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.

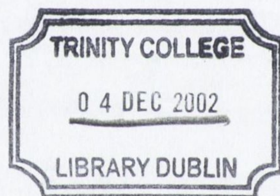
ENDOGENOUS ACETYLCHOLINE MODULATES SYNAPTIC PLASTICITY IN THE HIPPOCAMPUS *IN VIVO*

By

Shaomin Li, MD, MSc.

A dissertation submitted for the degree of Doctor of Philosophy of the University of Dublin, Trinity College, Dublin 2, Ireland.
This research was conducted in the Department of Pharmacology and Therapeutics in the Faculty of Health Sciences.

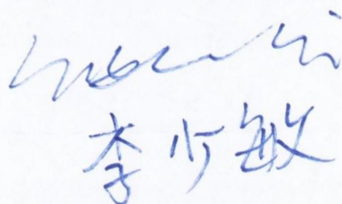
April 2002


TRINITY COLLEGE
0 4 DEC 2002
LIBRARY DUBLIN
THESIS
7006

Declaration

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

Shaomin Li, MD., MSc.



Acknowledgements

I would like to thank Prof. Michael J. Rowan, my supervisor, for giving me the occasion to carry out this research in his laboratory and for his financial support, also for his patience, encouragement and guidance. Appreciation is also extended to Prof. Roger Anwyl of the Department of Physiology for his kindness and constructive advice in physiology, pharmacology and electrophysiology.

I am sincere be grateful to Dr. Liam Cullen who as always there to help when I need, like as something wrong or everything I need for these experiments. I would like to thank Dr. Igor Klyubin and Dr. Jianqun Wu for their valuable scientific advice and discussion and also for their friendship.

Thanks to all the remaining colleagues of the Department of Pharmacology & Therapeutics and the staff of the Bio Resoures Unit for their help and all the corporation.

Finally, I would like to thank my parents for their love and support for so many years, and my wife for her encouragement and endurance throughout my time here in Ireland.

Table of Contents

Declaration -- i

Acknowledgments --- ii

Table of contents --- iii

Lists of Figures and Tables --- vii

Summary -- ix

Abbreviations -- xi

I. Introduction -- 1

1.1 Anatomy of hippocampus --- 1

1.2 Synaptic plasticity --- 2

1.2.1 Short-term plasticity --- 2

1.2.2 Long-term potentiation -- 3

1.2.2.1 The locus of LTP expression and retrograde signaling --------------- 4

1.2.2.2 Glutamate receptors and LTP -- 5

1.2.2.3 Intracellular biochemical events and LTP ---------------------------- 6

1.2.3 Long-term depression -- 7

1.2.4 LTP, LTD and metaplasticity -- 8

1.3 Electrophysiological studies of learning ------------------------------------ 9

1.3.1 Hippocampal activity in relation to natural behaviour -------------------- 9

1.3.2 LTP and mechanisms of memory formation ---------------------------- 10

1.4 The role of acetylcholine in hippocampal plasticity ------------------------ 12

1.4.1 Cholinergic modulation of hippocampal plasticity ---------------------- 13

1.4.1.1 ACh release in the hippocampus ------------------------------------- 13

1.4.1.2 ACh induced LTP-like phenomenon -------------------------------- 14

1.4.1.3 ACh influences synaptic plasticity in area CA1 ---------------------- 14

1.4.2 ACh has similar effects to LTP mechanisms ---------------------------- 15

1.4.2.1 Ca^{2+} influx and glutamate release ----------------------------------- 15

1.4.2.2 The activation of AChRs trigger intracellular cascades ------------ 16

1.4.3 Different mAChRs subtypes mediated different functions in brain ------ 17

1.4.3.1 M_1 muscarinic receptor subtype -- 17

1.4.3.2 M_2 muscarinic receptor subtype -- 17

1.4.3.3 M_3 muscarinic receptor subtype -- 18

II. Material and methods -- 20

2.1 Animals -- 20

2.2 Surgery -- 21

2.2.1 Surgical preparation -- 21

2.2.2 Electrode implantation -- 21

2.2.3 Intracerebroventricular (i.c.v.) cannulation --------------------------- 22

2.2.4 Location of recording and stimulating electrodes --------------------- 23

2.2.5 Recovery from surgery --- 26

2.3 Recording apparatus -- 26

2.4 Electrophysiological recording -- 27

2.5 Drugs -- 28

2.6 Histology -- 30

2.7 Data analysis --- 31

III. Results -- 33

3.1 Muscarinic agonists elicit biphasic responses --------------------------------- 33

3.1.1 Low dose of muscarine induced a transient increase of the EPSP-------- 33

3.1.2 High dose of muscarine depressed the EPSP *in vivo* --------------------- 33

3.1.3 M_1 agonist, YM796, had no effect on the baseline EPSP ---------------- 33

3.2 Endogenous acetylcholine enhanced EPSPs ----------------------------------- 35

3.2.1 Non-selective antagonist, scopolamine, had no effect on baseline ------ 35

3.2.2 M_1 antagonist, telenzepine, had no effect on EPSP baseline ------------ 35

3.2.3 M_2 antagonist, methoctramine, enhanced the EPSP --------------------- 36

3.2.4 M_3 antagonist, 4-DAMP, had no effect on the baseline ---------------- 36

3.3 Blockade of M_2 receptors induced a long-lasting potentiation ------------- 36

3.3.1 M_2 antagonist, methoctramine, induced an LTP-like phenomenon ----- 36

3.3.2 M_2 antagonist, AF-DX 116, induced an LTP-like phenomenon --------- 37

3.3.3 Methoctramine induced enhancement of the EPSP is dose-dependent -- 37

3.4 Methoctramine-induced LTP is dependent on muscarinic receptors -------- 41

3.4.1 Scopolamine blocked induction of methoctramine-induced 'LTP' ------ 41

3.4.2 Scopolamine had no effect on the maintenance of methoctramine-LTP -- 41

3.4.3 Mecamylamine failed to block methoctramine-'LTP' -------------------- 41

3.5 Non-M_2 muscarinic receptors are involved in the methoctramine-'LTP' --- 44

3.5.1 M_1 antagonist, telenzepine, partially blocked methoctramine-'LTP'--- 44

3.5.2 M_3 antagonist, 4-DAMP, blocked methoctramine-'LTP'--------------- 44

3.6 Methoctramine-'LTP' is dependent on nitric oxide synthase ---------------- 46

3.6.1 Methoctramine-'LTP' is associated with a reduction of PPF ---------- 46

3.6.2 NOS inhibitor, TRIM, blocked methoctramine-'LTP' ------------------ 46

3.6.3 L-arginine, reversed TRIM block of methoctramine-'LTP'------------- 47

3.7 NMDA receptors, not VDCCs, are involved in methoctramine-'LTP'------ 50

3.7.1 NMDAR antagonist, D-AP5, blocked methoctramine-'LTP' partially-- 50

3.7.2 VDCC antagonist, mibefradil, failed to block methoctramine-'LTP'--- 50

3.8 Second messengers involved in methoctramine-'LTP'----------------------- 52

3.8.1 PKC inhibitor, BIM, blocked methoctramine-'LTP' ------------------- 52

3.8.2 PKA inhibitor, Rp-cAMPs, blocked methoctramine-'LTP' ----------- 52

3.9 Methoctramine did not occlude tetanus-induced LTP ----------------------- 54

3.9.1 Methoctramine did not occlude tetanus-induced LTP ---------------- 54

3.9.2 BIM failed to prevent tetanus-induced LTP after methoctramine ------ 54

3.9.3 Tetanus-induced LTP occludes later methoctramine-induced 'LTP' -- 54

3.10 Methoctramine-'LTP' is not dependent on synaptic stimulation ---------- 57

3.10.1 Methoctramine-'LTP' is widespread ----------------------------------- 57

3.10.2 Stopping stimulation failed to prevent methoctramine-'LTP' -------- 57

3.10.3 Medial septal injection had no effect on hippocampal EPSPs --------57

3.11 Scopolamine failed to block tetanus-induced LTP in the intact brain ----- 59

3.11.1 Urethane-anaesthetized rats -- 59

3.11.2 Freely behaving rats -- 59

3.12 Novelty exposure facilitated LTP doesn't relate to ACh ------------------ 61

3.12.1 Novelty exposure facilitated LTP in behaving animals ------------- 61

3.12.2 Novelty facilitated LTP does not involve mAChRs ----------------- 66

3.12.3 Novelty facilitated LTP does not involve β-adrenoceptor ----------- 66

3.12.4 Novelty facilitated LTP is dependent on dopamine receptors ------- 66

IV. Discussion --- 71

4.1 Endogenous ACh and glutamate release induced by mAChR antagonists --71

4.2 Muscarinic receptor subtypes and synaptic plasticity in the CA1 region ----72

4.3 Role of NMDA receptor activation and VDCCs in muscarinic LTP --------74

4.4 Nitric oxide dependent of LTP ---74

4.5 Role of protein kinase activation in LTP -------------------------------------- 76

4.6 $M_1$and $M_{3/5}$ receptors may have different functions in the regulation of synaptic plasticity -- 77

4.4 Novelty exploration facilitates LTP -- 78

V. Conclusion -- 82

VI. Bibliography --- 83

Lists of Figures and Tables

Fig. 2-1. Laminar profile of field potentials recorded from CA1 region during the electrode locations.

Fig. 2-2. Schematic diagram of coronal hippocampus and the input/output curve of the field EPSP.

Fig. 3-1. Muscarine elicits biphasic effects on baseline synaptic transmission.

Fig. 3-2. Effects of different mucarinic cholinergic receptor antagonists on baseline synaptic transmission and short-term plasticity.

Fig. 3-3. Blockade of M_2 muscarinic receptor induces a fast onset long-lasting potentiation in the hippocampal CA1 region *in vivo*.

Fig. 3-4. Dose-dependent increase of field EPSP slope by administration of methoctramine.

Fig. 3-5. The methoctramine-induced persistent facilitation is muscarinic receptor-dependent.

Fig. 3-6. The induction of M_2 receptor antagonist induced-'LTP' is not nicotinic receptor-dependent.

Fig. 3-7. Non-M_2 receptor subtype mediate the methoctramine-induced 'LTP'.

Fig. 3-8. Methoctramine induced long-lasting potentiation is associated with a reduction in PPF in the hippocampal CA1 region *in vivo*.

Fig. 3-9. Nitric oxide is required for the induction of LTP induced both by HFS and methoctramine.

Fig. 3-10. The induction of M_2 receptor antagonist induced-LTP is partially NMDA receptor-dependent, but not VDCC-dependent.

Fig. 3-11. The activation of PKA and PKC is required for the methoctramine induced-persistent enhancement.

Fig. 3-12. Methoctramine did not occlude later tetanus(HFS)-induced LTP.

Fig. 3-13. A subthreshold dose of methoctramine had no effect on tetanus-induced LTP and methoctramine failed to affect the response to a subthreshold tetanus.

Fig. 3-14. Methoctramine induced 'LTP' is not dependent on synaptic stimulation.

Fig. 3-15. Scopolamine fails to block tetanus induced LTP in the intact brain.

Fig. 3-16. Brief exposure to a novel environment facilitates the induction of LTP at hippocampal CA1 synapses in freely behaving rats.

Fig. 3-17. Time window for the facilitation of the induction of LTP by novelty exposure.

Fig. 3-18. Novelty exploration facilitated weak tetanus induced-LTP involved dopamine receptors, but not mAChR or beta-adrenoceptor.

Table 3-1. Different drugs' effect on methoctramine induced-'LTP'

Table 3-2. Different drugs' effect on tetanus induced-LTP

Table 3-3. Novelty exploration effect on the weak tetanus induced-LTP

Endogenous Acetylcholine Modulates Synaptic Plasticity in the Hippocampus *in vivo*

By
Shaomin Li, MD, MSc.

Summary

Long-term potentiation (LTP) provides a model for the synaptic mechanisms underlying learning and memory. Acetylcholine (ACh) is an important excitatory transmitter in the hippocampus, and cholinergic activity is essential for learning in a range of behavioral tasks. Thus, the search for the physiological actions of ACh may be of major importance for understanding the role of ACh in cognitive functions.

The role of ACh in synaptic plasticity was assessed in anaesthetised and freely behaving adult rats. Electrodes were implanted into the CA1 area of the hippocampus, and field excitatory postsynaptic potential (EPSP) slope was measured. Drugs were injected via a cannula into the lateral cerebral ventricle. The effects of M_2 muscarinic ACh receptor (mAChR) antagonists on glutamatergic synaptic transmission and possible mechanisms were investigated.

Among mAChR antagonists, the M_2 receptor subtype selective antagonist methoctramine (12.5 μg), but not M_1, M_3 or non-selective antagonist, significantly increased synaptic transmissions. Blockade of M_2 receptors induced a fast onset, long-lasting potentiation (methoctramine-'LTP'). The magnitude of the methoctramine enhancement of the EPSP was dose-dependent. The induction, but not the maintenance, of the methoctramine-'LTP' was blocked by scopolamine, a non-selective mAChR antagonist. Further study showed that an M_1 receptor antagonist, telenzepine (12.5 μg) partially blocked and an M_3 receptor antagonist, 4-DAMP (6.25 μg) fully blocked this induction. The nicotinic receptor antagonist, mecamylamine (2 mg/kg. i.p.), failed to block the induction of methoctramine-'LTP'.

Like tetanus-induced LTP, this long-lasting enhancement of the field EPSP was associated with a reduction in paired-pulse facilitation and required nitric oxide synthase (NOS) activation, because pre-treatment of TRIM (75 mg/kg, i.p.), a NOS inhibitor, completely blocked methoctramine induced 'LTP'. Unlike tetanic LTP, the methoctramine-'LTP' was only partially blocked by the NMDA receptor antagonist

AP-5 (10 μg), and blocked completely by a PKC inhibitor, Bisindolylmaleimide (40 μg), and a PKA inhibitor, Rp-cAMPs (90 μg). Methoctramine did not occlude tetanus-induced LTP, while tetanus-induced LTP occluded later methoctramine-'LTP'. However, subthreshold methoctramine (1.56 μg) failed to evoke facilitation and standard methoctramine (12.5 μg) have no effect on the ability of a weak tetanus protocol (WTS) to induce LTP.

Since spatial memory is dependent on hippocampal function, the present study also examined the effect of exposure of freely behaving rats to a novel, non-stressful, environment on the ability to induce LTP in the CA1 area. A weak tetanus stimulation (WTS) didn't induce LTP of the field EPSP in the familiar recording box. When the same weak tetanus was applied 5 min after exploration of a novel recording box (for 5 min), significant LTP was induced. In contrast WTS applied 5 min before or 30 min after novelty exposure failed to induce LTP. Further studies showed that whereas intracerebroventricular pre-treatment with an mAChR (scopolamine, 5μg) or beta-adrenoceptor (propranolol, 2μg) antagonist failed to block the facilitation, an antagonist of dopamine receptors (SCH-23390, 15μg) prevented the effect.

From the results obtained it can be concluded that blockade of M_2 mAChR subtype induced an LTP-like persistent enhancement of transmission in the hippocampal CA1 region *in vivo*. This enhancement required the activation of non-M_2 receptors, and involved a presynaptic/retrograde messenger mechanism and PKC/PKA activation. ACh appeared to have little or no role in standard tetanus-induced LTP in the CA1 area. Brief novelty exploration lowered the threshold for the induction of LTP via a mechanism dependent on activation of dopaminergic transmission.

Abbreviations

ACh	acetylcholine
AChE	acetylcholinesterase
AD	Alzheimer's disease
AF-DX 116	11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one
AMPA	α–amino-3-hydroxy-5-methyl-4-isoazole propionic acid
AMPAR	AMPA receptor
D-AP-5	2-Amino-5-phosphonopentanoic acid
BDNF	brain-derived neurotrophic factor
BIM	Bisindolylmaleimide I hydrochloride
CA1	cornus ammonis area 1
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	adenosine 3',5'-cyclic monophosphate
ChAT	choline acetyltransferase
CNS	central nervous system
CPP	3-((R,S)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid
CREB	cAMP response element-binding protein
4-DAMP	4-Diphenylacetoxy-N-methylpiperidine methiodide
DMSO	dimethylsulphoxide
EEG	electroencephalogram
EPSP	excitatory postsynaptic potentials
ERK	extracellular signal-regulated kinase
GABA	γ-aminobutyric acid
cGMP	cyclic Guanosine monophosphate
H-7	(±)-1-(5-isoquinolinylsulfonyl)-2-methylpiperazine dihydrochloride
HFS	high frequency stimulation
IP_3	inositol 1,4,5-trisphosphate
ISI	interstimulus interval

LFS	low frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
LTPm	muscarinic long-term potentiation
mAChR	muscarinic acetylcholine receptors
MAPK	mitogen-activated protein kinase
mGluRs	metabotropic glutamate receptors
MSDB	medial septum and diagonal band of Broca
nAChR	nicotinic acetylcholine receptors
NE	norepinephrine
NMDA	N-methyl-D-aspartate
NMDAR	NMDA receptor
NO	nitric oxide
NOS	nitric oxide synthase
PIP_2	phosphatidylinositol 4,5-bisphosphate
PKA	cyclic AMP-dependent protein kinase
PKC	calcium-dependent protein kinase
PKG	cyclic GMP-dependent protein kinase
PP-1	protein phosphatase 1
PPF	paired-pulse facilitation
Rp-cAMPs	Rp-Adenosine 3',5'-cyclic monophosphothioate Triethylamine
SCH-23390	(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride
SKF-38393	(±)-1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrobromide
STP	short-term potentiation
TRIM	1-(2-trifluoromethylphenyl) imidazole
VDCCs	voltage-dependent calcium channels
WTS	weak tetanus stimulation
YM796	(-)-(S)-2, 8-dimethyl-3-methylene-1-oxa-8-azaspiro [4,5] decane L-tartrate monohydrate.

I. Introduction

It has been proposed that memory formation involves activity-dependent synaptic plasticity, such as long-term potentiation (LTP) of excitatory synaptic transmission (Bliss & Collingride 1993; Malenka & Nicoll 1999; McGaugh 2000). The most efficient stimulation patterns for induction of LTP are reminiscent of the natural firing of presynaptic neurons during learning (Larson & Lynch 1986; Dobrunz & Stevens 1999). Acetylcholine (ACh) is an important excitatory transmitter in the hippocampus, and cholinergic activity is essential for learning in a range of behavioral tasks (Hasselmo 1995). In addition, cholinergic deficits have been found in aged, demented patients, and cholinergic drugs have been used to modulate memory in rats (Bartus et al., 1982; Coyle et al.,1983; Molchan et al., 1992). Thus, the search for the physiological actions of ACh may be of major importance for understanding the role of ACh in cognitive functions (Bartus et al., 1982; Markram & Segal 1992).

1.1 Anatomy of hippocampus

The hippocampal formation is a useful preparation with which to study general neuroscience problems because it demonstrates a number of organizational principles. There are three synaptic paths (trisynaptic pathway) that undergo analytical investigations because of the simplicity of their monosynaptic connections: (1) the fibers from the entorhinal cortex via the perforant path make synapses on to the apical dendrites of granule cells of the dentate area; (2) the axons of granule cells make synapses on the apical dendrites of CA3 pyramidal cells; (3) the Schaffer axon collaterals of the CA3 cells make synapses on the apical dendrites of CA1 pyramidal cells. The CA1 pyramidal cells are the major output cells for the hippocampus. The Schaffer collaterals are probably the best-studied synaptic pathway in the hippocampus. Each Schaffer collateral axon synapses onto thousands of CA1 pyramidal neurons, but usually with only one or two synaptic contacts per neuron (Sorra & Harris 1993). The Schaffer collateral pathway has been studied extensively

because of interest in the various forms of synaptic plasticity occurring at this synapse.

1.2 Synaptic plasticity

Most of the excitatory synapses in the hippocampus exhibit various forms of use- or activity-dependent synaptic plasticity. A variety of data suggest that multiple forms of plasticity exist in the mammalian brain. These include short-term plasticity and long-term plasticity, the latter refers to the use dependent increases or decreases in synaptic strength and are known as long-term potentiation (LTP) or long-term depression (LTD), respectively.

1.2.1 Short-term plasticity

The strength of a synapse can vary with frequency of activation. This variation known as short-term plasticity, can be an increase or decrease which lasts just tens of milliseconds or as long as several minutes. Short-term synaptic plasticity has been a subject of intense study for several decades (Zucker 1999), and serves in filtering functions in temporal information processing (Lisman 1997; Fortune & Rose 2000). The most common form of short-term plasticity is paired-pulse facilitation (PPF) which is believed to provide a sensitive indicator of presynaptic transmitter release properties. PPF comprise an increase in the second " testing" postsynaptic response elicited shortly after a first " conditioning" response. When two stimuli are delivered and a smaller second response than the first is produced, this phenomenon is known as paired-pulse depression (PPD). Although PPF and PPD can be observed in many preparations under various experimental conditions, it is striking and unexpected to find that such processes co-exist at the same synapse. The dependence of these forms of short-term plasticity on the quantal content of a preceding presynaptic action potential implies that transmitter release at a given synapse will be a function of its recently history. That is, the synapses with a high degree of facilitation will transmit high frequencies of presynaptic discharge most faithfully, whereas synapses displaying depression will transmit low frequencies of presynaptic discharge better (Zucker 1999). Therefore, the synapse which displaying PPF or PPD by changes in release probability will affect the information processing properties of the hippocampus by influencing its filtering characteristics.

PPF is traditionally accounted for by the residual Ca^{2+} hypothesis (Wu & Saggau, 1994; Zucker, 1999), while recent study showed that PPF is thought to arise from the partial saturation of a Ca^{2+} buffer (Rozov et al. 2001) or relates to postsynaptic changes (Rozov & Burnashev 1999). The other forms of short-term increases in synaptic strength, which are due to the accumulation of residual Ca^{2+} in the presynaptic terminal as a result of one or more preceding action potentials, include facilitation, augmentation and posttetanic potentiation. (Fisher et al. 1997).

1.2.2. Long-term potentiation

Bliss and Lomo (1973) first demonstrated that a brief high frequency train of stimulation (HFS) given to any one of the three pathways in the hippocampus produces an increase of the amplitude/slope of the EPSP recorded in a postsynaptic pyramidal neuron. This use-dependent increase of synaptic efficacy was called LTP, which has been found to occur ubiquitously throughout the nervous system (Shors & Matzel 1997). Bliss and Lomo defined LTP as potentiation that lasted longer than 30 min, although they observed LTP for several hours. Later studies showed that LTP recorded in animals with permanent indwelling electrodes lasted from weeks to months (Barnes 1979). LTP can be induced in a number of ways, of which the most common technique is delivering a high frequency tetanus (e.g. 10 burst of 200 Hz for 75 ms with an inter-bursts interval of 10 second *in vivo*, or 100 Hz for 1 second repeated three times with 20 s interval *in vitro*). Beside tetanus-induced LTP, other forms of LTP have also reported, such as tetraethylammonium (TEA) (Hanse & Gustafsson 1994), (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) (Anwyl 1999) or others (Bliss & Collingridge 1993).

The phenomenon of LTP is characterized by three major properties: (1), input-specificity, only the inputs active at the time of tetanization are usually potentiated; (2) cooperativity, there is a certain intensity threshold for the production of LTP; (3) associativity, LTP at a weakly tetanized input is produced by concurrent activation of a weak and strong synaptic input. This is an implicit property of the Hebbian synapse. Although there has been enormous interest in LTP this decade, the exact mechanism

of LTP still remains to be clarified, I will restrict the presentation to LTP of synaptic responses of CA1 pyramidal neurons to Schaffer collateral stimulation.

1.2.2.1. The locus of LTP expression and retrograde signaling

There is general agreement for a postsynaptic locus for the induction of LTP. Whether the cellular change underlying synaptic modification occurs in the pre- or postsynaptic cell has been a long-lasting debate. Several experiments have suggested that postsynaptic experimental manipulations may be sufficient to induce LTP (Liedo et al. 1995; Yang et al. 1999) and numbers of results in the literature have strongly suggested that the expression of LTP is predominantly postsynaptic (Malenka & Nicoll 1999). Recent studies have been shown that AMPA receptors are inserted into or removed from the postsynaptic membrane after induction of LTP or LTD, respectively (Shi et al. 1999; Hayashi et al. 2000).

However, opposite results were shown by similar techniques in other studies which suggested a presynaptic involvement in LTP expression by observation, through quantal analysis, that LTP is associated with increased transmitter release (Kullmann et al. 1996), an increase in the frequency of miniature synaptic currents and an increase in the probability of transmitter release determined by PPF (Schulz et al. 1994; Li et al. 2000). Recently, fluorescent imaging provided direct evidence that tetanic (200 Hz)-LTP is expressed presynaptically (Zakharenko et al. 2001).

The presynaptic and postsynaptic sites involved in the different phases of LTP suggest that the postsynaptic neuron must send one or more retrograde messengers to the presynaptic terminals. The most convincing data has suggested that either nitric oxide (NO), carbon monoxide (CO) (Zhuo et al. 1993, 1998; Williams 1996) or brain-derived neurotrophic factor (BDNF) (Schinder et al., 2000) acts as a retrograde messenger during LTP in the hippocampus. For example, NO was found to facilitate the induction of LTP (Bohme et al. 1991; O’ Dell et al. 1991; Schuman & Madison 1991; Arancio et al. 1996; Haley et al. 1996; Doyle et al. 1996; Zhou; 1993, 1998. But see Bannerman et al. 1994; Cummings et al. 1994). Exogenously applied NO enhances transmitter release in an activity-dependent, NMDAR-independent manner (Zhou et al., 1993), whereas extracellular application of a membrane-impermeable NO scavenger inhibited the induction of LTP (O’ Dell et al., 1991; Schuman &

Madison 1991). Consistent with the role of retrograde messenger, postsynaptic injection of an NO synthase inhibitor blocked induction of LTP (Schuman & Madison 1991). Potentiation induced by NO released from a postsynaptically, but not presynaptically, injected donor is blocked by the scavenger (Arancio et al., 1996).

1.2.2.2 Glutamate receptors and LTP

It is well accepted that LTP induction is triggered by NMDA receptor activation that subsequently can lead to increased AMPA receptor activation in CA1 region (Bliss & Collingridge, 1993; Malenka & Nicoll, 1999). Metabotropic glutamate receptors (mGluRs) may also be involved in CA1 LTP and are capable of facilitating both induction and maintenance (Anwyl 1999).

The fundamental role of NMDARs in the induction of many forms of LTP is well established. The function of NMDARs is regulated by protein phosphorylation through serine/threonine and tyrosine kinases (Smart 1997), including the nonreceptor tyrosine kinase Src (Yu et al. 1997). NMDARs, activated at synapses that are proximal to active sites of depolarization may be depolarized sufficiently to remove Mg^{2+} block and initiate the cascade of events that leads to LTP induction. On the other hand, a non-NMDAR mediated LTP can be induced in area CA1 of the hippocampus in presence of the NMDA antagonist AP-5 by application of repeated strong tetanus (200 Hz) to the Schaffer collaterals (Cavus & Teyler 1996; Morgan & Teyler 1999). This AP-5-resistant LTP is blocked by antagonizing L-type voltage-dependent calcium channels (VDCCs) and is referred to as VDCC-LTP (Morgan & Teyler 1999). VDCC-LTP is blocked by the application of tyrosine kinase inhibitors, whereas NMDA-LTP is blocked by serine-threonine kinase (Cavus & Teyler 1996).

AMPA receptors (AMPARs) mediate the vast majority of fast excitatory responses in the hippocampus. There is now a growing body of evidence supporting the AMPAR as being responsible for the expression of LTP in CA1 region. For example, the phosphorylation of specific AMPAR subunits is believed to regulate channel function and synaptic plasticity (Barria et al. 1997; Lee et al., 2000). The use of biochemical and molecular techniques has identified phosphorylation sites within the GluR1 (PKA-Ser^{845}; PKC/CaMKII-Ser^{831}), GluR2(PKC-$Ser^{863/880}$) and GluR4 (Ser^{842}) subunits (Roche et al., 1996; Carvalho et al., 1999; Derkach et al., 1999; McDonald et al., 2001). The induction of LTP can cause transport of new AMPAR

molecules to activated spines (Shi et al., 1999). The redistribution of AMPARs to, and away from, the integral membrane proteins occurs via vesicular excytosis and endocytosis (Carroll et al., 2001). Therefore, AMPARs undergo a cycle of internalization and reinsertion into the postsynaptic membrane, eventually, increasing the total number of synapses (Luscher et al. 2000). This can lead to structural modification of the postsynaptic membrane and the production of new dendritic spines (Engert and Bonhoeffer 1999; Toni et al., 2001).

1.2.2.3 Intracellular biochemical events and LTP

The induction of LTP is dependent on increased intracellular Ca^{2+}, activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMK-II), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) (Malenka & Nicoll 1999). The consolidation of LTP requires the protein kinase A (PKA), MAPK and cAMP response element-binding protein (CREB) (Sweatt 1999).

Usually LTP depends on Ca^{2+} influx through postsynaptic NMDA-type glutamate receptors and subsequent activation of CaMKII. After CaMKII activation by Ca^{2+}/calmodulin, its can become autophosphorylated on threonine 286/287 causing the enzyme to remain active independent of Ca^{2+} /calmodulin binding and increase its affinity for Ca^{2+}/calmodulin. LTP induction can be blocked by pharmacologically inhibiting CaMKII (Otmakhov et al. 1997; Bortolotto & Collingridge 1998), or genetically removing it (Silva et al. 1992; Giese et al.1998). Other experiments also showed that introducing an active form of CaMKII into the postsynaptic neuron induced a potentiation which prevents subsequent LTP induction (Lledo et al. 1995). Thus, CaMKII is necessary and sufficient for LTP induction.

Activation of PKC is believed to be one of the requisite biochemical steps necessary for LTP, as selective PKC inhibitors can block induction of LTP (Hvalby et al. 1994; Stanton 1995; Chen et al. 1997; Routtenberg 1999) and measuring PKC substrate phosphorylation biochemicallly suggested the involvement of PKC in LTP (Chen et al. 1997; Ramaker et al. 1999). The importance of the protein phosphorylation cascades has also been demonstrated in mutant mice lacking the γ isoform of PKC which showed normal synaptic transmission but greatly diminished late LTP (Abeliovich et al. 1993).

PKA is believed to play a critical role in the expression of long-lasting forms of hippocampal LTP. Late LTP, beginning ~3 hr after induction, is generally accepted to depend on cAMP and protein synthesis (Frey et al. 1993; Huang & Kandel 1994; Winder et al. 1998), probably related to the formation of new synapses (Bolshakov et al. 1997; Ma et al. 1999). However the early phase (Otmakhova et al. 2000) and intermediate phase (Winder et al. 1998) of LTP have also been demonstrated to depend on cAMP. Transgenic mice with genetically reduced hippocampal PKA activity also show impairment of tetra-burst LTP (Woo et al. 2000). In agreement with this theory, the induction of LTP was found to produce a rise in cAMP level (Chetkovich & Sweatt 1993), activation of PKA (Roberson & Sweatt 1996) and phosphorylation of some PKA substrates (Blitzer et al. 1998). Activation of the cAMP cascade leads to secondary activation of MAPK in hippocampal area CA1 (Roberson et al. 1999).

Many of the signaling cascades involved in the induction of NMDAR-dependent (Sweatt 2001) and NMDAR-independent (Kanterewicz, et al. 2000) LTP in area CA1 were demonstrated to converge on and activate p42 MAPK (also referred to as extracellular signal-regulated kinase 2, ERK2) (Roberson et al. 1999). Hippocampal MAPK activation is regulated by a wide variety of neurotransmitter receptors coupled to either PKA or PKC: NMDARs, adrenergic receptors, dopamine receptors, muscarinic ACh receptors and mGluRs (Roberson et al. 1999; Sweatt 2001). MAPK is also known to phosphorylate transcription factors, including CREB and E1K-1 (Impey et al. 1998; Davis et al. 2000), which may contribute to the long-lasting, protein synthesis-dependent stages of long-term memory and synaptic potentiation. Moreover, MAPK may regulate Kv4.2 activation through enhancing the Ca^{2+} influx and thereby facilitate LTP induction (Sweatt 2001). Thus, MAPK signaling pathway, as a critical intermediary, not only regulates short-term synaptic function but may also promote the transcription and translation of new protein required for the late phase of LTP.

1.2.3 Long-term depression

A depressive counterpart to LTP has considerable theoretical significance with respect to memory saturation and possible causes for forgetting. An activity-dependent persistent decrease in slope and amplitude of the evoked response in naï ve pathways is called LTD, which is typically obtained by applying minutes-long trains

of single pulse stimulation at a rate of 1-3 Hz (see Bear & Malenka 1994). The induction of LTD, using the standard 900 stimuli at 1Hz, in the adult hippocampus *in vitro* has proved somewhat problematic. Some reports have demonstrated its induction (Dudek & Bear 1992; Staubli & Ji 1996), whilst others, under normal conditions, have failed to do so (Wagner & Alger 1995; Norris et al. 1996). Thiels et al (1994) has previously demonstrated the induction of LTD using a paired pulse paradigm (25 ms paired pulse interval) in adult rat CA1 *in vivo*. Two forms of LTD have been suggested to rely on different mechanisms and stimulus protocols in CA1 region (Oliet et al. 1997; Kemp et al. 2000). A protocol with 200 paired stimuli (25 ms interstimulus interval, ISI) at 0.5 Hz (Thiels et al. 1994) and 450 paired stimuli with 50 ms ISI in 1 Hz (Kemp & Bashir 1997) induced NMDAR-dependent LTD; while 900 paired stimuli (50 ms ISI) at 1 Hz induced LTD dependent on the activation of AMPA/kainate and mGlu receptors (Kemp & Bashir 1999). LTD can also involve CaMKII (Mayford et al. 1995), PKC (Bolskakov & Siegelbaum 1994; Oliet et al. 1997), PKA (Kameyama et al. 1998) and MAPK (Norman et al. 2000) activation.

1.2.4 LTP, LTD and metaplasticity

There have been numerous studies exploring multiple signaling cascades that underlie synaptic plasticity. The increase in synaptic strength that occurs after LTP induction has been reported to be accompanied by an increase in glutamate receptor phosphorylation (Lee et al. 2000), increase in activity of various protein kinases and a decrease in basal protein phosphatase activity (Sanes & Lichtman 1999; Sweatt 1999; Soderling & Derkach 2000). In contrast, a decrease in synaptic strength, i.e. LTD, is accompanied by a decrease the glutamate receptor phosphorylation (Lee et al. 2000) and an increase in the activity of serine/threonine protein phosphatases (Thiels et al. 1998).

