKI, J., CHESNUT, L.C., KUROSE, H., RAYMOND, J.R., CARON, M.G. & LEFKOWITZ, R.J. (1992). Sites in the third intracellular loop of the alpha 2A-adrenergic receptor confer short term agonist-promoted desensitization. Evidence for a receptor kinase-mediated mechanism. *Journal of Biological Chemistry* **267**, 4740-4746.

LIN, C.H., YEH, S.H., LIN, C.H., LU, K.T., LEU, T.H., CHANG, W.C. & GEAN, P.W. (2001). A role for the PI-3 kinase signaling pathway in fear conditioning and synaptic plasticity in the amygdala. *Neuron* **31**, 841-851.

LIN, T.A., KONG, X., HAYSTEAD, T.A., PAUSE, A., BELSHAM, G., SONENBERG, N. & LAWRENCE, J.C.Jr. (1994). PHAS-I as a link between mitogen-activated protein kinase and translation initiation. *Science* **266**, 653-656.

LIN, Y.W., MIN, M.Y., CHIU, T.H. & YANG, H.W. (2003). Enhancement of associative long-term potentiation by activation of beta-adrenergic receptors at CA1 synapses in rat hippocampal slices. *Journal of Neuroscience* **23**, 4173-4181.

LINDEN, D.J. (1999). The return of the spike: postsynaptic action potentials and the induction of LTP and LTD. *Neuron* **22**, 661-666.

LING, D.S., BENARDO, L.S., SERRANO, P.A., BLACE, N., KELLY, M.T.,

CRARY, J.F. & SACKTOR, T.C. (2002). Protein kinase Mzeta is necessary and sufficient for LTP maintenance. *Nature Neuroscience* **5**, 295-296.

LING, D.S., BENARDO, L.S., SERRANO, P.A., BLACE, N., KELLY, M.T., CRARY, J.F. & SACKTOR, T.C. (2002). Protein kinase Mzeta is necessary and sufficient for LTP maintenance. *Nature Neuroscience* **5**, 295-296.

LISMAN, J., SCHULMAN, H. & CLINE, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Reviews Neuroscience* **3**, 175-190.

LISMAN, J.E. (1985). A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 3055-3057.

LISMAN, J.E. & ZHABOTINSKY, A.M. (2001). A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. *Neuron* **31**, 191-201.

LITTLE, Z., GROVER, L.M. & TEYLER, T.J. (1995). Metabotropic glutamate receptor antagonist, (R,S)-alpha-methyl-4-carboxyphenyglycine, blocks two distinct forms of long-term potentiation in area CA1 of rat hippocampus. *Neuroscience Letters* **201**, 73-76.

LIU, F.C. & GRAYBIEL, A.M. (1996). Spatiotemporal dynamics of CREB phosphorylation: transient versus sustained phosphorylation in the developing striatum. *Neuron* **17**, 1133-1144.

LIU, J., FUKUNAGA, K., YAMAMOTO, H., NISHI, K. & MIYAMOTO, E. (1999). Differential roles of Ca(2+)/calmodulin-dependent protein kinase II and mitogen-activated protein kinase activation in hippocampal long-term potentiation. *Journal of Neuroscience* **19**, 8292-8299.

LIU, Y.C. & STORM, D.R. (1990). Regulation of free calmodulin levels by neuromodulin: neuron growth and regeneration. *Trends in Pharmacological*

Science **11**, 107-111.

LLEDO, P.M., HJELMSTAD, G.O., MUKHERJI, S., SODERLING, T.R., MALENKA, R.C. & NICOLL, R.A. (1995). Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 11175-11179.

LLEDO, P.M., ZHANG, X., SUDHOF, T.C., MALENKA, R.C. & NICOLL, R.A. (1998). Postsynaptic membrane fusion and long-term potentiation. *Science* **279**, 399-403.

LOEWI, O. (1921). Uber humorale Ubertragbarkeit der Herznervenwirkung. *Pluger's Archiv fur die gesamte Phsiologie* **189**, 201-213.

LOMELI, H., WISDEN, W., KOHLER, M., KEINANEN, K., SOMMER, B. & SEEBURG, P.H. (1992). High-affinity kainate and domoate receptors in rat brain. *FEBS Letters* **307**, 139-143.

LORENTE DE NO, R. (1934). Studies on the structure of the cerebral cortex - II. Continuation of the study of the ammonic system. *Journal Psychology and Neurology, Leipzig* **46**, 113-177.

LOVINGER, D.M., WONG, K.L., MURAKAMI, K. & ROUTTENBERG, A. (1987). Protein kinase C inhibitors eliminate hippocampal long-term potentiation. *Brain Research* **436**, 177-183.

LOY, R., KOZIELL, D.A., LINDSEY, J.D. & MOORE, R.Y. (1980). Noradrenergic innervation of the adult rat hippocampal formation. *Journal of Comparative Neurology* **189**, 699-710.

LU, Y.M., JIA, Z., JANUS, C., HENDERSON, J.T., GERLAI, R., WOJTOWICZ, J.M. & RODER, J.C. (1997). Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. *Journal of Neuroscience* **17**, 5196-5205.

LUJAN, R., NUSSER, Z., ROBERTS, J.D., SHIGEMOTO, R. & SOMOGYI, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *European Journal of Neuroscience* **8**, 1488-1500.

LUJAN, R., ROBERTS, J.D., SHIGEMOTO, R., OHISHI, H. & SOMOGYI, P. (1997). Differential plasma membrane distribution of metabotropic glutamate receptors mGluR1 alpha, mGluR2 and mGluR5, relative to neurotransmitter release sites. *Journal of Chemical Neuroanatomy* **13**, 219-241.

LUO, J.H., FU, Z.Y., LOSI, G., KIM, B.G., PRYBYLOWSKI, K., VISSEL, B. & VICINI, S. (2002). Functional expression of distinct NMDA channel subunits tagged with green fluorescent protein in hippocampal neurons in culture. *Neuropharmacology* **42**, 306-318.

LUSCHER, C., MALENKA, R.C. & NICOLL, R.A. (1998). Monitoring glutamate release during LTP with glial transporter currents. *Neuron* **21**, 465-441.

LUTHI, A., GAHWILER, B.H. & GERBER, U. (1994). Potentiation of a metabotropic glutamatergic response following NMDA receptor activation in rat hippocampus. *Pflugers Archiv* **427**, 197-202.

LUTTRELL, L.M., FERGUSON, S.S., DAAKA, Y., MILLER, W.E., MAUDSLEY, S., DELLA ROCCA, G.J., LIN, F., KAWAKATSU, H., OWADA, K., LUTTREL, l.D.K., CARON, M.G. & LEFKOWITZ, R.J. (1999). Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* **283**, 655-661.

LYNCH, G. & BAUDRY, M. (1984). The biochemistry of memory: a new and specific hypothesis. *Science* **224**, 1057-1063.

LYNCH, G., LARSON, J., KELSO, S., BARRIONUEVO, G. & SCHOTTLER, F. (1983). Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature* **305** , 719-721.

LYNCH, G.S., DUNWIDDIE, T. & GRIBKOFF, V. (1977). Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. *Nature* **266**, 737-739.

LYNCH, M.A., VOSS, K.L., RODRIGUEZ, J. & BLISS, T.V. (1994). Increase in synaptic vesicle proteins accompanies long-term potentiation in the dentate gyrus. *Neuroscience* **60**, 1-5.

MA, L., ZABLOW, L., KANDEL, E.R. & SIEGELBAUM, S.A. (1999). Cyclic AMP induces functional presynaptic boutons in hippocampal CA3-CA1 neuronal cultures. *Nature Neurosience* **2** , 24-30.

MACDERMOTT, A.B., MAYER, M.L., WESTBROOK, G.L., SMITH, S.J. & BARKER, J.L. (1986). NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* **321**, 519-522.

MADISON, D.V., LANCASTER, B. & NICOLL, R.A. (1987). Voltage clamp analysis of cholinergic action in the hippocampus. *Journal of Neuroscience* **7**, 733-741.

MADISON, D.V. & NICOLL, R.A. (1982). Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature* **299**, 636-638.

MAGEE, J.C. & JOHNSTON, D. (1997). A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* **275**, 209-213.

MAINEN, Z.F., JIA, Z., RODER. J. & MALINOW, R. (1998). Use-dependent AMPA receptor block in mice lacking GluR2 suggests postsynaptic site for LTP expression. *Nature Neuroscience* **1**, 579-586.

MAJEWSKA, A., BROWN, E., ROSS, J. & YUSTE, R. (2000). Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization. *Journal of Neuroscience* **20**, 1722-1734.

MAKHINSON, M., CHOTINER, J.K., WATSON, J.B. & O'DELL, T.J. (1999).

Adenylyl cyclase activation modulates activity-dependent changes in synaptic strength and Ca2+/calmodulin-dependent kinase II autophosphorylation. *Journal of Neuroscience* **19**, 2500-2510.

MALENKA, R.C. (1991). Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron* **6**, 53-60.

MALENKA, R.C. (1994). Synaptic plasticity in the hippocampus: LTP and LTD. *Cell* **78**, 535-538.

MALENKA, R.C., KAUER, J.A., ZUCKER, R.S. & NICOLL, R.A. (1988). Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science* **242**, 81-84.

MALENKA, R.C., LANCASTER, B. & ZUCKER, R.S. (1992). Temporal limits on the rise in postsynaptic calcium required for the induction of long-term potentiation. *Neuron* **9**, 121-128.

MALENKA, R.C., MADISON, D.V. & NICOLL, R.A. (1986). Potentiation of synaptic transmission in the hippocampus by phorbol esters. *Nature* **321**, 175-177.

MALENKA, R.C. & NICOLL, R.A. (1999). Long-term potentiation-a decade of progress? *Science* **285**, 1870-1874.

MALETIC-SAVATIC, M., KOOTHAN, T. & MALINOW, R. (1998). Calcium-evoked dendritic exocytosis in cultured hippocampal neurons. Part II: mediation by calcium/calmodulin-dependent protein kinase II. *Journal of Neuroscience* **18**, 6814-6821.

MALETIC-SAVATIC, M., MALINOW, R. & SVOBODA, K. (1999). Rapid dendritic morphogenesis in CA1 hippocampal dendrites induced by synaptic activity. *Science* **283**, 1923-1927.

MALGAROLI, A., TING, A.E., WENDLAND, B., BERGAMASCHI, A., VILLA, A., TSIEN, R.W. & SCHELLER, R.H. (1995). Presynaptic component of long-term

potentiation visualized at individual hippocampal synapses. *Science* **268**, 1624-1628.

MALINOW, R. (2003). AMPA receptor trafficking and long-term potentiation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **358**, 707-714.

MALINOW, R., MADISON, D.V. & TSIEN, R.W. (1988). Persistent protein kinase activity underlying long-term potentiation. *Nature* **335**, 820-824.

MALINOW, R. & MALENKA, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annual Review of Neuroscience* **25**, 103-126.

MALINOW, R., SCHULMAN, H. & TSIEN, R.W. (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* **245**, 862-866.

MALINOW, R. & TSIEN, R.W. (1990). Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* **346**, 177-180.

MAMMEN, A.L., KAMEYAMA, K., ROCHE, K.W. & HUGANIR, R.L. (1997). Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *Journal of Biological Chemistry* **272**, 32528-32533.

MANABE, T. & NICOLL, R.A. (1994). Long-term potentiation: evidence against an increase in transmitter release probability in the CA1 region of the hippocampus. *Science* **265**, 1888-1892.

MANABE, T., WYLLIE, D.J., PERKEL, D.J. & NICOLL, R.A. (1993). Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *Journal of Neurophysiology* **70**, 1451-1459.

MANAHAN-VAUGHAN, D. (1997). Group 1 and 2 metabotropic glutamate receptors play differential roles in hippocampal long-term depression and long-term potentiation in freely moving rats. *Journal of Neuroscience* **17**, 3303-3311.

MANAHAN-VAUGHAN, D. (1998). Priming of group 2 metabotropic glutamate receptors facilitates induction of long-term depression in the dentate gyrus of freely moving rats. *Neuropharmacology* **37**, 1459-1464.

MANAHAN-VAUGHAN, D., BRAUNEWELL, K.H. & REYMANN, K.G. (1998). Subtype-specific involvement of metabotropic glutamate receptors in two forms of long-term potentiation in the dentate gyrus of freely moving rats. *Neuroscience* **86**, 709-721.