High Ca^{2+} elevations might trigger LTP, whereas more moderate Ca^{2+} elevations might trigger LTD (Cormier et al. 2001) and it has been suggested that a presynaptic CaMKII must be activated to induce LTD, whilst postsynaptic CaMKII stimulation is needed to evoked LTP (Stanton & Gage 1996). Furthermore, the activation of conventional NR2A/B-containing NMDARs may contribute to LTP, whereas NR2C/D-containing NMDARs may contribute to LTD (Hrabetova et al. 2000). LTP has been related to the phosphorylation of Ser^{831}, a CaMKII-dependent

phosphorylation site, on GluR1 subunit of AMPAR while LTD to the dephosphorylation of Ser^{845}, a PKA-dependent phosphorylation site, on the GluR1 subunit (Lee et al. 2000; Soderling & Derkach 2000); A regulation of AMPARs via SNARE-dependent exocytosis or endocytosis has also been shown in LTP or LTD, respectively (Carroll et al. 2001). Active PP-1 and PP2A may contribute to the induction of LTD (Thiels et al.1998) whereas inhibition of PP-1 promotes LTP induction (Blitzer et al. 1998); In the MAPK cascade, p42/p44 MAPK pathway can regulate LTP (Sweatt 2001), while p38 MAPK pathway may mediate the induction of mGluR-dependent LTD at CA3-CA1 synapses (Bolshakov et al. 2000).

The stimulation protocol used to induce LTD can also reverse the potentiated state of LTP, i.e. depotentiation, which has some similarities and disparities with LTD (Wager & Alger 1996; Kemp & Bashir 2001). The induction of LTD or LTP is also sensitive to the state generated by previous patterns of pre- and postsynaptic activity. The activity-dependent modulation of subsequent synaptic plasticity has been termed "metaplasticity" (Abraham & Bear 1996). Autophosphorylation of CaMKII has been implicated in metaplasticity (Mayford et al. 1995). Neuromodulators, including stress hormones, are found to induce metaplastic changes. Stressed animals exhibit impaired LTP and facilitated LTD, which is dependent on glucocorticoid receptor activation (Xu et al. 1997, 1998; Kim & Yoon 1998). Therefore, this theoretical model is useful for actually events, like as afferent containing modulatory neurotransmitters such as norepinephrine, dopamine, serotonin and acetylcholine can regulate the initial induction of synaptic plasticity.

1.3 Electrophysiological studies of learning

1.3.1 Hippocampal activity in relation to natural behaviour

Spontaneous electrical activity of the hippocampus is very similar across mammalian species. In rodents, monkeys and humans, there are two alternating patterns of hippocampal electrical activity: irregular and regular activity (Bland 1986). The regular hippocampal activity is a large amplitude, sinusoidal wave termed theta or rhythmic slow activity that occurs during attentive immobility and exploratory behaviour in the rat, and it is thought to be involved in memory formation (Hargreaves et al.1990; Markowska et al. 1995; Kinney et al. 1999). Thus, the associated 4-12 Hz membrane potential oscillation has been proposed to play a role in

learning and memory (Huerta & Lisman 1993). These oscillations are dependent on interneuronal activity and occur in intracellularly recorded interneurons (Williams & Kauer 1997). Theta rhythm in the behaving rat was inferred to consist of atropine-sensitive and atropine-resistant components (Bland, 1986), which generated by the cholinergic afferents from the medial septum/diagonal band of Broca (Bland 1986) and the serotonergic afferents from the raphe (Nitz & McNaughton 1999), respectively. The other rhythms, like as γ (30 ¨ C100 Hz) and β (10-25 Hz) oscillations were reported play an important role in information processing (Jefferys et al. 1996; Traub et al. 1999).

1.3.2 LTP and mechanisms of memory formation

Storage of information in the brain may rely on activity-dependent synaptic plasticity. LTP satisfies many of the computational requirements of a memory-inducing cellular mechanism, and for this very reason there is now a huge body of literature dealing with the mechanisms of hippocampal LTP (Bliss & Collingride, 1993; Malenka & Nicoll, 1999).

The results of many memory studies parallel those of LTP studies: (1) selective antagonists of AMPA, NMDA or mGlu receptors (Tsien et al. 1996), CaMKII inhibitors (Cammarota et al. 1998), PKC inhibitors (Paratcha et al. 2000), PKA inhibitors (Bernabeu et al. 1997; Duffy et al. 2001) and MAPK inhibitors (Imper et al. 1999) impair memory or learning; (2) The activity of PKC, CaMKII and the phosphorylation of GAP43 and GluR1 increase in the CA1 region shortly after training (Cammarota et al. 1998; Paratcha et al. 2000), whereas the activity of PKA and MAPK increases in two peaks that are concomitant with increases in the concentration of nuclear CREB phosphorylated at Ser^{135} (Bernabeu et al. 1997); (3) Consistent with all of the effects of glutamate receptor antagonists or inhibitors of either CaMKII, PKC or PKA on memory and LTP, transgenic mice that either lack or express altered forms of these receptors or proteins are amnestic for spatial and other tasks (McGaugh 2000).

However, there are also some important differences between memory and LTP-based findings. For examples, the rat with hippocampal Kv1.4 mRNA knockdown had no effect on rat spatial maze memory or exploratory behaviour , but eliminated both early- and late-phase CA1 LTP (Meiri et al. 1998). In adult GluR-$A^{-/-}$ mice,

despite the lack of LTP at Schaffer collateral-CA1 synapses, spatial learning was normal (Zamanillo et al. 1999). Other examples of a dichotomy between LTP and learning were reported (Montkowski & Holsboer 1997; Okabe et al. 1998).

Two approaches have been used to investigate the relations between LTP and memory processes: (1) LTP saturation, i.e. maximal expression of LTP in the entire population of relevant synapses should produce hippocampus-dependent learning deficits (Moser et al. 1998; Moser & Moser 1999); (2) behavioral LTP, i.e., LTP-like changes are reflected in hippocampal evoked potentials as a result of natural learning (Hargreaves et al. 1990). Both of these approaches are still contraversial.

Based on above studies, several hypotheses about the role of LTP/LTD in learning/memory have been proposed: (1) null hypothesis, LTP/LTD has nothing to do with memory; (2) attentional hypothesis, LTP/LTD plays a role in attentional rather than memory processes (Shors & Matzel 1997); (3) synaptic plasticity and memory hypothesis : "activity-dependent synaptic plasticity is induced at appropriate synapses during memory formation and is both necessary and sufficient for the information storage underlying the type of memory mediated by the brain area in which that plasticity is observed" (Martin et al., 2000). This hypothesis has taken some of newly discovered properties of LTP/LTD, such as metaplasticity (**1.2.4**), naturalistic patterns of stimulation (Holscher et al. 1997) or back-propagating dendritic action potentials (Markram et al. 1997) for the induction of LTP, synaptic gain or temporal redistribution (Markram & Tsodyks 1996; Selig et al. 1999), the input specificity of LTP (Engert & Bonhoeffer 1997), the concept of silent synapses (Petersen et al. 1998) and the synaptic tagging hypothesis (Frey & Morris 1997).

There is extensive literature on the effects of manipulations of the cholinergic system in cognitive processes of hippocampal learning and memory (Steckler et al. 1998; see **1.3.1**). Evidence for cholinergic malfunctioning as a major hallmark of Alzheimer's disease and aging encouraged interest in the cholinergic forebrain system, starting off the still continuing era of cholinergic therapeutics.

One of the most common causes of dementia in elderly people is Alzheimer's disease (AD). It is associated with degeneration of forebrain cholinergic neurons and the result depletion of cholinergic markers in neocortex and hippocampus (Francis et al. 1999). In animal models, lesions of the brain's cholinergic system impair

performance in learning and memory tasks (Blokland 1996; Steckler et al. 1998). For example, a highly selective cholinergic neurotoxin, 192 IgG-saporin, which binds specifically to the low-affinity p75 nerve growth factor receptor that is localized on cholinergic cells predominantly, induced lesions in the forebrain cholinergic neurons and produced cognitive deficits (Waite et al., 1995)

There are numerous pharmacological studies which have evaluated the effects of cholinergic antagonists and cholinomimetics on learning and memory performance. The most widely studied cholinergic drug is the muscarinic antagonist scopolamine (Steckler et al. 1998). The anticholinergic drug disrupts short-term or working memory in humans (Beatty et al. 1986) and experimental animals (Blokand 1996; Bejar et al. 1999). Meanwhile, the cholinesterase inhibitors, like as physostigmine, tacrine, donepezil, heptylphysostigmine and rivastigmine, were able to reverse the scopolamine induced deficit (Braida et al. 1996; Bejar, et al. 1999), indicating that the cognitive deficit is cholinergic in nature. Clinically, the AChE inhibitors, including tacrine, donepezil and rivastigmine, have been used to improve cognitive function in patients with AD (Francis et al. 1999).

1.4 The role of acetylcholine in hippocampal plasticity

Acetylcholine (ACh) was the first compound to be identified as a neurotransmitter for synapses in the CNS. The synthesis of ACh takes place in the axonal terminals and is catalyzed by the cytosolic enzyme choline acetyltransferase (ChAT). In 1949, Koelle and Friedenwald developed the first histochemical method to visualize acetylcholinesterase (AChE) present in cholinergic neurons (van der Zee & Luiten 1999). ChAT appeared to be solely restricted to cholinergic neurons, whereas AChE is not an exclusive cholinergic marker.

The cholinergic innervation of the hippocampus arises solely from the medial septum and diagonal band of Broca (MSDB) (Fibiger 1982). These are heterogenous nuclei which also contain GABAergic neurons that innervate the hippocampus. The neurotransmitter ACh is release from vesicles in presynaptic nerve terminals and influences functional and behavioral states through its actions at metabotropic muscarinic acetylcholine receptors (mAChRs) and/or ionotropic nicotinic acetylcholine receptors (nAChRs). To date five different types of muscarinic

receptors (M_1-M_5) have been identified (Caulfield & Birdsall, 1998), and at least 11 different nAChR subunits (α2-α9 and β2-β4) occur in the brain (Lindstrom 1997). Different receptor subtypes are believed to mediate distinct functions.

1.4.1 The cholinergic system modulates hippocampal plasticity

1.4.1.1 ACh release in the hippocampus

In freely moving animals, sensory stimulation and motor behaviour were found to be accompanied by increased release of ACh in hippocampus (Marrosu et al., 1995). For example, novelty exposure induced higher ACh release in rats (Aloisi et al., 1997). Furthermore, electrical stimulation of septum can evoke release of ACh from the CA1 region (Smith 1974). The hippocampus can also be stimulated chemically by various neurotransmitter agonists and antagonists. For example, glutamate and its agonists are effective in evoking ACh release, that is, application of glutamate analogues (AMPA, quisqualate) by intracelebroventricular (ICV) injection produced a strong increase (200-215%) in ACh release from the hippocampus (Giovannini et al. 1998). NMDA antagonists also produce an increase in ACh release which maybe due to decreased activation of GABAergic interneurons (Giovannini et al. 1994). Similarly, the activation of dopamine (Imperato et al. 1993), serotonin (Consolo et al. 1994) and adenonsine (Cunha et al. 1995) receptors also facilitates *in vivo* ACh release from hippocampus.

Muscarinic ACh receptor antagonists, atropine and scopolamine, administrated systemically caused a significant increase in ACh release from hippocampus (Koppen et al. 1997; Scali et al. 1995; Vannucchi & Pepeu 1995; Vannucchi et al. 1997). More selectively, the muscarinic M_2 receptors antagonists, like as AF-DX 384, AF-DX 116, AQ-RA 741, BIBN-161 and methoctramine, significantly and concentration-dependently facilitate *in vivo* ACh release (Stillman et al. 1993, 1996; Vannucchi et al. 1997; Kitaichi et al.1999), but not *in vitro* (Vannucchi & Pepeu 1995). In freely moving rats, nicotine caused an enhancement of ACh release from the hippocampus and the effect was blocked by nAChR antagonist, mecamylamine (Tani et al 1998). Thus suggested that nicotine evoked ACh release may be attributed to activation of nAChR.

1.4.1.2. ACh induced LTP-like phenomenon

It was reported that pharmacological activation of mAChRs (Ito et al. 1988; Auerbach & Segal 1994, 1996; Kojima & Onodera 1998) or nAChRs (Nishizaki et al. 1999) produces LTP-like increase of synaptic transmission in CA1 region. Following physostigmine administration (0.1 mg/kg, i.v.), the population spike amplitude significantly increased (167%) and remained stable for 4 hours (Ito et al. 1988). This LTP-like potentiation is through a transient, abrupt increase in excitability and the subsequent block of recurrent GABAergic inhibition in the hippocampus. Similarly, another AChE inhibitor, NIK-247, which was demonstrated to ameliorate the impairment of learning (Suzuki et al. 1995), is able to induce a sustained increase in the amplitude of population spikes and that lasts at least 2 hrs *in vitro* (Kojima & Onodera 1998). This enhancement was completely blocked sensitively by atropine but not by pirenzepine suggesting that this LTP-like response is mediated by M_2 muscarinic receptors.

Auerbach and Segal (1994, 1996) have shown that low doses (<1 μM) of carbachol induced a slow onset, long-lasting potentiation of synaptic responses in hippocampal slices, which they called muscarinic LTP (LTPm). This effect is mediated by a postsynaptic M_2 receptor, and it shares several properties with the more familiar tetanic LTP. For example, LTPm involves a rise of Ca^{2+} concentration and an activation of both a tyrosine kinase and a serine/threonine kinase. This LTPm is likely to be mediated by a genuine long-term change in postsynaptic reactivity to activation of the AMPAR. However, LTPm depends on cholinergic stimulation for its initiation but not for its maintenance and is independent of activation of NMDARs. Somewhat similarly, nefiractam, a nootropic agent, caused an LTP-like response is via activation of nAChR and PKC pathway, but independent of NMDAR activation (Nishizaki et al. 1999).

1.4.1.3 ACh influences synaptic plasticity in area CA1

In a number of brain regions, pharmacological activation of mAChRs facilitates the induction of LTP (Blitzer et al. 1990; Burgard & Sarvey 1990;Brocher et al. 1992; Boyd et al. 2000) and LTD (Kirkwood et al. 1999). For example, LTP in the CA1 region was reported to be enhanced by muscarinic agonists and/or inhibitors of AChE *in vitro* (Tanaka et al. 1989; Blitzer et al. 1990; Huerta & Lisman 1993; Sokolov &

Kleschevnikov 1995; Shimoshige et al. 1997; Ye et al. 2001) and *in vivo* (Iga et al. 1996). Muscarinic antagonists, such as scopolamine or atropine, blocked the action of the cholinergic agonists, but when applied alone didn' t influence LTP induction in CA1 (Chavez-Noriega et al. 1989; Tanaka et al. 1989; Sokolov & Kleschevniko 1995; Yun et al. 2000). Some different studies showed scopolamine suppressed potentiation of the CA1 population spikes (Hirotsu et al. 1989; Iga et al. 1996; Ye et al.,2001). Others even reported an absence of an ACh effect on LTP induction in CA1 *in vitro* (Chavez-Noriega et al. 1989; Jouvenceau et al. 1996).

Calebresi et al (1998a,b) demonstrated that endogenous ACh induced corticostriatal LTP is NMDAR-dependent via M_1-like muscarinic receptors and PKC activation, which can be enhanced by M_2 muscarinic receptor antagonists. Another group reported that nicotine not only facilitates the induction of LTP which mediated by activation of non-$\alpha7$ nAChRs and inactivation of $\alpha7$ nAChR (Fujii et al., 2000), but also enhances depotentiation and LTD in the hippocampal CA1 region (Fujii & Sumikawa 2001). These results suggest that activation of nicotinic, as well as muscarinic receptors, may represent the cellular mechanisms underlying ACh-induced enhancement of learning and memory.

1.4.2 ACh has similar effects to LTP mechanisms

1.4.2.1 Ca^{2+} influx and glutamate release

One important function of AChRs localized on or near presynaptic terminals is the modulation of transmitter release (Caulfield 1993). Muscarinic AChRs show dual actions in CNS, that is they can either increase or decrease transmitter release (Baba et al. 1998), depending on the mAChRs subtypes and the type of G-protein to which the muscarinic receptor is linked (Brown et al. 1997). The mAChRs in the hippocampus can regulate neuronal excitability by modulating ionic conductances (Cole & Nicoll 1983) and neurotransmitter release (Hounsgaard 1978). The mAChRs can either directly or indirectly modulate K^+, Cl^-, Na^+ and VDCCs (Caulfield 1993; Brown et al. 1997). Muscarinic receptors-mediate enhanced Ca^{2+} influx through VDCCs has been reported in entorhinal cortex (Klink & Alonso 1997) or enhanced synaptic Ca^{2+} signals by direct augmentation of NMDAR-mediated currents in CA1 (Egorov et al. 1999). An increase in cytoplasmic Ca^{2+} concentration by release of intracellular Ca^{2+} stores or Ca^{2+} influx through VDCCs will trigger neurotransmitter

release (Sheng et al. 1996). In contrast, muscarine-mediated inhibition does not involve changes in Ca^{2+} influx (Scanziani et al.1995).

The types of muscarinic receptors involved in inhibition or enhancement of release are not fully resolved. For example, several authors have suggested that activation of the M_2 receptor is responsible for the inhibitory effect of muscarine (Stillman et al., 1993, 1996; Vannucchi et al., 1997; Kitaichi et al.,1999), while others have suggested the M_4 receptor (Hasuo et al. 1995; Vannucchi & Pepeu 1995) is involved. Hsu et al (1995) have suggested that the M_3 receptor is responsible for the inhibitory effect. Concerning the enhancement of ACh release, studies have shown that the M_1 receptor is involved (Jones 1996).

1.4.2.2 The activation of AChRs trigger intracellular cascades

mAChRs-induced raised intracellular PKA and PKC levels and increased dendritic action-potential amplitude (Hoffman & Johnston, 1999), may be a crucial in the induction of synaptic plasticity. It has been reported that three subtypes of mAChRs (M_1, M_3, M_5) stimulate phosphatidylinositol 4,5-bisphosphate (PIP_2) breakdown while the others (M_2, M_4) inhibit adenylyl cyclase (Sandmann et al. 1991). The classic signaling cascade initiated by activation of PIP_2-coupled receptors entails hydrolysis of PIP_2 to form diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) with subsequent activation of PKC. PIP_2-coupled mAChRs induce tyrosine phosorylation of a number of proteins including PKCδ, phospholipase Cγ, the delayed rectifier potassium channel Kv1.2, and the G protein Gαq/11 subunits (Huang et al. 1993; Soltoff & Toker 1995; Umemori et al. 1997). The activation of mAChRs can activate tyrosine kinases in hippocampal slices (Stratton et al.1989; Siciliano et al. 1994).

It is now apparent that mAChRs also activate other kinases, including MAPK (Wan et al. 1996; Rosenblum et al. 2000). Muscarinic receptors were able to stimulate K^+ selective channel in neurons from dentate gyrus, which involves pertussis toxin-insensitive G proteins and IP_3-stimulated release of intracellular (Nabekura et al., 1993). Muscarinic transmission also facilitates intradendritic accumulation of free Ca^{2+} from activation of small-conductance inhibitory K^+ channel mediating accommodation of firing and a slow afterhyperpolarization following repetitive discharge (Kohler et al., 1996).

1.4.3 Different mAChRs subtypes mediate different functions in brain

1.4.3.1 M_1 muscarinic receptor subtype

The M_1 receptor is by far the most abundant in the hippocampal formation, and this receptor is especially enriched in hippocampal area CA1 (Levey et al., 1995). The M_1 subtype is found commonly at postsynaptic sites on pyramidal and granule cell somata and dendrites (Levey et al., 1995; Rouse et al., 1997,1999), but is also localized presynaptically (Vannucchi & Pepeu, 1995). Interestingly, the M_1 and the NR1 subunit of the NMDAR are colocalized in CA1 pyramidal neurons (Rouse et al., 1999), indicating an appropriate spatial relationship for the M_1 subtype to modulate the activity of the NMDA receptor. In agreement with this finding, an M_1 mediated muscarinic enhancement of NMDA currents has been reported in CA1 pyramidal neurons in hippocampal slices (Markram & Segal 1992; Marino et al. 1998).

Muscarine and the M_1 receptor agonist McN-A-343, but not the M_2 receptor agonist oxotremorine, cause a dose-dependent increase in the membrane depolarizations/inward currents produced in striatal spiny neurons by brief applications of NMDA but not of AMPA. The selective facilitatory effect of M_1 receptor activation on NMDA currents is mimicked by nesotigmine, an AChE inhibitor, and the facilitatory effects are fully antagonized by scopolamine or prienzepine, but not by methoctramine (Calabresi et al. 1998a,b).

Behavioural studies showed that M_1 agonists (YM796, AF102B) significantly reduced the impairment of learning in drug (cycloheximide and anticholinergic drugs) treated and aged mice (Suzuki et al. 1995; Iga et al. 1996) and M_1 agonists are more selective for anti-amnestic effects than AChE inhibitors (Wanibuchi et al., 1994). On the other hand, the administration of M_1 receptor antagonist, pirenzepine, impaired accuracy of memory performance (Andrews et al., 1994; Aura et al., 1997), but not telenzepine (Herremans et al., 1996).

1.4.3.2 M_2 muscarinic receptor subtype

A high concentration of M_2 receptors is found on somata of stratum oriens and alveus interneurons (Rouse et al. 1997). Anatomical studies have localized M_2

receptors to the dendrites of a sub-population of GABAergic neurons in the stratum oriens/stratum radiatum (Levey et al. 1995; Hajos et al. 1998). Therefore, the M_2 subtype may indeed function not only as a cholinergic autoreceptor in the hippocampus (Rouse et al. 1999, 2000), but also as a presynaptic heteroreceptor to regulate GABA release (Hajos et al. 1998). However, the M_2 receptor was also found postsynaptically in cells of the basal forebrain and interneurones in the hippocampus (Rouse et al. 1997).

Auerbach and Segal (1996) described an LTP form dependent on activation of M_2 postsynaptic receptors in hippocampus, this may be the M_2 receptors which reside on the pyramidal neurons (Rouse et al. 1997).

M_2 muscarinic receptor antagonists have been proposed to improve mnemonic processess and cognitive performance (Packard et al. 1990; Pike & Hamm 1995; Quirion et al.1995; Aura et al. 1997; Vannucchi et al. 1997). For example, the M_2 antagonist, methoctramine, resulted in an invert U-shaped dose response curve in the stimulation of spatial short-term memory performance, a test that is known to be sensitive to cholinergic activity (Dunnett et al. 1985; Aura et al. 1997), although some reported a lack of effect of M_2 muscarinic antagonists after ICV infusion in delayed matching to position (DMTP) tasks (Andrews et al. 1994; Herremans et al. 1996).

1.4.3.3 M_3 muscarinic receptor subtype

Much less is known about the precise localization and functions of the other mAChR subtypes (M_3-M_5) in the hippocampus. The M_3 receptors are present in various neuronal populations throughout the brain in a low level (Levey 1996), while abundant in CA1 region and contribute to release of Ca^{2+} from intracellular pool (Wakamori et al. 1993). The M_3 receptor subtype excites septohippocampal neurons (Liu et al., 1998), the excitatory neurons are GABA-type, not cholinergic neurons (Guo & Chiappinelli 2000; Wu et al., 2000).

Based on above available literature, selective blockade of the M_2 receptor may provide a more physiological approach to cholinergic enhancement than AChE inhibitors or postsynaptic mAChR stimulation as blockade of the M_2 autoreceptor serves to amplify normal cholinergic release. In contrast, AChE inhibitors while

increasing synaptic ACh concentrations, also enhance ACh stimulation at presynaptic M_2 autoreceptor. In addition, muscarinic agonists targeted at the postsynaptic M_1 site may only increase the neuronal signal-to-noise ratio while diminishing activity-dependent neuronal stimulation which may be important to learning and memory processes (Sarter et al. 1990). Although M_2 muscarinic receptor antagonists can improve mnemonic processess and cognitive performance (Packard et al, 1990; Quirion et al, 1995; Aura et al, 1997 and Vannucchi et al, 1997), little is known regarding the neurophysiological effects of M_2 muscarinic receptor antagonists in the CA1 area. In addition, exposure to novelty significantly increases ACh release in freely moving rats (Acquas et al., 1996; Aloisi et al., 1997), and it has been reported that exploration of an novel, non-stressful, environment did not affect the induction of LTP by a strong tetanus whereas pre-established LTP was reversed (Xu et al., 1998). Whether novelty can affect induction of LTP by a weak tetanus via ACh mechanism is not known. Therefore, the present study focussed on the effects of M_2 antagonists on glutamatergic synaptic transmission in the CA1 region in anaesthetised rats and the possible mechanisms involved. A study on freely behaving rats examined the effect of novelty on induction of LTP by a weak tetanus and the possible involvement of acetylcholine and other transmitters.

II. Materials and methods

2.1 Animals

All animals used in these studies were adult male Wistar rats (inbred strain, BioResources Unit, Trinity College Dublin), weighing 250-350 g which corresponds to an age of 2-4 months. Animals were group housed 7 or less in cages and were maintained in an air-conditioned and temperature controlled room (21±2 °C) on a 12-h light/dark cycle. Water and food (standard rodent chow) were freely available except during the testing periods. All rats were weighed before use to determine the dose of anaesthetic required to induce unconsciousness.

For the acute experiment, the body temperature of the rat was assessed by measuring rectal temperature using an anal thermometer (Ama-digit, range: -40 —120°C, Germany). With the use of an externally controlled heating blanket, the temperature was maintained at 36.5±1 °C for the duration of the experiment.

For chronic studies, animals were housed individually after surgery. This cage was modified by placing clear Plexiglas over the top, sides and front to prevent the rat catching the dental cement cap in the bars. All rats were allowed 8-10 days recovery after surgery before the electrophysiological experiments in freely moving animals. The rats were transported from their home cage to the laboratory at a similar time each day (1000-1030) and handled in a similar manner to limit possible changes in response to being transferred. Before the experiment in which animals were exposed to novelty environment, the rats were put in a darkly lit recording box (familiar environment) for 3 hrs at same time each day and the socket assembly on the animals' head was connected to a preamplifier by a flexible wire. One week later, there was no evidence of behavioural "freezing" and the rats became very tame and well used to being handled.

All experiments were performed in compliance with the relevant laws and guidelines and the animal care and experimental protocol was licensed by the Department of Health, Ireland.

2.2 Surgery

2.2.1 Surgical preparation

Rats were anaesthetized with urethane (ethyl carbamate 1.5 mg/kg, i.p.) for acute experiments. The animal remained in an immobilised, anaesthetised state for the next 4-5 hours or longer. For chronical implantation of electrodes, the animals were anaesthetized with sodium pentobarbitone (Sagatal, 60 mg/kg, as initial dose, supplemented by 0.3 (1.8 mg) ¨ C0.5 ml (3mg) intraperitoneal injections as necessary). To test for unconciousness, the rat’ s paw was pinched to detemine if there was any muscle response to the pinch. The hair over the scalp was removed with a curved scissors from the neck to the nose and 0.5 ml of Norocaine was injected subcutaneously under the skin to the skull. The scalp was longitudinally incised from a point between the eyes to the back of the skull, and skull was leveled between lambda and bregma. The periosteum was removed by scraping with a scalpel blade revealing the skull plates. Any excess tissue was cut out and the area sterilized with ethanol and dried out with tissue paper before marking the skull.

2.2.2 Electrode implantation

Bipolar stimulating and monopolar recording electrodes were constructed prior to each experiment. These were made by soldering two pieces of Teflon (PTFE) insulated tungsten wire (bare wire diameter 0.050mm, Nominal o.d. of coated wire 0.075mm, Advent Research Materials Ltd, Oxford, UK) to a dual pin socket connector. These were twisted, glued together with super glue (Loctite) and supported at the point where the wires join the socket with dental (acrylic) cement. The electrical continuity of each wire was checked before use to ensure that the electrode was working. The ends of the wires were cut at an angle exposing the tips so that one was marginally below the other. Separate reference and ground electrodes each

consisted of small stainless steel screws (1.5 mm diameter, Bilaney Consultants, Ltd. Germany) to which single pins had been soldered.

A mark was made with a waterproof pen at the intersection of the midline plate and bregma to serve as the reference zero point. The stimulating electrode position was measured at 4.2 mm posterior to bregma and 3.8 mm lateral to the midline. The recording electrode position was measured as 3.4 mm posterior to bregma and 2.5 mm lateral to the midline. The reference electrode was located at 8 mm anterior to bregma and 1 mm lateral on the opposite hemisphere (left) to that used for electrode implantation. The ground electrode was located at 7 mm posterior to bregma and 5 mm lateral (left) to the midline. Coordinates for the electrodes position were obtained by referring to the rat brain atlas of Paxinos and Watson (1998).

Burr holes of 1.5 mm diameter were drilled in the skull for the placement of electrodes. Care was taken not to disturb the underlying dura mater or cortical hemispheres when the drill bit penetrated the skull. The two modified screws were put in place and the dura mater pierced with a sharp sterile syringe needle. The rat was then transferred to the stereotaxic recording apparatus (KOPF instrument). The electrodes were lowered into the CA1 region of the dorsal hippocampus until the desired response was found (Fig.2-1). A drop of super glue was then placed around the electrode and the whole assembly was fixed in place with dental cement.

2.2.3 Intracerebroventricular (i.c.v.) cannulation

In order to deliver drugs which would not cross the blood brain barrier directly to the hippocampal formation through the ventricular space and minimize an aversive stimulus (handled injection) in behaving animals, cannulae were constructed for these purposes.

A stainless steel guide cannula was implanted into the right lateral cerebroventricle using the following coordinates relative to bregma: lateral 0.5 mm, ventral 4.0 mm from the surface of the skull (Paxinos and Watson, 1998). For the medial septum and diagonal band of Broca (MSDB) injection, the guide cannula was lowered into MSDB at the steretaxic coordinates 0.5 mm anterior to bregma in the middle line and

6.5 mm ventral from the skull. Verification of the positioning of the cannula was carried out postmortem by checking the spread of dye (Indian ink) after i.c.v. injection. The cannula assembly was implanted above the right lateral cerebral ventricle under anaesthesia and sealed into place with super glue and acrylic cement before the electrodes were implanted.

For the acute experiment, the cannula was assembled using a stainless steel hypodermic needle (22 gauge, 0.7 mm outer diameter) which was cut to 13 mm in length. The bevelled end of the needle was ground down to a length of 1.5 mm to reduce the angle of the exposed tip. An internal plug was also prepared from 28 gauge (0.36 mm diameter) stainless steel wire. This was kept in the cannula when it was not in use to prevent blockages occurring. For microinjections the plug was removed and stainless steel injection cannula (0.40 mm outer diameter) was lowered through the guide cannula to the site of injection. The injection cannula was connected to a 25μl Hamilton syringe with polythylene tubing. A volume of 2.5 μl per site of vehicle or one of the drug doses was delivered over 2 min. The injector was left in place for an additional 1 min to allow for drug diffusion and avoid reflux, then removed and the plug replaced.

For freely behaving rats, the cannula was 4.5 mm in length (0.8 mm outer diameter) with 3.5 mm head (2.5 mm outer diameter and 1.5 mm inner diameter). The head of the cannula was filled with silicon. The injection cannula (0.4 mm o.d.) had a handle bolt which one end is 9.0 mm long needle which was inserted into the guide cannula and the other end was connected via a polythylene tube (in 40 cm length) to a 20 μl Hamilton microinjector (CR-700-20, Hamilton Co., Reno, Nevada). For injection, the injection cannula was inserted into the guide cannula, then the microinjector was twisted slowly to ensure the drug was delivered over 4 min. The injection cannula was removed with the electrodes after the experiment.

2.2.4 Location of recording and stimulating electrodes

The positions of the electrodes moving through the cortical and hippocampal layers to the dendrites of stratum radiatum of CA1 region were constantly monitored

as they were lowered through the tissue. This was carried out by generating a 0.1 ms duration, 2 ms delay, 2.4 V pulse through the stimulating electrode at a frequency of 0.1 Hz. Evoked responses were recorded by the monopolar electrode and were displayed on a computer screen. Because of the hippocampal laminar structures, a local depolarisation such as occurs during an excitatory postsynaptic potential (EPSP) can act as an electron "sink" along a vertical superficial-deep axis. A phase reversal was encountered when this dipole was crossed, indicating that this was the area generating the electromotive force and the response recorded was not from a distal site by voltage conduction. From this method it was possible to determine which layer the electrodes were in by referring to the electrophysiological criteria determined for each region of the hippocampus as defined by Leung (1979).

The bipolar stimulating electrode was lowered to a depth of 1.5 mm from the surface of the dura mater using the coarse manipulator control, and followed to the same depth by the monopolar recording electrode. Occasionally a transient cortical potential was seen. The recording electrode was then lowered in 10-20 μm increments to approximately 2.5 mm below the surface. The stimulating electrode was then lowered by similar increments.

The first potential recorded was a small positive deflecting waveform which peaked at 8-15 ms latency after the stimulus, and increase in amplitude as the distance between the recording and stimulating electrodes decreased which was the evoked response recorded as the stimulating electrode penetrated the alveus (Fig. 2-1-A). The large second potential was the evoked response observed from stimulating the stratum oriens (Fig. 2-1-B). As the stimulating electrode approached the cell body layer, the amplitude of the evoked response decreased and became biphasic (Fig. 2-1-C). At this point the stimulating electrode passed through the cell body layer of stratum radiatum and into the dendritic layer, where the response reversed to a negative deflecting EPSP (Fig. 2-1-D). The stimulating electrode was adjusted downwards in increments of 10 μm to maximize the EPSP amplitude. When this amplitude was at a maximum, the recording electrode was then adjusted to further maximize the response. The recording electrode was averaged lowered approximately 2.5 mm, while the stimulating electrode was in 2.7 mm below the surface.

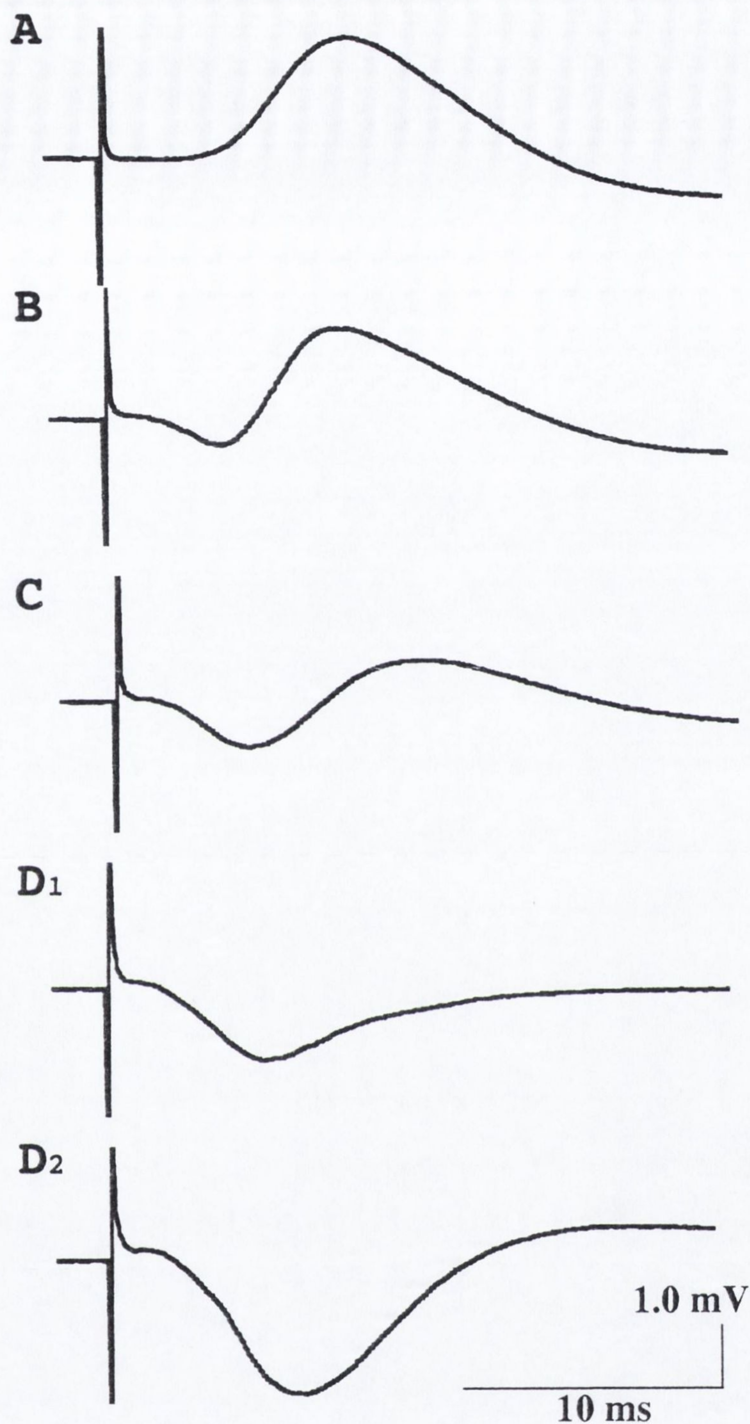


Fig. 2-1. Representative EPSP traces from a single experiment during which the electrodes were lowered into place. Monitoring the characteristic shape indicated position of electrodes in the hippocampus. (**A**). Potential recorded from the alveus; (**B**). Potential recorded from the cell body layer; (**C**). Reversal of potential as electrodes enters the CA1 layer; (**D**). Adjustment of electrode to obtain the maximum EPSP.

2.2.5 Recovery from surgery

Anaesthetised animals were removed from the stereotaxic ear-bars and an EEG sweep taken over 24 seconds. The animals were kept warm by electric blanket to recover over a 2 hrs period. Recovery was normally assessed by observing a rightward shift in the frequency of the hippocampal EEG. The animals for chronically implanted electrodes were housed individually after they had recovered from surgery, and returned to the animal house. Experiments were carried out only on animals they were eating and drinking normally and in which the baseline test EPSPs were stable over three consecutive days. The skin contact to the cap became swollen in some animals 3-4 days after surgery, which normally subsided with time. An antibiotic powder, cicatrin, was sprinkled over this area during the recovery period to minimise infection.

Training was carried out for 8-14 days after surgery. The animals were placed in the dark recording box and connected to the pre-amplifier via flexible leads every day in order to familiarize them. An input/output (I/O) curve was measured every day at the start of recording and used to assess if a there was a shift in the electrode site. A consistent I/O curve over 3-4 days was taken as a benchmark for inclusion in an experiment. The site was lost temporarily in some animals where oedema had taken place after surgery, but it would be recovered when the swelling subsided. The chronically implanted animals were given 30 minutes in the experimental set-up to settle down prior to experiments and a fan was on all the time to provide background noise. The rats were awake but relatively motionless while recording took place. Recordings were taken at same time each day for awake (1100-1600) and anaesthetised (1300-1800) animals.

2.3 Recording apparatus

The electrophysiological recording chamber was surrounded by a Faraday cage to isolate the signal from environmental interference. All instruments in the cage were grounded to a central point to eliminate 50 cycle noise. In both acute and chronic experiments, test EPSPs were evoked by a single square wave pulse of current which

generated by a constant current isolation unit (ISO-Flex) to the bipolar stimulating electrode embedded in the dendrites of stratum radiatum of the CA1 hippocampal region. Chronically implanted rats were connected to a pre-amplifier via flexible leads (40 cm long) to allow the rats free movement in the recording box. For the acute recording, the leads were 10 cm long. This evoked a response which was transmitted via a pre-amplifier (gain 11) with a broad band setting of 4 Hz to 6 kHz, to an analogue-to-digital converter (MacLab/2e, Analog Digital Instruments). This was a digitized system linked to an Apple Macintosh computer (Classic II for acute recording, Performa 6200 for chronic recording) which interfaced with the converter via a specifically written software package (Scope, version 3.5.4). This was customized to control both the generation of the square wave pulses and recording of evoked potentials. The field EPSPs were therefore displayed on-line and could be analyzed at the time of recording or at later date.

Experiments were carried out in a well lit (~750 lux, fluorescent lighting) room. The overall dimensions of the recording box were 55× 24 × 24 cm (length, width, height). The box which made of perspex was further divided into two chambers – the red (27× 24 × 24 cm, for familiar environment) and white (27× 24 × 24 cm, for novel environment) – by an opaque barrier through which rats could traverse. The familiar box was covered with a thin sheet of plastic which acted as a red filter (>600 nm, filter factor ~3 ×), bedding with wood-flakes. The white box as a novelty had no bedding (Novel-1). To minimize the behaviour stress and increase animal exploration, some toys (balls, blocks, some plastic chow, rope and wire) were placed in the white box and yellow paper covered the perspex (Novel-2). Behavioural evidence that the animals acquired information about the new environment was provided by the observation that the animals explored less on re-exposure to the novel box on consecutive days. Entry into the novel box did not elicit any observable stress responses like as behavioural freezing, piloerection or defecation typical of stress.

2.4 Electrophysiological recording

Population field excitatory postsynaptic potentials (EPSPs) were evoked in the stratum radiatum of the CA1 region by stimulating the Schaffer

collaterals/commissural fibers. An input/output curve (stimulus intensity versus EPSP amplitude) was recorded at the start of each experiment to determine the maximum EPSP amplitude (Fig. 2-2). The test EPSP used for baseline and subsequent measurements in the experiment was 50% of this maximum value. In most of experiments, an intensity evoking half maximum response was chosen for paired-pulse stimulation. The conditioning (and test) stimuli were delivered at a rate of 0.033 Hz (square wave constant-current pulses of 0.1 ms duration, 40 ms interstimulus interval). Recordings were taken for at least 1 h to ensure stability of the baseline. The high-frequency stimulation (HFS) protocol for inducing LTP consisted of 10 trains of 20 stimuli: interstimulus interval, 5 ms (200 Hz); intertrain interval, 2 s. The stimulation intensity was raised to given an EPSP of 75% maximum during the HFS. Weak tetanus stimulation (WTS) protocol consisted of 10 trains of 10 stimuli with an interstimulus interval of 10 ms (100 Hz) and an intertrain interval of 2 s, while the intensity was the same as the test stimulus (50% maximum).

2.5 Drugs*

AF-DX 116	{11-[[2-[(Diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one} (Tocris)[♣] dissolved in dimethylsulph oxide and diluted in a saline.
D-AP-5	D-(-)-2-Amino-5-phosphonovaleric acid (Tocris)
L-arginine	(Sigma) [♫]
BIM	Bisindolylmaleimide I hydrochloride (Calbiochem, CN Biosciences (UK) Ltd., Boulevard Industrial Park, Beeston, Nottingham, NG9 2JR UK, www.cnbiosciences.co.uk).
CPP	3-((R,S)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (RBI) [☼]
4-DAMP	4-Diphenylacetoxy-N-methylpiperidine methiodide (RBI)
Dental acrylic liquid	Associated Dental Products Ltd., Purton, Swindon, Wiltshire

	SN5 9HT., UK
Dental acrylic powder	Associated Dental Products Ltd., Purton, Swindon, Wiltshire SN5 9HT., UK
H-7	[(±)-1-(5-isoquinolinylsulfonyl)-2-methylpiperazine] dihydrochloride (Tocris)
Indian ink	Geoge T. Gurr Ltd., London, SW6, UK
Mecamylamine	N,2,3,3-Tetramethylbicyclo(2.2.1)heptan-2-amine Hydrochloride (Sigma)
Methoctramine	N,N' -*bis* [6-[[(2-Methoxyphenyl)methyl]amino]hexyl]-1,8-octane diamine tetrahydrochloride (RBI),
Mibefradil	F.Hffmann-La Roche Ltd., (Pharma. Division, CH-4070 Basel, Switzerland)
Muscarine	DL-muscarine chloride (Sigma)
Norocaine	Lidocaine hydrochloride with adrenaline (Norbrook Laboratories Ltd., Newry BT35 6JP, UK)
Propranolol	1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride (Sigma)
Rp-cAMPs	Rp-Cyclic 3',5'-hydrogen phosphorothioate adenosine Triethylammonium salt (RBI)
Saggital	Pentobarbitone sodium B.P. (Rhone Merieux Ltd., Harlow, Essex CM19 5TS, UK)
Saline	Sodium chloride injection (Antigen Pharmaceuticals Ltd., Roscrea, Ireland)
SCH-23390	(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine Hydrochloride (RBI)
Scopolamine	Scopine tropate Hydrobromide (Sigma)
SKF-38393	(±)-1-Phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol Hydrobromide (Tocris)

Telenzepine	4,9-Dihydro-3-methyl-4-[(4-methyl-1-piperazinyl)acetyl]-10H-thieno[3,4-b][1,5]benzodiazepin-10-one dihydrochloride (RBI)
TRIM	1-(2-trifluoromethylphenyl) imidazole (Lancaster, Eastgate, White Lund, Morecambe, England)
Urethane	Ethyl carbamate (Sigma)
YM 796	((-)-(S)-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4,5]decane L-tartrate monohydrate (Yamanouchi Pharmaceutical Co. Tokyo, Japan).

Notes: *: All the drugs for the experiments were dissolved in distilled water or saline, except for mentioned.

☼: Research Biochemicals International, One Strathmore Road, Natick, MA 01760-2447 USA, (www.sigma-aldrich.com).

♫ : Sigma, 3050 Spruce Street, Saint Louis, Missouri 63103 USA, (www.sigma-aldrich.com)

♣ : Tocris Cookson Ltd. Northpoint Fourth Way, Avonmouth, Bristol, BS11 8TA UK (www.tocris.com).

2.6 Histology

Verification of the electrode and cannula positions was carried out postmortem. Ten μl of Indian ink was injected into the ventricles via the cannula before the animal was scarified. On dissection of the brain, it could be seen clearly that the ink had migrated from the injection site along the ventricular space reaching the hippocampus on both sides of the brain validating this method as a route for drug administration in all animals.

The animals were killed by overdosed with sodium pentobarbitone (for freely moving rats) or neck dislocation (for urethane-anaesthetised rats). In each case the brain was removed from the skull and preserved in a 10% formalin solution for histological examination. Brains were sectioned by hand to check for electrode tracts visible in the CA1 region. Blocks of tissue 3 mm from the cannula or electrode sites were cut and embedded in paraffin wax. A microtome (American Optical Company, Spencer 820 Rotary microtome) was used to prepare 10 or 300 μm thick sections which were then stained with thionin (1% aqueous solution) for 3-4 minutes and mounted in distrene plastiziser xylene. The precise location of electrodes and the

cannula could be seen using a light microscope. The electrode and cannula sites verified before the result of that brain was included in the analysis.

2.7 Data analysis

The amplitude and slope of the field EPSP were measured but it was found that there is a direct linear correlation between the two (Fig.2-2). Slope, rather than peak amplitude, is used to extracellular measure changes in synaptic efficacy because peak amplitude, but not initial slope, can be reduced by a population spike occurring at the field EPSP peak, or altered by EPSP-spike potentiation (Abraham,et al., 1987). Therefore the slope of the response was taken as the main indicator of excitatory synaptic transmission in these off-line analysis.

The initial slope of the EPSP and PPF values were calculated by taking the average of 5-min epochs. Slopes of the initial part of single field potentials were measured within a 0.6-1.0 ms widow of the declining phase (Fig.2-2B). The magnitude of LTP was expressed as the percentage of pre-HFS (or pre-methoctramine administration) baseline EPSP slope±S.E.M. (standard error of means). Paired-pulse facilitation (PPF) was expressed as the percentage of the second EPSP (EPSP2) minus the first (EPSP1) divided by the first.

Statistical significance of the difference between means was estimated using two different statistical measures:

- Analysis of variance (ANOVA) with repeated measures.
- A two-tailed paired/unpaired Student's t-test.

The data were analyzed using JMP IN 3.2.1 statistical software on a Macintosh Performa 5260. Statistical significance was taken at the 95% level (P-values <0.05). All data in individual experiments within a set were grouped and averaged with the means and S.E.M. calculated using Microsoft Excel 6.0.

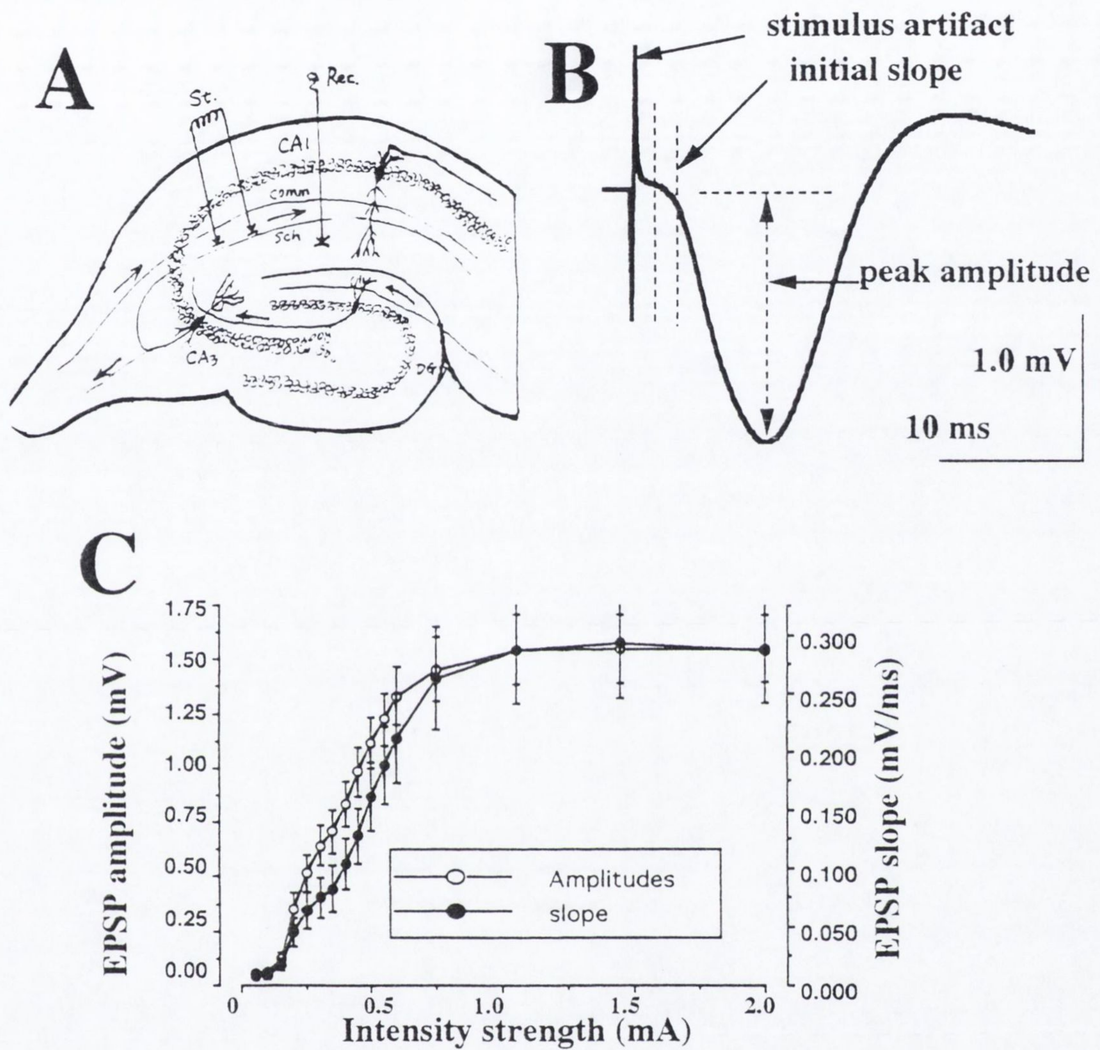


Fig. 2-2. Schematic diagram of coronal hippocampus and the input/output curve of the field EPSP. (**A**). Schematic diagram of coronal section of the rat hippocampus shows the stimulation and recording sites; (**B**). A represent example of the field EPSP recordings from the CA1 used to make the measurements; (**C**). Input/output curve of initial slope and peak amplitude of EPSP in present study (n=14).

III. Results

3.1 Muscarinic agonists elicit biphasic responses

3.1.1 Low dose of muscarine induced a transient increase of the EPSP

The muscarinic agonist, carbachol, has been reported to induce muscarinic LTP (LTPm) in low concentrations (<1 μM) and to depress EPSPs in high doses (>5 μM) in the isolated hippocampal slice (Auerbach and Segal 1994, 1996; Yun, et al, 2000). In order to verify the biphasic effect of ACh, muscarine, a mAChR agonist, was injected into the lateral cerebral ventricle (i.c.v.) in a low concentration (1 μM) in urethane-anaesthetized rats. In contrast with the previous reports, this dose (1 μM) of muscarine did not induce a long lasting potentiation; instead it transiently increased the field EPSPs (117±4.8% of baseline at 30 min, n=5, P<0.05) (Fig.3-1).

3.1.2 High dose of muscarine depressed the EPSP *in vivo*

Subsequently, a relatively high concentration (10 μM) of muscarine was injected i.c.v. Consistent with the previous report *in vitro*, the high dose of muscarine (n=5) depressed the EPSPs: the slope of EPSP was reduced to 73.1±10.3% of baseline at 30 min (P<0.05) (Fig.3-1).

3.1.3 M_1 agonist, YM796, had no effect on the baseline EPSP

As the M_1 subtype receptor activation was reported to reverse cognitive impairment in passive avoidance tasks in rats (Fisher et al. 1991), we monitored baseline synaptic transmission after YM 796, a predominately M_1 receptor agonist (Wanibuchi et al. 1994), was administered by intraperitoneal injection (0.5 mg/kg) in urethane-anaesthetized rats. Neither amplitude or slope of the EPSP showed any significant change (n=4).

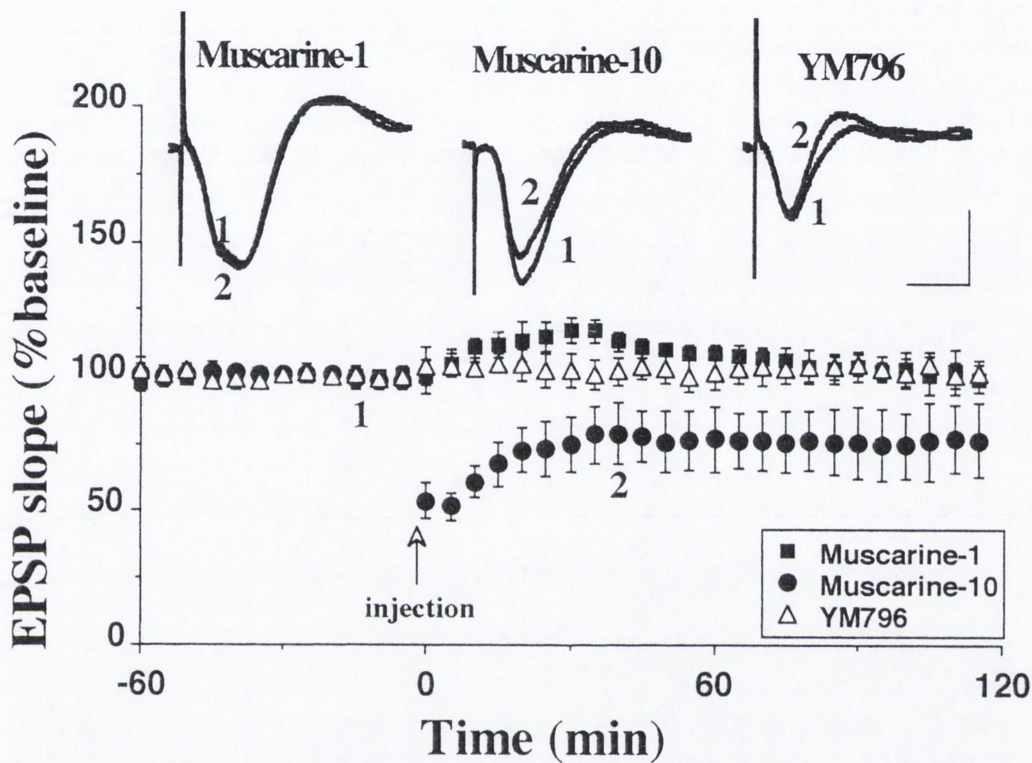


Fig. 3-1. Muscarine elicits biphasic effects on baseline synaptic transmission. A low concentration of muscarine (1μM) increased the slope of the field EPSP transiently (n=5, ■); a high dose of muscarine (10 μM) depressed the field EPSPs (n=5, ●); YM-796 (n=4, Δ) injection did not change the baseline level of synaptic transmission. Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.2 Endogenous acetylcholine enhanced EPSPs

The mAChRs in the hippocampus can regulate neuronal excitability by modulating ionic conductance (Cole & Nicoll 1983) and neurotransmitter release (Hounsgaard 1978). The types of muscarinic receptors involved in inhibition or enhancement of release are not fully resolved. We first examined the effects of different mAChR antagonists on baseline synaptic transmission and short term plasticity by monitoring changes in EPSP slope and PPF in urethane-anaesthetized rats.(Fig. 3-2).

3.2.1 Non-selective antagonist, scopolamine, had no effect on EPSP baseline

Muscarinic ACh receptor antagonists, atropine and scopolamine, administered systemically have been reported to cause a significant increase in ACh release from the hippocampus (Koppen et al. 1997; Scali et al. 1995; Vannucchi & Pepeu 1995; Vannucchi et al. 1997). In the present study, scopolamine (5 μg) was given i.c.v. PPF decreased from 70.9±9.9% to 57.7±8.1% (n=12, $P<0.05$) which is consistent with an increase in neurotransmitter release. However, no significant effect on the EPSP slope was observed (95.3±2.9% to 108.7±4.8%, $P>0.05$).

3.2.2 M_1 antagonist, telenzepine, had no effect on EPSP baseline

The M_1 receptor is by far the most abundant in the hippocampal formation, and this receptor is especially enriched in hippocampal area CA1 (Levey et al., 1995). The M_1 subtype is mainly confined to the postsynaptic site in pyramidal and granule cell somata and dendrites (Levey et al., 1995; Rouse et al., 1997,1999). Consistent with this, the M_1 antagonist, telenzepine (12.5 μg, icv), had no effect on the EPSP or PPF. (Fig.3-2).

3.2.3 M_2 antagonist, methoctramine, enhanced the EPSP (see also 3.3.1 and 3.6.1)

The muscarinic M_2 receptors antagonists, such as AF-DX 384, AF-DX 116, AQ-RA 741, BIBN-161 and methoctramine, have been reported to significantly and concentration-dependently facilitate *in vivo* ACh release (Stillman et al. 1993, 1996; Vannucchi et al. 1997; Kitaichi et al.1999), but not *in vitro* (Vannucchi & Pepeu 1995). In the present study, injection of methoctramine (12.5 μg, icv) increased the EPSP slope (98.9±4.0% to 148.7±9.5%, n=14, P<0.01) and decreased PPF (57.3±12.4% to 42.4±10.8%, n=14, P<0.05).

3.2.4 M_3 antagonist, 4-DAMP, had no effect on the baseline

Much less is known about the precise localization and functions of the other mAChR subtypes (M_3-M_5) in the hippocampus. In the present study, the M_3 antagonist 4-DAMP (6.5 μg, icv) had a similar effect on the EPSP slope and PPF as scopolamine, but, the effect was not significant (n=7, P>0.05).

3.3 Blockade of M_2 receptors induced a long-lasting potentiation

3.3.1 M_2 antagonist, methoctramine, induced an LTP-like phenomenon

Methoctramine, a relatively selective M_2 /M_4 receptor antagonist (Michel and Whiting, 1988; Caulfield, 1993), significantly enhanced field EPSPs (see **3.2.3**). The increase in EPSP slope was fast onset and long lasting similar to tetanus-induced LTP (Fig. 3-3, black circles). This LTP-like change induced by methoctramine (12.5 μg) was stable and lasted at least 3 hours in our recording. The field EPSP slope was 145.7±12.3% and 160.1±12.2% of baseline at 10 and 120 min after i.c.v. injection of methoctramine, respectively (n=8, P<0.05 at 10 min and P<0.01 at 120 min).

3.3.2 M_2 antagonist, AF-DX 116, induced an LTP-like phenomenon

In order to confirm the LTP-like phenomenon was induced by M_2 mAChR antagonism, another M_2 antagonist, AF-DX 116 (Galvan et al., 1989; Caulfield, 1993) was injected i.c.v.(8 μM in 0.1% DMSO). The field EPSP showed a similar change to that seen with methoctramine (Fig. 3-3, white circles, n=5). Vehicle injection i.c.v. had no effect on the EPSP slope in the same pathway (n=6). Thus, these results suggest that blockade of M_2 muscarinic receptor subtype by antagonists can induce a fast onset 'LTP' *in vivo*.

3.3.3 Methoctramine induced enhancement of the EPSP is dose-dependent

Methoctramine had been reported to affect rat behaviour in concentrations of 2-100 μg (2.7-137 nmol) i.c.v. (Aura, et al 1997; Puolivali et al, 1998; Mayorga, et al, 1999) and 250 nmol- 1μmol *in vitro* preparations (Calabresi, et al, 1998; Liu, et al,1998). We first tested baseline changes in synaptic transmission after methoctramine administration at a dose of 12.5 μg (17 nmol)/rat. The EPSP amplitude and slope increased significantly (n=8, $P<0.05$, Fig.3-4). Following this we progressively reduced the concentration by half (6.25 μg, 3.12 μg and 1.56 μg) (n=5, respectively). The increased of the EPSP slope was dose-dependent, the dose of 1.56 μg (2.1 nmol) being inactive.

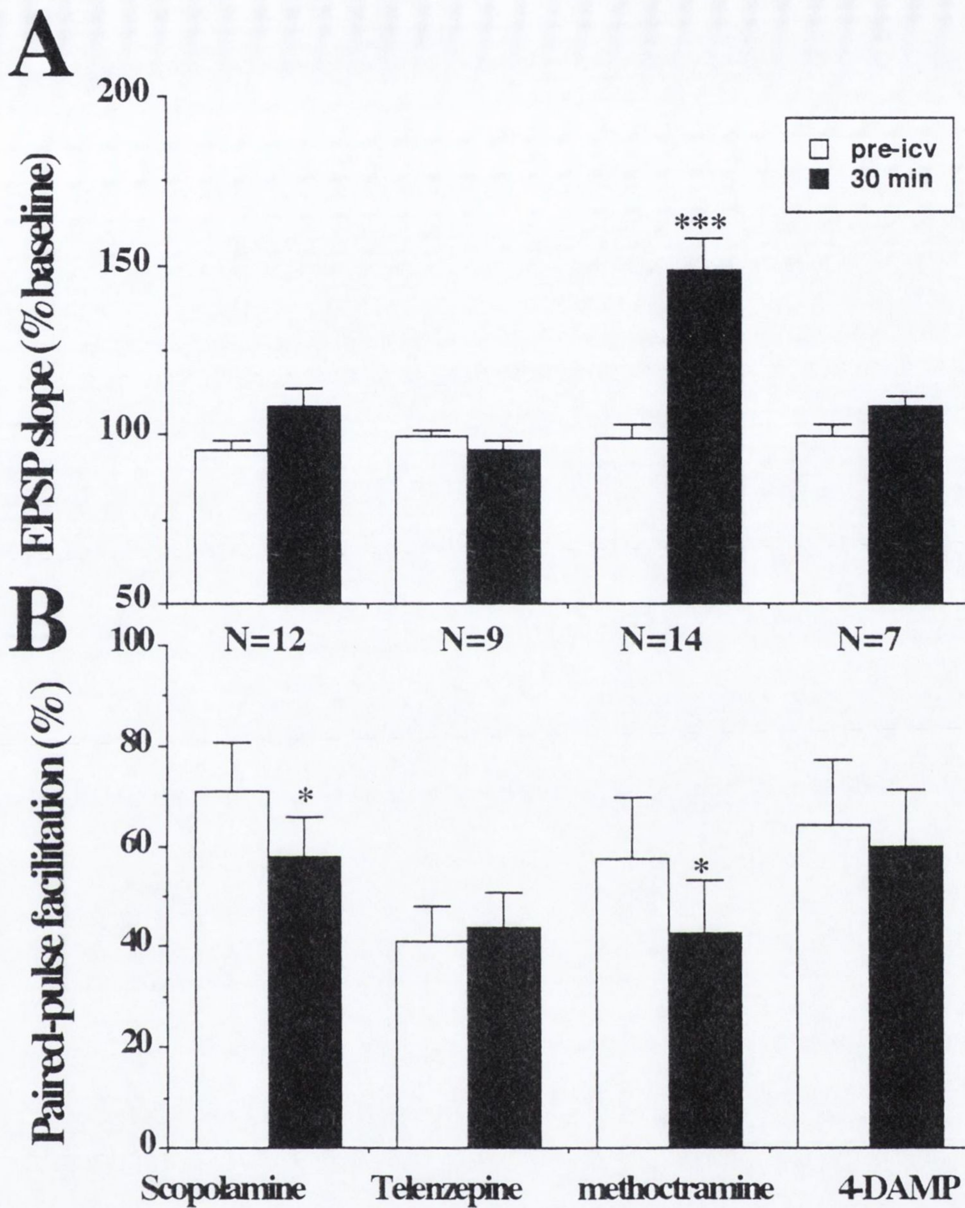


Fig. 3-2. Effects of different muscarinic cholinergic receptor antagonists on baseline synaptic transmission and short-term plasticity. (**A**). Field EPSP change after mAChRs antagonists administrated i.c.v.; (**B**). PPF changes.