MANAHAN-VAUGHAN, D. & REYMANN, K.G. (1995). 1S,3R-ACPD dose-dependently induces a slow-onset potentiation in the rat hippocampal CA1 region in vivo. *Neuropharmacology* **34**, 1103-1105.

MANAHAN-VAUGHAN, D. & REYMANN, K.G. (1997). Group 1 metabotropic glutamate receptors contribute to slow-onset potentiation in the rat CA1 region in vivo. *Neuropharmacology* **36**, 11-12.

MANAHAN-VAUGHAN, D. & REYMANN, K.G. (1996). Metabotropic glutamate receptor subtype agonists facilitate long-term potentiation within a distinct time window in the dentate gyrus in vivo. *Neuroscience* **74**, 723-731.

MANZONI, O.J., WEISSKOPF, M.G. & NICOLL, R.A. (1994). MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. *European Journal of Neuroscience* **6**, 1050-1054.

MARAVALL, M., MAINEN, Z.F., SABATINI, B.L. & SVOBODA, K. (2000). Estimating intracellular calcium concentrations and buffering without wavelength ratioing. *Biophysical Journal* **78**, 2655-2667.

MARINO, M.J., ROUSE, S.T., LEVEY, A.I., POTTER, L.T. & CONN, P.J. (1998). Activation of the genetically defined m1 muscarinic receptor potentiates N-

methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 11465-11470.

MARKRAM, H., HELM, P.J. & SAKMANN, B. (1995). Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *Journal of Physiology* **485**, 1-20.

MARKRAM, H., LUBKE, J., FROTSCHER, M. & SAKMANN, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* **275**, 213-215.

MARKRAM, H., ROTH, A. & HELMCHEN, F. (1998). Competitive calcium binding: implications for dendritic calcium signaling. *Journal of Computational Neuroscience* **5**, 331-348.

MARKRAM, H. & SEGAL, M. (1992). The inositol 1,4,5-trisphosphate pathway mediates cholinergic potentiation of rat hippocampal neuronal responses to NMDA. *Journal of Physiology* **447**, 513-533.

MARKRAM, H. & SEGAL, M. (1990). Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. *Journal of Physiology* **427**, 381-393.

MARTIN, K.C., MICHAEL, D., ROSE, J.C., BARAD, M., CASADIO, A., ZHU, H. & KANDEL, E.R. (1997). MAP kinase translocates into the nucleus of the presynaptic cell and is required for long-term facilitation in Aplysia. *Neuron* **18**, 899-912.

MARTIN, L.J., BLACKSTONE, C.D., LEVEY, A.I., HUGANIR, R.L. & PRICE, D.L. (1993). AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* **53**, 327-358.

MARTIN, S.J. & MORRIS, R.G. (1997). (R,S)-alpha-methyl-4-carboxyphenylglycine (MCPG) fails to block long-term potentiation under urethane anaesthesia in

vivo. *Neuropharmacology* **36**, 139-1354.

MARY, S., GOMEZA, J., PREZEAU, L., BOCKAERT, J. & PIN, J.P. (1998). A cluster of basic residues in the carboxyl-terminal tail of the short metabotropic glutamate receptor 1 variants impairs their coupling to phospholipase C. *Journal of Biological Chemistry* **273**, 425-432.

MASU, M., NAKAJIMA, Y., MORIYOSHI, K., ISHII, T., AKAZAWA, C. & NAKANASHI, S. (1993). Molecular characterization of NMDA and metabotropic glutamate receptors. *Annual New York Academy of Sciences* **707**, 153-164.

MASU, M., TANABE, Y., TSUCHIDA, K., SHIGEMOTO, R. & NAKANISHI, S. (1991). Sequence and expression of a metabotropic glutamate receptor. *Nature* **349**, 760-765.

MATSUDA K, K.Y.M.S.Y.M. (2002). Cloning and characterization of a novel NMDA receptor subunit NR3B: a dominant subunit that reduces calcium permeability. *Molecular Brain Research* **100**, 43-52.

MATSUSHITA, M., TOMIZAWA, K., MORIWAKI, A., LI, S.T., TERADA, H. & MATSUI, H. (2001). A high-efficiency protein transduction system demonstrating the role of PKA in long-lasting long-term potentiation. *Journal of Neuroscience* **21**, 6000-6007.

MATSUZAKI, M., ELLIS-DAVIES, G.C., NEMOTO, T., MIYASHITA, Y., IINO, M. & KASAI, H. (2001). Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* **4**, 1086-1092.

MATTHEWS, R.P., GUTHRIE, C.R., WAILES, L.M., ZHAO, X., MEANS, A.R. & MCKNIGHT, G.S. (1994). Calcium/calmodulin-dependent protein kinase types II and IV differentially regulate CREB-dependent gene expression. *Molecular Cell Biology* **14**, 6107-6116.

MATTHIES, H. (1989). Neurobiological aspects of learning and memory. *Annual Review of Psychology* **40**, 381-404.

MATTHIES, H., FREY, U., REYMANN, K., KRUG, M., JORK, R. & SCHROEDER, H. (1990). Different mechanisms and multiple stages of LTP. *Advances in Experimental Medicine and Biology* **268**,

MATTHIES, H. & REYMANN, K.G. (1993). Protein kinase A inhibitors prevent the maintenance of hippocampal long-term potentiation. *NeuroReport* **4**, 712-714.

MATTHIES, H.J., BEHNISCH, T., KASE, H., MATTHIES, H. & REYMANN, K.G. (1991). Differential effects of protein kinase inhibitors on pre-established long-term potentiation in rat hippocampal neurons in vitro. *Neuroscience Letters* **121**, 259-262.

MATTSON, M.P., LAFERLA, F.M., CHAN, S.L., LEISSRING, M.A., SHEPEL, P.N. & GEIGER, J.D. (2000). Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. *Trends in Neuroscience* **23**, 222-229.

MAYER, M.L., VYKLICKY, L.J. & WESTBROOK, G.L. (1989). Modulation of excitatory amino acid receptors by group IIB metal cations in cultured mouse hippocampal neurones. *Journal of Physiology* **415**, 329-350.

MAYER, M.L. & WESTBROOK, G.L. (1987). Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *Journal of Physiology* **394**, 501-527.

MAYER, M.L., WESTBROOK, G.L. & GUTHRIE, P.B. (1984). Voltage-dependent block by Mg2+ of NMDA responses in spinal cord neurones. *Nature* **309**, 261-263.

MAYFORD, M., BACH, M.E., HUANG, Y.Y., WANG, L., HAWKINS, R.D. & KANDEL, E.R. (1996). Control of memory formation through regulated expression of a CaMKII transgene. *Science* **274**, 1678-1683.

MCCAFFERY, B., BORTOLOTTO, Z.A., BASHIR, Z.I., COLLET, V.J., GASPARINI, F., FLOR, P.J., JANE, D.E., CONQUET, F. & COLLINGRIDGE, G.L. (1998). Effects of metabotropic glutamate receptor mGluR1 antanonists on long-term potentiation in area CA1 of the hippocampus. *In: Metabotropic Glutamate Receptors and Brain Function. Eds. Moronie, F., Nicoletti, F., Pellegrini-Giampietro, D.E. London: Portland Press Ltd.* 139-145.

MCCLELLAND, J.L., MCNAUGHTON, B.L. & O'REILLY, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychological Review* **101** , 419-457.

MCCUNE, S.K., VOIGT, M.M. & HILL, J.M. (1993). Expression of multiple alpha adrenergic receptor subtype messenger RNAs in the adult rat brain. *Neuroscience* **57**, 143-51.

MCGAHON, B., MAGUIRE, C., KELLY, A. & LYNCH, M.A. (1999). Activation of p42 mitogen-activated protein kinase by arachidonic acid and trans-1-amino-cyclopentyl-1,3- dicarboxylate impacts on long-term potentiation in the dentate gyrus in the rat: analysis of age-related changes. *Neuroscience* **90**, 1167-1175.

MCGUINNESS, N., ANWYL, R. & ROWAN, M. (1991). Trans-ACPD enhances long-term potentiation in the hippocampus. *European Journal of Pharmacology* **197**, 231-232.

MCNAUGHTON, B.L. (1980). Evidence for two physiologically distinct perforant pathways to the fascia dentata. *Brain Research* **199**, 1-19.

MCNAUGHTON, B.L. (1982). Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. *Journal of Physiology* **324**, 249-262.

MCNAUGHTON, B.L., DOUGLAS, R.M. & GODDARD, G.V. (1978). Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain*

Research **157**, 277-293.

MEAKIN, S.O., MACDONALD, J.I., GRYZ, E.A., KUBU, C.J. & VERDI, J.M. (1999). The signaling adapter FRS-2 competes with Shc for binding to the nerve growth factor receptor TrkA. A model for discriminating proliferation and differentiation. *Journal of Biological Chemistry* **274**, 9861-9870.

MEGURO, H., MORI, H., ARAKI, K., KUSHIYA, E., KUTSUWADA, T., YAMAZAKI, M., KUMANISHI, T., ARAKAWA, M., SAKIMURA, K. & MISHINA, M. (1992). Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature* **357**, 70-74.

MELLOR, H. & PARKER, P.J. (1998). The extended protein kinase C superfamily. *Biochemical Journal* **332**, 281-292.

MEYER, A. (1971). Historical Aspects of Cerebral Anatomy. *London: Oxford University Press*

MIGEON, J.C. & NATHANSON, N.M. (1994). Differential regulation of cAMP-mediated gene transcription by m1 and m4 muscarinic acetylcholine receptors. Preferential coupling of m4 receptors to Gi alpha-2. *Journal of Biological Chemistry* **269**, 9767-9773.

MILLER, S.G. & KENNEDY, M.B. (1986). Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. *Cell* **44** , 861-870.

MILNER, T.A., SHAH, P. & PIERCE, J.P. (2000). beta-adrenergic receptors primarily are located on the dendrites of granule cells and interneurons but also are found on astrocytes and a few presynaptic profiles in the rat dentate gyrus. *Synapse* **36**, 178-193.

MINAKAMI, R., JINNAI, N. & SUGIYAMA, H. (1997). Phosphorylation and calmodulin binding of the metabotropic glutamate receptor subtype 5 (mGluR5) are antagonistic in vitro. *Journal of Biological Chemistry* **272**, 20291-20298.

MINAKAMI, R., KATSUKI, F. & SUGIYAMA, H. (1993). A variant of metabotropic glutamate receptor subtype 5: an evolutionally conserved insertion with no termination codon. *Biochemical and Biophysical Research Communications* **194**, 622-627.

MITCHELL, J.B., LUPICA, C.R. & DUNWIDDIE, T.V. (1993). Activity-dependent release of endogenous adenosine modulates synaptic responses in the rat hippocampus. *Journal of Neuroscience* **13**, 3439-3447.

MIZUMORI, S.J., ROSENZWEIG, M.R. & BENNETT, E.L. (1985). Long-term working memory in the rat: effects of hippocampally applied anisomycin. *Behavioral Neuroscience* **99**, 220-232.

MOCHIDA, H., SATO, K., SASAKI, S., YAZAWA, I., KAMINO, K. & MOMOSE-SATO, Y. (2001). Effects of anisomycin on LTP in the hippocampal CA1: long-term analysis using optical recording. *NeuroReport* **12**, 987-991.

MOCHLY-ROSEN, D., KHANER, H. & LOPEZ, J. (1991). Identification of intracellular receptor proteins for activated protein kinase C. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 3997-4000.

MODY, I., BAIMBRIDGE, K.G. & MILLER, J.J. (1984). Blockade of tetanic- and calcium-induced long-term potentiation in the hippocampal slice preparation by neuroleptics. *Neuropharmacology* **23**, 625-631.

MOLLOY, S.S. & KENNEDY, M.B. (1991). Autophosphorylation of type II Ca2+/calmodulin-dependent protein kinase in cultures of postnatal rat hippocampal slices. *Proceedings of the National Academy of Sciences of the United States of America* **88**, 4756-4760.

MOLNAR, E., BAUDE, A., RICHMOND, S.A., PATEL, P.B., SOMOGYI, P. & MCILHINNEY, R.A. (1993). Biochemical and immunocytochemical characterization of antipeptide antibodies to a cloned GluR1 glutamate receptor subunit: cellular and subcellular distribution in the rat forebrain. *Neuroscience*

53, 307-326.

MOMIYAMA, A., FELDMEYER, D. & CULL-CANDY, S.G. (1996). Identification of a native low-conductance NMDA channel with reduced sensitivity to Mg2+ in rat central neurones. *Journal of Physiology* **494**, 479-492.

MONAGHAN, D.T. & COTMAN, C.W. (1985). Distribution of N-methyl-D-aspartate-sensitive L-[3H]glutamate-binding sites in rat brain. *Journal of Neuroscience* **5**, 2909-2919.

MONS, N., HARRY, A., DUBOURG, P., PREMONT, R.T., IYENGAR, R. & COOPER, D.M. (1995). Immunohistochemical localization of adenylyl cyclase in rat brain indicates a highly selective concentration at synapses. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 8473-8477.

MONTAGUE, P.R. & SEJNOWSKI, T.J. (1994). The predictive brain: temporal coincidence and temporal order in synaptic learning mechanisms. *Learning and Memory* **1**, 1-33.

MONTAROLO, P.G., GOELET, P., CASTELLUCCI, V.F., MORGAN, J., KANDEL, E.R. & SCHACHER, S. (1986). A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in Aplysia. *Science* **234**, 1249-1254.

MONTGOMERY, J.M., PAVLIDIS, P. & MADISON, D.V. (2001). Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation. *Neuron* **29**, 691-701.

MONYER, H., SPRENGEL, R., SCHOEPFER, R., HERB, A., HIGUCHI, M., LOMELI, H., BURNASHEV, N., SAKMANN, B. & SEEBURG, P.H. (1992). Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science* **256**, 1217-1221.

MOODY, T.D., CARLISLE, H.J. & O'DELL, T.J. (1999). A nitric oxide-independent and beta-adrenergic receptor-sensitive form of metaplasticity limits theta-

frequency stimulation-induced LTP in the hippocampal CA1 region. *Learning and Memory* **6**, 619-633.

MOORE, R.Y. & BLOOM, F.E. (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual Review of Neuroscience* **2**, 113-168.

MORGAN, S.L. & TEYLER, T.J. (2001). Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP. *Journal of Neurophysiology* **96**, 1289-1296.

MORI, H., MASAKI, H., YAMAKURA, T. & MISHINA, M. (1992). Identification by mutagenesis of a Mg(2+)-block site of the NMDA receptor channel. *Nature* **358**, 673-675.

MORIYOSHI, K., MASU, M., ISHII, T., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. *Nature* **354**, 31-37.

MUDA, M., BOSCHERT, U., DICKINSON, R., MARTINOU, J.C., MARTINOU, I., CAMPS, M., SCHLEGEL, W. & ARKINSTALL, S. (1996b). MKP-3, a novel cytosolic protein-tyrosine phosphatase that exemplifies a new class of mitogen-activated protein kinase phosphatase. *Journal of Biological Chemistry* **271**, 4319-4326.

MUDA, M., THEODOSIOU, A., RODRIGUES, N., BOSCHERT, U., CAMPS, M., GILLIERON, C., DAVIES, K., ASHWORTH, A. & ARKINSTALL, S. (1996a). The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *Journal of Biological Chemistry* **271**, 27205-27208.

MULLER, D., BITTAR, P. & BODDEKE, H. (1992). Induction of stable long-term potentiation in the presence of the protein kinase C antagonist staurosporine. *Neuroscience Letters* **135**, 18-22.

MULLER, D., JOLY, M. & LYNCH, G. (1988). Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. *Science* **242**, 1694-1697.

MULLER, D. & LYNCH, G. (1989). Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in hippocampus. *Brain Research* **479**, 290-299.

MULLER, D., TURNBULL, J., BAUDRY, M. & LYNCH, G. (1988). Phorbol ester-induced synaptic facilitation is different than long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **18**,

MULLER, W. & MISGELD, U. (1989). Carbachol and pirenzepine discriminate effects mediated by two muscarinic receptor subtypes on hippocampal neurons in vitro. *EXS* **57**, 114-122.

MURPHY, S.N. & MILLER, R.J. (1988). A glutamate receptor regulates Ca2+ mobilization in hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **85**, 8737-8741.

MURPHY, T.H., BLATTER, L.A., BHAT, R.V., FIORE, R.S., WIER, W.G. & BARABAN, J.M. (1994). Differential regulation of calcium/calmodulin-dependent protein kinase II and p42 MAP kinase activity by synaptic transmission. *Journal of Neuroscience* **3 Pt 1**,

NADASDY, Z., HIRASE, H., CZURKO, A., CSICSVARI, J. & BUZSAKI, G. (1999). Replay and time compression of recurring spike sequences in the hippocampus. *Journal of Neuroscience* **19**, 9497-9507.

NAKAHARA, K., OKADA, M. & NAKANISHI, S. (1997). The metabotropic glutamate receptor mGluR5 induces calcium oscillations in cultured astrocytes via protein kinase C phosphorylation. *Journal of Neurochemistry* **69**, 1467-1475.

NAKAJIMA, Y., IWAKABE, H., AKAZAWA, C., NAWA, H., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *Journal of Biological Chemistry* **268**, 11868-11873.

NAKANISHI, S. (1992). Molecular diversity ofglutamate receptors and implications for brain function. *Science* **258**, 597-603.

NAKANISHI, S. (1995). Second-order neurones and receptor mechanisms in visual- and olfactory-information processing. *Trends in Neuroscience* **18**, 359-364.

NAKANISHI, S., MASU, M., BESSHO, Y., NAKAJIMA, Y., HAYASHI, Y. & SHIGEMOTO, R. (1994). Molecular diversity of glutamate receptors and their physiological functions. *EXS* **71**, 71-80.

NAMGUNG, U., VALCOURT, E. & ROUTTENBERG, A. (1995). Long-term potentiation in vivo in the intact mouse hippocampus. *Brain Research* **689**, 85-92.

NATHANSON, N.M. (1987). Molecular properties of the muscarinic acetylcholine receptor. *Annual Review of Neuroscience* **10**, 195-236.

NAYAK, A., ZASTROW, D.J., LICKTEIG, R., ZAHNISER, N.R. & BROWNING, M.D. (1998). Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. *Nature* **394**, 680-683.

NEVE, R.L., COOPERSMITH, R., MCPHIE, D.L., SANTEUFEMIO, C., PRATT, K.G., MURPHY, C.J. & LYNN, S.D. (1998). The neuronal growth-associated protein GAP-43 interacts with rabaptin-5 and participates in endocytosis. *Journal of Neuroscience* **18**, 7757-7767.

NGUYEN, P.V., ABEL, T. & KANDEL, E.R. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* **265**, 1104-1107.

NGUYEN, P.V. & KANDEL, E.R. (1996). A macromolecular synthesis-dependent late phase of long-term potentiation requiring cAMP in the medial perforant pathway of rat hippocampal slices. *Journal of Neuroscience* **16**, 3189-3198.

NICHOLS, A.J. (1991). α–Adrenoceptor signal transduction mechanism. *In R.R. Ruggolo (Ed.), α–Adrenoceptors: Molecular Biology, Biochemistry and Pharmacology. Progress in Basic Clinical Pharmacology, Basel and Krager* 44-74.

NICOLL, R.A. & MALENKA, R.C. (1995). Contrasting properties of two forms of long-term potentiation in the hippocampus. *Nature* **377**, 115-118.

NILSSON, O.G., BRUNDIN, P. & BJORKLUND, A. (1990). Amelioration of spatial memory impairment by intrahippocampal grafts of mixed septal and raphe tissue in rats with combined cholinergic and serotonergic denervation of the forebrain. *Brain Research* **515** , 193-206.

NISHIZUKA, Y. (1995). Protein kinase C and lipid signaling for sustained cellular responses. *FASEB Journal* **9**, 484-496.

NORRIS, C.M., HALPAIN, S. & FOSTER, T.C. (1998). Reversal of age-related alterations in synaptic plasticity by blockade of L-type Ca2+ channels. *Journal of Neuroscience* **18**, 3171-3179.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462-465.

NUSSER, Z., LUJAN, R., LAUBE, G., ROBERTS, J.D., MOLNAR, E. & SOMOGYI, P. (1998). Cell type and pathway dependence of synaptic AMPA receptor number and variability in the hippocampus. *Neuron* **21**, 545-559.

O'CARROLL, R.E., DRYSDALE, E., CAHILL, L., SHAJAHAN, P. & EBMEIER, K.P. (1999). Memory for emotional material: a comparison of central versus

peripheral beta blockade. *Journal of Psychopharmacology* **13**, 32-39.

O'CONNOR, J.J., ROWAN, M.J. & ANWYL, R. (1994). Long-lasting enhancement of NMDA receptor-mediated synaptic transmission by metabotropic glutamate receptor activation. *Nature* **10**, 6463

O'CONNOR, J.J., ROWAN, M.J. & ANWYL, R. (1995). Tetanically induced LTP involves a similar increase in the AMPA and NMDA receptor components of the excitatory postsynaptic current: investigations of the involvement of mGlu receptors. *Journal of Neuroscience* **15**, 2013-2020.

O'DELL, T.J. & KANDEL, E.R. (1994). Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. *Learning and Memory* **1**, 129-139.

O'HARA, P.J., SHEPPARD, P.O., THOGERSEN, H., VENEZIA, D., HALDEMAN, B.A., MCGRANE, V., HOUAMED, K.M., THOMSEN, C., GILBERT, T.L. & MULVIHILL, E.R. (1993). The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. *Neuron* **11**, 41-52.

O'KEEFE, J., NADEL, L., KEIGHTLEY, S. & KILL, D. (1975). Fornix lesions selectively abolish place learning in the rat. *Experimental Neurology* **48**, 152-166.

O'LEARY, D.M. & O'CONNOR, J.J. (1997). Potentiation of synaptic transmission in the rat dentate gyrus in vitro by (S)-3,5-dihydroxyphenylglycine ((S)-DHPG). *Neuroscience Letters* **229**, 29-32.

O'MARA, S.M., ROWAN, M.J. & ANWYL, R. (1995). Metabotropic glutamate receptor-induced homosynaptic long-term depression and depotentiation in the dentate gyrus of the rat hippocampus in vitro. *Neuropharmacology* **34**, 983-989.

OBENAUS, A., MODY, I. & BAIMBRIDGE, K.G. (1989). Dantrolene-Na (Dantrium)

blocks induction of long-term potentiation in hippocampal slices. *Neuroscience Letters* **98**, 172-178.

OESTREICHER, A.B., DE GRAAN, P.N., GISPEN, W.H., VERHAAGEN, J. & SCHRAMA, L.H. (1997). B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. *Progress in Neurobiology* **53**, 627-686.

OKAMOTO, N., HORI, S., AKAZAWA, C., HAYASHI, Y., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1994). Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *Journal of Biological Chemisty* **269**, 1231-1236.

OKAMOTO, T., SEKIYAMA, N., OTSU, M., , S.Y., SATO, A., NAKANISHI, S. & JINGAMI, H. (1998). Expression and purification of the extracellular ligand binding region of metabotropic glutamate receptor subtype 1. *Journal of Biological Chemistry* **273**, 13089-13096.

OLESKEVICH, S., DESCARRIES, L. & LACAILLE, J.C. (1989). Quantified distribution of the noradrenaline innervation in the hippocampus of adult rat. *Journal of Neuroscience* **9**, 3803-3815.

OLIET, S.H., MALENKA, R.C. & NICOLL, R.A. (1997). Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. *Neuron* **18**, 969-982.

OLSON, N.J., MASSE, T., SUZUKI, T., CHEN, J., ALAM, D. & KELLY, P.T. (1995). Functional identification of the promoter for the gene encoding the alpha subunit of calcium/calmodulin-dependent protein kinase II. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 1659-1663.

OPAZO, P., WATABE, A.M., GRANT, S.G. & O'DELL, T.J. (2003). Phosphatidylinositol 3-kinase regulates the induction of long-term potentiation

through extracellular signal-related kinase-independent mechanisms. *Journal of Neuroscience* **23**, 3679-3688.

ORBAN, P.C., CHAPMAN, P.F. & BRAMBILLA, R. (1999). Is the Ras-MAPK signalling pathway necessary for long-term memory formation? *Trends in Neuroscience* **22**, 38-44.

ORR, J.W. & NEWTON, A.C. (1994). Intrapeptide regulation of protein kinase C. *Journal of Biological Chemistry* **269**, 8383-8387.

OSTEN, P., VALSAMIS, L., HARRIS, A. & SACKTOR, T.C. (1996). Protein synthesis-dependent formation of protein kinase Mzeta in long-term potentiation. *Journal of Neuroscience* **16**, 2444-2451.