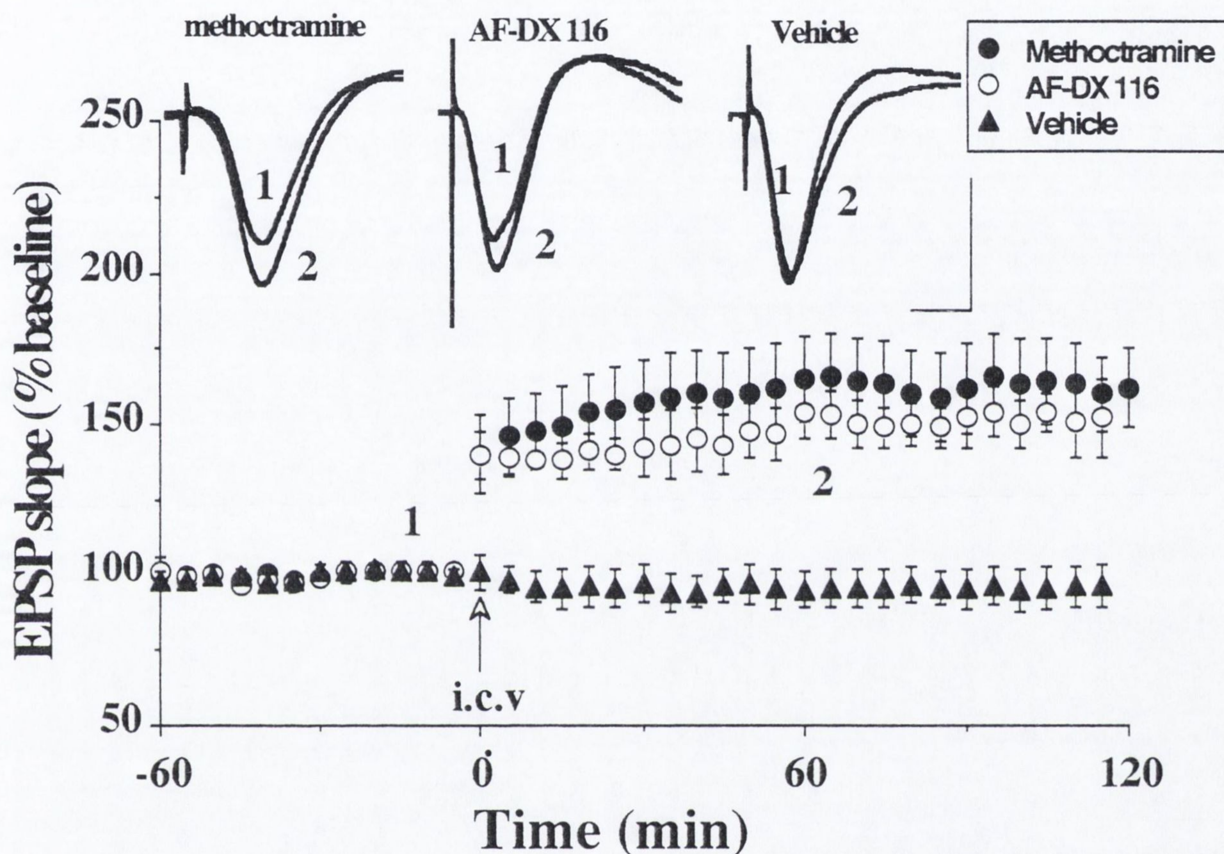


Fig. 3-3. Blockade of M_2 muscarinic receptor induces a fast onset long-lasting potentiation in the hippocampal CA1 region *in vivo*. Methoctramine injection i.c.v. (17 nmol) significantly increased the EPSP slope (n=8) for at least 3 hours ($P<0.05$; ●); AF-DX 116 injection (8 μM) also caused a similar potentiation (n=5, $P<0.05$; ○). Saline vehicle injection i.c.v had no effect on the baseline (n=6, $P>0.05$; ▲). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

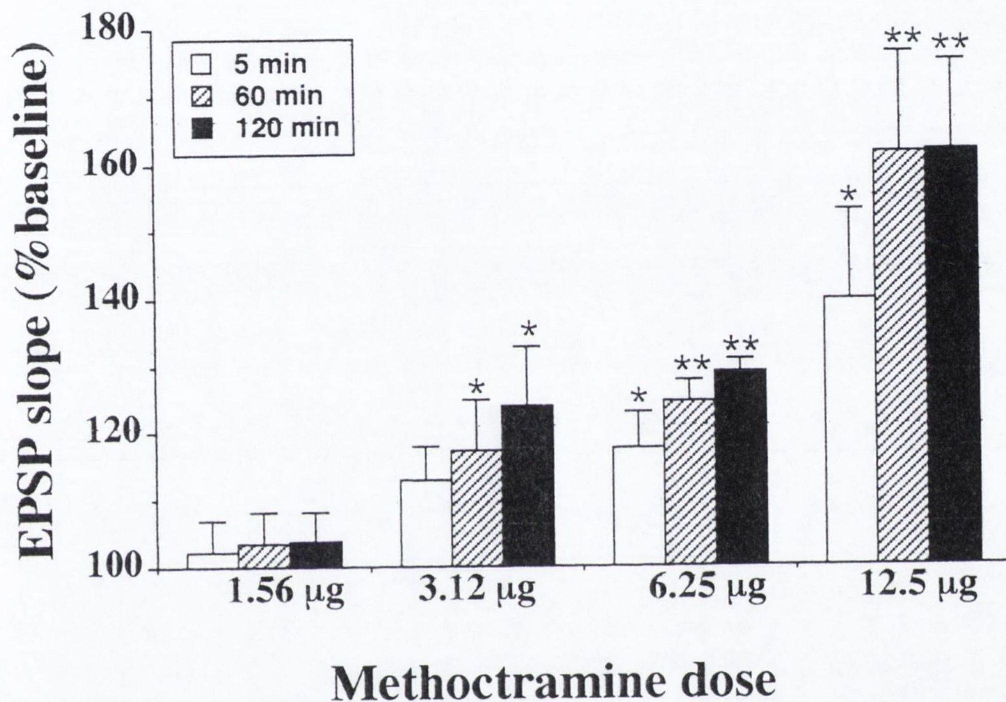


Fig. 3-4. Dose-dependent increase of field EPSP slope by administration of methoctramine. A dose of 3.12 μg (4.2 nmol, i.c.v.) induced a significant enhancement of the EPSP in CA1 region (n=5, P<0.05). A lower dose (2.1 nmol) failed to increase the field EPSP (n=5, P>0.05). Higher doses (8.5 and 17 nmols) caused a further increase (n=5 for 8.5 nmol, n=8 for 17 nmol). *:P<0.05; **:P<0.01.

3.4 Methoctramine-induced 'LTP' is dependent on muscarinic receptors

3.4.1 Scopolamine blocked the induction of methoctramine-induced 'LTP'

Muscarinic M_2 subtype receptor is considered to be the major autoreceptor in the hippocampus (Quirion, et al, 1995; Stillman et al, 1996; Kitaichi, et al, 1999; Rouse, et al, 2000a). M_2 antagonists significantly increase ACh release in hippocampus (Stillman, et al, 1993; 1996). As ACh release in the presence of methoctramine might activate other muscarinic receptor subtypes, a non-selective muscarinic antagonist, scopolamine, was given in combination with methoctramine. Scopolamine was injected 30 min (5μg/rat,13 nmol, i.c.v.) before methoctramine administration. The LTP-like response was blocked (n=7, $P>0.05$, compared with pre-methoctramine injection, Fig.3-5A). Subsequent high frequency stimulation still induced a robust LTP ($P<0.01$), which is consistent with previous reports that cholinergic antagonists have no effect on LTP in CA1 (Tanaka et al.,1989; Sokolov and Kleschevnikov,1995; Yun, et al, 2000). Similarly, when scopolamine was systemically injected (1.0mg/kg, i.p., n=5) 30 min before methoctramine administration, the methoctramine-induced increase in the field EPSPs was completed blocked (104.8±18.8% of baseline at 60 min, n=5, $P>0.05$).

3.4.2 Scopolamine had no effect on the maintenance of methoctramine-'LTP'

Although scopolamine blocked the induction of the methoctramine induced LTP-like phenomenon, we wanted to know whether it affected the maintenance of this LTP. Scopolamine failed to reverse the LTP-like responses when it was injected (13 nmol, i.c.v.) 60 min after methoctramine administration (n=5, Fig.3-5B). These results suggest that the induction of the LTP-like phenomenon by M_2 antagonists was muscarinic dependent, but that maintenance does not need mAChR activation.

3.4.3 Mecamylamine failed to block methoctramine-'LTP'

To verify that the LTP-like phenomenon is muscarinic receptor rather than nicotinic receptor-dependent, mecamylamine, a nicotinic antagonist, was applied 30 min (2mg/kg, i.p.) before methoctramine. A significant potentiation of EPSP slope was still evoked (153.9±8.7% of baseline at 60 min, n=6, $P<0.05$) (Fig. 3-6). In additional experiments, 3 rats were injected with mecamylamine by i.c.v. (10 μg) before methoctramine administration. A robust LTP was still evoked (158.1±11.4% of baseline at 60 min, $P<0.05$).

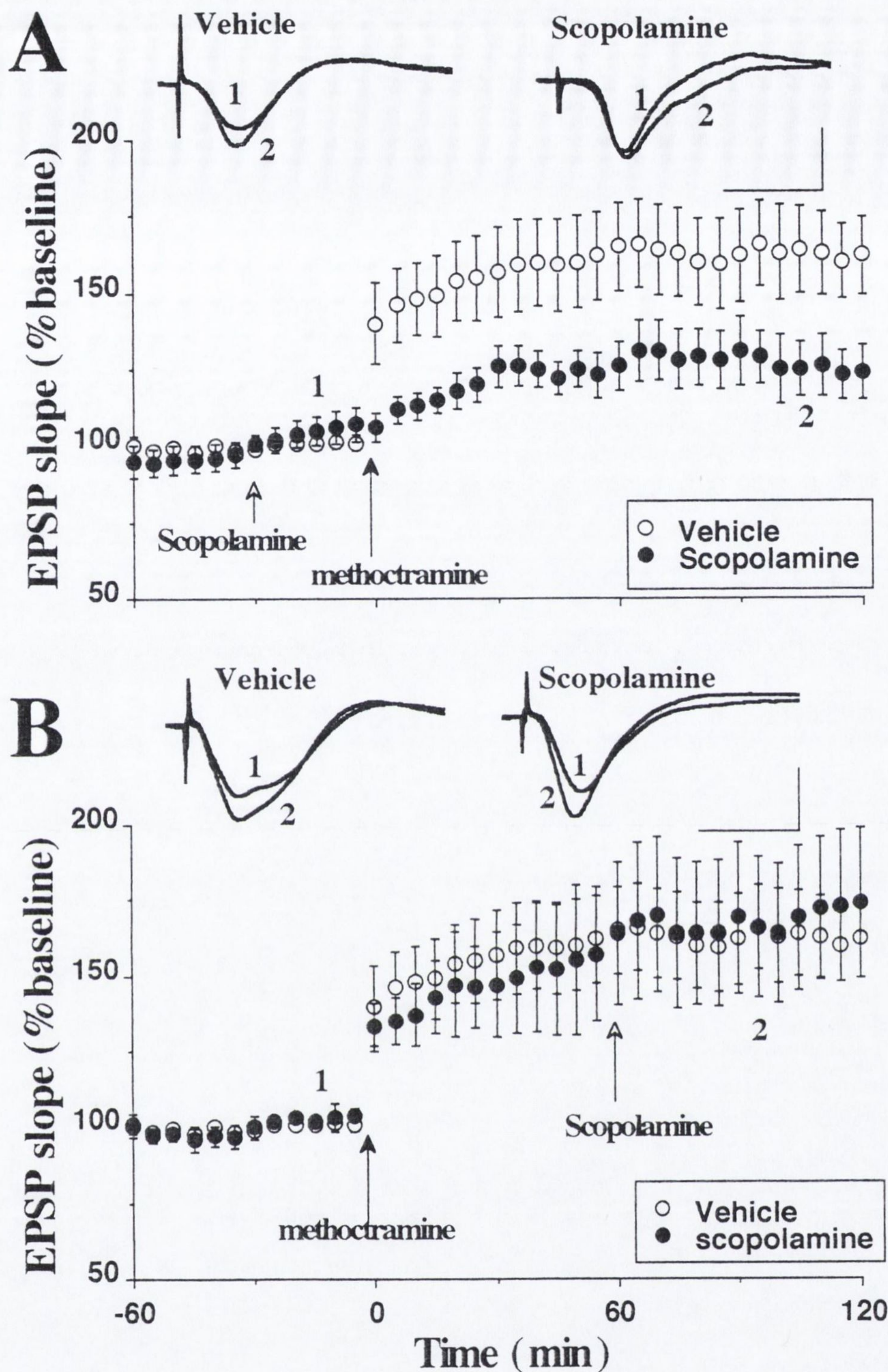


Fig. 3-5. The methoctramine-induced persistent facilitation is muscarinic receptor-dependent. (**A**) Pre-treatment with scopolamine (13 nmol i.c.v.) inhibited the effect of methoctramine (17 nmol i.c.v.) (n=6, P<0.05). (**B**) Injection of scopolamine (13 nmol i.c.v.) after methoctramine (17 nmol i.c.v.) did not affect the persistent facilitation (n=6, P>0.05). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

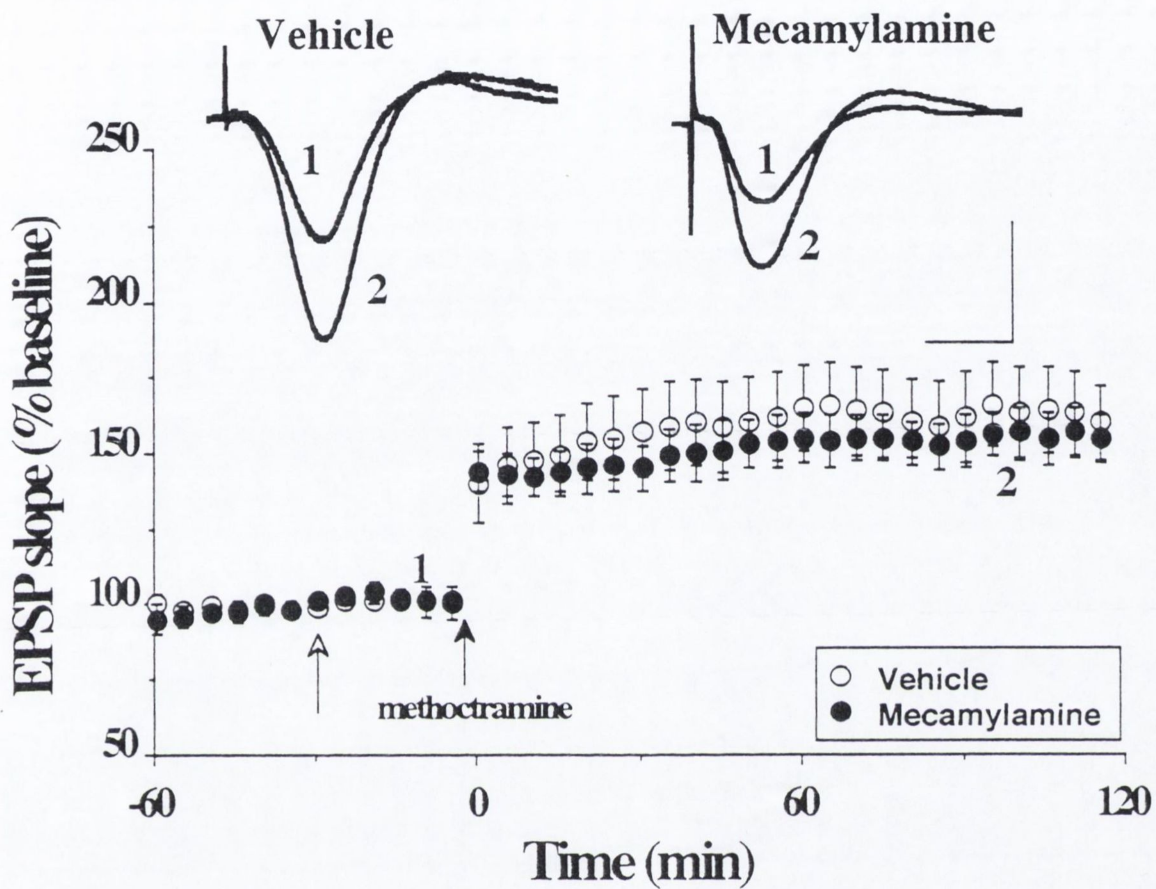


Fig. 3-6. The induction of M_2 receptor antagonist induced-LTP is not nicotinic receptor-dependent. The nicotinic receptor antagonist mecamylamine had no effect on the methoctramine induced LTP-like response (n=6). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.5 Non-M_2 muscarinic receptors are involved in the methoctramine-'LTP'

3.5.1 M_1 antagonist, telenzepine, partially blocked methoctramine-'LTP'

As there are no very selective antagonists for mAChR subtypes, different relatively selective antagonists were used to determine if they could prevent the methoctramine-'LTP'. Telenzepine (12.5 μg/rat, 28 nmol, i.c.v.), a relatively selective M_1 subtype receptor antagonist (Schudt, et al, 1988; Galvan, et al, 1989), did not block the methoctramine-'LTP' completely (113.7±5.3% at 10 min, n=9, $P<0.05$, Fig. 3-7, black circles). However, the magnitude of LTP induced by methoctramine after telenzepine was smaller than that of the methoctramine alone group ($P< 0.05$). The apparent decrease of LTP magnitude may be due to partial block of non-M_1 subtypes, such as M_3/M_5.

3.5.2 M_3 antagonist, 4-DAMP, blocked methoctramine-'LTP'

To test idea that non-M_1 receptors may be responsible, a relatively selective M_3 subtype receptor antagonist, 4-DAMP (Michel, et al, 1989; Caulfield, 1993) was given (6.25 μg/rat,14 nmol, i.c.v.) to the rats before methoctramine administration. Initially the methoctramine-'LTP' was blocked (111.3±4.6% at 10 min, n=7, $P>0.05$, Fig.3-7, white triangles). Later, the magnitude of the EPSP increased somewhat (compared with methoctramine, $P>0.05$, while compared with pre-4DAMP, $P<0.05$). Interestingly, in both groups a subsequent tetanus induced LTP. These results together with the finding that the relatively M_1 selective agonist YM 796 failed to potentiate the EPSP responses (Fig. 3-1), suggest that M_3 and possibly M_5 receptor subtypes mediate the methoctramine-'LTP' in the CA1 area *in vivo*.

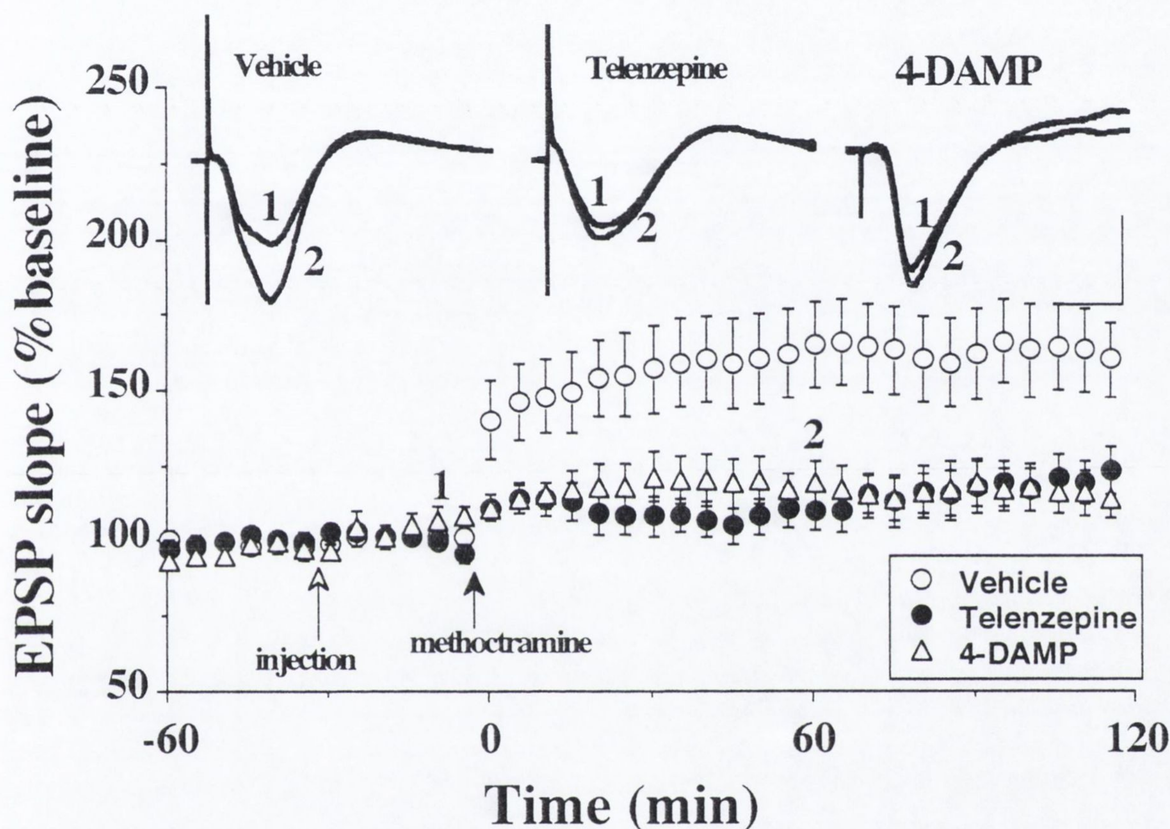


Fig. 3-7. Non-M_2 receptor subtypes mediate the methoctramine-induced 'LTP'. (**A**) Telenzepine, an M_1 receptor-preferring antagonist, partially blocked the methoctramine-induced 'LTP' (n=9, •); (**B**). 4-DAMP, an M_3/M_1 receptor antagonist, also partially blocked the induction of methoctramine-induced 'LTP' (n=7, Δ). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.6 Methoctramine-'LTP' is dependent on nitric oxide synthase

3.6.1 Methoctramine-'LTP' is associated with a reduction of PPF

The LTP-like change induced by methoctramine was stable, lasting at least 3 hours. The field EPSP slope response to the first stimulus pulse was 139.9±13.2%, 161.6±14.6% and 168.2±26.7% at 5, 60 and 180 min after ICV injection of methoctramine, respectively (n=8, P<0.05; Fig.3-3). This was accompanied by an increase in the second pulse response to 130.9±8.1%, 150.8±13.6% and 147.8±15.3% at 5, 60 and 180 min, respectively. As the group of scopolamine effect on the expression of methoctramine-'LTP' is the same before scopolamine injection (see **3.4.2.**; Fig.3-5B), the data of these two groups were pooled. The EPSP slope response to the first stimulus was 157.4±12.2% at 60 min (n=14, Fig.3-8A). Paired-pulse facilitation (PPF) was decreased after methoctramine injection (Fig.3-8B). In the first group injected with 12.5 μg (17 nmol) PPF did not decrease significantly (n=8, P>0.05). The PPF was significantly decreased when we pooled this first group and scopolamine group which also shows a PPF decrease following methoctramine administration (57.3±12.4% reduced to 39.3±9.9% at 60 min, n=14, P<0.05), these results suggest this LTP induced by methoctramine involve a presynaptic mechanism (Li et al, 2000).

3.6.2 NOS inhibitor, TRIM, blocked methoctramine-'LTP'

We tested whether the HFS-induced LTP was dependent on nitric oxide synthesis (NOS) by using the NOS inhibitor, TRIM (Handy et al., 1996). The administration of TRIM (75 mg/kg, i.p.) 30 min before HFS blocked HFS-induced LTP (119.7±6.33% and 104.2±10.1% of baseline at 10 and 120 min after HFS, n=7, P<0.01 and >0.05, respectively; P<0.05 compared with vehicle group of 173.5±18.5% and 168.9±16.2%, n=10, ANOVA) (Fig. 3-9A). The increase in the field EPSP slope lasted 1 hour (111.0±7.1%, P<0.05, compared with baseline; P<0.05, with the vehicle). This

indicates that the NOS inhibitor TRIM blocked tetanus-induced LTP, but spared short-term potentiation (STP).

In order to determine if the M_2 mAChR antagonist induced facilitation shared common mechanisms with LTP, the same dose and protocol of TRIM administration as was used in the tetanus-induced LTP group was applied to the methoctramine induced-'LTP'. Interestingly, TRIM completely blocked methoctramine-'LTP' (102.1±3.6% and 101.7±8.7% of baseline at 10 and 120 min after methoctramine administration, n=5, P>0.05) (Fig. 3-9B).

3.6.3 L-arginine, reversed TRIM block of methoctramine-'LTP'

To verify the possible involvement of NO in tetanus-induced LTP in the CA1 area, a precursor of NO, L-arginine, which is used by NOS for NO production, was given (250 mg/kg, i.p.) one minute before application of TRIM. A potentiation of the field EPSP slope was found indicating that increased substrate for NOS had overcome the inhibitory effect of TRIM on the induction of LTP (133.1±10.5% and 155.6±10.8% of baseline at 10 and 120 min after HFS, n=6, P<0.05 and P<0.01, respectively; P>0.05 compared with vehicle group, ANOVA) (Fig. 3-9A). The reversal of LTP in the presence of L-arginine was significant compared with the group of rats injected solely with TRIM (P<0.05). The inhibitory effect of TRIM on methoctramine-'LTP' was partially reservsal by pretreatment of animals with L-arginine (114.6±4.5% and 121.5±7.6% of baseline at 10 and 120 min, n=7, P<0.05; compared with TRIM group or vehicle group, P<0.05, ANOVA), Therefore, the methoctramine-'LTP' is dependent on NO production.

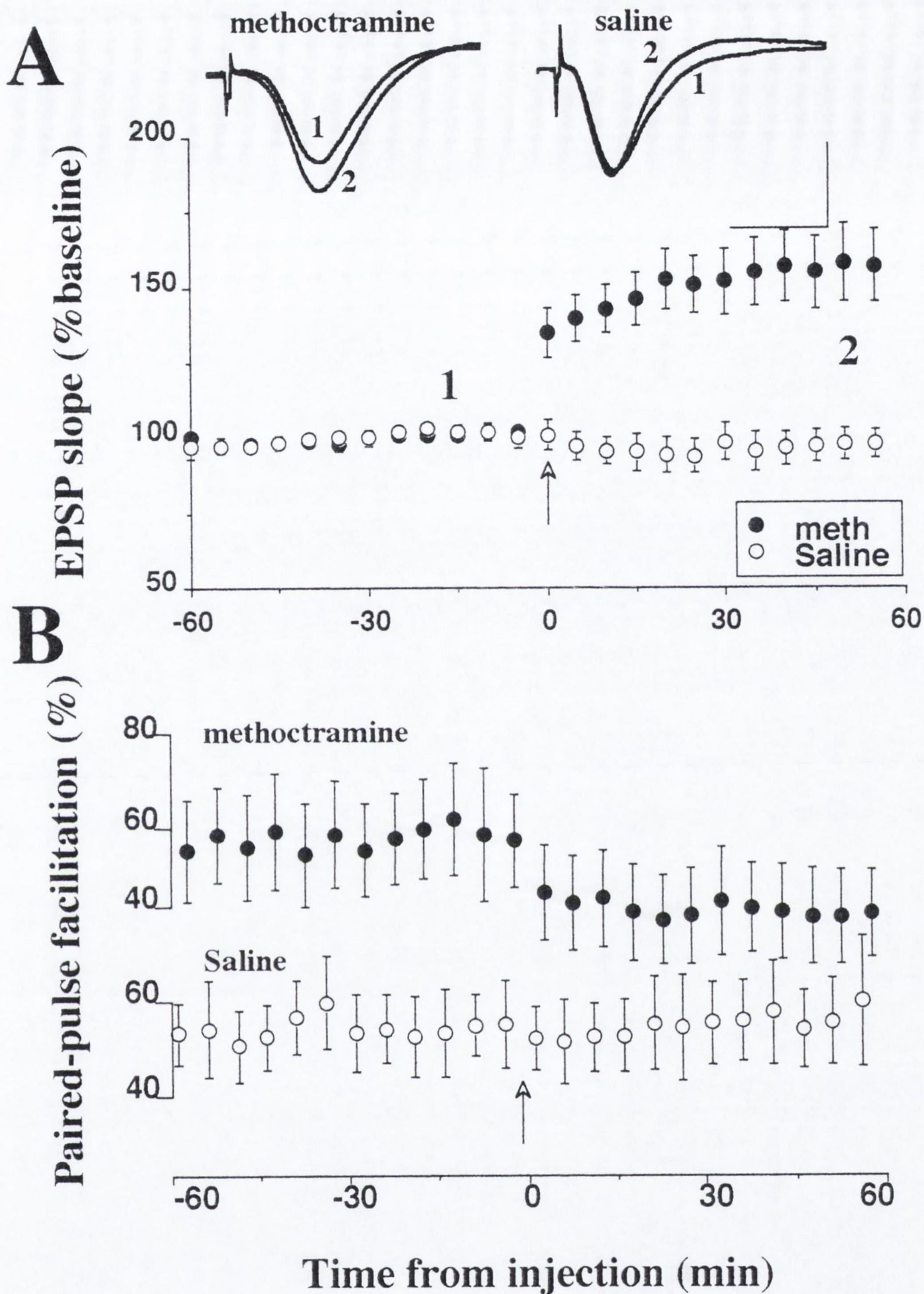


Fig. 3-8. Methoctramine induced long-lasting potentiation is associated with a reduction in PPF in the hippocampal CA1 region *in vivo*. (**A**) Methoctramine injection (17 nmol i.c.v.) caused a significant persistent increase in the EPSP (n=14, P<0.05) while saline injection had no effect (n=6, P>0.05). (**B**) Methoctramine significantly reduced paired-pulse facilitation (n=14, P<0.05). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 0.5 mV.

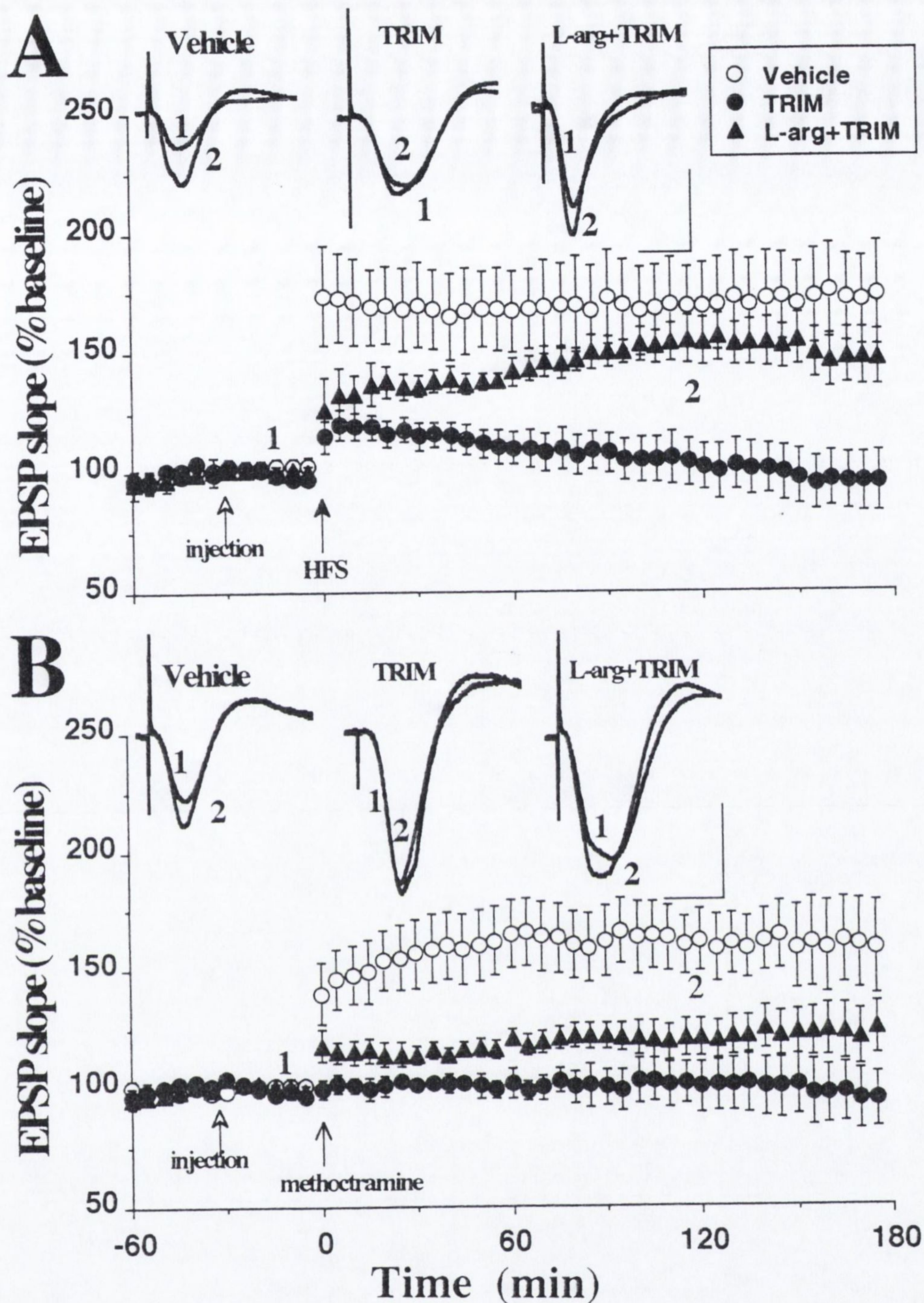


Fig. 3-9. NO is required for the induction of LTP induced both by (**A**) HFS and (**B**) methoctramine. TRIM (75 mg/kg, i.p., ●) completely blocked the potentiation caused by methoctramine (**B**, n=5) and converted tetanus-induced LTP into a short-term potentiation (**A**, n=7). The block was reduced by pretreatment with L-arginine (250 mg/kg, i.p., 1 minute before TRIM administration, n=7, ▲). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.7 NMDA receptors, not VDCCs, are involved in methoctramine-'LTP'

3.7.1 NMDAR antagonist, D-AP5, blocked methoctramine-'LTP' partially

It is well accepted that tetanic induction of LTP in the CA1 area is NMDA receptor-dependent (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). D-AP5, a strong and specific NMDA receptor antagonist, blocked tetanus-induced LTP in urethane anaesthetized rats (111.8±6.3% of baseline at 60 min, n=4, $P>0.05$). When this drug (10 μg) was injected i.c.v. 10 min before methoctramine, a small LTP was still elicited (121.6±5.2% of baseline at 60 min, n=6, $P<0.05$) (Fig.3-10, black circles). CPP, another NMDA receptor antagonist, systemically injected (10 mg/kg, i.p.) also blocked tetanus-induced LTP (106.7±9.3% of baseline at 60 min, n=8, $P>0.05$). However, when CPP was systemically injected 45 min before methoctramine, the methoctramine-'LTP' was still recorded (143.9±12.3% of baseline at 60 min, n=4, $P<0.05$), this indicated the LTP induced by M_2 antagonist was only partially NMDA receptor-dependent.

3.7.2 VDCC antagonist, mibefradil, failed to block methoctramine-'LTP'

Although LTP usually depends on Ca^{2+} influx through postsynaptic NMDA-type glutamate receptors, a non-NMDAR mediated LTP can be induced in area CA1 of the hippocampus in presence of the NMDA receptor antagonist AP-5 by application of repeated strong tetanus (200 Hz) to the Schaffer collaterals (Cavus & Teyler 1996; Morgan & Teyler 1999). This AP-5-resistant LTP is blocked by antagonizing L-type voltage-dependent calcium channels (VDCCs) and is referred to as VDCC-LTP (Morgan & Teyler 1999). So, a VDCC antagonist, mibefradil, was used to determine if the methoctramine-'LTP' was dependent on the VDCCs. Mibefradil (10 mM) was injected i.c.v. 10 min before methoctramine administration. A significant LTP-like response was produced in these rats (159.1±25.3% of baseline at 1 hour after methoctramine injection, n=5, $P<0.05$) (Fig. 3-10, black triangles). Thus, the methoctramine-'LTP' is VDCC-independent.