OSTROFF, L.E., FIALA, J.C., ALLWARDT, B. & HARRIS, K.M. (2002). Polyribosomes redistribute from dendritic shafts into spines with enlarged synapses during LTP in developing rat hippocampal slices. *Neuron* **35**, 535-545.

OTANI, S. & ABRAHAM, W.C. (1989). Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats. *Neuroscience Letters* **106**, 175-180.

OTANI, S. & BEN-ARI, Y. (1993). Biochemical correlates of long-term potentiation in hippocampal synapses. *International Review of Neurobiology* **35**, 1-41.

OTANI, S., BEN-ARI, Y. & ROISIN-LALLEMAND, M.P. (1993). Metabotropic receptor stimulation coupled to weak tetanus leads to long-term potentiation and a rapid elevation of cytosolic protein kinase C activity. *Brain Research* **613**, 1-9.

OTANI, S. & CONNOR, J.A. (1998). Requirement of rapid Ca2+ entry and synaptic activation of metabotropic glutamate receptors for the induction of long-term depression in adult rat hippocampus. *Journal of Physiology* **511**, 761-770.

OTANI, S., MARSHALL, C.J., TATE, W.P., GODDARD, G.V. & ABRAHAM, W.C. (1989). Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not messenger RNA synthesis immediately post-tetanization. *Neuroscience* **28**, 519-526.

OTANI, S., ROISIN-LALLEMAND, M.P. & BEN-ARI, Y. (1992). Enhancement of extracellular protein concentrations during long-term potentiation in the rat hippocampal slice. *Neuroscience* **47**, 265-272.

OTMAKHOV, N., GRIFFITH, L.C. & LISMAN, J.E. (1997). Postsynaptic inhibitors of calcium/calmodulin-dependent protein kinase type II block induction but not maintenance of pairing-induced long-term potentiation. *Journal of Neuroscience* **17**, 5357-5365.

OTMAKHOVA, N.A. & LISMAN, J.E. (1998). D1/D5 dopamine receptors inhibit depotentiation at CA1 synapses via cAMP-dependent mechanism. *Journal of Neuroscience* **18**, 1270-1279.

OTMAKHOVA, N.A., OTMAKHOV, N., MORTENSON, L.H. & LISMAN, J.E. (2000). Inhibition of the cAMP pathway decreases early long-term potentiation at CA1 hippocampal synapses. *Journal of Neuroscience* **20**, 4446-4451.

OUYANG, Y., KANTOR, D., HARRIS, K.M., SCHUMAN, E.M. & KENNEDY, M.B. (1997). Visualization of the distribution of autophosphorylated calcium/calmodulin-dependent protein kinase II after tetanic stimulation in the CA1 area of the hippocampus. *Journal of Neuroscience* **17**, 5416-5427.

OUYANG, Y., ROSENSTEIN, A., KREIMAN, G., SCHUMAN, E.M. & KENNEDY, M.B. (1999). Tetanic stimulation leads to increased accumulation of Ca(2+)/calmodulin-dependent protein kinase II via dendritic protein synthesis in hippocampal neurons. *Journal of Neuroscience* **19**, 7823-7833.

OVERSTREET, L.S., PASTERNAK, J.F., COLLEY, P.A., SLATER, N.T. & TROMMER, B.L. (1997). Metabotropic glutamate receptor mediated long-term

depression in developing hippocampus. *Neuropharmacology* **36**, 831-844.

PALMER, M.J., IRVING, A.J., SEABROOK, G.R., JANE, D.E. & COLLINGRIDGE, G.L. (1997). The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. *Neuropharmacology* **36**, 1517-1532.

PANG, K., WILLIAMS, M.J. & OLTON, D.S. (1993). Activation of the medial septal area attenuates LTP of the lateral perforant path and enhances heterosynaptic LTD of themedial perforant path in aged rats. *Brain Research* **632**, 150-160.

PANG, L., SAWADA, T., DECKER, S.J. & SALTIEL, A.R. (1995). Inhibition of MAP kinase kinase blocks the differentiation of PC-12 cells induced by nerve growth factor. *Journal of Biological Chemistry* **270**, 13585-13588.

PARFITT, K.D., DOZE, V.A., MADISON, D.V. & BROWNING, M.D. (1992). Isoproterenol increases the phosphorylation of the synapsins and increases synaptic transmission in dentate gyrus, but not in area CA1, of the hippocampus. *Hippocampus* **2**, 59-64.

PARK, C.H., LEE, Y.J., LEE, S.H., CHOI, S.H., KIM, H.S., JEONG, S.J., KIM, S.S. & SUH, Y.H. (2000). Dehydroevodiamine.HCl prevents impairment of learning and memory and neuronal loss in rat models of cognitive disturbance. *Journal of Neurochemistry* **74**, 244-253.

PASINELLI, P., RAMAKERS, G.M., URBAN, I.J., HENS, J.J., OESTREICHER, A.B., DE GRAAN, P.N. & GISPEN, W.H. (1995). Long-term potentiation and synaptic protein phosphorylation. *Behavioral Brain Research* **66**, 53-59.

PASSAFARO, M., PIECH, V. & SHENG, M. (2001). Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nature Neuroscience* **4**, 917-926.

PATNEAU, D.K. & MAYER, M.L. (1990). Structure-activity relationships for amino acid transmitter candidates acting at N-methyl-D-aspartate and quisqualate

receptors. *Journal of Neuroscience* **10**, 2385-2399.

PATTERSON, S.L., PITTENGER, C., MOROZOV, A., MARTIN, K.C., SCANLIN, H., DRAKE, C. & KANDEL, E.R. (2001). Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron* **32**, 123-140.

PATTON, P.E. & MCNAUGHTON, B. (1995). Connection matrix of the hippocampal formation: I. The dentate gyrus. *Hippocampus* **5**, 245-86.

PAVLIDES, C., GREENSTEIN, Y.J., GRUDMAN, M. & WINSON, J. (1988). Long-term potentiation in the dentate gyrus is induced preferentially on the positive phase of theta-rhythm. *Brain Research* **439**, 383-387.

PAWSON, T. (1994). Tyrosine kinase signalling pathways. *Princess Takamatsu Symposium* **24**, 303-322.

PAWSON, T. & SCOTT, J.D. (1997). Signaling through scaffold, anchoring, and adaptor proteins. *Science* **278**, 2075-2080.

PEARS, C., STABEL, S., CAZUBON, S. & PARKER, P.J. (1992). Studies on the phosphorylation of protein kinase C-α. *Biochemical Journal* **283**, 515-518.

PELLETIER, M.R., KIRKBY, R.D., JONES, S.J. & CORCORAN, M.E. (1994). Pathway specificity of noradrenergic plasticity in the dentate gyrus. *Hippocampus* **4**, 181-188.

PELTEKOVA, V., HAN, G., SOLEYMANLOU, N. & HAMPSON, D.R. (2000). Constraints on proper folding of the amino terminal domains of group III metabotropic glutamate receptors. *Molecular Brain Research* **76**, 180-190.

PENTTONEN, M., KAMONDI, A., SIK, A., ACSADY, L. & BUZSAKI, G. (1997). Feed-forward and feed-back activation of the dentate gyrus in vivo during dentate spikes and sharp wave bursts. *Hippocampus* **7**, 437-450.

PERKEL, D.J. & NICOLL, R.A. (1993). Evidence for all-or-none regulation of neurotransmitter release: implications for long-term potentiation. *Journal of Physiology* **471**, 481-500.

PERKINTON, M.S., IP, J.K., WOOD, G.L., CROSSTHWAITE, A.J. & WILLIAMS, R.J. (2002). Phosphatidylinositol 3-kinase is a central mediator of NMDA receptor signalling to MAP kinase (Erk1/2), Akt/PKB and CREB in striatal neurones. *Journal of Neurochemistry* **80**, 239-254.

PETRALIA, R.S., ESTEBAN, J.A., WANG, Y.X., PARTRIDGE, J.G., ZHAO, H.M., WENTHOLD, R.J. & MALINOW, R. (1999). Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neuroscience* **2**, 31-36.

PETRALIA, R.S., WANG, Y.X., NIEDZIELSKI, A.S. & WENTHOLD, R.J. (1996). The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. *Neuroscience* **71**, 949-976.

PETRALIA, R.S. & WENTHOLD, R.J. (1992). Light and electron immunocytochemical localization of AMPA-selective glutamate receptors in the rat brain. *Journal of Comparative Neurology* **318**, 329-354.

PETROZZINO, J.J., POZZO MILLER, L.D. & CONNOR, J.A. (1995). Micromolar Ca2+ transients in dendritic spines of hippocampal pyramidal neurons in brain slice. *Neuron* **14**, 1223-1231.

PETTIT, D.L., PERLMAN, S. & MALINOW, R. (1994). Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. *Science* **266**, 1881-1885.

PIERCE, J.P., VAN LEYEN, K. & MCCARTHY, J.B. (2000). Translocation machinery for synthesis of integral membrane and secretory proteins in dendritic spines. *Nature Neuroscience* **3**, 311-313.

PIERIBONE, V.A., NICHOLAS, A.P., DAGERLIND, A. & HOKFELT, T. (1994).

Distribution of alpha 1 adrenoceptors in rat brain revealed by in situ hybridization experiments utilizing subtype-specific probes. *Journal of Neuroscience* **14**, 4252-4268.

PIN, J.P., JOLY, C., HEINEMANN, S.F. & BOCKAERT, J. (1994). Domains involved in the specificity of G protein activation in phospholipase C-coupled metabotropic glutamate receptors. *EMBO* **13**, 642-348.

PIN, J.P., WAEBER, C., PREZEAU, L., BOCKAERT, J. & HEINEMANN, S.F. (1992). Alternative splicing generates metabotropic glutamate receptors inducing different patterns of calcium release in Xenopus oocytes. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 10331-10335.

PIPPIG, S., ANDEXINGER, S., DANIEL, K., PUZICHA, M., CARON, M.G., LEFKOWITZ, R.J. & LOHSE, M.J. (1993). Overexpression of beta-arrestin and beta-adrenergic receptor kinase augment desensitization of beta 2-adrenergic receptors. *Journal of Biological Chemistry* **268**, 3201-3208.

PISA, M. & FIBIGER, H.C. (1983). Intact selective attention in rats with lesions of the dorsal noradrenergic bundle. *Behavioural Neurscience* **97**, 519-529.

POLSTER, M.R., NADEL, L. & SCHACHER, D.L. (1991). Cognitive neuroscience. Analysis of memory: a historical perspective. *Journal of Cognitive Neuroscience* **3**, 95-116.

PREMKUMAR, L.S., QIN, F. & AUERBACH, A. (1997). Subconductance states of a mutant NMDA receptor channel kinetics, calcium, and voltage dependence. *Journal of General Physiology* **109**, 181-189.

PUSSINEN, R. & SIRVIO, J. (1998). Minor role for alpha1-adrenoceptors in the facilitation of induction and early maintenance of long-term potentiation in the CA1 field of the hippocampus. *Journal of Neuroscience Research* **51**, 309-315.

QI, M., ZHUO, M., SKALHEGG, B.S., BRANDON, E.P., KANDEL, E.R.,

MCKNIGHT, G.S. & IDZERDA, R.L. (1996). Impaired hippocampal plasticity in mice lacking the Cbeta1 catalytic subunit of cAMP-dependent protein kinase. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 1571-1576.

QIAN, X., RICCIO, A., ZHANG, Y. & GINTY, D.D. (1998). Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* **21**, 1017-1029.

RAABE, T., RIESGO-ESCOVAR, J., LIU, X., BAUSENWEIN, B.S., DEAK, P., MAROY, P. & HAFEN, E. (1996). DOS, a novel pleckstrin homology domain-containing protein required for signal transduction between sevenless and Ras1 in Drosophila. *Cell* **85**, 6

RAITERI, M., MARCHI, M., MAURA, G. & BONANNO, G. (1989). Presynaptic regulation of acetylcholine release in the CNS. *Cell Biology International Report* **13**, 1109-1118.

RAMAKERS, G.M., DE GRAAN, P.N., URBAN, I.J., KRAAY, D., TANG, T., PASINELLI, P., OESTREICHER, A.B. & GISPEN, W.H. (1995). Temporal differences in the phosphorylation state of pre- and postsynaptic protein kinase C substrates B-50/GAP-43 and neurogranin during long-term potentiation. *Journal of Biological Chemistry* **270**, 13892-13898.

RAMAKERS, G.M., GERENDASY, D.D. & DE GRAAN, P.N. (1999). Substrate phosphorylation in the protein kinase Cgamma knockout mouse. *Journal of Biological Chemistry* **274**, 1873-1874.

RAMAKERS, G.M., PASINELLI, P., HENS, J.J., GISPEN, W.H. & DE GRAAN, P.N. (1997). Protein kinase C in synaptic plasticity: changes in the in situ phosphorylation state of identified pre- and postsynaptic substrates. *Progress in Neuropsychopharmacology and Biological Psychiatry* **21**, 455-486.

RAMAN, I.M., TONG, G. & JAHR, C.E. (1996). Beta-adrenergic regulation of

synaptic NMDA receptors by cAMP-dependent protein kinase. *Neuron* **16**, 415-421.

RAUGHT, B. & GINGRAS, A.C. (1999). eIF4E activity is regulated at multiple levels. *The International Journal of Biochemistry and Cell Biology* **31**, 43-57.

RAUGHT, B., GINGRAS, A.C. & SONENBERG, N. (2001). The target of rapamycin (TOR) proteins. *Proceedings of the National Academy of Sciences of the United States of America* **98** , 7037-7044.

RAY, L.B. & STURGILL, T.W. (1988). Characterization of insulin-stimulated microtubule-associated protein kinase. Rapid isolation and stabilization of a novel serine/threonine kinase from 3T3-L1 cells. *Journal of Biological Chemsitry* **263**, 12721-12727.

RAYMOND, C.R. & REDMAN, S.J. (2002). Different calcium sources are narrowly tuned to the induction of different forms of LTP. *Journal of Neurophysiology* **88**, 249-255.

RAYMOND, C.R., REDMAN, S.J. & CROUCH, M.F. (2002). The phosphoinositide 3-kinase and p70 S6 kinase regulate long-term potentiation in hippocampal neurons. *Neuroscience* **109**, 531-536.

RAYMOND, C.R., THOMPSON, V.L., TATE, W.P. & ABRAHAM, W.C. (2000). Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong long-term potentiation. *Journal of Neuroscience* **20**, 969-976.

REID, S.N., DAW, N.W., GREGORY, D.S. & FLAVIN, H. (1996). cAMP levels increased by activation of metabotropic glutamate receptors correlate with visual plasticity. *Journal of Neurscience* **16**, 7619-7626.

REIMANN, E.M., WALSH, D.A. & KREBS, E.G. (1971). Purification and properties of rabbit skeletal muscle of cAMP-dependent protein kinases. *Journal of Biological Chemistry* **246**, 1986-1995.

REPRESA, A., DELOULME, J.C., SENSENBRENNER, M., BEN-ARI, Y. & BAUDIER, J. (1990). Neurogranin: immunocytochemical localization of a brain-specific protein kinase C substrate. *Journal of Neuroscience* **10**, 3782-3792.

RESCORLA, R.A. (1988). Behavioral studies of Pavlovian conditioning. *Annual Review of Neuroscience* **11**, 329-359.

REYMANN, K.G. & MATTHIES, H. (1989). 2-Amino-4-phosphonobutyrate selectively eliminates late phases of long-term potentiation in rat hippocampus. *Neuroscience Letters* **98**, 166-171.

RIBER, T.J., RODRIGUIZ, R.M., KHIROUG, L., WETSEL, W.C., AUGUSTINE, G.J. & MEANS, A.R. (2000). Cerebellar defects in Ca2+/calmodulin kinase IV-deficient mice. *Journal of Neuroscience* **20**, RC107

RICHTER-LEVIN, G., ERRINGTON, M.L., MAEGAWA, H. & BLISS, T.V. (1994). Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning. *Neuropharmacology* **33**, 853-857.

RIEDEL, G. & REYMANN, K. (1993). An antagonist of the metabotropic glutamate receptor prevents LTP in the dentate gyrus of freely moving rats. *Neuropharmacology* **32**, 929-931.

ROBERSON, E.D., ENGLISH, J.D., ADAMS, J.P., SELCHER, J.C., KONDRATICK, C. & SWEATT, J.D. (1999). The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. *Journal of Neuroscience* **19**, 4337-4348.

ROBERSON, E.D. & SWEATT, J.D. (2001). Memory-forming chemical reactions. *Review of Neuroscience* **12**, 41-50.

ROBERSON, E.D. & SWEATT, J.D. (1996). Transient activation of cyclic AMP-

dependent protein kinase during hippocampal long-term potentiation. *Journal of Biological Chemistry* **271**, 30436-30441.

ROBINSON, G.B. & RACINE, R.J. (1982). Heterosynaptic interactions between septal and entorhinal inputs to the dentate gyrus: long-term potentiation effects. *Brain Research* **249**, 162-166.

ROBINSON, G.B. & RACINE, R.J. (1985). Long-term potentiation in the dentate gyrus: effects of noradrenaline depletion in the awake rat. *Brain Research* **325**, 71-78.

ROBINSON, P.J. (1991). The role of protein kinase C and its neuronal substrates dephosphin, B-50, and MARCKS in neurotransmitter release. *Molecular Neurobiology* **5**, 87-130.

ROOK, M.S., LU, M. & KOSIK, K.S. (2000). CaMKIIalpha 3' untranslated region-directed mRNA translocation in living neurons: visualization by GFP linkage. *Journal of Neuroscience* **20**, 6385-6393.

ROSE, G.M. & DUNWIDDIE, T.V. (1986). Induction of hippocampal long-term potentiation using physiologically patterned stimulation. *Neuroscience Letters* **69**, 244-248.

ROSENBLUM, K., FUTTER, M., JONES, M., HULME, E.C. & BLISS, T.V. (2000). ERKI/II regulation by the muscarinic acetylcholine receptors in neurons. *Journal of Neuroscience* **20**, 977-985.

ROSIN, D.L., TALLEY, E.M., LEE, A., STORNETTA, R.L., GAYLINN, B.D., GUYENET, P.G. & LYNCH, K.R. (1996). Distribution of alpha 2C-adrenergic receptor-like immunoreactivity in the rat central nervous system. *Journal of Comparative Neurology* **372**, 135-165.

ROUSE, S.T. & LEVEY, A.I. (1997). Muscarinic acetylcholine receptor immunoreactivity after hippocampal commissural/associational pathway lesions: evidence for multiple presynaptic receptor subtypes. *Journal of*

Comparative Neurology **380**, 382-394.

ROUSE, S.T., MARINO, M.J., POTTER, L.T., CONN, P.J. & LEVEY, A.I. (1999). Muscarinic receptor subtypes involved in hippocampal circuits. *Life Sciences* **64**, 501-509.

ROUTTENBERG, A., CANTALLOPS, I., ZAFFUTO, S., SERRANO, P. & NAMGUNG, U. (2000). Enhanced learning after genetic overexpression of a brain growth protein. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 7657-7662.

RUMPEL, S., HATT, H. & GOTTMANN, K. (1998). Silent synapses in the developing rat visual cortex: evidence for postsynaptic expression of synaptic plasticity. *Journal of Neuroscience* **18**, 8863-8874.

RUSH, A.M., ROWAN, M.J. & ANWYL, R. (2001a). Application of N-methyl-D-aspartate induces long-term potentiation in the medial perforant path and long-term depression in the lateral perforant path of the rat dentate gyrus in vitro. *Neuroscience Letters* **298**, 175-178.

RUSH, A.M., WU, J., ROWAN, M.J. & ANWYL, R. (2001b). Activation of group II metabotropic glutamate receptors results in long-term potentiation following preconditioning stimulation in the dentate gyrus. *Neuroscience* **105**, 335-341.

RUSH, D.K. (1988). Scopolamine amnesia of passive avoidance: a deficit of information acquisition. *Behavioral and Neural Biology* **50**, 255-274.

RYAN, T.A., ZIV, N.E. & SMITH, S.J. (1996). Potentiation of evoked vesicle turnover at individually resolved synaptic boutons. *Neuron* **17**, 125-134.

SABATINI, B.L., OERTNER, T.G. & SVOBODA, K. (2002). The life cycle of Ca(2+) ions in dendritic spines. *Neuron* **33**, 439-452.

SACKTOR, T.C., OSTEN, P., VALSAMIS, H., JIANG, X., NAIK, M.U. & SUBLETTE, E. (1993). Persistent activation of the zeta isoform of protein

kinase C in the maintenance of long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 8342-8346.

SAH, P. & BEKKERS, J.M. (1996). Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. *Journal of Neuroscience* **16**, 4537-4542.

SAITO, N. (1995). Differential involvement of PKC subspecies in neuronal function. *Nippon Yakurigaku Zasshi.* **105**, 127-136.

SAITOH, T. & SCHWARTZ, J.H. (1985). Phosphorylation-dependent subcellular translocation of a Ca2+/calmodulin-dependent protein kinase produces an autonomous enzyme in Aplysia neurons. *Journal of Biological Chemistry* **100**, 835-842.

SAKURADA, K., MASU, M. & NAKANISHI, S. (1993). Alteration of Ca2+ permeability and sensitivity to Mg2+ and channel blockers by a single amino acid substitution in the N-methyl-D-aspartate receptor. *Journal of Biological Chemistry* **268**, 410-415.

SAMAMA, P., COTECCHIA, S., COSTA, T. & LEFKOWITZ, R.J. (1993). A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. *Journal of Biological Chemistry* **268**, 4625-4636.

SANNA, P.P., CAMMALLERI, M., BERTON, F., SIMPSON, C., LUTJENS, R., BLOOM, F.E. & FRANCESCONI, W. (2002). Phosphatidylinositol 3-kinase is required for the expression but not for the induction or the maintenance of long-term potentiation in the hippocampal CA1 region. *Journal of Neuroscience* **22**, 3359-3365.

SARVEY, J.M. & VOULALAS, P.J. (1995). *Society of Neuroscience* **21**, 1322

SASTRY, B.R., GOH, J.W. & AUYEUNG, A. (1986). Associative induction of posttetanic and long-term potentiation in CA1 neurons of rat hippocampus.

Science **232**, 988-990.

SAUERWALD, A., HOESCHE, C., OSCHWALD, R. & KILIMANN, M.W. (1990). The 5'-flanking region of the synapsin I gene. A G+C-rich, TATA- and CAAT-less, phylogenetically conserved sequence with cell type-specific promoter function. *Journal of Biological Chemistry* **265**, 14932-14937.

SAUGSTAD, J.A., SEGERSON, T.P. & WESTBROOK, G.L. (1996). Metabotropic glutamate receptors activate G-protein-coupled inwardly rectifying potassium channels in Xenopus oocytes. *Journal of Neuroscience* **16**, 5979-5985.

SCANZIANI, M., SALIN, P.A., VOGT, K.E., MALENKA, R.C. & NICOLL, R.A. (1997). Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors. *Nature* **385**, 630-634.

SCHARF, M.T., WOO, N.H., LATTAL, K.M., YOUNG, J.Z., NGUYEN, P.V. & ABEL, T. (2001). Protein synthesis is required for the enhancement of long-term potentiation and long-term memory by spaced training. *Journal of Neurophysiology* **87**, 2770-2777.

SCHEININ, M., LOMASNEY, J.W., HAYDEN-HIXSON, D.M., SCHAMBRA, U.B., CARON, M.G., LEFKOWITZ, R.J. & FREMEAU, R.T.Jr. (1994). Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Molecular Brain Research* **21**, 133-149.

SCHILLER, J., SCHILLE, Y. & CLAPHAM, D.E. (1998). NMDA receptors amplify calcium influx into dendritic spines during associative pre- and postsynaptic activation. *Nature Neuroscience* **1**, 114-118.

SCHOEPP, D.D. & JOHNSON, B.G. (1993). Pharmacology of metabotropic glutamate receptor inhibition of cyclic AMP formation in the adult rat hippocampus. *Neurochemistry International* **22**, 277-283.

SCHULZ, P.E., COOK, E.P. & JOHNSTON, D. (1994). Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. *Journal*

of Neuroscience **14**, 5325-5337.

SCHULZ, S., SIEMER, H., KRUG, M. & HOLLT, V. (1999). Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. *Journal of Neuroscience* **19**, 5683-5692.

SCHUMAN, E.M. (1999). mRNA trafficking and local protein synthesis at the synapse. *Neuron* **23**, 645-648.