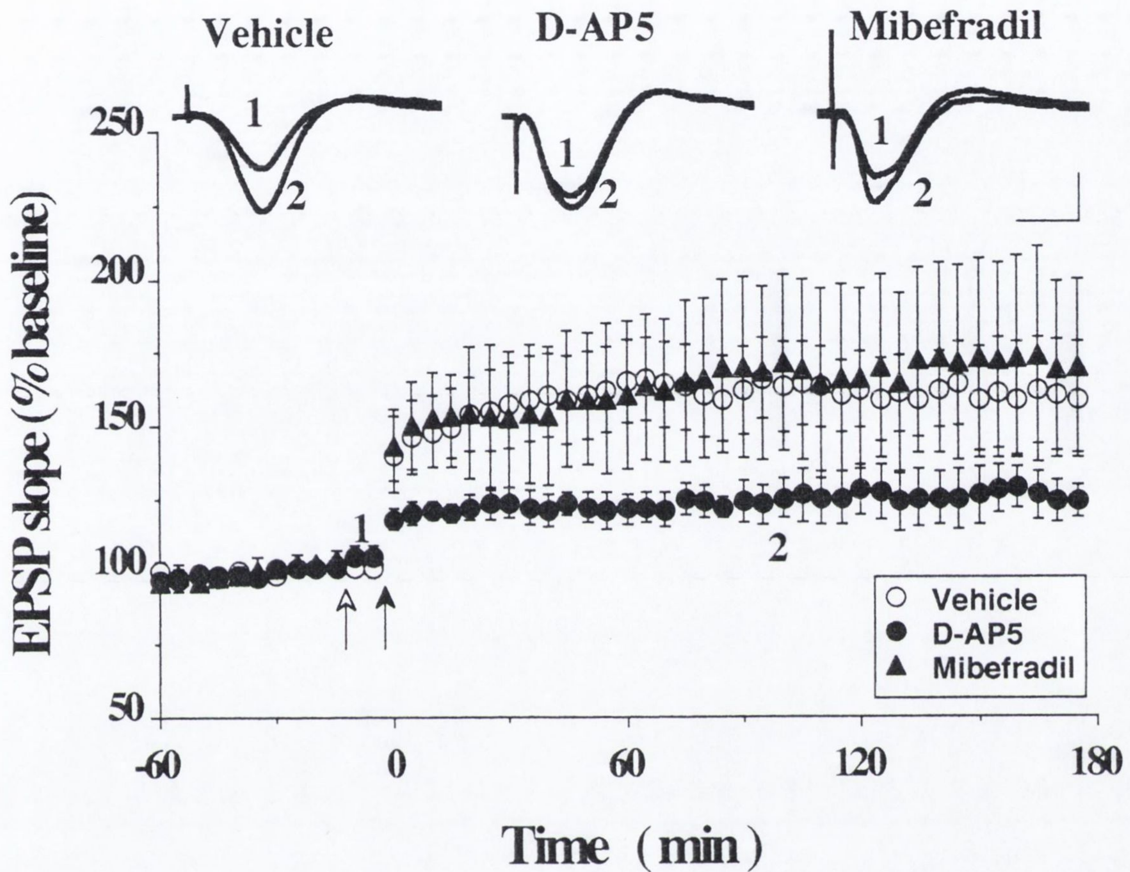


Fig. 3-10. The induction of M_2 receptor antagonist induced-'LTP' is NMDA receptor-dependent partially, but not VDCC-dependent. D-AP5 (10 μM, i.c.v., n=6, ●) depressed the magnitude of methoctramine-induced 'LTP'; The VDCCs antagonist, mibefradil (10 mM, i.c.v.) had no effect on the methoctramine induced LTP-like response (n=5, ▲). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.8 Second messengers involved in methoctramine-'LTP'

It is clear that Ca^{2+} -dependent protein kinases are involved the induction of LTP by HFS (Malenka and Nicoll, 1999). We first tested the effect of H-7, a non-specific serine-threonine protein kinase inhibitor (Hidaka et al., 1984), on the induction of LTP by tetanus and methoctramine. H-7 (40 μg/rat , i.c.v.) was injected 30 min before tetanus or methoctramine (12.5 μg/rat, i.c.v.). It failed to block tetanus-induced LTP (EPSP slope 158.4±13.5% and 150.0±8.7% of baseline at 10 and 120 min after HFS, respectively, P<0.01, n=5). Methoctramine-'LTP' was partially blocked by H-7 (132.6±12.3% and 128.0±13.3% of baseline at 10 and 120 min after methoctramine, respectively, n=8, P<0.05). This suggested that one or more protein kinase (PKA, PKC, PKG), is involved in the methoctramine-'LTP'.

3.8.1 PKC inhibitor, BIM, blocked methoctramine-'LTP'

Bisindolylmaleimide I (BIM), a selective and potent PKC inhibitor (Toullec et al., 1991), did not block the potentiation of the EPSP slope induced by HFS (159.7±11.8% and 140.2±12.9% of baseline at 10 and 120 min after HFS, respectively, n=6, P<0.01; and P>0.05 compared with vehicle, ANOVA) (Fig.3-11A). However, the same dose of BIM (40 μg) blocked the M_2 antagonist induced-LTP (110.1±9.0 % and 103.9±12.6% at 10 and 120 min after methoctramine, respectively, n=6, P>0.05 compared to baseline; P<0.05 compared to the vehicle, ANOVA) (Fig.3-11B) (Table 3-1). This finding suggests that methoctramine induced-'LTP' requires activation of the PKC signal pathway.

3.8.2 PKA inhibitor, Rp-cAMPs, blocked methoctramine-'LTP'

When Rp-cAMPs, a selective and potent PKA inhibitor, was injected i.c.v. (90 μg), the potentiation of the EPSP slope induced by HFS was partially blocked (114.3±7.1% and 119.7±6.9% at 10 and 120 min after HFS, respectively, n=6, P<0.05; and P>0.05 compared with vehicle, ANOVA) (Fig.3-11A). Rp-cAMPs blocked the M_2 antagonist induced-'LTP' (94.3±1.9 % and 96.6±4.0% at 10 and 120 min after methoctramine, respectively, n=6, P>0.05 compared with baseline; P<0.05 compared with the vehicle, ANOVA) (Fig.3-11B). Therefore, methoctramine induced-'LTP' also requires activation of the PKA signal pathway.

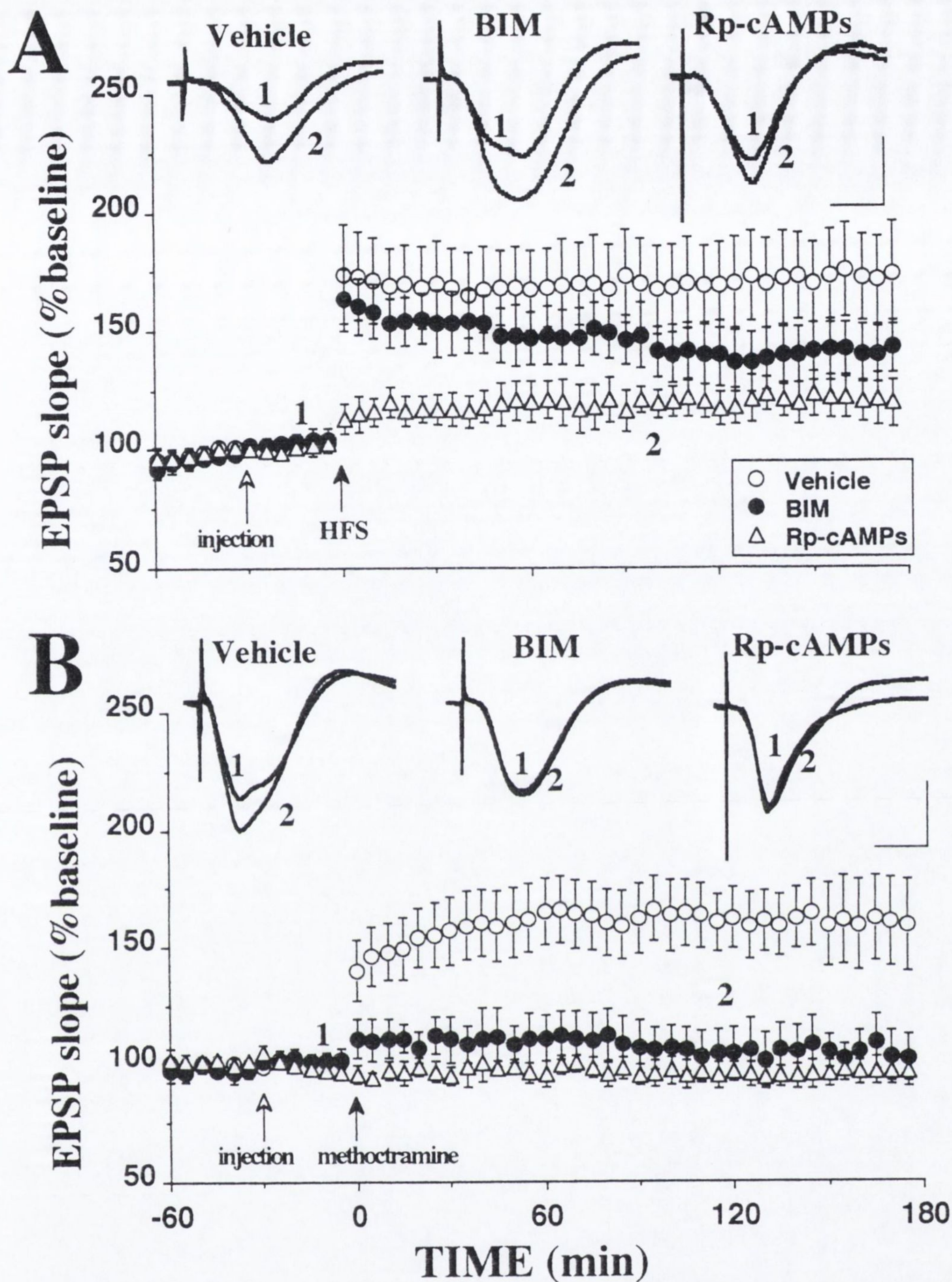


Fig. 3-11. The activation of PKA and PKC is required for the methoctramine induced-persistent enhancement. **(A)** BIM (40 µg, i.c.v., •), a selective PKC inhibitor, failed to block tetanus-induced LTP. The Rp-cAMPs (90 µg, i.c.v., Δ) PKA inhibitor, partially blocked it (n=6, P>0.05 versus Vehicle, n=10); **(B),** BIM (40 µg, n=6) and Rp-cAMPs (90 µg , n=7) completely blocked the methoctramine-induced persistent enhancement (P<0.05 versus Vehicle, n=8). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.9 Methoctramine did not occlude tetanus-induced LTP

3.9.1 Methoctramine did not occlude tetanus-induced LTP

Using the dose (12.5 μg) of methoctramine which increased baseline synaptic transmission, we tested its effect on the tetanus-induced LTP in the same pathway. Results showed that methoctramine did not occlude later tetanus-induced LTP (352.4±44.7% after HFS compared with 174.8±10.9% pre-HFS, n=7, P<0.01) (Fig. 3-12.A, white circles), the magnitude of HFS-LTP rebasline from pre-HFS is 202.3±24.5% vs. control HFS-LTP 140.6±13%, n=6, Fig.3-12B). A low dose (1.56μg) of methoctramine had no effect on LTP induced by a strong tetanus (3 sets of 200 Hz HFS given 10 min apart) (n=6, Fig.3-13A). Weak tetanus stimulation (WTS) did not evoke LTP in urethane-anaesthetised rats (n=8, Fig.3-13B), and methoctramine failed to facilitate LTP induction by WTS (n=6, Fig.3-13C).

3.9.2 BIM failed to prevent the tetanus-induced LTP after methoctramine

Because PKC is required for methoctramine induced-'LTP', we wanted to determine if the tetanic-induced LTP after methoctramine was effect by PKC. BIM, a selective PKC inhibitor, was applied prior to methoctramine. The tetanus-induced LTP was not blocked by BIM (173.2±26.4% after HFS vs 97.1±4.7% of BIM with methoctramine, n=6, P<0.05). When the baseline was calculated from the responses just before the HFS, the magnitude of tetanus-induced LTP was 202.3±24.5% and 175.8±20.5% at 10 min (P>0.05, ANOVA). The tetanus-induced LTP in the BIM with methoctramine group was similar to that found in animals given BIM alone (P>0.05) (Fig.3-12.A, black circles). This is further evidence that PKC inhibitors have no effect on tetanus-induced LTP.

3.9.3 Tetanus-induced LTP occludes later methoctramine-induced 'LTP'

Although methoctramine did not occlude tetanus-induced LTP, standard HFS-induced LTP was not blocked by a PKC inhibitor. The possibility that the methoctramine effect shared common expression mechanisms with standard HFS-induced LTP was next studied. When methoctramine was injected 2 hour after robust LTP induction, the magnitude of the EPSP slope did not change (Fig. 3-12.B). These results suggest that methoctramine can facilitate later tetanus-induced LTP, but tetanus-induced LTP occludes later methoctramine-'LTP'. It may suggest that the methoctramine-'LTP' and tetanus-induced LTP require overlapping expression mechanisms but different induction mechanisms.

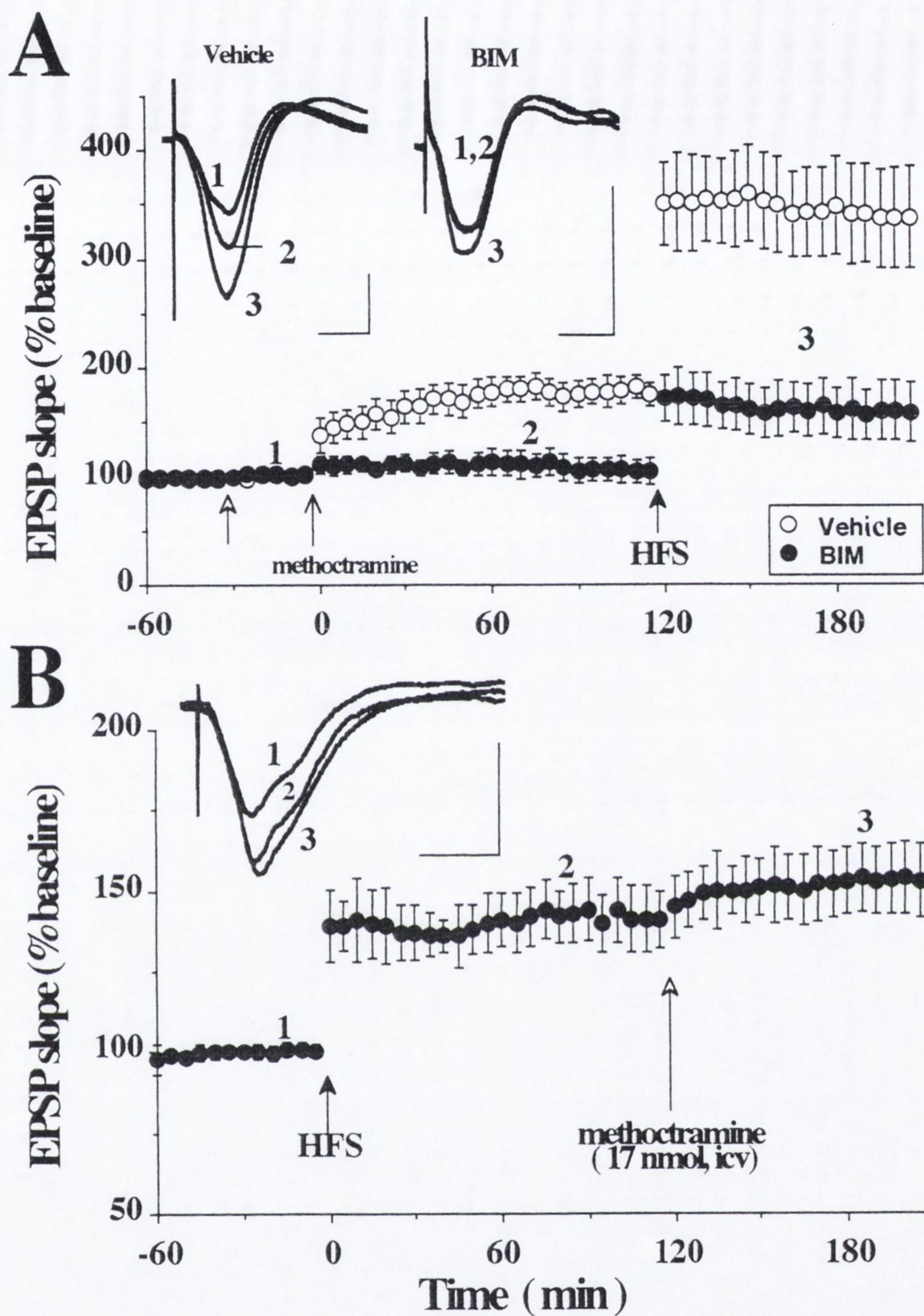


Fig. 3-12. Methoctramine did not occlude tetanus-induced LTP. (**A**), LTP was induced by HFS (200 Hz) in rats pretreated with methoctramine (n=7, ○);the PKC inhibitor, BIM, did not block this LTP (n=6, •); (**B**), Tetanus-induced LTP occluded methoctramine-induced LTP in the same pathway. Insets in A and B show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

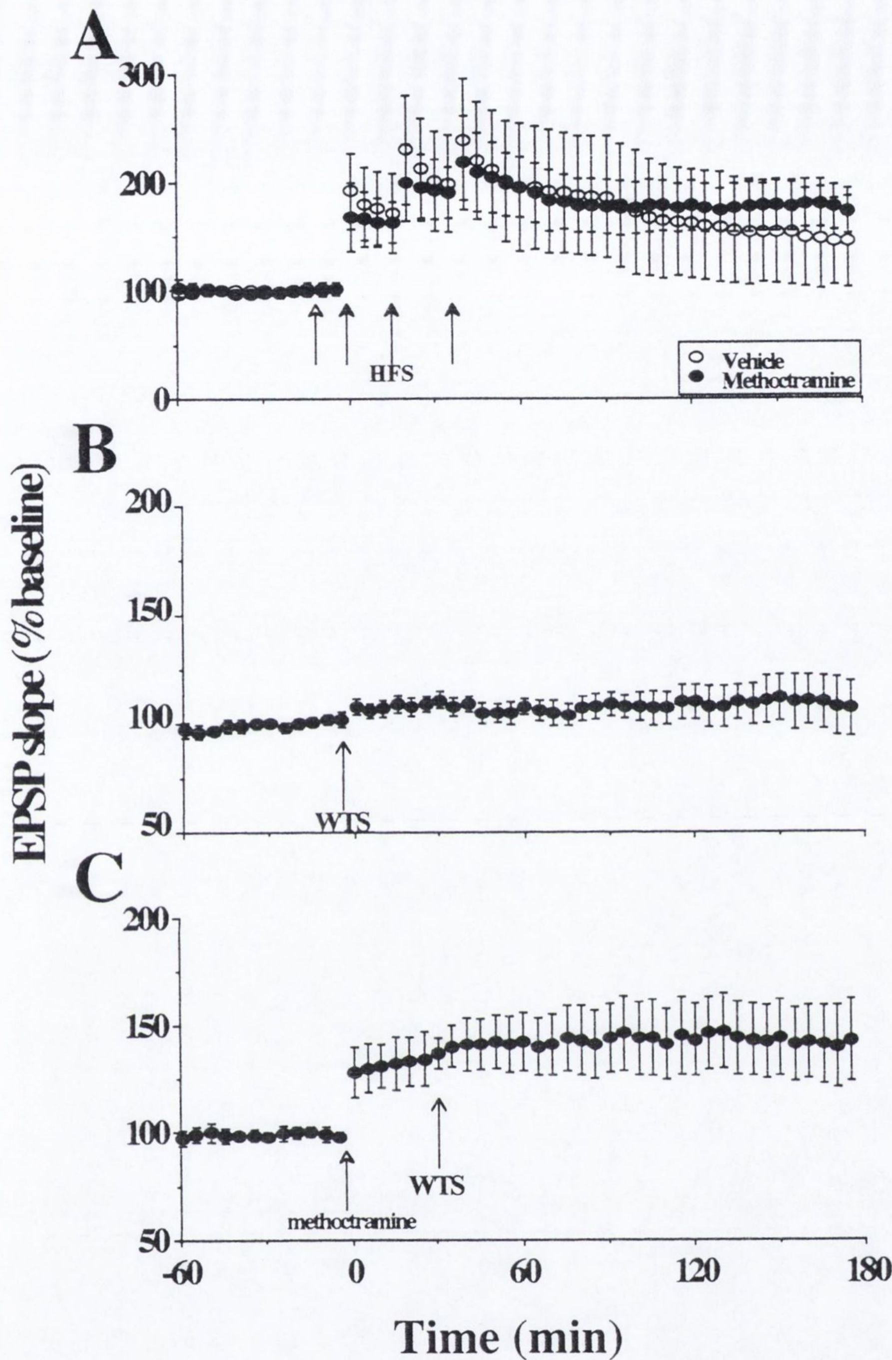


Fig. 3-13. A subthreshold dose of methoctramine had no effect on tetanus-induced LTP and methoctramine failed to affect the response to a subthreshold tetanus. (**A**), Subthreshold methoctramine (white arrow) failed to affect strong tetanus (3 sets, black arrow) induced LTP (methoctramine, n=7 vs. vehicle n=5, P>0.05); (**B**). Weak tetanus stimulation (WTS, 100 Hz) failed to induce LTP in urethane anaesthetized rats (n=7); (**C**), The standard dose of methoctramine (12.5 μg) failed to affect the response to weak tetanus stimulation (WTS) (n=6).

3.10 Methoctramine-'LTP' is not dependent on synaptic stimulation

3.10.1 Methoctramine-'LTP' is widespread

In order to examine if the methoctramine induced-'LTP' was specific to the hippocampus closest to the cannula, both test (ipsilateral) pathway and control (contralateral) pathway field EPSP responses were recorded (n=4). In contrast to previous study on tetanus-LTP (Bliss & Lomo, 1973), methoctramine induced-'LTP' was recorded in both pathways (Fig. 3-14.A). This result suggests that injection icv has widespread persistent functioning effects on synaptic transmission bilaterally in the hippocampal CA1 areas in anaesthetized rats.

3.10.2 Stopping stimulation failed to prevent methoctramine-'LTP'

To determine if stimulation affected the methoctramine induced-LTP, suspension of stimulation was included in the protocol. After at least 1 hour stable baseline recording, the standard dose (12.5 μg) of methoctramine was administrated i.c.v. After a delay of 90 min without any stimulation, scopolamine was given i.c.v. to prevent activation of muscarinic receptors. On re-commencing stimulation the EPSP slope was significantly increased. Thus the methoctramine-'LTP' is not stimulation-dependent (Fig. 3-14.B). This result also confirmed the finding that scopolamine failed to reverse the expression of LTP induced by methoctramine (Fig. 3-5.B).

3.10.3 Medial septal injection of methoctramine had no effect on hippocampal EPSPs

The cholinergic innervation of the hippocampus arises solely from the medial septum and diagonal band of Broca (MSDB) (Fibiger 1982). To answer whether methoctramine induced 'LTP' is initiated in the MSDB, methoctramine (6.25μg/1.0μl) was locally injected into the MSDB. No significant change of the field EPSP was recorded in the CA1 area (97.0±6.9% at 30 min, n=6, vs 98.0±4.0% of saline injection, n=3, $P>0.05$). Similarly, injection of a higher dose (12.5 μg/1.0μl, n=3, 101.2±4.8%) or lower dose (3.12 μg/1.0μl, n=2, 96.7±5.7%) of MSDB had no effect on the hippocampal EPSP slope. Thus, the methoctramine induced LTP did not appear to originate from the septum.

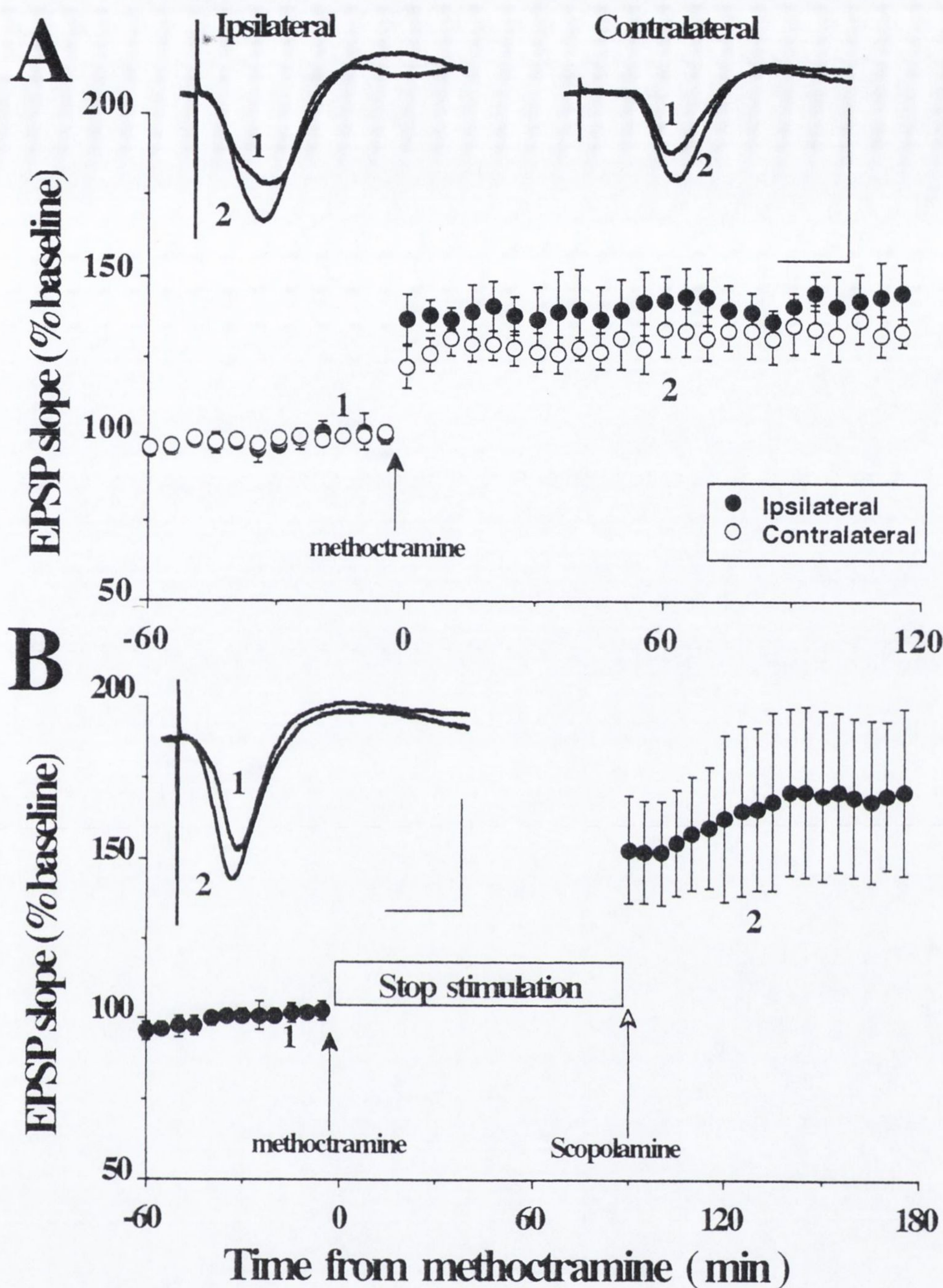


Fig. 3-14. Methoctramine-induced 'LTP'not dependent on synaptic stimulation. (**A**), Methoctramine-LTP was not localized to one hippocampus (n=4); (**B**), Stopping stimulation failed to prevent methoctramine-induced 'LTP' (n=8). Insets show traces of field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.11 Scopolamine failed to block tetanus-induced LTP in the intact brain

Muscarinic antagonists can block the action of cholinergic agonists at doses that do not influence LTP induction by HFS in the CA1 region (Tanaka et al.,1989; Sokolov and Kleschevnikov,1995; Yun, et al, 2000). Consistent with this, robust LTP was induced 150 min after scopolamine, telenzepine and 4-DAMP in the present work (**3.4.1, 3.5.1, 3.5.2**). It may be argued that the brain levels of these drugs was reduced when the tetanus was applied. Here the tetanus (200 Hz) was applied 30 min after scopolamine administration both in urethane-anaesthetized and freely behaving rats.

3.11.1 Urethane-anaesthetized rats

Consistent with previous report (Tanaka et al.,1989; Sokolov and Kleschevnikov, 1995; Yun, et al, 2000), scopolamine failed to block the induction of LTP by HFS (Fig.3-15.A). Thus, similar to the methoctramine did not occlude tetanus-induced LTP, scopolamine had no effect on tetanus LTP.

3.11.2 Freely behaving rats

Anaesthesia is a reversible depression of the central nervous system by anaesthetics. Urethane can non-selectively reduce activation of NMDA, kainate and quisqualate receptors (Evans & Smith, 1982). To examine the effect of scopolamine on the tetanus-induced LTP independent of anaesthesia, freely behaving animals were used in this study. In agreement with the results from urethane anaesthetized animals, scopolamine failed to block the tetanus-induced LTP in acclimatized freely moving rats (Fig. 3-15.B). This result supports previous reports that muscarinic receptor activation is not required for LTP induction in CA1 region by standard HFS protocols (Tanaka et al.,1989; Sokolov and Kleschevnikov,1995; Yun, et al, 2000).

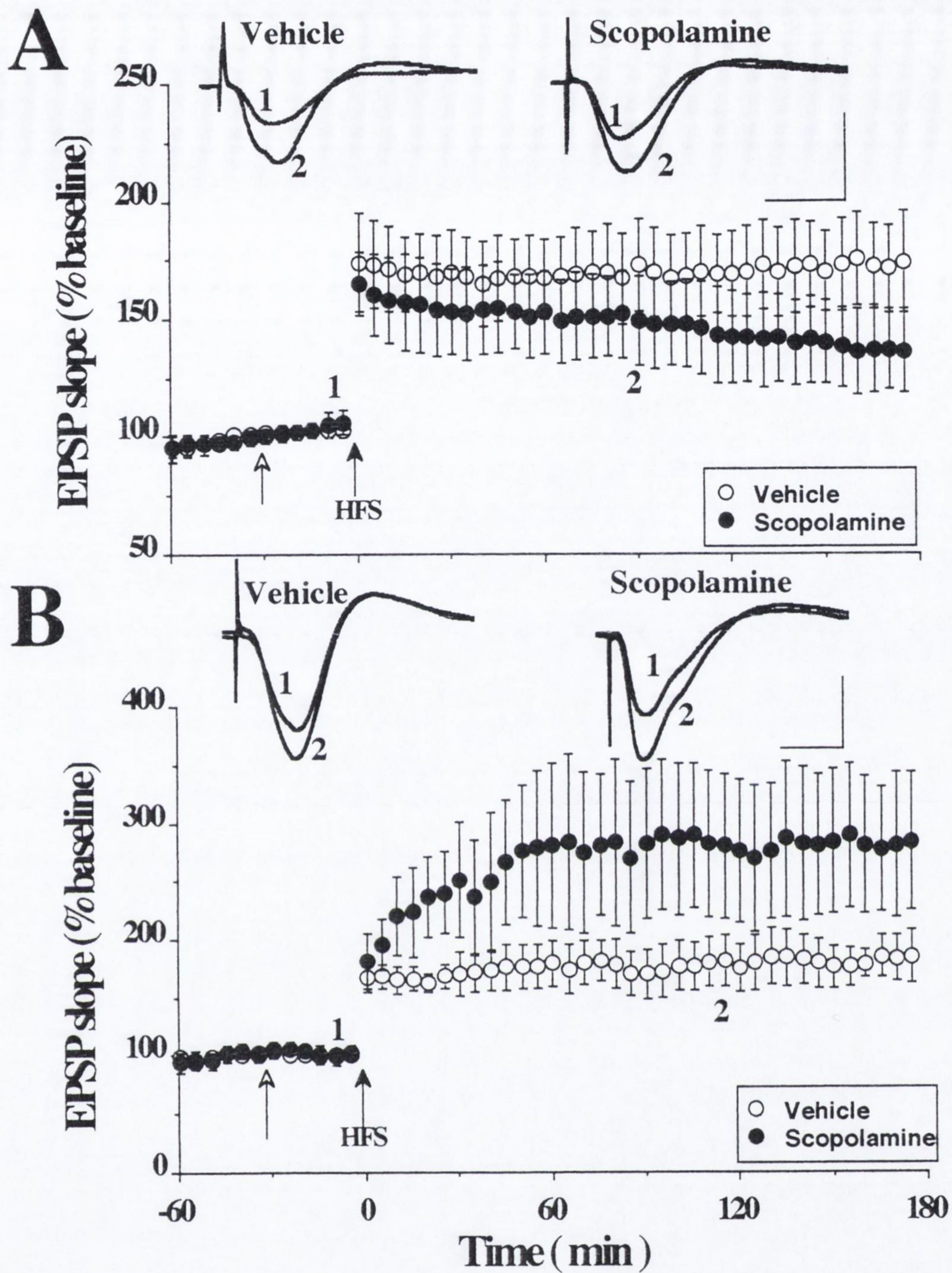


Fig. 3-15. Scopolamine fails to block tetanus induced LTP in the intact brain. (**A**), Urethane-anaesthetised rats (n=5); (**B**), Freely behaving rats (n=5). Insets show traces of the field EPSPs recorded at the times indicated. Horizontal bar, 10 ms; vertical bar, 1.0 mV.

3.12 Novelty exposure facilitation of LTP induction is not dependent on muscarinic receptor activation

Hippocampal long-term potentiation (LTP) of excitatory synaptic transmission provides a model of the synaptic mechanisms underlying information storage. The link between LTP and learning/memory can best be examined in freely behaving animals where there are intact excitatory and inhibitory circuits of neurones. Since spatial memory is dependent on hippocampal function. It has been shown previously that exploration of an unfamiliar non-stressful environment did not affect the induction of LTP by a strong tetanus whereas pre-established LTP was reversed (Xu et al., 1998). The present study examined the effect of exposure of freely behaving rats to a novel environment on the ability to induce LTP in the CA1 area. Here the response to a weak tetanus was evaluated. (results summarised in Table 3-3).

3.12.1 Novelty exposure facilitates LTP induction in behaving animals

To evaluate the novelty effect on the ability to induce LTP, a weak tetanus stimulation (WTS) which consisted of a set of 10 trains of 10 stimuli at 100 Hz, inter-train interval 2 s, was applied in the familiar recording box. When the animals crossed from the familiar to the novel environment, they explored continuously for at least 5 min. The ability of a WTS to induce LTP was then studied 5 min after the animals had returned to the familiar environment, a time when the animals were in a quiescent but alert state. This allowed us to compare the effects of conditioning stimulation with and without novelty exposure under an equivalent behavioural state thereby minimising any potential confounding effect of concurrent transient activity-related changes in excitability (Leung 1980; Hargreaves et al., 1990; Moser et al., 1993a) on LTP induction. The application of WTS to animals in a still alert state that had remained in the familiar environment throughout the recording period failed to produce a persistent change in synaptic strength (109.9±5.6, 111.1±6 and 110.1±9.6% of pre-conditioning baseline EPSP slope ± s.e.m. at 10 min, 1 h and 3h, respectively, $P>0.05$ compared to baseline, $n=7$) (Fig. 3-16A). Remarkably, if the animals were allowed to explore the novel environment (Novel-2, see methods) for a 5 min period and then an identical WTS applied while the animals were in a still alert state 5 min

after returning to the familiar environment, a rapid and robust LTP was now induced (176.1±19.1%, 155.3±11.8% and 151.1±10.8% at 10 min, 1 and 3 h, respectively, P<0.05, n=7) (Fig.3-16B).