SCHWORER, C.M., COLBRAN, R.J., KEEFER, J.R. & SODERLING, T.R. (1988). Ca2+/calmodulin-dependent protein kinase II. Identification of a regulatory autophosphorylation site adjacent to the inhibitory and calmodulin-binding domains. *Journal of Biological Chemistry* **25**, 27

SCOTT, J.D., STOFKO, R.E., MCDONALD, J.R., COMER, J.D., VITALIS, E.A. & MANGILI, J.A. (1990). Type II regulatory subunit dimerization determines the subcellular localization of the cAMP-dependent protein kinase. *Journal of Biological Chemistry* **265**, 21561-21566.

SCOVILLE, W.B. & MILNER, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery and Psychiatry* **20**, 11-21.

SEDMAN, G.L., JEFFREY, P.L., AUSTIN, L. & ROSTAS, J.A. (1986). The metabolic turnover of the major proteins of the postsynaptic density. *Brain Research* **387**, 221-230.

SEGAL, M. (1992). Acetylcholine enhances NMDA-evoked calcium rise in hippocampal neurons. *Brain Research* **587**, 83-87.

SEGAL, M., GREENBERGER, V. & MARKRAM, H. (1989). Presynaptic cholinergic action in the hippocampus. *EXS* **57**, 88-96.

SEGAL, M., MARKRAM, H. & RICHTER-LEVIN, G. (1991). Actions of

norepinephrine in the rat hippocampus. *Progress in Brain Research* **88**, 323-330.

SELCHER, J.C., NEKRASOVA, T., PAYLOR, R., LANDRETH, G.E. & SWEATT, J.D. (2001). Mice lacking the ERK1 isoform of MAP kinase are unimpaired in emotional learning. *Learning and Memory* **8**, 11-19.

SELIG, D.K., HJELMSTAD, G.O., HERRON, C., NICOLL, R.A. & MALENKA, R.C. (1995b). Independent mechanisms for long-term depression of AMPA and NMDA responses. *Neuron* **15**, 417-426.

SELIG, D.K., LEE, H.K., BEAR, M.F. & MALENKA, R.C. (1995a). Reexamination of the effects of MCPG on hippocampal LTP, LTD, and depotentiation. *Journal of Neurophysiology* **74**, 1075-1082.

SERGUEEVA, O.A., FEDOROV, N.B. & REYMANN, K.G. (1993). An antagonist of glutamate metabotropic receptors, (RS)-alpha-methyl-4-carboxyphenylglycine, prevents the LTP-related increase in postsynaptic AMPA sensitivity in hippocampal slices. *Neuropharmacology* **32**, 933-935.

SERODIO, P., KENTROS, C. & RUDY, B. (1994). Identification of molecular components of A-type channels activating at subthreshold potentials. *Journal of Neurophysiology* **72**, 1516-1529.

SHANNON, H.E., BEMIS, K.G., HENDRIX, J.C. & WARD, J.S. (1990). Interactions between scopolamine and muscarinic cholinergic agonists or cholinesterase inhibitors on spatial alternation performance in rats. *Journal of Pharmacology and Experimental Therapeutics* **255**, 1071-1077.

SHEARDOWN, M.J., NIELSEN, E.O., HANSEN, A.J., JACOBSEN, P. & HONORE, T. (1990). 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline: a neuroprotectant for cerebral ischemia. *Science* **247**, 571-574.

SHEARMANN, M.S., SHINOMURA, T., ODA, T. & NISHIZUKA, Y. (1991). Synaptosomal protein kinase C subspecies: A dynamic change in the

hippocampus and cerebellar cortex conconitant with synaptogenesis. *Journal of Neurochemistry* **56**, 1565-1572.

SHEN, K. & MEYER, T. (1999). Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science* **284**, 162-166.

SHEN, K., TERUEL, M.N., CONNOR, J.H., SHENOLIKAR, S. & MEYER, T. (2000). Molecular memory by reversible translocation of calcium/calmodulin-dependent protein kinase II. *Nature Neuroscience* **3**, 881-886.

SHENG, M. (2001). Molecular organization of the postsynaptic specialization. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 7058-7061.

SHI, S.H., HAYASHI, Y., PETRALIA, R.S., ZAMAN, S.H., WENTHOLD, R.J., SVOBODA, K. & MALINOW, R. (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811-1816.

SHIGEMOTO, R., KINOSHITA, A., WADA, E., NOMURA, S., OHISHI, H., TAKADA, M., FLOR, P.J., NEKI, A., ABE, T., NAKANISHI, S. & MIZUNO, N. (1997). Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *Journal of Neuroscience* **17**, 7503-7522.

SHIMOSHIGE, Y., MAEDA, T., KANEKO, S., AKAIKE, A. & SATOH, M. (1997). Involvement of M2 receptor in an enhancement of long-term potentiation by carbachol in Schaffer collateral-CA1 synapses of hippocampal slices. *Neuroscience Research* **27**, 175-180.

SHIRKE, A.M. & MALINOW, R. (1997). Mechanisms of potentiation by calcium-calmodulin kinase II of postsynaptic sensitivity in rat hippocampal CA1 neurons. *Journal of Neurophysiology* **78**, 2682-2692.

SIAPAS, A.G. & WILSON, M.A. (1998). Coordinated interactions between hippocampal ripples and cortical spindles during slow-wave sleep. *Neuron* **21**, 1123-1128.

SILVA, A.J., PAYLOR, R., WEHNER, J.M. & TONEGAWA, S. (1992a). Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257**, 206-211.

SILVA, A.J., STEVENS, C.F., TONEGAWA, S. & WANG, Y. (1992b). Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. *Science* **257**, 201-206.

SIMPSON, P.B., CHALLISS, R.A. & NAHORSKI, S.R. (1995). Neuronal Ca2+ stores: activation and function. *Trends in Neuroscience* **18**, 299-306.

SLADECZEK, F., PIN, J.P., RECASENS, M., BOCKAERT, J. & WEISS, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* **317**, 717-719.

SMITH, W.B., AAKALU, G. & SCHUMAN, E.M. (2001). Local protein synthesis in neurons. *Current Biology* **11**, R901-903.

SODERLING, TR., CHANG, B. & BRICKEY, D. (2001). Cellular signaling through multifunctional Ca2+/calmodulin-dependent protein kinase II. *Journal of Biological Chemistry* **276**, 3719-3722.

SOKOLOV, M.V. & KLESCHEVNIKOV, A.M. (1995). Atropine suppresses associative LTP in the CA1 region of rat hippocampal slices. *Brain Research* **672**, 281-284.

SOLTESZ, I., BOURASSA, J. & DESCHENES, M. (1993). The behavior of mossy cells of the rat dentate gyrus during theta oscillations in vivo. *Neuroscience* **57**, 555-564.

SOMMER B, K.K.V.T.W.W.B.N.H.A.K.M.T.T.S.B.S.PH. (1990). Flip and flop: a cell-

specific functional switch in glutamate-operated channels of the CNS. *Science* **249**, 1580-1585.

SONENBERG, N. (1996). In Translation Control (Herschey, J., Matthews, M. and Sonenberg, N., Eds). *Cold Spring Harbor Press, Cold Spring Harbor, NY* 245-270.

SPENCER, S.A., SCHUH, S.M., LIU, W.S. & WILLARD, M.B. (1992). GAP-43, a protein associated with axon growth, is phosphorylated at three sites in cultured neurons and rat brain. *Journal of Biological Chemistry* **267**, 9059-9064.

SPOOREN, W.P., GASPARINI, F., BERGMANN, R. & KUHN, R. (2000). Effects of the prototypical mGlu(5) receptor antagonist 2-methyl-6-(phenylethynyl)-pyridine on rotarod, locomotor activity and rotational responses in unilateral 6-OHDA-lesioned rats. *European Journal of Pharmacology* **406**, 403-410.

SPRUSTON, N., JONAS, P. & SAKMANN, B. (1995). Dendritic glutamate receptor channels in rat hippocampal CA3 and CA1 pyramidal neurons. *Journal of Physiology* **482**, 325-352.

STAAK, S., BEHNISCH, T. & ANGENSTEIN, F. (1995). Hippocampal long-term potentiation: transient increase but no persistent translocation of protein kinase C isoenzymes alpha and beta. *Brain Research* **682**, 55-62.

STANTON, P.K. (1995). Transient protein kinase C activation primes long-term depression and suppresses long-term potentiation of synaptic transmission in hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 1724-1728.

STANTON, P.K. & SARVEY, J.M. (1984). Blockade of long-term potentiation in rat hippocampal CA1 region by inhibitors of protein synthesis. *Journal of Neuroscience* **4**, 3080-3088.

STANTON, P.K. & SARVEY, J.M. (1985). Depletion of norepinephrine, but not serotonin, reduces long-term potentiation in the dentate gyrus of rat

hippocampal slices. *Journal of Neuroscience* **5**, 2169-2176.

STAUBLI, U., FARADAY, R. & LYNCH, G. (1985). Pharmacological dissociation of memory: anisomycin, a protein synthesis inhibitor, and leupeptin, a protease inhibitor, block different learning tasks. *Behavioral and Neural Biology* **43**, 287-297.

STAUBLI, U. & LYNCH, G. (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Research* **435**, 227-234.

STERN, P., BEHE, P., SCHOEPFER, R. & COLQUHOUN, D. (1992). Single-channel conductances of NMDA receptors expressed from cloned cDNAs: comparison with native receptors. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **250**, 271-277.

STERN, P., CIK, M., COLQUHOUN, D. & STEPHENSON, F.A. (1994). Single channel properties of cloned NMDA receptors in a human cell line: comparison with results from Xenopus oocytes. *Journal of Physiology* **476**, 391-397.

STETTER, M., LANG, E.W. & OBERMAYER, K. (1998). Unspecific long-term potentiation can evoke functional segregation in a model of area 17. *NeuroReport* **9**, 2697-2702.

STEWARD, O. (1997). mRNA localization in neurons: a multipurpose mechanism? *Neuron* **18**, 9-12.

STEWARD, O. & LEVY, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *Journal of Neuroscience* **2**, 284-291.

STEWARD, O. & SCHUMAN, E.M. (2001). Protein synthesis at synaptic sites on dendrites. *Annual Review of Neuroscience* **24**,

STEWARD, O. & WORLEY, P. (2002). Local synthesis of proteins at synaptic sites on dendrites: role in synaptic plasticity and memory consolidation? *Neurobiology*

of Learning and Memory **78**, 508-527.

STRACK, S., BARBAN, M.A., WADZINSKI, B.E. & COLBRAN, R.J. (1997). Differential inactivation of postsynaptic density-associated and soluble Ca2+/calmodulin-dependent protein kinase II by protein phosphatases 1 and 2A. *Journal of Neurochemistry* **68**, 2119-2128.

STRATTON, K.R., WORLEY, P.F., HUGANIR, R.L. & BARABAN, J.M. (1989). Muscarinic agonists and phorbol esters increase tyrosine phosphorylation of a 40-kilodalton protein in hippocampal slices. *Proceedings of the National Academy of Sciences of the United States of America* **86**, 2498-2501.

STRAUBE, T. & FREY, J.U. (2003). Involvement of beta-adrenergic receptors in protein synthesis-dependent late long-term potentiation (LTP) in the dentate gyrus of freely moving rats: the critical role of the LTP induction strength. *Neuroscience* **119**, 473-479.

STRITTMATTER, S.M., VARTANIAN, T. & FISHMAN, M.C. (1992). GAP-43 as a plasticity protein in neuronal form and repair. *Journal of Neurobiology* **23**, 507-520.

SUGIYAMA, H., ITO, I. & HIRONO, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature* **325**, 531-533.

SUN, H., CHARLES, C.H., LAU, L.F. & TONKS, N.K. (1993). MKP-1 (3CH134), an immediate early gene product, is a dual specificity phosphatase that dephosphorylates MAP kinase in vivo. *Cell* **75**, 487-493.

SUN, P., ENSLEN, H., MYUNG, P.S. & MAURER, R.A. (1994). Differential activation of CREB by Ca2+/calmodulin-dependent protein kinases type II and type IV involves phosphorylation of a site that negatively regulates activity. *Genes and Development* **21**,

SUN, Z., MEANS, R.L., LEMAGUERESSE, B. & MEANS, A.R. (1995). Organization and analysis of the complete rat calmodulin-dependent protein

kinase IV gene. *Journal of Biological Chemistry* **270**, 29507-29514.

SUTHERLAND, R.J., WHISHAW, I.Q. & KOLB, B. (1983). A behavioural analysis of spatial localization following electrolytic, kainate- or colchicine-induced damage to the hippocampal formation in the rat. *Behavioural Brain Research* **7**, 33-153.

SUZUKI, S.S. & SMITH, G.K. (1987). Spontaneous EEG spikes in the normal hippocampus. I. Behavioral correlates, laminar profiles and bilateral synchrony. *Electroencephalography and Clinical Neurophysiology* **67**, 348-359.

SVENSSON, S.P., BAILEY, T.J., PEPPERL, D.J., GRUNDSTROM, N., ALA-UOTILA, S., SCHEININ, M., KARLSSON, J.O. & REGAN, J.W. (1993). Cloning and expression of a fish alpha 2-adrenoceptor. *British Journal of Pharmacology* **110**, 54-60.

SWANSON, L., KOHLER, C. & BJORKLUND, A. (1987). The limbic region. I. The septohippocampal system. *In Integrated Systems of the CNS (Eds. Bjorklund, A., Hokfelt, T. and Swanson, L.) Elsevier Science, Amsterdam.* 125-269.

SWANSON-PARK, J.L., COUSSENS, C.M., MASON-PARKER, S.E., RAYMOND, C.R., HARGREAVES, E.L., DRAGUNOW, M., COHEN, A.S. & ABRAHAM, W.C. (1999). A double dissociation within the hippocampus of dopamine D1/D5 receptor and beta-adrenergic receptor contributions to the persistence of long-term potentiation. *Neuroscience* **92**, 485-497.

SWEATT, J.D. (2001). The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry* **76**, 1-10.

TAKUMI, Y., RAMIREZ-LEON, V., LAAKE, P., RINVIK, E. & OTTERSEN, O.P. (1999). Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neuroscience* **2**, 618-624.

TANABE, Y., MASU, M., ISHII, T., SHIGEMOTO, R. & NAKANISHI, S. (1992). A

family of metabotropic glutamate receptors. *Neuron* **8**, 169-179.

TANAKA, C. & NISHIZUKA, Y. (1994). The protein kinase C family for neuronal signaling. *Annual Review of Neuroscience* **17**,

TANG, C.M., SHI, Q.Y., KATCHMAN, A. & LYNCH, G. (1991). Modulation of the time course of fast EPSCs and glutamate channel kinetics by aniracetam. *Science* **254**, 288-290.

TANG, S.J., REIS, G., KANG, H., GINGRAS, A.C., SONENBERG, N. & SCHUMAN, E.M. (2002). A rapamycin-sensitive signaling pathway contributes to long-term synaptic plasticity in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 467-472.

TANG, W.J. & GILMAN, A.G. (1991). Type-specific regulation of adenylyl cyclase by G protein beta gamma subunits. *Science* **254**, 1500-1503.

TEYLER, T.J. (1989). Comparative aspects of hippocampal and neocortical long-term potentiation. *Journal of Neuroscience Methods* **28**, 101-108.

TEYLER, T.J. & DISCENNA, P. (1986). The hippocampal memory indexing theory. *Behavioral Neuroscience* **100**, 147-154.

THASTRUP, O., CULLEN, P.J., DROBAK, B.K., HANLEY, M.R. & DAWSON, A.P. (1990). Thapsigargin, a tumor promoter, discharges intracellular Ca2+ stores by specific inhibition of the endoplasmic reticulum Ca2(+)-ATPase. *Proceedings of the National Academy of Sciences of the United States of America* **87**, 2466-2470.

THEOFAN, G. & NORMAN, A.W. (1986). Effects of alpha-amanitin and cycloheximide on 1,25-dihydroxyvitamin D3-dependent calbindin-D28K and its mRNA in vitamin D3-replete chick intestine. *Journal of Biological Chemistry* **261**, 7311-7315.

THOMAS, K.L., LAROCHE, S., ERRINGTON, M.L., BLISS, T.V. & HUNT, S.P.

(1994). Spatial and temporal changes in signal transduction pathways during LTP. *Neuron* **13**, 737-745.

THOMAS, M.J., MOODY, T.D., MAKHINSON, M. & O'DELL, T.J. (1996). Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. *Neuron* **17**, 475-482.

THOMAS, M.J. & O'DELL, T.J. (1995). The molecular switch hypothesis fails to explain the inconsistent effects of the metabotropic glutamate receptor antagonist MCPG on long-term potentiation. *Brain Research* **695**, 45-52.

THOMAS, M.J., WATABE, A.M., MOODY, T.D., MAKHINSON, M. & O'DELL, T.J. (1998). Postsynaptic complex spike bursting enables the induction of LTP by theta frequency synaptic stimulation. *Journal of Neuroscience* **18**, 7118-7126.

THOMSEN, C., KRISTENSEN, P., MULVIHILL, E., HALDEMAN, B. & SUZDAK, P.D. (1992). L-2-amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase. *European Journal of Pharmacology* **227**, 361-362.

THOMSON, A.M. (1986). A magnesium-sensitive post-synaptic potential in rat cerebral cortex resembles neuronal responses to N-methylaspartate. *Journal of Physiology* **370**, 531-549.

TIEDGE, H. & BROSIUS, J. (1996). Translational machinery in dendrites of hippocampal neurons in culture. *Journal of Neuroscience* **16**, 7171-7181.

TOKUMITSU, H., CHIJIWA, T., HAGIWARA, M., MIZUTANI, A., TERASAWA, M. & HIDAKA, H. (1990). KN-62, 1-[N,O-bis(5-isoquinolinesulfonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazi ne, a specific inhibitor of Ca2+/calmodulin-dependent protein kinase II. *Journal of Biological Chemistry* **265**, 4315-4320.

TOMS, N.J., JANE, D.E., KEMP, M.C., BEDINGFIELD, J.S. & ROBERTS, P.J. (1996). The effects of (RS)-alpha-cyclopropyl-4-phosphonophenylglycine

((RS)-CPPG), a potent and selective metabotropic glutamate receptor antagonist. *British Journal of Pharmacology* **119**, 851-854.

TONGIORGI, E., RIGHI, M. & CATTANEO, A. (1997). Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. *Journal of Neuroscience* **17**, 9492-9505.

TONI, N., BUCHS, P.A., NIKONENKO, I., BRON, C.R. & MULLER, D. (1999). LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. *Nature* **402**, 421-425.

TORRE, E.R. & STEWARD, O. (1996). Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. *Journal of Neuroscience* **16**, 5967-5978.

TRUDEAU, L.E., EMERY, D.G. & HAYDON, P.G. (1996). Direct modulation of the secretory machinery underlies PKA-dependent synaptic facilitation in hippocampal neurons. *Neuron* **17**, 789-797.

TRUSSELL, L.O. & FISCHBACH, G.D. (1989). Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* **3**, 209-218.

TSIEN, R.W., LIPSCOMBE, D., MADISON, D.V., BLEY, K.R. & FOX, A.P. (1988). Multiple types of neuronal calcium channels and their selective modulation. *Trends in Neuroscience* **11**, 431-438.

TULLY, T., PREAT, T., BOYNTON, S.C. & DEL VECCHIO, M. (1994). Genetic dissection of consolidated memory in Drosophila. *Cell* **79**, 35-47.

VAN BIESEN, T., HAWES, B.E., RAYMOND, J.R., LUTTRELL, L.M., KOCH, W.J. & LEFKOWITZ, R.J. (1996). G(o)-protein alpha-subunits activate mitogen-activated protein kinase via a novel protein kinase C-dependent mechanism. *Journal of Biological Chemistry* **271**, 1266-1269.

VAN CALKER, D., MULLER, M. & HAMPRECHT, B. (1979). Adenosine regulates

via two different types of receptors, the accumulation of cyclic AMP in cultured brain cells. *Journal of Neurochemistry* **33**, 999-1005.

VAN STEGEREN, A.H., EVERAERD, W., CAHILL, L., MCGAUGH, J.L. & GOOREN, L.J. (1998). Memory for emotional events: differential effects of centrally versus peripherally acting beta-blocking agents. *Psychopharmacology* **1383-4**,

VANDERWOLF, C.H. (1969). Hippocampal electical activity and voluntary movement in the rat. *Electroencephalography and Clinical Neurophysiology* **26**, 407-418.

VAZQUEZ, D. (1974). Inhibitors of protein synthesis. *FEBS Letters* **40**, S63-S84

VIGNES, M. & COLLINGRIDGE, G.L. (1997). The synaptic activation of kainate receptors. *Nature* **388**, 179-182.

VILLACRES, E.C., WONG, S.T., CHAVKIN, C. & STORM, D.R. (1998). Type I adenylyl cyclase mutant mice have impaired mossy fiber long-term potentiation. *Journal of Neuroscience* **18**, 3186-3194.

VON EULER (1946). The presence of a sympathomimetic substance in extracts of mammalian heart. *Journal of Physiology* **105**, 38-44.

VOSSLER, M.R., YAO, H., YORK, R.D., PAN, M.G., RIM, C.S. & STORK, P.J. (1997). cAMP activates MAP kinase and Elk-1 through a B-Raf- and Rap1-dependent pathway. *Cell* **89**, 73-82.

VOULALAS, P.J. & SARVEY, J.M. (1995). Forskolin-induced potentiation in hippocampal dentate gyrus increases phosphorylation of mitogen-activated protein kinases. *Society of Neuroscience Abstracts* **21**, 601

VYKLICKY, L.Jr., PATNEAU, D.K. & MAYER, M.L. (1991). Modulation of excitatory synaptic transmission by drugs that reduce desensitization at AMPA/kainate receptors. *Neuron* **7** , 971-984.

WALAAS, S.I., LAI, Y., GORELICK, F.S., DECAMILLI, P., MORETTI, M. & GREENGARD, P. (1988). Cell-specific localization of the alpha-subunit of calcium/calmodulin-dependent protein kinase II in Purkinje cells in rodent cerebellum. *Brain Research* **464**, 233-242.

WANG, J.H. & FENG, D.P. (1992). Postsynaptic protein kinase C essential to induction and maintenance of long-term potentiation in the hippocampal CA1 region. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 2576-2580.

WANG, J.H. & KELLY, P.T. (1995). Postsynaptic injection of CA2+/CaM induces synaptic potentiation requiring CaMKII and PKC activity. *Neuron* **15**, 443-452.

WANG, L.Y., SALTER, M.W. & MACDONALD, J.F. (1991). Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. *Science* **253**, 1132-1135.

WANG, S.J., CHENG, L.L. & GEAN, P.W. (1999). Cross-modulation of synaptic plasticity by beta-adrenergic and 5-HT1A receptors in the rat basolateral amygdala. *Journal of Neuroscience* **19**, 570-577.

WANG, Y. & DURKIN, J.P. (1995). alpha-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, but not N-methyl-D-aspartate, activates mitogen-activated protein kinase through G-protein beta gamma subunits in rat cortical neurons. *Journal of Biological Chemistry* **270**, 22783-22787.

WANG, Y., ROWAN, M.J. & ANWYL, R. (1995). (RS)-alpha-Methyl-4-carboxyphenylglycine inhibits long-term potentiation only following the application of low frequency stimulation in the rat dentate gyrus in vitro. *Neuroscience Letters* **197**, 207-210.

WANG, Y., WU, J., ROWAN, M.J. & ANWYL, R. (1998). Role of protein kinase C in the induction of homosynaptic long-term depression by brief low frequency

stimulation in the dentate gyrus of the rat hippocampus in vitro. *Journal of Physiology* **513**, 467-475.

WANG, Y., WU, J., ROWAN, M.J. & ANWYL, R. (1996). Ryanodine produces a low frequency stimulation-induced NMDA receptor-independent long-term potentiation in the rat dentate gyrus in vitro. *Journal of Physiology* **495**, 755-767.

WASKIEWICZ, A.J., JOHNSON, J.C., PENN, B., MAHALINGAM, M., KIMBALL, S.R. & COOPER, J.A. (1999). Phosphorylation of the cap-binding protein eukaryotic translation initiation factor 4E by protein kinase Mnk1 in vivo. *Molecular Cell Biology* **19**, 1871-1880.

WATABE, A.M., ZAKI, P.A. & O'DELL, T.J. (2000). Coactivation of beta-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal CA1 region. *Journal of Neuroscience* **20**, 5924-5931.

WATANABE, T., HUANG, H.B., HORIUCHI, A., DA CRUZE SILVA, E.F., HSIEH-WILSON, L., ALLEN, P.B., SHENOLIKAR, S., GREENGARD, P. & NAIRN, A.C. (2001). Protein phosphatase 1 regulation by inhibitors and targeting subunits. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 3080-3085.