If, as hypothesised, the facilitation of LTP induction was due to the novel environment triggering a state in the hippocampus favoring the storage of new information, it was reasoned that if the animals were habituated to the novel environment that re-exposure to such an environment should have little effect on LTP induction. A group of animals was familiarised with the novel environment by allowing exploration for a 30 min period on 3 consecutive days. Subsequent application of the WTS on the fourth or fifth day after the animals had visited the now familiar environment for 5 min did not result in the facilitation of LTP (116.9±6.7, 120.9±9.4 and 116.6±11.5% at 10 min, 1 and 3 h, respectively, P>0.05, n=5) (Fig. 3-16C). These experiments show that the novelty aspect of the new environment plays a crucial role in the facilitation of LTP induction.

In order to verify this facilitation, another novel, non-stressful, environment (Novel-1, see methods) was used. The animals explored such a novel environment (Novel-1) to the same extent as when the objects were present i.e. continuously during the 5 min exposure period and LTP was induced by the WTS after they had returned to the familiar environment (144.3±10.7, 143.3±7 and 142.6±7.9 at 10 min, 1 and 3 h, respectively, P<0.05, n=7) (Fig. 3-16D). These experiments demonstrate that the presence of the novel objects was not essential and that relatively simple novelty in the environment was sufficient to trigger novelty exploration and to facilitate LTP induction.

It was important to examine the time window for the facilitation of LTP induction by novelty exposure in order to measure the temporal constraints on information encoding by the hippocampus. In one set of experiments, the animals were allowed remain in the novel environment for 25 min. The animals explored actively for approximately 15 min and then became quiescent. Five min later, after returning to the familiar environment, application of the WTS failed to facilitate LTP (124.5±10.5% at 1 h, P>0.05, n=5) (Fig. 3-17A). A similar lack of LTP facilitation was observed when the animals were allowed to explore the novel environment for 5 min, the weak tetanus being applied 25 min later in the familiar environment (98.9±6.1%, P>0.05, n=4) (Fig. 3-17B). Thus, neither a prolonged (~15 min) nor a

brief (5 min) period of exploration facilitated LTP induction if there was a 30 min delay between the animals commencing exploration and the application of the conditioning stimulation. I also examined the effect of allowing the animals to explore the novel environment 5 min after applying the WTS. In contrast to the experiments where the exploration took place prior to the weak tetanus, no LTP was induced (118.9±7.4%, P>0.05, n=5) (Fig. 3-17C). Taken together, these experiments show that the time window for the facilitation of LTP by novelty exposure is relatively narrow.

It has been previously reported that, in manmals, exposure to a novel, complex environment enhanced field EPSP in the dentate gyrus in a mammal similar to tetanus induced-LTP (Sharp et al., 1985; Moser et al., 1993b). However, novelty exploration following WTS did not enable LTP in present study (Fig.3-16B). Exposure to Novel-2 for 5 min before WTS slightly increased field EPSP slope (115.6±9.0%, P>0.05), but not significantly. Consistent with previous report that paired-pulse facilitation (PPF) reduced accompanied with the LTP expression in freely behaving rats (Li et al., 2000). When the facilitation data pooled for both Novel-2 and Novel-1 groups PPF, a presynaptic mechanism indicator, was found to be significantly reduced (57.5±13.3% reduced to 39.6±9.3% at 60 min, n=14, P<0.05).

Next the study addressed which neurotransmitter may be involved in this type of facilitation of LTP by exposure to novelty.

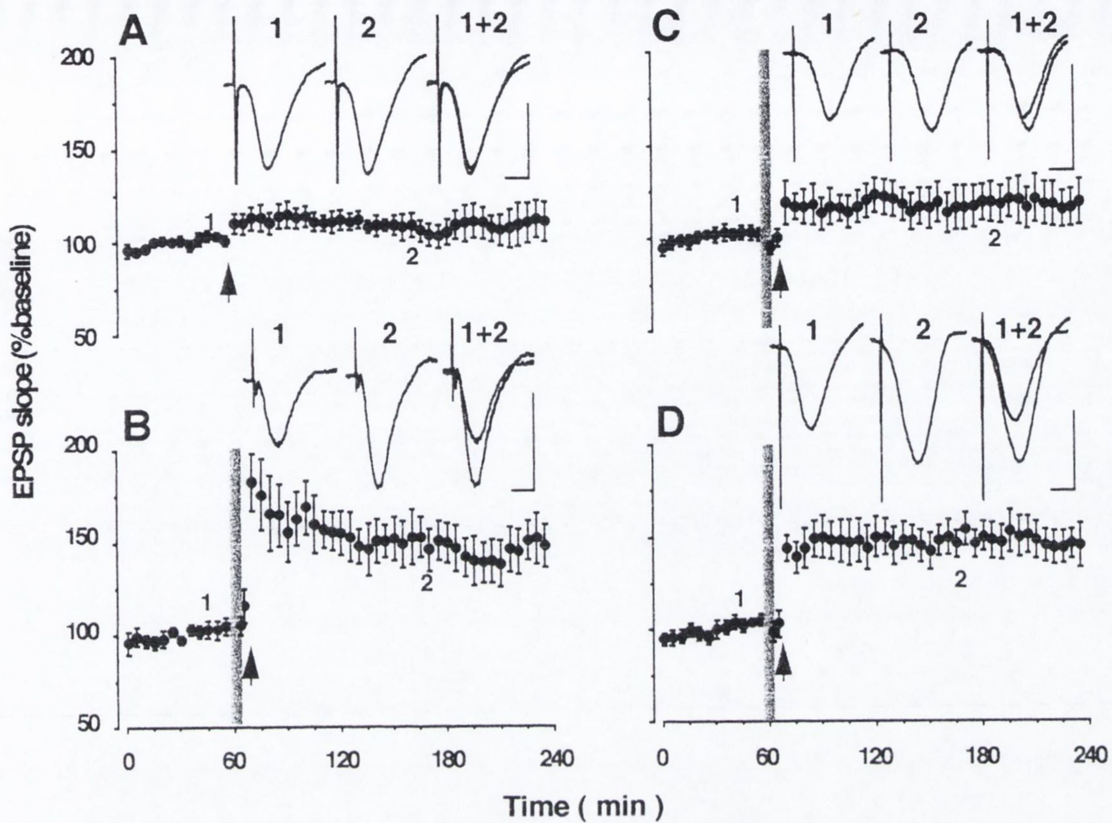


Fig. 3-16. Brief exposure to a novel environment facilitates the induction of LTP at hippocampal CA1 synapses in freely behaving rats. **(A),** Application of weak tetanus stimulation (arrow) failed to induce LTP if the animal remained in a familiar environment throughout the recording period (n=7). **(B),** The application of the same conditioning protocol 5 min after exploration of novel environment containing objects for 5 min (grey bar) now induced LTP (n=7). **(C),** This protocol did not induce LTP if the animal had been habituated to the novel environment on three previous days (n=5). **(D),** Exposure to the novel environment even in the absence of the objects (grey bar) also facilitated the induction of LTP (n=7). Insets show representative field EPSP traces at the time points indicated by the numbers. Horizontal bar, 5 ms; vertical bar 1 mV.

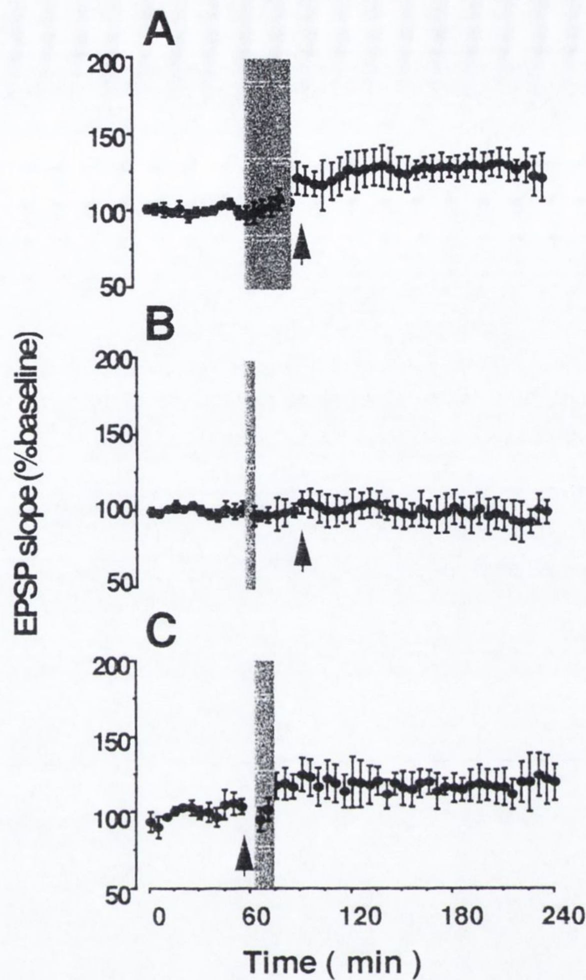


Fig. 3-17. Time window for the facilitation of the induction of LTP by novelty exposure. **(A),** Exposure to the novel environment for 25 min (grey bar) prior to the application of the weak conditioning stimulation (' arrow) did not facilitate LTP (n=5). **(B),** Similarly, LTP was not induced if the animal explored the novel environment for 5 min and the weak conditioning stimulation was applied 25 min later (n=4). **(C),** Application of the weak conditioning stimulation 5 min before exposure to the novel environment did not induce LTP (n=5).

3.12.2 Novelty facilitated LTP did not involve in mAChRs

It has been previously reported that environmental transitions increase the incidence of exploratory behaviours and hippocampal theta activity (Green et al.,1990). Novelty exposure is also associated with increased cholinergic (Acquas et al., 1996) drive to the hippocampus and activation of muscarinic acetylcholine receptors (Sah & Bekkers, 1996) can facilitate LTP induction. Therefore the effects of selective receptor antagonists for these receptors were examined. Injection of muscarinic receptor antagonist scopolamine (5 μg in 5 μl) 10 min prior to novelty exposure failed to block the facilitation of LTP induction (217.4±17.2% at 1 h, $P<0.05$, n=4) (Fig. 3-18A).

3.12.3 Novelty facilitated LTP did not involve β-adrenergic receptors

Norepinephrine (NE) has been found to increase during behavioural arousal and to facilitate the induction and maintenance of LTP via activation of β-adrenergic receptors (Thomas et al., 1996; Watabe et al., 2000). The present study also examined the effect of ß-adrenergic receptor antagonist on this facilitation of LTP induction by novelty exposure. In the presence of the β-adrenergic receptors antagonist, propranolol (2 μg in 5μl), the weak conditioning stimulation still induced LTP after novelty exposure (139.6±17.2% at 1 hr, n=5, $P<0.05$) (Fig. 3-18B). This suggests that the facilitation by novelty is not dependent on NE.

3.12.4 Novelty facilitated LTP is mediated by the dopamine receptors

Exposure to novelty is known to activate mesolimbic dopaminergic neurones which supply brain areas, including the hippocampus (Schultz et al., 1993; Ihalainen et al., 1999; Schultz 2000). We therefore assessed the role of dopamine on the facilitation of LTP induction observed in the present studies. As LTP at CA1 synapses can be regulated by D1/D5 dopamine receptor activation (Frey et al., 1993; Huang & Kandel 1995; Otmakhova & Lisman 1996; Swanson-Park et al., 1999), we examined the ability of the selective antagonist SCH23390 to affect novelty facilitated LTP induction. In animals pre-injected with SCH23390 (15 μg in 5μl, i.c.v.) 50 min prior to the introduction of the animal to the novel environment, the

WTS protocol failed to induce LTP even though the animal continuously explored the novel environment for the 5 min exposure period (118.4±3.3% at 1 h, $P>0.05$, $n=6$) (Fig. 3-18C). It was important to examine the effect of D1/D5 receptor activation by an agonist on the ability to induce LTP with WTS as most previous reports have focussed on the contribution of dopamine to LTP induced by relatively strong high frequency stimulation in the CA1 area (Frey et al., 1991; Otmakhova & Lisman 1996; Swanson-Park et al., 1999; Pittenger et al., 2002). The same WTS protocol as we used in the studies on the awake animals failed to affect baseline synaptic transmission in urethane anaesthetised animals (Fig. 3-18D, open circles, see also Fig.3-13B). However, the injection of the D1/D5 agonist SKF38393, at a dose that had no effect on baseline transmission (2.5 mg/kg, i.p., 106.8±17.9% at 3 h after injection, $P>0.05$, $n=6$), facilitated LTP induction (Fig. 3-18D, closed circles). Thus, in the intact hippocampus dopamine receptor activation is not only necessary for the facilitation of LTP by novelty exposure, but also may be sufficient to mediate it.

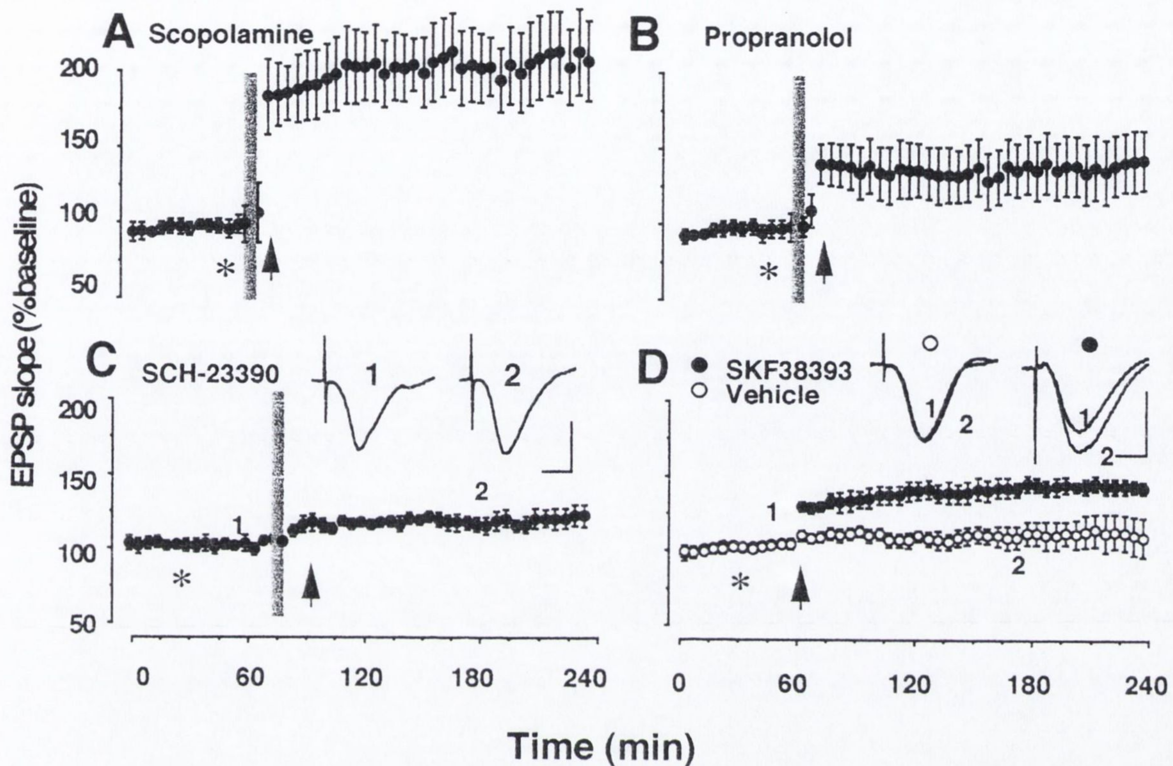


Fig. 3-18. Novelty exploration facilitated weak tetanus induced-LTP involved dopamine receptors. **(A),** Block of muscarinic acetylcholine receptor with the selective antagonist scopolamine (5 μg i.c.v., asterisk, n=4) in freely behaving rats did not prevent the facilitation of LTP induction by novelty exposure; **(B),** Similarly, LTP was induced by the WTS when applied following exploration of the novel environment if ß-adrenoceptors were blocked with the selective antagonist propranolol (2 μ g i.c.v., asterisk, n=5); **(C),** Application of the WTS (arrow) following exploration of the novel environment (grey bar) failed to induce LTP in freely behaving rats in which D1/D5 receptors were blocked with the selective antagonist SCH23390 (15 μg i.c.v., asterisk, n=6). **(D),** Injection of anaesthetised animals with the selective D1/D5 dopamine receptor agonist SKF38393 (2.5 mg/kg, i.p., asterisk) 30 min prior to WTS facilitated the induction of LTP (n=7). Horizontal bar, 5 ms; vertical bar 1 mV.

Table 3-1 Different drugs' effect on the methoctramine induced-LTP

Compounds	doses	samples	pre-meth	methoctramine administration 10 min	60 min	120 min
Vehicle (meth)	12.5 μg	8	100.9±2.8	145.7±12.3**	161.6±14.6**	163.5±13.5**
Scopolamine	5.0 μg	7	108.1±7.7	112.1±4.4 (*)	122.9±7.3 (*)	125.8±10.0 (*)
Telenzepine	12.5μg	9	99.7±2.5	113.7±5.3 *(*)	110.9±6.6 (*)	118.8±7.1*(**)
4-DAMP	6.5 μg	7	108.3±3.2	111.3±4.6 (*)	117.5±5.8 (*)	116.0±7.6 (*)
Mecamylamine	2 mg/kg	6	104.8±5.5	141.4±6.8**	153.9±8.7**	155.3±7.8**
D-AP5	10 μg	6	106.0±1.3	120.2±4.1*	121.6±5.2*(*)	128.5±8.6*
Mibefradil	10 mM	5	105.6±1.9	149.9±14.9*	159.1±25.3*	166.2±30.7*
TRIM	75 mg/kg	5	98.5±2.9	102.2±3.6(*)	99.6±3.5 (**)	101.7±8.6(**)
L-arginine	250 mg/kg	7	99.0±2.2	114.6±4.5*(*)	120.9±3.7**(*)	121.5±7.7**(*)
BIM	40 μg	7	101.4±3.8	110.1±7.6(*)	112.3±8.9(*)	105.9±9.5(**)
Rp-cAMPs	90 μg	6	96.5±1.7	94.3±1.9 (**)	96.1±4.4(**)	96.6±4.0(**)

Note: significant level: * for P<0.05; ** for P<0.01; inside of brackets are compare with the vehicle group.

Table 3-2. Different drugs' effect on the tetanus induced-LTP

Compounds	doses	samples	pre-HFS	After HFS 10 min	60 min	120 min
Vehicle (saline)		12	105.1±3.2	169.8±19.7**	169.5±18.3**	177.1±19.8**
Scopolamine	5.0 μg	5	109.4±5.0	158.6±17.4*	151.3±17.7*	142.0±18.4*
TRIM	75 mg/kg	7	95.9±1.8	119.7±6.3*	110.7±7.1(*)	102.5±9.6(*)
L-arginine	250 mg/kg	6	99.2±2.5	133.1±10.4*	138.3±3.1**	155.5±10.8**
BIM	40 μg	6	106.4±1.3	159.7±11.7**	148.5±11.5*	140.1±13.6
Rp-cAMPs	90 μg	6	100.7±3.2	114.3±7.1*	120.6±9.8*	119.7±6.9*

Note: significant level: * for P<0.05; ** for P<0.01; inside of brackets are compare with the vehicle group.

Table 3-3. Novelty exploration effect on the weak tetanus induced-LTP

Protocol	time or dose	numbers	pre-WTS	After Weak Tetanus Stimulation (WTS) 10 min	60 min	180 min
Familiar	0	7	102.5±3.5	109.9±5.6	111.1±6.0	110.1±9.6
Novel-2	+ 5 min	7	103.2±4.7	176.1±19.1*	155.3±11.8*	151.1±10.8*
Novel-1	+ 5 min	7	104.3±2.4	144.3±10.7*	143.3±7.0*	142.6±7.9*
Novel-2	+30 min	5	104.0±3.5	119.4±9.3	124.5±10.5	128.4±11.5
Novel-2	- 10 min	4	104.0±4.7	117.4±8.0	118.9±7.4	117.1±11.4
Novel-2	+5 min/Td	5	101.8±3.5	116.9±6.7	120.9±9.3	116.6±11.5
Novel-2	5N+25F	4	100.8±5.2	105.2±7.4	98.9±6.1	99.2±7.7
Scopolamine	5 μg	4	100.6±10.9	188.4±21.0*	217.4±17.2*	219.3±12.7*
Propranolol	2 μg	5	103.4±7.8	140.9±14.5*	139.6±17.2*	146.6±17.8*
SCH-23390	15 μg	6	101.1±5.4	114.4±5.6	118.4±3.3	120.1±7.2
SKF-38393	2.5mg/kg,i.p	7	103.8±1.9	126.0±2.8*	138.0±6.6*	140.6±4.4*
Vehicle (Urethane)		8	101.6±3.6	106.3±3.3	107.6±3.6	107.8±13.1

Note: significant level: * for $P<0.05$; ** for $P<0.01$

IV. Discussion

The present data show that M_2 muscarinic receptor subtype antagonists can induce an LTP-like phenomenon at Schaffer collateral-CA1 synapses in the intact hippocampus. The magnitude of LTP was dependent on the dose of M_2 receptor antagonist and was blocked by non-M_2 muscarinic and NMDA receptor antagonists, but not by nicotinic receptors and VDCC antagnists. This long-lasting potentiation of the field EPSP appeared to be mediated by NO, PKC and PKA. Whereas inhibition of PKC failed to block tetanus-induced LTP, tetanus-induced LTP occluded the M_2 antagonist induced LTP (methoctramine-'LTP') in the same pathway. In contrast, methoctramine-'LTP' enhanced the magnitude of tetanus-induced LTP via a PKC-dependent mechanism. Furthermore, tetanus-induced LTP in the intact hippocampus was not blocked by the muscarinic receptor antagonist scopolamine. Brief exposure to a novel environment facilitates the induction of LTP at hippocampal CA1 synapses in freely behaving rats, which involved dopamine receptors, not muscarinic or β-adrenergic receptors. It is concluded that tetanus-induced LTP and methoctramine-'LTP' require different but interacting mechanisms, Brief novelty exploration lowers the threshold for the induction of LTP via a mechanism dependent on activation of dopaminergic transmission.

4.1 Endogenous ACh and glutamate release induced by mAChR antagonists

ACh is one of several neuromodulators in the hippocampus and most of its known effects are mediated by the muscarinic family of receptors (M_1—M_5). Whereas some of these receptors appear to be preferentially positively coupled to the hydrolysis of phosphoinositides (M_1, M_3, and M_5) others are negatively coupled to adenylate cyclase (M_2 and M_4) (Wall et al, 1992). The M_2 mAChR is believed to be a presynaptic inhibitory autoreceptor that modulates ACh release from the presynaptic cholinergic terminal (Quirion, et al, 1995; Stillman et al, 1996; Kitaichi, et al, 1999; Rouse, et al, 2000a; but see, Vannucchi and Pepeu, 1995). Indeed, investigations have demonstrated elevated ACh levels following M_2 antagonists administration *in vitro* (Hoss, et al, 1990; Torocsik and Vizi, 1991) and *in vivo* (Stillman, et al, 1993; 1996)

supporting this hypothesis. Muscarinic ACh receptor antagonists, atropine and scopolamine, administrated systemically caused a significant increase in ACh release from hippocampus (Koppen et al. 1997; Scali et al. 1995; Vannucchi & Pepeu 1995; Vannucchi et al. 1997).

Electrophysiologically, in the central nervous system, excitatory responses to muscarinic agonists have been found to be mediated via M_1 (Jones 1996; Wang and McKinnon 1996) and M_3 receptors (Givens and Olton, 1990; Hasuo et al., 1996) and inhibitory responses have been reported to be mediated via M_2 (Bellingham and Berger, 1996) and M_4 receptor subtypes (Bernheim et al., 1992; Hasuo et al., 1995).

The M_2-like receptor has also been reported to act as a heteroreceptor to regulate the release of an excitatory amino acid transmitter and γ-aminobutyric acid (GABA) (Rouse et al., 1999; 2000a). Using PPF as an indicator of presynaptic release (Li, et al., 2000), the results of the present study are consistent with these previous reports. That is, both scopolamine and methoctramine significantly reduced PPF indicating that they increased presynaptic excitatory amino acid neurotransmitter release (Fig. 3-2). However, in the case of scopolamine there was no significant change in the synaptic response to the first pulse.

Taken together, it is hypothesised that the administration of M_2 antagonists increased ACh and glutamate release in the hippocampus and thereby induced the LTP-like phenomenon.

4.2 Muscarinic receptor subtypes and synaptic plasticity in the CA1 region

Carbachol, an mAChR agonist, causes a slow onset muscarinic LTP (LTPm) in low concentrations (0.20-0.50 μM) and depresses the field EPSPs in high concentrations (>5 μM) in the CA1 area of hippocampal slices (Auerbach and Segal; 1996; Yun et al., 2000). The present data show that a low dose of muscarine increased the field EPSPs temporarily and that a higher dose depressed transmission *in vivo*, are somewhat consistent with these reports. The LTPm was accompanied by an increase in the responsiveness of postsynaptic AMPA receptors and was hypothesised to be mediated by activation of postsynaptic M_2 mAChR (Auerbach and Segal 1996). In contrast to these reports, in the present studies methoctramine, an M_2 receptor

antagonist, induced a fast onset LTP (methoctramine-'LTP') which appeared to involve a presynaptic mechanism, at least partly. The induction, but not maintenance, of this LTP was muscarinic receptor-dependent. Thus, the non-selective muscarinic receptor antagonist, scopolamine, and the M_1/M_3 antagonists telenzepine and 4-DAMP, blocked the methoctramine induced rapid increase of the EPSP. Application of scopolamine after methoctramine failed to affect methoctramine-'LTP'. This supports the idea that induction but not maintenance of methoctramin-'LTP' requires ACh to act at non-M_2 muscarinic receptors, possibly M_1 or M_3. The difference between the previously reported LTPm and the currently described methoctramine-'LTP' may be due to the difference between the slice preparation and the intact hippocampus. In addition, in the present study endogenously released ACh interacting with synaptic muscarinic receptors may be responsible whereas exogenously applied carbachol, which has a higher affinity for M_2 over M_3 and M_1 receptor subtypes (Auerbach and Segal, 1996) may decrease endogenous ACh release and act at extrasynaptic muscarinic receptors.

How can one explain the apparently contradictory findings with the different ligands for mAChR subtypes, including the facilitation of tetanic LTP by activation of M_1 receptors (Iga et al., 1996), induction of LTPm by M_2 subtype receptor activation (Auerbach and Segal, 1994, 1996) and the induction of methoctramine-'LTP' by the M_2 receptor antagonists in the present study? One possible explanation is that cholinergic receptors can activate a variety of signalling pathways that trigger or overlap with pathways required for synaptic plasticity of excitatory amino acid-mediated transmission (for example Ca^{2+} release, PKC and MAPK) (Rosenblum et al., 2000). It is likely that the ACh activated pathways modulate NMDA/AMPA receptor-mediated transmission through different mAChR subtypes which thereby play different roles in synaptic plasticity modulation. Thus, M_1 receptor activation may enhance NMDA responses and thereby facilitate normal tetanus-induced LTP, M_2 receptor activation may increase AMPA receptor sensitivity to glutamate resulting in a slow onset potentiation, M_3/M_5 receptor activation may stimulate a different pathway and thereby trigger a rapid onset potentiation of EPSPs, different from LTPm. It is important to note that mAChR antagonists have no effect on the induction of tetanic-LTP in CA1 (Tanaka et al.,1989; Sokolov and Kleschevnikov,1995; Yun et al., 2000; present study) while they can block or depress the different forms of muscarinic LTP (both LTPm and methoctramine-'LTP') (Auerbach and Segal, 1996, present study). The block of both the slow onset and fast onset forms of muscarinic

LTP by non-subtype selective mAChR antagonists may cause impaired cognitive function and thereby provide a link between synaptic enhancement and learning/memory (Steckler et al., 1998).

4.3 Role of NMDA receptor activation and VDCCs in muscarinic LTP

LTP may be one of the cellular mechanisms underlying learning and memory (Bliss and Collingridge,1993). The induction of LTP is triggered by NMDA receptor (NMDA-LTP) or voltage dependent calcium channel (VDCC-LTP) activation and/or Ca^{2+} release. The subsequent rise in intracellular Ca^{2+} can lead to activation of CaMKII, PKC and mitogen-activated protein kinase which then cause an increase in AMPA receptor mediated responses (Malenka and Nicoll, 1999). Consistent with a possible modulation of LTP by mAChR, activation of mAChR was demonstrated to potentiate NMDA receptor mediated current responses / enhance NMDA receptor function (Marino et al.,1998; Egorov et al.,1999; Rouse et al, 1999). Moreover, muscarinic activation modulates membrane excitability and synaptic Ca^{2+} signals subcellularly possibly through suppression of the after-hyperpolarization (Egorov et al., 1999). The closure of one or more of a number of K^{+} channels (Brown, et al, 1997) enhances neuronal excitability. The present finding of an NMDA receptor dependence of the methoctramine-induced 'LTP' is consistent with the idea that increased mAChR activation may have produced sufficient depolarisation to enhance NMDA receptor mediated Ca^{2+} entry. In contrast, Ye et al (2001) demonstrated that ACh enhanced-LTP is independent of NMDA receptors and is mediated by GABA neurons.

Many reports showed that mAChR activation can enhance calcium influx through voltage gated calcium channels (Felder, 1995; Klink and Alonso, 1997) and release of Ca^{2+} from intracellular stores (Berridge and Irvine, 1989). In the present study the VDCC blocker mibefradil failed to affect the induction of the methoctramine-induced 'LTP'.

4.4 Nitric oxide dependence of LTP

Both LTP and memory processes share common molecular mechanisms in the hippocampus, including second messenger systems (Izquierdo and Medina, 1997; Paratcha et al., 2000). Many of the effects of inhibitors of second messenger systems on memory and LTP are similar to each other (Fukunaga and Miyamoto, 2000). They are also consistent with the findings of experiments on transgenic mice that either lack or express altered forms of these proteins and are amnestic for spatial and other tasks (Izquiredo and Medina , 1997; McGaugh, 2000). Importantly, both LTP and memory are modulated by ACh and NO, as mentioned in the introduction. The NO synthase inhibitor, TRIM, blocked tetanus-induced LTP. This supports previous reports with inhibitors (Bohme et al., 1991; O'Dell et al., 1991; Schuman and Madison 1991; Haley et al., 1996; Doyle et al., 1996;Zhou et al., 1993, 1998). The finding that the amino acid L-arginine, a precursor of NO, prevented this block of LTP is in agreement with previous studies in which the blocking action of the NOS inhibitors TRIM and 7-NI (7-nitro indazole) on LTP induction was prevented by L-arginine (Doyle et al., 1996; Wu et al., 1997). Since NO is synthesised from L-arginine, the prevention of the blocking effect of NOS inhibitors by L-arginine strongly suggests that these agents are inhibiting the induction of LTP by a specific block of NOS.

Consistent with the retrograde messenger hypothesis, tetanus-induced LTP in the intact hippocampus is often accompanied by a decrease of PPF indicating an increase in presynaptic neurotransmitter release (Li et al., 2000). The reduction in PPF was prevented by TRIM, and L-arginine overcame the effect of TRIM. This greatly supports the proposal that NO may act presynaptically to trigger enhanced transmitter release during LTP (O'Dell et al., 1991, Arancio et al., 1996; but see Ko and Kelly, 1999). The administration of the NOS inhibitor left a residual short-term potentiation perhaps suggesting that NO is more involved in the induction of a late phase of tetanus LTP (Lu et al., 1999). Interestingly, M_2 receptor antagonist induced-LTP also required the activation of the NO pathway. The same dose of TRIM that prevented tetanus-LTP totally blocked methoctramine induced long-lasting potentiation. L-Arginine also reversed this potentiation but apparently to a lesser degree. These findings may mean that the involvement of the NO pathway is somewhat different for tetanus and M_2 receptor antagonist induced LTP. It is possible that NO contributes importantly to 'LTP' induced by M_2 receptor antagonists whereas other messengers contribute more to tetanus-LTP.

The M_2 receptor antagonist induced LTP-like phenomenon was muscarinic receptor-dependent. Previously the mAChR agonist carbachol was found to have a biphasic action on NO synthase activity: low concentrations enhanced NOS activation and high ones inhibited enzymatic activity in rat cerebral frontal cortex (Borda et al., 1998). These results are similar to the EPSP changes induced by carbachol in the CA1 area of the hippocampal slice, where it caused muscarinic LTP (LTPm) at low concentrations and depressed field EPSPs at high concentrations (Auerbach and Segal; 1996; Yun et al., 2000). Muscarinic receptor activation can induce inward Ca^{2+} currents that may preferentially stimulate NOS and subsequently enhance cGMP production leading to even greater Ca^{2+} influx in a positive feedback pathway (Mathes and Thompson, 1996). Actually, the NO dependence of LTP was thought to be mediated by the NO-cGMP-PKG pathway (Lu et al., 1999; Zhou et al., 1998). Thus a muscarinic receptor activation of a positive feedback circuit dependent on the rapid production of cGMP could possibly explain the M_2 antagonist induced LTP-like phenomenon. Furthermore, previous studies have shown that NO enhances ACh release in hippocampus (Suzuki et al., 1997; Prast and Philippu, 2001).

4.5 Role of protein kinase activation in LTP

ACh activates second messenger systems involving phospholipases, PKC and inositol trisphosphate (IP_3) (Smith et al., 1989; Cantrell et al., 1996). Activated PKC is a key enzyme for signal transduction and various neuronal plasticity mechanisms. Activation of PKC may be a mechanism for generating long-term cell regulation induced by transient cell signals and second messengers (Nelsestuen and Bazz, 1991). PKC phosphorylates many substrates, among others mAChRs, and the phosphorylation of mAChRs by PKC appears to be subtype-specific, with M_1, M_3 and M_5 as the most likely substrates (Haga et al., 1993). It is now apparent that muscarinic receptors also activate other kinases, including mitogen-activated protein kinase (MAPK) (Wan et al., 1996; Rosenblum et al., 2000; Slack 2000). All of these effects of ACh on the above signalling mechanisms could favour the induction of LTP.