WATKINS, J.C. & COLLINGRIDGE, G.L. (1989). The NMDA receptor. *Oxford University Press: Oxford*

WATSON, J.B., SUTCLIFFE, J.G. & FISHER, R.S. (1992). Localization of the protein kinase C phosphorylation/calmodulin-binding substrate RC3 in dendritic spines of neostriatal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 8581-8585.

WEISSKOPF, M.G., CASTILLO, P.E., ZALUTSKY, R.A. & NICOLL, R.A. (1994). Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP.

Science **265**, 1878-1882.

WENG, G., BHALLA, U.S. & IYENGAR, R. (1999). Complexity in biological signaling systems. *Science* **284**, 92-96.

WENK, G., HUGHEY, D., BOUNDY, V., KIM, A., WALKER, L. & OLTON, D. (1987). Neurotransmitters and memory: role of cholinergic, serotonergic, and noradrenergic systems. *Behavioural Neuroscience* **101**, 325-332.

WENZEL, J., DESMOND, N.L. & LEVY, W.B. (1993). Somatic ribosomal changes induced by long-term potentiation of the perforant path-hippocampal CA1 synapses. *Brain Research* **619**, 331-333.

WESS, J. (1996). Molecular biology of muscarinic acetylcholine receptors. *Critical Review of Neurobiology* **10**, 69-99.

WESTPHAL, R.S., TAVALIN, S.J., LIN, J.W., ALTO, N.M., FRASER, I.D., LANGEBERG, L.K., SHENG, M. & SCOTT, J.D. (1999). Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* **285**, 93-96.

WEXLER, E.M. & STANTON, P.K. (1993). Priming of homosynaptic long-term depression in hippocampus by previous synaptic activity. *NeuroReport* **4**, 591-594.

WHITEHURST, V.E., VICK, J.A., ALLEVA, F.R., ZHANG, J., JOSEPH, X. & BALAZS, T. (1999). Reversal of propranolol blockade of adrenergic receptors and related toxicity with drugs that increase cyclic AMP. *Proceedings of the Society of Experimental Biology and Medicine* **221**, 382-385.

WHITTON, P.S. (1997). Glutamatergic control over brain dopamine release in vivo and in vitro. *Neuroscience Biobehavioral Review* **21**, 481-488.

WIGSTROM, H., GUSTAFSSON, B., HUANG, Y.Y. & ABRAHAM, W.C. (1986). Hippocampal long-term potentiation is induced by pairing single afferent

volleys with intracellularly injected depolarizing current pulses. *Acta Physiologica Scandinavica* **126**, 317-319.

WILDING, T.J. & HUETTNER, J.E. (1996). Antagonist pharmacology of kainate- and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-preferring receptors. *Molecular Pharmacology* **49**, 540-546.

WILSCH, V.W., BEHNISCH, T., JAGER, T., REYMANN, K.G. & BALSCHUN, D. (1998). When are class I metabotropic glutamate receptors necessary for long-term potentiation? *Journal of Neuroscience* **18**, 6071-6080.

WILSON, M.A. & MCNAUGHTON, B.L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science* **265**, 676-679.

WINDER, D.G. & CONN, P.J. (1993). Activation of metabotropic glutamate receptors increases cAMP accumulation in hippocampus by potentiating responses to endogenous adenosine. *Journal of Neuroscience* **13**, 38-44.

WINDER, D.G., MANSUY, I.M., OSMAN, M., MOALLEM, T.M. & KANDEL, E.R. (1998). Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* **92**, 25-37.

WINDER, D.G., MARTIN, K.C., MUZZIO, I.A., ROHRER, D., CHRUSCINSKI, A., KOBILKA, B. & KANDEL, E.R. (1999). ERK plays a regulatory role in induction of LTP by theta frequency stimulation and its modulation by beta-adrenergic receptors. *Neuron* **24**, 715-726.

WISDEN, W. & SEEBURG, P.H. (1993). A complex mosaic of high-affinity kainate receptors in rat brain. *Journal of Neuroscience* **13**, 3582-3598.

WO, Z.G. & OSWALD, R.E. (1995). Unraveling the modular design of glutamate-gated ion channels. *Trends in Neuroscience* **18**, 161-168.

WONG, E.H., KEMP, J.A., PRIESTLEY, T., KNIGHT, A.R., WOODRUFF, G.N. & IVERSEN, L.L. (1986). The anticonvulsant MK-801 is a potent N-methyl-D-

aspartate antagonist. *Proceedings of the National Academy of Sciences of the United States of America* **83**, 7104-7108.

WONG, L.A., MAYER, M.L., JANE, D.E. & WATKINS, J.C. (1994). Willardiines differentiate agonist binding sites for kainate- versus AMPA-preferring glutamate receptors in DRG and hippocampal neurons. *Journal of Neuroscience* **14**, 3881-3897.

WONG, S.T., ATHOS, J., FIGUEROA, X.A., PINEDA, V.V., SCHAEFER, M.L., CHAVKIN, C.C., MUGLIA, L.J. & STORM, D.R. (1999). Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. *Neuron* **23**, 787-798.

WOO, N.H., DUFFY, S.N., ABEL, T. & NGUYEN, P.V. (2000). Genetic and pharmacological demonstration of differential recruitment of cAMP-dependent protein kinases by synaptic activity. *Journal of Neurophysiology* **84**, 2739-2745.

WOO, N.H., DUFFY, S.N., ABEL, T. & NGUYEN, P.V. (2003). Temporal spacing of synaptic stimulation critically modulates the dependence of LTP on cyclic AMP-dependent protein kinase. *Hippocampus* **13**, 293-300.

WOOD, M.W., VANDONGEN, H.M. & VANDONGEN, A.M. (1995). Structural conservation of ion conduction pathways in K channels and glutamate receptors. *Proceedings of the National Academy of Sciences of the United States of America* **92**, 4882-4886.

WOODSIDE, B.L., HAMMONDS, M.D. & TEYLER, T.J. (1999). VdccLTP and nmdaLTP mediate different aspects of acquisition and retention in the 4/8 radial arm maze task. *Society for Neuroscience Abstracts* **25**, A354.6

WRIGHT, R.A. & SCHOEPP, D.D. (1996). Differentiation of group 2 and group 3 metabotropic glutamate receptor cAMP responses in the rat hippocampus. *European Journal of Pharmacology* **287**, 275-282.

WU, G.Y., DEISSEROTH, K. & TSIEN, R.W. (2001b). Activity-dependent CREB phosphorylation: convergence of a fast, sensitive calmodulin kinase pathway and a slow, less sensitive mitogen-activated protein kinase pathway. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 2808-2813.

WU, G.Y., DEISSEROTH, K. & TSIEN, R.W. (2001a). Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nature Neuroscience* **4**, 151-158.

WU, S.P., LU, K.T., CHANG, W.C. & GEAN, P.W. (1999). Involvement of mitogen-activated protein kinase in hippocampal long-term potentiation. *Journal of Biomedical Science* **6**, 409-417.

WYLLIE, D.J., BEHE, P. & COLQUHOUN, D. (1998). Single-channel activations and concentration jumps: comparison of recombinant NR1a/NR2A and NR1a/NR2D NMDA receptors. *Journal of Physiology* **510**, 1-18.

WYLLIE, D.J., BEHE, P., NASSAR, M., SCHOEPFER, R. & COLQUHOUN, D. (1996). Single-channel currents from recombinant NMDA NR1a/NR2D receptors expressed in Xenopus oocytes. *Proceedings of the Royal Society of London. Series B, Biological Sciences* **263**, 1079-1086.

XIA, Z., DUDEK, H., MIRANTI, C.K. & GREENBERG, M.E. (1996). Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *Journal of Neuroscience* **16**, 5425-5436.

XIE, X., BERGER, T.W. & BARRIONUEVO, G. (1992). Isolated NMDA receptor-mediated synaptic responses express both LTP and LTD. *Journal of Neurophysiology* **67**, 1009-1013.

XING, J., GINTY, D.D. & GREENBERG, M.E. (1996). Coupling of the RAS-MAPK pathway to gene activation by RSK2, a growth factor-regulated CREB kinase. *Science* **273**, 959-963.

YAMADA, K.A. & TANG, C.M. (1993). Benzothiadiazides inhibit rapid glutamate receptor desensitization and enhance glutamatergic synaptic currents. *Journal of Neuroscience* **13** , 3904-3915.

YANAGIHASHI, R. & ISHIKAWA, T. (1992). Studies on long-term potentiation of the population spike component of hippocampal field potential by the tetanic stimulation of the perforant path rats: effects of a dopamine agonist, SKF-38393. *Brain Research* **579**, 79-86.

YANG, S.N., TANG, Y.G. & ZUCKER, R.S. (1999). Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. *Journal of Neurophysiology* **81**, 781-787.

YANG, S.N., WU, J.N., LIU, D. & TUNG, C.S. (1998). Metabotropic glutamate receptors are involved in calcium-induced LTP of AMPA and NMDA receptor-mediated responsesin the rat hippocampus. *Brain Research Bulletin* **46**, 505-512.

YIN, J.C., WALLACH, J.S., DEL VECCHIO, M., WILDER, E.L., ZHOU, H., QUINN, W.G. & TULLY, T. (1994). Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. *Cell* **79**, 19-58.

YLINEN, A., BRAGIN, A., NADASDY, Z., JANDO, G., SZABO, I., SIK, A. & BUZSAKI, G. (1995). Sharp wave-associated high-frequency oscillation (200 Hz) in the intact hippocampus: network and intracellular mechanisms. *Journal of Neuroscience* **15**, 30-46.

YOKOI, M., KOBAYASHI, K., MANABE, T., TAKAHASHI, T., SAKAGUCHI, I., KATSUURA, G., SHIGEMOTO, R., OHISHI, H., NOMURA, S., NAKAMURA, K., NAKAO, K., KATSUKI, M. & NAKANISHI, S. (1996). Impairment of hippocampal mossy fiber LTD in mice lacking mGluR2. *Science* **273**, 645-647.

YOSHIMURA, Y., SOGAWA, Y. & YAMAUCHI, T. (1999). Protein phosphatase 1 is

involved in the dissociation of Ca2+/calmodulin-dependent protein kinase II from postsynaptic densities. *FEBS Letters* **446**, 239-242.

YU, S.S., LEFKOWITZ, R.J. & HAUSDORFF, W.P. (1993). Beta-adrenergic receptor sequestration. A potential mechanism of receptor resensitization. *Journal of Biological Chemistry* **268**, 337-341.

YUSTE, R. & DENK, W. (1995). Dendritic spines as basic functional units of neuronal integration. *Nature* **375**, 682-684.

ZAKHARENKO, S.S., ZABLOW, L. & SIEGELBAUM, S.A. (2001). Visualization of changes in presynaptic function during long-term synaptic plasticity. *Nature Neuroscience* **4**, 711-717.

ZALUTSKY, R.A. & NICOLL, R.A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* **248**, 1619-1624.

ZHABOTINSKY, A.M. (2000). Bistability in the Ca(2+)/calmodulin-dependent protein kinase-phosphatase system. *Biophysical Journal* **79**, 2211-2221.

ZHENG, C.F. & GUAN, K.L. (1993). Properties of MEKs, the kinases that phosphorylate and activate the extracellular signal-regulated kinases. *Journal of Biological Chemistry* **268**, 23933-23939.

ZHOU, G., BAO, Z.Q. & DIXON, J.E. (1995). Components of a new human protein kinase signal transduction pathway. *Journal of Biological Chemistry* **270**, 12665-12669.

ZHU, J.J., ESTEBAN, J.A., HAYASHI, Y. & MALINOW, R. (2000). Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nature Neuroscience* **3**, 1098-1106.

ZILLES, K., GROSS, G., SCHLEICHER, A., SCHILDGEN, S., BAUER, A., BAHRO, M., SCHWENDEMANN, G., ZECH, K. & KOLASSA, N. (1991). Regional and laminar distributions of alpha 1-adrenoceptors and their subtypes in human

and rat hippocampus. *Neuroscience* **40**, 307-320.

ZUCCHI, R. & RONCA-TESTONI, S. (1997). The sarcoplasmic reticulum Ca2+ channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacological Review* **49**, 1-51.

ZUCKER, R.S. (1999). Calcium- and activity-dependent synaptic plasticity. *Current Opinions in Neurobiology* **9**, 305-313.