Activation of PKC is considered by many to be one of the requisite biochemical steps necessary for LTP since some selective PKC inhibitors can block induction of LTP (Hvalby et al., 1994; Stanton 1995; Chen et al., 1997; Routtenberg 1999). However, the present results demonstrated that H-7, a non-selective protein kinase inhibitor, and Bisindolymaleimide I, a selective PKC inhibitor, failed to block the

induction of tetanus-induced LTP, but did prevent M_2 receptor antagonist induced-LTP *in vivo*. The difference between the present data and previous reports on tetanus-induced LTP may be accounted for by the different experimental conditions or inhibitors. It is also known that hippocampal glutamatergic neurons express multiple PKC isoforms (Majewski and Iannazzo, 1998). The different subtypes of PKC are differentially distributed at the cellular and subcellular levels in the brain and exhibit somewhat different physiological functions (Dekker and Parker, 1994).

PKA is believed to play a critical role in the expression of long-lasting forms of hippocampal LTP. Late LTP is generally accepted to be dependent on cAMP and protein synthesis (Frey et al. 1993; Huang & Kandel 1994; Winder et al. 1998), probably related to the formation of new synapses (Bolshakov et al. 1997; Ma et al. 1999). This study showed that Rp-cAMPs, a selective PKA inhibitor, reduced the magnitude of tetanus-induced LTP, and completely blocked methoctramine-induced 'LTP', suggesting that PKA activation contributes to the induction of both forms of LTP.

Tetanic stimulation induced a further enhancement of EPSPs after the M_2 antagonist had induced a robust long-lasting potentiation (methoctramine-'LTP'). In contrast, the enhancement induced by tetanus occluded the M_2 antagonist induced potentiation. This indicates that the LTP induced by M_2 antagonists and tetanus may require different but overlapping pathways: M_2 antagonist induced-'LTP' requires PKC, PKA and NO pathway activation whereas tetanus induced-LTP appears to require more messengers than PKC, PKA and NO.

4.6 M_1 and $M_{3/5}$ receptors may have different functions in the regulation of synaptic plasticity

The mechanisms by which muscarinic receptors are involved in learning and memory are not known. The M_1 mAChR is by far the most abundant in the hippocampal formation and this receptor is especially enriched in the CA1 region (Levey et al., 1995). Consequently, it has been assumed that the M_1 receptor is responsible for mediating the mAChR-induced increase in excitability of pyramidal cells by inhibition of several K^+ conductances. However, recent evidence indicates that this may not be the case (Rouse et al., 2000b). A small LTP was still induced by methoctramine following telenzepine injection and the M_1 agonist, YM 796 failed to

induce potentiation of EPSPs. Taken together with the finding that 4-DAMP, which is approximately equally effective at M_1 and M_3 sites (Caulfield, 1993), prevented the induction of methoctramine-'LTP', this may suggest that M_3 and possibly M_5 subtypes are involved in inducing the long lasting potentiation of synaptic transmission.

Aura et al (1997) demonstrated that muscarinic M_2 receptor antagonists may be effective in enhancing behavioural function that depend on the activity of the septohippocampal cholinergic system. Intriguingly, the excitation of septohippocampal cholinergic neurons has been reported to be mediated via non-M_1 subtype muscarinic receptors (M_3/M_5 subtypes) (Liu et al., 1998). Furthermore, the excitation of septohippocampal cholinergic pathway was via GABAergic neurons (Liu et al, 1998; Wu et al., 2000). However, in the present study intraseptal injection of methoctramine failed to induce LTP in the CA1 area.

Other evidence indicates that the activation of M_1 mAChR does not regulate the release of glutamate or AMPA-mediated transmission at corticostriatal synapses (Calabresi et al., 1998) and that the M_3 receptor, which is abundant in the CA1 region, contributes to the release of Ca^{2+} from intracellular pools (Wakamori et al., 1993). This suggests that the M_3 (and/or M_5) receptor subtype may mediate the mAChR rapid onset LTP. Significantly, studies *in vivo* have shown that the injection of mAChR antagonists into the brain tissue of rats results in a significant increase in M_3 receptor density (Wall et al., 1992). In conclusion, the present study demonstrates that activation of a non-M_2 receptor subtype is necessary to induce this form of LTP (methoctramine-'LTP').

4.7 Novelty exploration facilitates LTP

There is a growing body of evidence that the detection of novelty is important in hippocampal information storage (Parkin 1997; Honey et al., 1998). Previous studies have shown that exposure to a novel, complex environment caused a persistent enhancement of field potentials recorded in the dentate gyrus, which appeared to be similar to tetanically induced-LTP (Sharp et al., 1985). Although environmental change increased exploratory behaviour and hippocampal theta activity, the persistent enhancement of the evoked field EPSP was not accounted for by either, and was suggested to be due to the variation in sensory input that accompanies exploration

(Green et al., 1990). Subsequently Moser et al., (1993b) showed that after correcting for changes in locomotion, theta activity and brain temperature, only a transient increase in synaptic transmission was detected in the dentate gyrus.

In the CA1 area, although exploration of a new, non-stressful environment had no long-lasting effect on baseline transmission it induced a complete and persistent reversal of the expression of high frequency stimulation induced LTP (Xu et al., 1998). This suggests that exploration of novelty can interact with artificially induced LTP.

The present data showed that brief exposure to a novel environment significantly facilitated the induction of LTP. The duration of exploration of novelty was found to be critical in a previous study of novelty reversal of LTP, that is, 10 min exposure to novelty failed to reverse LTP while 20 min exploration induced persistent depotentiation (Li, Anwyl & Rowan, unpublished observation). The different effects of exposure to novelty on LTP induction and maintenance at different time periods may be accounted for by differential neurotransmitter release, including ACh (Acquas et al., 1996; Aloisi et al., 1997). Exposure to certain types of novelty can induce stress-like behavioural changes. Physical and psychological stressors provoke the secretion of catecholamines rapidly which typically return to baseline levels within 10 min, while glucocorticoid secretion is usually much slower (De Boer et al., 1990; McEwen & Sapolsky 1995). Stressful novelty has been shown to block LTP induction (Xu et al., 1997). Another explanation is that novelty exposure elicits behavioural arousal (Acquas et al., 1996) and hippocampal theta activity (Xu et al., 1998).

Since PPF was reduced significantly after the expression of novelty facilitated LTP the present data strongly suggest a presynaptic mechanism may be involved (Li et al., 2000). Further study showed that the LTP induction required activation of dopamine receptors, but not the mAChRs or β-adrenergic receptors (Fig. 3-18). It was surprising that the novelty exploration facilitated-LTP did not involve a mAChR-dependent mechanism, since novelty is known to increase ACh release in hippocampus (Acquas et al., 1996; Aloisi et al., 1997) and endogenous ACh release can induce an LTP-like response and significantly facilitate tetanus-LTP in anaesthetised animals (present data). A possible explanation is that the increase in ACh release in response to novelty exposure is a secondary or minor change. The

finding that mAChRs are not required for tetanus-induced LTP in the CA1 region in awake and anaesthetised rats is consistent with a previous *in vitro* report (Yun et al., 2000).

Norepinephrine (NE) may be released directly into the hippocampus from ascending terminals of the locus coeruleus upon arousal and stress, which in turn may facilitate the induction and maintenance of LTP (Hopkins & Johnston 1984; Sah & Bekkers 1996; Thomas et al., 1996). The NE facilitated-LTP is contingent upon activation of β-adrenergic receptors. The present data showed that a β-adrenergic receptor antagonist, propranolol, failed to block the novelty facilitated-LTP. This suggests that NE is not involved in this form of LTP. Recent studies demonstrating that NE had little or no effect on the induction of LTP in CA1 region are consistent with the present results (Katsuki et al., 1997; Swanson-Park et al., 1999).

SCH-23390, a dopamine D1/D5 receptor antagonist, blocked novelty facilitated LTP induced by weak tetanic stimulation suggesting that endogenous dopamine is necessary for novelty facilitated LTP in area CA1. This is consistent with previous work that LTP at the CA1 Schaffer collateral synapses is facilitated by D1/D5 dopamine receptors (Frey et al., 1993; Huang & Kandel 1995; Otmakhova & Lisman 1996; Swanson-Park et al., 1999). Behavioural experiments also demonstrated that dopaminergic actions are important: intrahippocampal dopamine agonists improve memory retention and antagonists decrease it (Bernabeu et al., 1997).

The present data also elucidate how novelty exposure can control learning by activation of mesolimbic dopaminergic neurones (Horvitz 2000; Schultz 2000;) . Indeed, consistent with our findings, a recent model posits a key role of novelty-activated dopaminergic drive in the promotion of the encoding of information at hippocampal CA3-CA1 synapses (Lisman & Otmakova 2001). Thus a salient environmental change, such as that used in the present studies, can trigger a dopamine-dependent state in the hippocampus that favours the synaptic storage of the recently encountered event. This provides an explanation for the known ability of hippocampal dopamine depletion to impair (Gasbarri et al., 1996), and dopamine D1 receptor agonists to promote, certain types of hippocampal-dependent learning (Hersi et al., 1995; Bach et al., 1999). In the present experiments the time window of novelty facilitation was found to be relatively transient, lasting for a few minutes, presumably rapidly changing as the behavioural relevance of the new information for learning

fluctuates. Since the CA1 area appears to act as both an information store and a mismatch detector (Lisman & Otmakova 2001), the interaction between novelty detection-triggered dopamine release and subsequent promotion of information encoding should be critical for the normal function of the intact hippocampus.

V. Conclusions

1. Administration of scopolamine(non-selective muscarinic receptor antagonist) and methoctramine (M_2 receptor antagonist) produced a decrease of the paired-pulse facilitation ratio, a presynaptic parameter, in the hippocampus *in vivo*.
2. Blockade of M_2 muscarinic receptor subtype induced an LTP-like persistent enhancement of transmission at Schaffer collateral-CA1 synapses in the hippocampus *in vivo*
3. Non-subtype selective block of muscarinic receptors prevented the induction but not the maintenance of the persistent enhancement, consistent with a requirement for activation of non- M_2 receptors
4. This fast onset LTP induced by the M_2 antagonist was blocked by AP-5 partially, an antagonist of NMDA, but not blocked by VDCCs antagonist, mibefradil.
5. Like tetanus-induced LTP, this long-lasting enhancement of the field EPSP was associated with a reduction in paired-pulse facilitation and required NO synthase activation, indicative of a role of presynaptic / retrograde messenger mechanisms
6. Unlike tetanus-induced LTP, the enhancement was dependent on PKC and PKA activation.
7. Whereas tetanus-induced LTP occluded the M_2 antagonist induced enhancement, induction of the M_2 antagonist enhancement did not occlude subsequent tetanus-induced LTP.
8. Brief novelty exploration lowers the threshold for the induction of LTP via a mechanism dependent on activation of dopaminergic transmission.

VI. Bibliography

Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S.(1993) Modified hippocampal long-term potentiation in PKC gamma-mutant mice. **Cell**. 75:1253-1262.

Abraham WC, Bear MF.(1996) Metaplasticity: the plasticity of synaptic plasticity. **Trends Neurosci.** 19:126-130.

Abraham WC, Gustafsson B, Wigstrom H. (1987) Long-term potentiation involves enhanced synaptic excitation relative to synaptic inhibition in guinea-pig hippocampus. **J Physiol**. 394:367-380.

Acquas E, Wilson C, Fibiger HC.(1996) Conditioned and unconditioned stimuli increase frontal cortical and hippocampal acetylcholine release: effects of novelty, habituation, and fear. **J Neurosci**. 16:3089-3096.

Aloisi AM, Casamenti F, Scali C, Pepeu G, Carli G.(1997) Effects of novelty, pain and stress on hippocampal extracellular acetylcholine levels in male rats. **Brain Res**. 748:219-226.

Andrews JS, Jansen JH, Linders S, Princen A.(1994) Effects of disrupting the cholinergic system on short-term spatial memory in rats. **Psychopharmacology** . 115:485-494.

Anwyl R.(1999) Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. **Brain Res Brain Res Rev**. 29:83-120.

Arancio O, Kiebler M, Lee CJ, Lev-Ram V, Tsien RY, Kandel ER, Hawkins RD.(1996) Nitric oxide acts directly in the presynaptic neuron to produce long-term potentiation in cultured Hippocampal neurons. **Cell**. 87:1025-1035.

Auerbach JM, Segal M.(1994) A novel cholinergic induction of long-term potentiation in rat hippocampus. **J Neurophysiol.** 72:2034-2040.

Auerbach JM, Segal M.(1996) Muscarinic receptors mediating depression and long-term potentiation in rat hippocampus. **J Physiol** (Lond). 492 :479-493.

Aura J, Sirvio J, Riekkinen P Jr.(1997) Methoctramine moderately improves memory but pirenzepine disrupts performance in delayed non-matching to position test. **Eur J Pharmacol**. 333:129-134.

Baba H, Kohno T, Okamoto M, Goldstein PA, Shimoji K, Yoshimura M. (1998) Muscarinic facilitation of GABA release in substantia gelatinosa of the rat spinal dorsal horn. **J Physiol.** 508: 83-93.

Bach, M., Barad, M., Son, H., Zhuo, M., Lu, Y., Shih, R., Mansuy, I., Hawkins, R. & Kandel, E. (1999) Age-related defects in spatial memory are correlated with defects in late phase of hippocampal long-term potentiation in vitro and are attenuated by drugs that enhance the cAMP signalling pathway. **Proc Nat Acad Sci USA** 96:5280-5285.

Bannerman DM, Chapman PF, Kelly PA, Butcher SP, Morris RG.(1994) Inhibition of nitric oxide synthase does not prevent the induction of long-term potentiation in vivo. **J Neurosci.** 14:7415-7425.

Barnes CA.(1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. **J Comp Physiol Psychol.** 93:74-104.

Barria A, Muller D, Derkach V, Griffith LC, Soderling TR.(1997) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. **Science.** 276:2042-2045.

Bartus RT, Dean RL 3d, Beer B, Lippa AS.(1982) The cholinergic hypothesis of geriatric memory dysfunction. **Science**. 217:408-414.

Bear MF, Abraham WC.(1996) Long-term depression in hippocampus. **Annu Rev Neurosci**. 19:437-462.

Bear MF, Malenka RC.(1994) Synaptic plasticity: LTP and LTD.**Curr Opin Neurobiol.** 4:389-399.

Beattie EC, Carroll RC, Yu X, Morishita W, Yasuda H, von Zastrow M, Malenka RC. (2000) Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. **Nat Neurosci**. 3:1291-1300.

Beatty WW, Butters N, Janowsky DS.(1986) Patterns of memory failure after scopolamine treatment: implications for cholinergic hypotheses of dementia. **Behav Neural Biol.** 45:196-211.

Bejar C, Wang RH, Weinstock M. (1999) Effect of rivastigmine on scopolamine-induced memory impairment in rats. **Eur J Pharmacol.** 383:231-240.

Bellingham MC, Berger AJ.(1996) Presynaptic depression of excitatory synaptic inputs to rat hypoglossal motoneurons by muscarinic M2 receptors. **J Neurophysiol.** 76:3758-3770.

Bernabeu R, Bevilaqua L, Ardenghi P, Bromberg E, Schmitz P, Bianchin M, Izquierdo I, Medina JH. (1997) Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning in rats. **Proc Natl Acad Sci U S A.** 94:7041-7046.

Bernheim L, Mathie A, Hille B.(1992) Characterization of muscarinic receptor subtypes inhibiting Ca^{2+} current and M current in rat sympathetic neurons. **Proc Natl Acad Sci U S A.** 89: 9544-9548.

Berridge MJ, Irvine RF.(1989) Inositol phosphates and cell signalling. **Nature** 341:197-205.

Bland BH.(1986) The physiology and pharmacology of hippocampal formation theta rhythms. **Prog Neurobiol**. 26:1-54.

Bliss TVP and Collingridge GL.(1993) A synaptic model for memory: long-term potentiation in the hippocampus. **Nature,** 361:31-39.

Bliss TV, Lomo T.(1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. **J Physiol**. 232:331-356.

Blitzer RD, Connor JH, Brown GP, Wong T, Shenolikar S, Iyengar R, Landau EM. (1998) Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. **Science.** 280:1940-1942.

Blitzer RD, Gil O, Landau EM.(1990) Cholinergic stimulation enhances long-term potentiation in the CA1 region of rat hippocampus. **Neurosci Lett**. 119:207-210.

Blokland A.(1996) Acetylcholine: a neurotransmitter for learning and memory? **Brain Res Brain Res Rev.** 21:285-300.

Bohme GA, Bon C, Stutzmann JM, Doble A, Blanchard JC. (1991) Possible involvement of nitric oxide in long-term potentiation. **Eur J Pharmacol.** 199:379-381.

Bolshakov VY, Carboni L, Cobb MH, Siegelbaum SA, Belardetti F.(2000) Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. **Nat Neurosci.** 3:1107-1112.

Bolshakov VY, Golan H, Kandel ER, Siegelbaum SA.(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.

Borda T, Genaro A, Sterin-Borda L, Cremaschi G.(1998) Involvement of endogenous nitric oxide signalling system in brain muscarinic acetylcholine receptor activation. **J Neural Transm.** 105:193-204.

Bortolotto ZA, Collingridge GL.(1998) Involvement of calcium/calmodulin-dependent protein kinases in the setting of a molecular switch involved in hippocampal LTP. **Neuropharmacology.** 37:535-544.

Boyd TE, Trepel C, Racine RJ.(2000) Cholinergic modulation of neocortical long-term potentiation in the awake, freely moving rat. **Brain Res**. 881:28-36.

Braida D, Paladini E, Griffini P, Lamperti M, Maggi A, Sala M.(1996) An inverted U-shaped curve for heptylphysostigmine on radial maze performance in rats: comparison with other cholinesterase inhibitors. **Eur J Pharmacol.** 302:13-20.

Brocher S, Artola A, Singer W. (1992) Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. **Brain Res**. 573:27-36.

Brown DA, Abogadie FC, Allen TG, Buckley NJ, Caulfield MP, Delmas P, Haley JE, Lamas JA, Selyanko AA.(1997) Muscarinic mechanisms in nerve cells. **Life Sci.** 60:1137-1144.

Burgard EC, Sarvey JM.(1990) Muscarinic receptor activation facilitates the induction of long-term potentiation (LTP) in the rat dentate gyrus. **Neurosci Lett**. 116:34-39.

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G.(1998a) Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. **Eur J Neurosci**.;10:2887-2895.

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G.(1998b) Blockade of M2 -like muscarinic receptors enhances long-term potentiation at corticostriatal synapses. **Eur J Neurosci.**;10:3020-3023.

Cammarota M, Bernabeu R, Levi De Stein M, Izquierdo I, Medina JH. (1998) Learning-specific, time-dependent increases in hippocampal Ca2+/calmodulin-dependent protein kinase II activity and AMPA GluR1 subunit immunoreactivity. **Eur J Neurosci.** 10:2669-2676.

Cantrell AR, Ma JY, Scheuer T, Catterall WA.(1996) Muscarinic modulation of sodium current by activation of protein kinase C in rat hippocampal neurons. **Neuron.**16:1019-1026.

Carroll RC, Beattie EC, von Zastrow M, Malenka RC.(2001) Role of AMPA receptor endocytosis in synaptic plasticity. **Nat Rev Neurosci.** 2:315-324.

Carvalho AL, Kameyama K, Huganir RL.(1999) Characterization of phosphorylation sites on the glutamate receptor 4 subunit of the AMPA receptors. **J Neurosci.** 19:4748-4754.

Caulfield MP.(1993) Muscarinic receptors--characterization, coupling and function. **Pharmacol Ther**.58:319-379.

Caulfield MP, Birdsall NJ.(1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. **Pharmacol Rev.** 50:279-290.

Cavus I and Teyler TJ. (1996) Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. **J Neurophysiol.** 76:3038-3047.

Chavez-Noriega LE, Bliss TV, Halliwell JV.(1989) The EPSP-spike (E-S) component of long-term potentiation in the rat hippocampal slice is modulated by GABAergic but not cholinergic mechanisms. **Neurosci Lett.** 104:58-64.

Chen SJ, Sweatt JD, Klann E.(1997) Enhanced phosphorylation of the postsynaptic protein kinase C substrate RC3/neurogranin during long-term potentiation. **Brain Res.** 749:181-187.

Chetkovich DM, Klann E, Sweatt JD. (1993) Nitric oxide synthase-independent long-term potentiation in area CA1 of hippocampus. **Neuroreport**. 4:919-922.

Chetkovich DM, Sweatt JD.(1993) NMDA receptor activation increases cyclic AMP in area CA1 of the hippocampus via calcium/calmodulin stimulation of adenylyl cyclase. **J Neurochem.** 61:1933-1942.

Cole AE, Nicoll RA.(1983) Acetylcholine mediates a slow synaptic potential in hippocampal pyramidal cells. **Science.** 221:1299-1301.

Consolo S, Bertorelli R, Russi G, Zambelli M, Ladinsky H.(1994) Serotonergic facilitation of acetylcholine release in vivo from rat dorsal hippocampus via serotonin 5-HT3 receptors. **J Neurochem.** 62:2254-2261.

Cormier RJ, Greenwood AC, Connor JA.(2001) Bidirectional synaptic plasticity correlated with the magnitude of dendritic calcium transients above a threshold. **J Neurophysiol**. 85:399-406.

Coyle JT, Price DL, DeLong MR.(1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. **Science**. 219:1184-1190.

Cummings JA, Nicola SM, Malenka RC.(1994) Induction in the rat hippocampus of long-term potentiation (LTP) and long-term depression (LTD) in the presence of a nitric oxide synthase inhibitor. **Neurosci Lett.** 176:110-114.

Cunha RA, Johansson B, Fredholm BB, Ribeiro JA, Sebastiao AM.(1995) Adenosine A2A receptors stimulate acetylcholine release from nerve terminals of the rat hippocampus. **Neurosci Lett.** 196:41-44.

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. **J Neurosci.** 20:4563-4572.

De Boer SF, Koopmans SJ, Slangen JL, Van der Gugten J. (1990) Plasma catecholamine, corticosterone and glucose responses to repeated stress in rats: effect of interstressor interval length. **Physiol Behav.** 47:1117-1124.

Dekker LV, Parker PJ.(1994) Protein kinase C--a question of specificity. **Trends Biochem Sci.** 19:73-77.

Derkach V, Barria A, Soderling TR.(1999) Ca2+/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. **Proc Natl Acad Sci U S A.** 96:3269-3274.

Dobrunz LE, Stevens CF.(1999) Response of hippocampal synapses to natural stimulation patterns. **Neuron.** 22:157-166.

Doyle C, Holscher C, Rowan MJ, Anwyl R.(1996) The selective neuronal NO synthase inhibitor 7-nitro-indazole blocks both long-term potentiation and depotentiation of field EPSPs in rat hippocampal CA1 in vivo.**J Neurosci**16:418-424.

Dudek SM, Bear MF.(1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. **Proc Natl Acad Sci U S A**.89:4363-4367.

Duffy SN, Craddock KJ, Abel T, Nguyen PV.(2001) Environmental enrichment modifies the PKA-dependence of hippocampal LTP and improves hippocampus-dependent memory. **Learn Mem.** 8:26-34.

Dunnett SB.(1985) Comparative effects of cholinergic drugs and lesions of nucleus basalis or fimbria-fornix on delayed matching in rats. **Psychopharmacology** 87:357-363.

Egorov AV, Gloveli T, Muller W.(1999) Muscarinic control of dendritic excitability and Ca(2+) signaling in CA1 pyramidal neurons in rat hippocampal slice. **J Neurophysiol**. 82:1909-1915.

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

Engert F, Bonhoeffer T.(1999) Dendritic spine changes associated with hippocampal long-term synaptic plasticity. **Nature.** 399:66-70.

Evans RH, Smith DA.(1982) Effect of urethane on synaptic and amino acid-induced excitation in isolated spinal cord preparations. **Neuropharmacology**. 21: 857-860.

Felder CC.(1995) Muscarinic acetylcholine receptors: signal transduction through multiple effectors. **FASEB J.** 9:619-625.

Fibiger HC.(1982) The organization and some projections of cholinergic neurons of the mammalian forebrain. **Brain Res**. 257:327-388.

Fisher A, Brandeis R, Karton I, Pittel Z, Gurwitz D, Haring R, Sapir M, Levy A, Heldman E. (1991) (+-)-cis-2-methyl-spiro(1,3-oxathiolane-5,3')quinuclidine, an M1 selective cholinergic agonist, attenuates cognitive dysfunctions in an animal model of Alzheimer's disease. **J Pharmacol Exp Ther**. 257:392-403.

Fisher SA, Fischer TM, Carew TJ.(1997) Multiple overlapping processes underlying short-term synaptic enhancement. **Trends Neurosci.** 20:170-177.

Fortune ES, Rose GJ.(2000) Short-term synaptic plasticity contributes to the temporal filtering of electrosensory information. **J Neurosci.** 20:7122-7130.

Foster TC, McNaughton BL.(1991) Long-term enhancement of CA1 synaptic transmission is due to increased quantal size, not quantal content. **Hippocampus**. 1:79-91.

Francis PT, Palmer AM, Snape M, Wilcock GK.(1999) The cholinergic hypothesis of Alzheimer's disease: a review of progress. **J Neurol Neurosurg Psychiatry.**66:137-147.

Frey, U., Matthies, H., Reyman, K. & Matthies, H. (1991) The effect of dopaminergic D1 blockade during tetanization on the expression of long-term potentiation in the rat CA1 region *in vitro*. **Neurosci Lett** 129: 111-114.

Frey U, Huang YY, Kandel ER.(1993) Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. **Science.** 260:1661-1664.

Frey U, Morris RG.(1997) Synaptic tagging and long-term potentiation. **Nature.** 385:533-536.

Fujii S, Ji Z, Sumikawa K.(2000) Inactivation of alpha7 ACh receptors and activation of non-alpha7 ACh receptors both contribute to long term potentiation induction in the hippocampal CA1 region. **Neurosci Lett.** 286:134-138.

Fujii S, Sumikawa K.(2001) Nicotine accelerates reversal of long-term potentiation and enhances long-term depression in the rat hippocampal CA1 region. **Brain Res.** 2001;894:340-346.

Fukunaga K, Miyamoto E. (2000) A working model of CaM kinase II activity in hippocampal long-term potentiation and memory. **Neurosci Res**. 38:3-17.

Galvan M, Boer R, Schudt C.(1989) Interaction of telenzepine with muscarinic receptors in mammalian sympathetic ganglia. **Eur J Pharmacol**. 167:1-10.

Gasbarri, A., Sulli, A., Innocenzi, R., Pacitti, C. & Brioni, D. (1996) Spatial memory impairment induced by lesion of the mesohippocampal dopaminergic system in the rat. **Neuroscience** 74: 1037-1044.

Giese KP, Fedorov NB, Filipkowski RK, Silva AJ.(1998) Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. **Science**. 279:870-873.

Giovannini MG, Mutolo D, Bianchi L, Michelassi A, Pepeu G.(1994) NMDA receptor antagonists decrease GABA outflow from the septum and increase acetylcholine outflow from the hippocampus: a microdialysis study. **J Neurosci.** 14:1358-1365.

Giovannini MG, Rakovska A, Della Corte L, Bianchi L, Pepeu G.(1998) Activation of non-NMDA receptors stimulates acetylcholine and GABA release from dorsal hippocampus: a microdialysis study in the rat. **Neurosci Lett.** 243:152-156.

Givens BS, Olton DS.(1990) Cholinergic and GABAergic modulation of medial septal area: effect on working memory. **Behav Neurosci.** 104:849-855.

Green EJ, McNaughton BL, Barnes CA.(1990) Exploration-dependent modulation of evoked responses in fascia dentata: dissociation of motor, EEG, and sensory factors and evidence for a synaptic efficacy change. **J Neurosci.**10:1455-1471.

Gribkoff VK, Lum-Ragan JT.(1992) Evidence for nitric oxide synthase inhibitor-sensitive and insensitive hippocampal synaptic potentiation. **J Neurophysiol**. 68:639-642.

Growdon JH(1997)Muscarinic agonists in Alzheimer's disease.**Life Sci.**60:993-998.

Guo JZ, Chiappinelli VA.(2000) Muscarinic receptors mediate enhancement of spontaneous GABA release in the chick brain. **Neuroscience.** 95:273-282.

Haga T, Haga K, Kameyama K, Nakata H.(1993) Phosphorylation of muscarinic receptors: regulation by G proteins. **Life Sci.** 52:421-428.

Hajos N, Papp EC, Acsady L, Levey AI, Freund TF.(1998) Distinct interneuron types express m2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. **Neuroscience.** 82:355-376.

Haley JE, Malen PL, Chapman PF.(1993) Nitric oxide synthase inhibitors block long-term potentiation induced by weak but not strong tetanic stimulation at physiological brain temperatures in rat hippocampal slices. **Neurosci Lett.** 160:85-88.

Haley JE, Schaible E, Pavlidis P, Murdock A, Madison DV.(1996) Basal and apical synapses of CA1 pyramidal cells employ different LTP induction mechanisms. **Learn Mem.** 3:289-295.

Handy RL, Harb HL, Wallace P, Gaffen Z, Whitehead KJ, Moore PK.(1996) Inhibition of nitric oxide synthase by 1-(2-trifluoromethylphenyl) imidazole (TRIM) in vitro: antinociceptive and cardiovascular effects. **Br J Pharmacol.** 119:423-431.

Hanse E, Gustafsson B.(1994) TEA elicits two distinct potentiations of synaptic transmission in the CA1 region of the hippocampal slice. **J Neurosci.** 14:5028-5034.

Hargreaves EL, Cain DP, Vanderwolf CH.(1990) Learning and behavioral-long-term potentiation: importance of controlling for motor activity. **J Neurosci**. 10:1472-1478.

Hasselmo ME.(1995) Neuromodulation and cortical function: modeling the physiological basis of behavior. **Behav Brain Res.** 67:1-27.

Hasuo H, Akasu T, Gallagher JP.(1995) Muscarine increases a voltage-independent potassium conductance through an M4 receptor in rat dorsolateral septal nucleus neurons. **Neurosci Lett.** 189:163-166.

Hasuo H, Akasu T, Gallagher JP.(1996) Muscarine activates a nonselective cation current through a M3 muscarinic receptor subtype in rat dorsolateral septal nucleus neurons. **J Neurophysiol.** 76:2221-2230.

Hayashi Y, Shi SH, Esteban JA, Piccini A, Poncer JC, Malinow R. (2000) Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. **Science.** 287:2262-2267.

Herremans AH, Hijzen TH, Welborn PF, Olivier B, Slangen JL.(1996) Effects of infusion of cholinergic drugs into the prefrontal cortex area on delayed matching to position performance in the rat. **Brain Res.** 711:102-111.

Hersi, A., Rowe, W., Gaudreau, P. & Quirion, R. (1995) Dopamine D1 ligands modulate performance and hippocampal acetylcholine release in memory-impaired aged rats. **Neuroscience** 69: 1067-1074.

Heynen AJ, Abraham WC, Bear MF.(1996) Bidirectional modification of CA1 synapses in the adult hippocampus in vivo. **Nature.** 1996;381:163-166.

Hidaka H, Inagaki M, Kawamoto S, Sasaki Y.(1984) Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. **Biochemistry.** 23:5036-5041.

Hirotsu I, Hori N, Katsuda N, Ishihara T.(1989) Effect of anticholinergic drug on long-term potentiation in rat hippocampal slices. **Brain Res**. 482:194-197.

Hoffman DA, Johnston D.(1999) Neuromodulation of dendritic action potentials. **J Neurophysiol**.81:408-411.

Holscher C, Anwyl R, Rowan MJ.(1997) Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can Be depotentiated by stimulation on the negative phase in area CA1 in vivo. **J Neurosci.** 17:6470-6477.

Honey RC, Watt A, Good M.(1998) Hippocampal lesions disrupt an associative mismatch process. **J Neurosci.** 18:2226-2230.

Hopkins WF, Johnston D.(1984) Frequency-dependent noradrenergic modulation of long-term potentiation in the hippocampus. **Science.** 226:350-352.

Horvitz, J. (2000) Mesolimbic and nigrostriatal dopamine responses to salient non-reward events. **Neuroscience** 96:651-656.

Hoss W, Messer WS Jr, Monsma FJ Jr, Miller MD, Ellerbrock BR, Scranton T, Ghodsi-Hovsepian S, Price MA, Balan S, et al. (1990) Biochemical and behavioral evidence for muscarinic autoreceptors in the CNS. **Brain Res**. 517: 195-201.

Hounsgaard J.(1978) Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus. **Exp Neurol.** 62:787-797.

Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, Monaghan DT, Sacktor TC.(2000) Distinct NMDA Receptor Subpopulations Contribute to Long-Term Potentiation and Long-Term Depression Induction. **J Neurosci** . 20:RC81(1-6).

Hsu KS, Huang CC, Gean PW.(1995) Muscarinic depression of excitatory synaptic transmission mediated by the presynaptic M3 receptors in the rat neostriatum. **Neurosci Lett**. 197:141-144.

Huang XY, Morielli AD, Peralta EG.(1993) Tyrosine kinase-dependent suppression of a potassium channel by the G protein-coupled m1 muscarinic acetylcholine receptor. **Cell**. 75:1145-1156.

Huang YY, Kandel ER.(1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. **Learn Mem.** 1:74-82.

Huang YY, Kandel ER.(1995) D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. **Proc Natl Acad Sci U S A**. 92:2446-2450.

Huerta PT, Lisman JE.(1993) Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. **Nature.** 364:723-725.

Hvalby O, Hemmings HC Jr, Paulsen O, et al.(1994) Specificity of protein kinase inhibitor peptides and induction of long-term potentiation. **Proc Natl Acad Sci U S A.** 91:4761-4765.

Iga Y, Arisawa H, Ise M, Yasuda H, Takeshita Y.(1996) Modulation of rhythmical slow activity, long-term potentiation and memory by muscarinic receptor agonists. **Eur J Pharmacol**. 308:13-19.

Ihalainen, J., Riekkinen, P. & Feenstra, M. (1999) Comparison of dopamine and noradrenaline release in mouse prefrontal cortex, striatum and hippocampus using microdialysis. **Neurosci Lett** 277: 71-74.

Imperato A, Obinu MC, Gessa GL.(1993) Stimulation of both dopamine D1 and D2 receptors facilitates in vivo acetylcholine release in the hippocampus. **Brain Res**. 618:341-345.

Impey S, Obrietan K, Storm DR.(1999) Making new connections: role of ERK/MAP kinase signaling in neuronal plasticity. **Neuron**. 23:11-14.

Impey S, Obrietan K, Wong ST, Poser S, Yano S, Wayman G, Deloulme JC, Chan G, Storm DR. (1998) Cross talk between ERK and PKA is required for Ca2+ stimulation of CREB-dependent transcription and ERK nuclear translocation. **Neuron**. 21:869-883.

Ito T, Miura Y, Kadokawa T.(1988) Physostigmine induces in rats a phenomenon resembling long-term potentiation. **Eur J Pharmacol.** 156:351-359.

Izquierdo I, Medina JH.(1997) Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. **Neurobiol Learn Mem.** 68:285-316.

Jefferys JG, Traub RD, Whittington MA.(1996) Neuronal networks for induced '40 Hz' rhythms. **Trends Neurosci.** 19:202-208.

Jones SV.(1996) Modulation of the inwardly rectifying potassium channel IRK1 by the m1 muscarinic receptor. **Mol Pharmacol**. 49:662-667.

Jouvenceau A, Billard JM, Lamour Y, Dutar P.(1996) Persistence of CA1 hippocampal LTP after selective cholinergic denervation. **Neuroreport.** 7:948-952.

Kameyama K, Lee HK, Bear MF, Huganir RL.(1998) Involvement of a postsynaptic protein kinase A substrate in the expression of homosynaptic long-term depression. **Neuron.** 21:1163-1175.

Kamiya H, Zucker RS.(1994) Residual Ca2+ and short-term synaptic plasticity. **Nature**. 371:603-606.

Kanterewicz BI, Urban NN, McMahon DB, Norman ED, Giffen LJ, Favata MF, Scherle PA, Trzskos JM, 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. **J Neurosci.**20:3057-3066.

Kato K, Zorumski CF.(1993) Nitric oxide inhibitors facilitate the induction of hippocampal long-term potentiation by modulating NMDA responses. **J Neurophysiol.** 70:1260-1263.

Katsuki H, Izumi Y, Zorumski CF.(1997) Noradrenergic regulation of synaptic plasticity in the hippocampal CA1 region. **J Neurophysiol.** 77:3013-3020.

Kemp N, Bashir ZI.(1997) NMDA receptor-dependent and -independent long-term depression in the CA1 region of the adult rat hippocampus in vitro. **Neuropharmacology.** 36:397-399.

Kemp N, Bashir ZI.(1999) Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. **Neuropharmacology**. 38:495-504.

Kemp N, Bashir ZI.(2001) Long-term depression: a cascade of induction and expression mechanisms. **Prog Neurobiol.** 65:339-365.

Kemp N, McQueen J, Faulkes S, Bashir ZI.(2000) Different forms of LTD in the CA1 region of the hippocampus: role of age and stimulus protocol. **Eur J Neurosci**. 12: 360-366.

Kim JJ, Yoon KS. (1998) Stress: metaplastic effects in the hippocampus. **Trends Neurosci**. 21:505-509.

Kinney GG, Patino P, Mermet-Bouvier Y, Starrett JE Jr, Gribkoff VK.(1999) Cognition-enhancing drugs increase stimulated hippocampal theta rhythm amplitude in the urethane-anesthetized rat. **J Pharmacol Exp Ther.** 291:99-106.

Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF.(1999) Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. **J Neurosci**. 19:1599-1609.

Kitaichi K, Day JC, Quirion R.(1999) A novel muscarinic M(4) receptor antagonist provides further evidence of an autoreceptor role for the muscarinic M(2) receptor sub-type. **Eur J Pharmacol.** 383:53-56.

Kleschevnikov AM, Routtenberg A.(2001) PKC activation rescues LTP from NMDA receptor blockade. **Hippocampus**. 11:168-175.

Klink R, Alonso A.(1997) Ionic mechanisms of muscarinic depolarization in entorhinal cortex layer II neurons. **J Neurophysiol**. 77:1829-1843.

Ko GY, Kelly PT. (1999) Nitric oxide acts as a postsynaptic signaling molecule in calcium/calmodulin-induced synaptic potentiation in hippocampal CA1 pyramidal neurons. **J Neurosci.** 19:6784-6794.

Kohler M, Hirschberg B, Bond CT, Kinzie JM, Marrion NV, Maylie J, Adelman JP. (1996) Small-conductance, calcium-activated potassium channels from mammalian brain. **Science.** 273:1709-1714.

Kojima J, Onodera K.(1998) NIK-247 induces long-term potentiation of synaptic transmission in the CA1 region of rat hippocampal slices through M2 muscarinic receptors. **Gen Pharmacol.** 31:297-300.

Koppen A, Klein J, Erb C, Loffelholz K.(1997) Acetylcholine release and choline availability in rat hippocampus: effects of exogenous choline and nicotinamide. **J Pharmacol Exp Ther.** 282:1139-1145.

Kullmann DM, 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.

Larson J, Lynch G.(1986) Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. **Science.** 232:985-958.

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL.(2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. **Nature.** 405:955-959.

Leung LS. (1979) Orthodromic activation of the hippocampal CA1 region in the rat. **Brain Res**. 176:49-63.

Leung, L. (1980) Behavior-dependent evoked potentials in the hippocampal CA1 region of the rat. 1. Correlation with behavior and EEG. **Brain Res.** 198: 95-117.

Levey AI.(1996) Muscarinic acetylcholine receptor expression in memory circuits: implications for treatment of Alzheimer disease. **Proc Natl Acad Sci U S A**. 93:13541-13546.

Levey AI, Edmunds SM, Koliatsos V, Wiley RG, Heilman CJ.(1995) Expression of m1-m4 muscarinic acetylcholine receptor proteins in rat hippocampus and regulation by cholinergic innervation. **J Neurosci.** 15:4077-4092.

Li S, Anwyl R, Rowan, MJ.(2000) A persistent reduction in short-term facilitation accompanies long-term potentiation in the CA1 area in the intact hippocampus. **Neuroscience**, 100:213-220.

Lindstrom J.(1997) Nicotinic acetylcholine receptors in health and disease. **Mol Neurobiol.**15:193-222.

Lisman JE.(1997) Bursts as a unit of neural information: making unreliable synapses reliable. **Trends Neurosci**. 20:38-43.

Lisman, J. & Otmakova, N.(2001) Storage, recall, and novelty detection of sequences by the hippocampus: elaborating on the SOCRATIC model to account for normal and aberrant effects of dopamine. **Hippocampus** 11**:** 551-558.

Liu W, Kumar A, Alreja M.(1998) Excitatory effects of muscarine on septohippocampal neurons: involvement of M3 receptors. **Brain Res.** 805:220-233.

Lledo PM, Hjelmstad GO, Mukherji S, Soderling TR, Malenka RC, Nicoll RA.(1995) Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. **Proc Natl Acad Sci USA**.;92:11175-11179.

Lu YF, Kandel ER, Hawkins RD.(1999) Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. **J Neurosci**. 19:10250-10261.

Luscher C, Nicoll RA, Malenka RC, Muller D.(2000) Synaptic plasticity and dynamic modulation of the postsynaptic membrane. **Nat Neurosci.** 3:545-550.

Ma L, Zablow L, Kandel ER, Siegelbaum SA.(1999) Cyclic AMP induces functional presynaptic boutons in hippocampal CA3-CA1 neuronal cultures. **Nat Neurosci.** 2:24-30.

Majewski H, Iannazzo L. (1998) Protein kinase C: a physiological mediator of enhanced transmitter output. **Prog Neurobiol**. 55(5):463-475.

Malen PL, Chapman PF.(1997) Nitric oxide facilitates long-term potentiation, but not long-term depression. **J Neurosci**. 17:2645-2651.

Malenka RC, Nicoll RA.(1999) Long-term potentiation--a decade of progress? **Science.** 285:1870-1874.

Marino MJ, Rouse ST, Levey AI, Potter LT, Conn PJ.(1998) Activation of the genetically defined m1 muscarinic receptor potentiates N-methyl-D-aspartate (NMDA) receptor currents in hippocampal pyramidal cells. **Proc Natl Acad Sci U S A**. 95:11465-11470.

Markowska AL, Olton DS, Givens B.(1995) Cholinergic manipulations in the medial septal area: age-related effects on working memory and hippocampal electrophysiology. **J Neurosci.** 15:2063-2073.

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, Segal M.(1992) The inositol 1,4,5-trisphosphate pathway mediates cholinergic potentiation of rat hippocampal neuronal responses to NMDA. **J Physiol**. 447:513-533.

Markram H, Tsodyks M.(1996) Redistribution of synaptic efficacy between neocortical pyramidal neurons. **Nature.** 382:807-810.

Marrosu F, Portas C, Mascia MS, Casu MA, Fa M, Giagheddu M, Imperato A, Gessa GL.(1995) Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep-wake cycle in freely moving cats. **Brain Res.** 671:329-332.

Martin SJ, Grimwood PD, Morris RG.(2000) Synaptic plasticity and memory: an evaluation of the hypothesis. **Annu Rev Neurosci**. 23:649-711.

Mathes C, Thompson SH.(1996) The nitric oxide/cGMP pathway couples muscarinic receptors to the activation of Ca2+ influx. **J Neurosci.** 16:1702-1709.

Mayford M, Wang J, Kandel ER, O'Dell TJ. (1995) CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. **Cell.** 81:891-904.

Mayorga AJ, Cousins MS, Trevitt JT, Conlan A, Gianutsos G, Salamone JD. (1999) Characterization of the muscarinic receptor subtype mediating pilocarpine-induced tremulous jaw movements in rats. **Eur J Pharmacol**. 364:7-11.

McDonald BJ, Chung HJ, Huganir RL.(2001) Identification of protein kinase C phosphorylation sites within the AMPA receptor GluR2 subunit. **Neuropharmacology**. 41:672-679.

McEwen BS, Sapolsky RM. (1995) Stress and cognitive function. **Curr Opin Neurobiol.** 5:205-216.

McGaugh JL.(2000) Memory--a century of consolidation. **Science**. 287:248-251.

Meiri N, Sun MK, Segal Z, Alkon DL.(1998) Memory and long-term potentiation (LTP) dissociated: normal spatial memory despite CA1 LTP elimination with Kv1.4 antisense. **Proc Natl Acad Sci U S A.** 95:15037-15042.

Michel AD, Stefanich E, Whiting RL.(1989) Direct labeling of rat M3-muscarinic receptors by [3H]4DAMP. **Eur J Pharmacol**. 166:459-466.

Michel AD, Whiting RL.(1988) Methoctramine, a polymethylene tetraamine, differentiates three subtypes of muscarinic receptor in direct binding studies. **Eur J Pharmacol.** 145:61-66.

Molchan SE, Martinez RA, Hill JL, Weingartner HJ, Thompson K, Vitiello B, Sunderland T. (1992) cognitive sensitivity to scopolamine with age and a perspective on the scopolamine model. **Brain Res Brain Res Rev**. 17:215-226.

Montkowski A, Holsboer F. (1997) Intact spatial learning and memory in transgenic mice with reduced BDNF. **Neuroreport.** 8:779-782.

Morgan SL, Teyler TJ.(1999) VDCCs and NMDARs underlie two forms of LTP in CA1 hippocampus in vivo. **J Neurophysiol**. 82:736-740.

Moser, E., Mathiesen, I. & Andersen, P. (1993a) Association between brain temperature and dentate field potentials in exploring and swimming rats. **Science** 259: 1324-1326.

Moser E, Moser MB, Andersen P.(1993b) Synaptic potentiation in the rat dentate gyrus during exploratory learning. **Neuroreport.** 5:317-320.

Moser EI, Krobert KA, Moser MB, Morris RG.(1998) Impaired spatial learning after saturation of long-term potentiation. **Science.** 281:2038-2042.

Moser EI, Moser MB.(1999) Is learning blocked by saturation of synaptic weights in the hippocampus? **Neurosci Biobehav Rev.** 23:661-672.

Nabekura J, Ebihara S, Akaike N.(1993) Muscarinic receptor activation of potassium channels in rat dentate gyrus neurons. **J Neurophysiol.** 70:1544-1552.

Nelsestuen GL, Bazzi MD.(1991) Activation and regulation of protein kinase C enzymes. **J Bioenerg Biomembr**. 23(1):43-61.

Nishizaki T, Matsuoka T, Nomura T, Matsuyama S, Watabe S, Shiotani T, Yoshii M. (1999) A 'long-term-potentiation-like' facilitation of hippocampal synaptic transmission induced by the nootropic nefiracetam. **Brain Res.** 826:281-288.

Nitz DA, McNaughton BL.(1999) Hippocampal EEG and unit activity responses to modulation of serotonergic median raphe neurons in the freely behaving rat. **Learn Mem**. 6:153-167.

Norman ED, Thiels E, Barrionuevo G, Klann E. (2000) Long-term depression in the hippocampus in vivo is associated with protein phosphatase-dependent alterations in extracellular signal-regulated kinase. **J Neurochem.** 74:192-198.

Norris CM, Korol DL, Foster TC.(1996) Increased susceptibility to induction of long-term depression and long-term potentiation reversal during aging. **J Neurosci.** 16:5382-5392

Ohno M, Yamamoto T, Watanabe S.(1994) Blockade of hippocampal M1 muscarinic receptors impairs working memory performance of rats. **Brain Res** 650:260-266.

O'Dell TJ, Hawkins RD, Kandel ER, Arancio O.(1991) Tests of the roles of two diffusible substances in long-term potentiation: evidence for nitric oxide as a possible early retrograde messenger. **Proc Natl Acad Sci U S A.**88:11285-11289.

Okabe S, Collin C, Auerbach JM, Meiri N, Bengzon J, Kennedy MB, Segal M, McKay RD.(1998) Hippocampal synaptic plasticity in mice overexpressing an embryonic subunit of the NMDA receptor. **J Neurosci**. 18:4177-4188.

Oliet SH, Malenka RC, Nicoll RA. (1997) Two distinct forms of long-term depression coexist in CA1 hippocampal pyramidal cells. **Neuron**. 18:969-982.

Otmakhov N, Griffith LC, Lisman JE.(1997) Postsynaptic inhibitors of calcium/calmodulin-dependent protein kinase type II block induction but not maintenance of pairing-induced long-term potentiation. **J Neurosci.** 17:5357-5365.

Otmakhova NA, Lisman JE.(1996) D1/D5 dopamine receptor activation increases the magnitude of early long-term potentiation at CA1 hippocampal synapses. **J Neurosci.** 16:7478-7486.

Otmakhova NA, Otmakhov N, Mortenson LH, Lisman JE.(2000) Inhibition of the cAMP pathway decreases early long-term potentiation at CA1 hippocampal synapses. **J Neurosci.** 20:4446-4451.

Packard MG, Regenold W, Quirion R, White NM.(1990) Post-training injection of the acetylcholine M2 receptor antagonist AF-DX 116 improves memory. **Brain Res.** 524:72-76.

Paratcha G, Furman M, Bevilaqua L, Cammarota M, Vianna M, de Stein ML, Izquierdo I, Medina JH.(2000) Involvement of hippocampal PKCbetaI isoform in the early phase of memory formation of an inhibitory avoidance learning. **Brain Res.** 855:199-205.

Parkin AJ.(1997) Human memory: novelty, association and the brain. **Curr Biol.** 7:R768-R769.

Paxinos G and Watson C. The rat brain in stereotaxic coordinates, 4th ed., San Diego: London, 1998.

Petersen CC, Malenka RC, Nicoll RA, Hopfield JJ.(1998) All-or-none potentiation at CA3-CA1 synapses. **Proc Natl Acad Sci U S A.** 95:4732-4737.

Pike BR, Hamm RJ.(1995) Post-injury administration of BIBN 99, a selective muscarinic M2 receptor antagonist, improves cognitive performance following traumatic brain injury in rats. **Brain Res.** 686:37-43.

Pittenger, C., Huang, Y., Paletzki, R., Bourtchouladze, R., Scanlin, H., Vronskaya, S. & Kandel, E. (2002) Reversible inhibition of CREB/ATF transcription factors in region CA1 of the dorsal hippocampus disrupts hippocampus-dependent spatial memory. **Neuron** 34: 447-462.

Powell CM, Johnston D, Sweatt JD.(1994) Autonomously active protein kinase C in the maintenance phase of N-methyl-D-aspartate receptor-independent long term potentiation. **J Biol Chem.** 269:27958-27963.

Prast H, Philippu A.(2001) Nitric oxide as modulator of neuronal function. **Prog Neurobiol.** 64:51-68.

Puolivali J, Jakala P, Koivisto E, Riekkinen P Jr.(1998) Muscarinic M1 and M2 receptor subtype selective drugs modulate neocortical EEG via thalamus. **Neuroreport.** 9:1685-1689.

Quirion R, Wilson A, Rowe W, Aubert I, Richard J, Doods H, Parent A, White N, Meaney MJ.(1995) Facilitation of acetylcholine release and cognitive performance by an M2-muscarinic receptor antagonist in aged memory-impaired.**J Neurosci**. 15:1455-1462.

Ramakers GM, McNamara RK, Lenox RH, De Graan PN.(1999) Differential changes in the phosphorylation of the protein kinase C substrates myristoylated alanine-rich C kinase substrate and growth-associated protein-43/B-50 following Schaffer collateral long-term potentiation and long-term depression. **J Neurochem**. 73:2175-2183.

Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, Sweatt JD.(1999) The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. **J Neurosci.** 19:4337-4348.

Roberson ED, Sweatt JD.(1996) Transient activation of cyclic AMP-dependent protein kinase during hippocampal long-term potentiation. **J Biol Chem.** 271:30436-30441.

Roche KW, O'Brien RJ, Mammen AL, Bernhardt J, Huganir RL.(1996) Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. **Neuron.** 16:1179-1188.

Rosenblum K, Futter M, Jones M, Hulme EC, Bliss TV. (2000) ERKI/II regulation by the muscarinic acetylcholine receptors in neurons. **J Neurosci**. 20:977-985.

Rouse ST, Edmunds SM, Yi H, Gilmor ML, Levey AI.(2000a) Localization of M(2) muscarinic acetylcholine receptor protein in cholinergic and non-cholinergic terminals in rat hippocampus. **Neurosci Lett.** 284:182-186.

Rouse ST, Hamilton SE, Potter LT, Nathanson NM, Conn PJ.(2000b) Muscarinic-induced modulation of potassium conductances is unchanged in mouse hippocampal pyramidal cells that lack functional M1 receptors. **Neurosci Lett.** 278:61-64.

Rouse ST, Marino MJ, Potter LT, Conn PJ, Levey AI. (1999) Muscarinic receptor subtypes involved in hippocampal circuits. **Life Sci**. 64:501-509.

Rouse ST, Thomas TM, Levey AI.(1997) Muscarinic acetylcholine receptor subtype, m2: diverse functional implications of differential synaptic localization. **Life Sci.** 60:1031-1038.

Routtenberg A.(1999) Tagging the Hebb synapse. **Trends Neurosci**. 22:255-256.

Rozov A, Burnashev N.(1999) Polyamine-dependent facilitation of postsynaptic AMPA receptors counteracts paired-pulse depression. **Nature.** 401:594-598.

Rozov A, Burnashev N, Sakmann B, Neher E.(2001) Transmitter release modulation by intracellular $Ca2+$ buffers in facilitating and depressing nerve terminals of pyramidal cells in layer 2/3 of the rat neocortex indicates a target cell-specific difference in presynaptic calcium dynamics. **J Physiol**. 531:807-826.

Sah P, Bekkers JM.(1996) Apical dendritic location of slow afterhyperpolarization current in hippocampal pyramidal neurons: implications for the integration of long-term potentiation. **J Neurosci.** 16:4537-4542.

Sandmann J, Peralta EG, Wurtman RJ.(1991) Coupling of transfected muscarinic acetylcholine receptor subtypes to phospholipase D. **J Biol Chem.** 266:6031-6034.

Sanes JR, Lichtman JW.(1999) Can molecules explain long-term potentiation? **Nat Neurosci.** 2:597-604.

Sarter M, Bruno JP, Dudchenko P.(1990) Activating the damaged basal forebrain cholinergic system: tonic stimulation versus signal amplification. **Psychopharmacology** (Berl). 101:1-17.

Scali C, Vannucchi MG, Pepeu G, Casamenti F.(1995) Peripherally injected scopolamine differentially modulates acetylcholine release in vivo in the young and aged rats. **Neurosci Lett**. 197:171-174.

Scanziani M, Gahwiler BH, Thompson SM.(1995) Presynaptic inhibition of excitatory synaptic transmission by muscarinic and metabotropic glutamate receptor activation in the hippocampus:are Ca2+ channels involved? **Neuropharmacology**. 34:1549-1557.

Schinder AF, Berninger B, Poo M.(2000) Postsynaptic target specificity of neurotrophin-induced presynaptic potentiation. **Neuron**.;25(1):151-63.

Schudt C, Auriga C, Birdsall NJ, Boer R.(1988) Does the prolonged occupancy of M1 receptors by telenzepine protect them against the action of vagally released acetylcholine? **Pharmacology.** 37 (Suppl 1):32-39.

Schultz, W. (2000) Multiple reward systems in the brain. **Nature Rev Neurosci, 1:**199-207.

Schultz, W., Apicella, P. & Ljungberg, T. (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. **J Neuroscience** 13:900-913.

Schulz PE, Cook EP, Johnston D.(1994) Changes in paired-pulse facilitation suggest presynaptic involvement in long-term potentiation. **J Neurosci.** 14:5325-5337.

Schuman EM, Madison DV.(1991) A requirement for the intercellular messenger nitric oxide in long-term potentiation. **Science**. 254:1503-1506.

Selig DK, Nicoll RA, Malenka RC.(1999) Hippocampal long-term potentiation preserves the fidelity of postsynaptic responses to presynaptic bursts. **J Neurosci.** 19:1236-1246.

Sharp PE, McNaughton BL, Barnes CA.(1985) Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. **Brain Res**. 339:361-365.

Sheng ZH, Rettig J, Cook T, Catterall WA.(1996) Calcium-dependent interaction of N-type calcium channels with the synaptic core complex. **Nature.** 379:451-454.

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K, Malinow R. (1999) Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. **Science**. 284:1811-1816.

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. **Neurosci Res**. 27:175-180.

Shors TJ, Matzel LD.(1997) Long-term potentiation: what's learning got to do with it? **Behav Brain Sci.** 20:597-614; discussion 614-655.

Siciliano JC, Gelman M, Girault JA.(1994) Depolarization and neurotransmitters increase neuronal protein tyrosine phosphorylation. **J Neurochem**. 62:950-959.

Silva AJ, Stevens CF, Tonegawa S, Wang Y.(1992) Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice.**Science.**257:201-206.

Smart TG.(1997) Regulation of excitatory and inhibitory neurotransmitter-gated ion channels by protein phosphorylation. **Curr Opin Neurobiol.** 7:358-367.

Smith CJ, Court JA, Keith AB, Perry EK.(1989) Increases in muscarinic stimulated hydrolysis of inositol phospholipids in rat hippocampus following cholinergic deafferentation are not parallelled by alterations in cholinergic receptor density. **Brain Res.** 485:317-324.

Soderling TR, Derkach VA. (2000) Postsynaptic protein phosphorylation and LTP. **Trends Neurosci.** 23:75-80.

Sokolov MV, Kleschevnikov AM.(1995) Atropine suppresses associative LTP in the CA1 region of rat hippocampal slices. **Brain Res**. 672:281-284.

Soltoff SP, Toker A.(1995) Carbachol, substance P, and phorbol ester promote the tyrosine phosphorylation of protein kinase C delta in salivary gland epithelial cells. **J Biol Chem**. 270:13490-13495.

Sorra KE, Harris KM.(1993) Occurrence and three-dimensional structure of multiple synapses between individual radiatum axons and their target pyramidal cells in hippocampal area CA1. **J Neurosci**. 13:3736-3748..

Stanton PK. (1995) Transient protein kinase C activation primes long-term depression and suppresses long-term potentiation of synaptic transmission in hippocampus. **Proc Natl Acad Sci USA**. 92:1724-1728.

Stanton PK, Gage AT.(1996) Distinct synaptic loci of Ca2+/calmodulin-dependent protein kinase II necessary for long-term potentiation and depression. **J Neurophysiol.** 76:2097-2101.

Staubli UV, Ji ZX.(1996) The induction of homo- vs. heterosynaptic LTD in area CA1 of hippocampal slices from adult rats. **Brain Res.** 714:169-176.

Steckler T, Sahgal A, Aggleton JP, Drinkenburg WH.(1998) Recognition memory in rats--III. Neurochemical substrates. **Prog Neurobiol.** 54:333-348.

Stillman MJ, Shukitt-Hale B, Galli RL, Levy A, Lieberman HR.(1996) Effects of M2 antagonists on in vivo hippocampal acetylcholine levels.**Brain Res Bull**.41:221-226.

Stillman MJ, Shukitt-Hale B, Kong RM, Levy A, Lieberman HR.(1993) Elevation of hippocampal extracellular acetylcholine levels by methoctramine. **Brain Res Bull**. 32:385-389.

Stratton KR, Worley PF, Huganir RL, Baraban JM.(1989) Muscarinic agonists and phorbol esters increase tyrosine phosphorylation of a 40-kilodalton protein in hippocampal slices. **Proc Natl Acad Sci U S A.** 86:2498-2501.

Suzuki M, Yamaguchi T, Ozawa Y, Ohyama M, Yamamoto M.(1995) Effects of (-)-S-2,8-dimethyl-3-methylene-1-oxa-8-azaspiro[4,5]decane L-tartrate monohydrate (YM796), a novel muscarinic agonist, on disturbance of passive avoidance learning behavior in drug-treated and senescence-accelerated mice. **J Pharmacol Exp Ther**. 275:728-736.

Swanson-Park JL, Coussens CM, Mason-Parker SE, Raymond CR, Hargreaves EL, Dragunow M, Cohen AS, Abraham WC.(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 JD. (1999) Toward a molecular explanation for long-term potentiation. **Learn Mem.** 6:399-416.

Sweatt JD.(2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. **J Neurochem**. 76:1-10.

Tanaka Y, Sakurai M, Hayashi S.(1989) Effect of scopolamine and HP 029, a cholinesterase inhibitor, on long-term potentiation in hippocampal slices of the guinea pig. **Neurosci Lett.** 98:179-183.

Tani Y, Saito K, Imoto M, Ohno T.(1998) Pharmacological characterization of nicotinic receptor-mediated acetylcholine release in rat brain--an in vivo microdialysis study. **Eur J Pharmacol.** 351:181-188.

Thiels E, Barrionuevo G, Berger TW.(1994) Excitatory stimulation during postsynaptic inhibition induces long-term depression in hippocampus in vivo. **J Neurophysiol.** 72:3009-3016.

Thiels E, Norman ED, Barrionuevo G, Klann E.(1998) Transient and persistent increases in protein phosphatase activity during long-term depression in the adult hippocampus in vivo. **Neuroscience**. 86:1023-1029.

Thiels E, Xie X, Yeckel MF, Barrionuevo G, Berger TW.(1996) NMDA receptor-dependent LTD in different subfields of hippocampus in vivo and in vitro. **Hippocampus**. 6:43-51.

Thomas MJ, Moody TD, Makhinson M, O'Dell TJ. (1996) Activity-dependent beta-adrenergic modulation of low frequency stimulation induced LTP in the hippocampal CA1 region. **Neuron.** 17:475-482.

Toni N, Buchs PA, Nikonenko I, Povilaitite P, Parisi L, Muller D.(2001) Remodeling of synaptic membranes after induction of long-term potentiation. **J Neurosci.** 21:6245-6251.

Torocsik A, Vizi ES.(1991) Presynaptic effects of methoctramine on release of acetylcholine. **Neuropharmacology**. 30(3):293-298.

Toullec D, Pianetti P, Coste H, et al. (1991) The bisindolylmaleimide GF 109203X is a potent and selective inhibitor of protein kinase C. **J Biol Chem**. 266:15771-15781.

Traub RD, Whittington MA, Buhl EH, Jefferys JG, Faulkner HJ. (1999) On the mechanism of the gamma --> beta frequency shift in neuronal oscillations induced in rat hippocampal slices by tetanic stimulation. **J Neurosci**. 19:1088-1105.

Tsien JZ, Huerta PT, Tonegawa S.(1996) The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. **Cell.** 87:1327-1338.

Umemori H, Inoue T, Kume S, et al.(1997) Activation of the G protein Gq/11 through tyrosine phosphorylation of the alpha subunit. **Science.** 276:1878-1881.

van der Zee EA, Luiten PG.(1999) Muscarinic acetylcholine receptors in the hippocampus, neocortex and amygdala: a review of immunocytochemical localization in relation to learning and memory. **Prog Neurobiol.** 58:409-471.

Vankov, A., Herve-Minevielle, A. & Sara, S. (1995) Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat. **Eur J Neurosci.** 7:1180-1187.

Vannucchi MG, Pepeu G.(1995) Muscarinic receptor modulation of acetylcholine release from rat cerebral cortex and hippocampus. **Neurosci Lett.** 190:53-56.

Vannucchi MG, Scali C, Kopf SR, Pepeu G, Casamenti F.(1997) Selective muscarinic antagonists differentially affect in vivo acetylcholine release and memory performances of young and aged rats. **Neuroscience.** 79:837-846.

Wagner JJ, Alger BE.(1995) GABAergic and developmental influences on homosynaptic LTD and depotentiation in rat hippocampus.**J Neurosci**.15:1577-1586.

Wagner JJ, Alger BE.(1996) Homosynaptic LTD and depotentiation: do they differ in name only? **Hippocampus**. 6:24-29.

Waite JJ, Chen AD, Wardlow ML, Wiley RG, Lappi DA, Thal LJ.(1995) 192 immunoglobulin G-saporin produces graded behavioral and biochemical changes accompanying the loss of cholinergic neurons of the basal forebrain and cerebellar Purkinje cells. **Neuroscience**. 65:463-476.

Wakamori M, Hidaka H, Akaike N.(1993) Hyperpolarizing muscarinic responses of freshly dissociated rat hippocampal CA1 neurones. **J Physiol**. 463:585-604.

Wall SJ, Yasuda RP, Li M, Ciesla W, Wolfe BB.(1992) Differential regulation of subtypes m1-m5 of muscarinic receptors in forebrain by chronic atropine administration. **J Pharmacol Exp Ther**. 262:584-588.

Wan Y, Kurosaki T, Huang XY. (1996) Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. **Nature**. 380:541-544.

Wang HS, McKinnon D.(1996) Modulation of inwardly rectifying currents in rat sympathetic neurones by muscarinic receptors. **J Physiol.** 492: 467-478.

Wanibuchi F, Nishida T, Yamashita H, Hidaka K, Koshiya K, Tsukamoto S, Usuda S. (1994) Characterization of a novel muscarinic receptor agonist, YM796: comparison with cholinesterase inhibitors in in vivo pharmacological studies. **Eur J Pharmacol.** 265:151-158.

Watabe, A., Zaki, P. & O'Dell, T. (2000) Coactivation of beta-adrenergic and cholinergic receptors enhances the induction of long-term potentiation in the hippocampal CA1 region. **J Neurosci.** 20:5924-5931.

Wenthold RJ, Petralia RS, Blahos J II, Niedzielski AS. (1996) Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. **J Neurosci** 16:1982-1989.

Williams JH, Kauer JA.(1997) Properties of carbachol-induced oscillatory activity in rat hippocampus. **J Neurophysiol**. 78:2631-2640.

Williams JH, Li YG, Nayak A, Errington ML, Murphy KP, Bliss TV.(1993) The suppression of long-term potentiation in rat hippocampus by inhibitors of nitric oxide synthase is temperature and age dependent. **Neuron**. 11:877-884.

Winder DG, Mansuy IM, Osman M, Moallem TM, Kandel ER.(1998) Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. **Cell.** 92:25-37.

Woo NH, Duffy SN, Abel T, Nguyen PV.(2000) Genetic and pharmacological demonstration of differential recruitment of cAMP-dependent protein kinases by synaptic activity. **J Neurophysiol.** 84:2739-2745.

Wu J, Wang Y, Rowan MJ, Anwyl R.(1997) Evidence for involvement of the neuronal isoform of nitric oxide synthase during induction of long-term potentiation and long-term depression in the rat dentate gyrus in vitro. **Neuroscience** 78:393-398.

Wu LG, Saggau P.(1994) Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. **J Neurosci**. 14:645-654.

Wu M, Shanabrough M, Leranth C, Alreja M.(2000) Cholinergic excitation of septohippocampal GABA but not cholinergic neurons: implications for learning and memory. **J Neurosci.** 20:3900-3908.

Xu L, Anwyl R, Rowan MJ. (1997) Behavioural stress facilitates the induction of long-term depression in the hippocampus. **Nature,** 387:497-500.

Xu L, Anwyl R, Rowan MJ.(1998) Spatial exploration induces a persistent reversal of long-term potentiation in rat hippocampus. **Nature.** 394:891-894.

Yang SN, Tang YG, Zucker RS.(1999) Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. **J Neurophysiol**. 81:781-787.

Ye L, Qi J, Qiao J.(2001) Long-term potentiation in hippocampus of rats is enhanced by endogenous acetylcholine in a way that is independent of N-methyl-D-aspartate receptors. **Neurosci Lett**. 300:145-148.

Yu XM, Askalan R, Keil GJ, Salter MW.(1997) NMDA channel regulation by channel-associated protein tyrosine kinase Src. **Science.** 275:674-678.

Yun SH, Cheong MY, Mook-Jung I, Huh K, Lee C, Jung MW. (2000) Cholinergic modulation of synaptic transmission and plasticity in entorhinal cortex and hippocampus of the rat. **Neuroscience.** 97:671-676.

Zakharenko SS, Zablow L, Siegelbaum SA.(2001) Visualization of changes in presynaptic function during long-term synaptic plasticity. **Nat Neurosci.** 4:711-717.

Zamanillo D, Sprengel R, Hvalby O, et al.(1999) Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. **Science.** 284:1805-1811.

Zhuo M, Laitinen JT, Li XC, Hawkins RD.(1998) On the respective roles of nitric oxide and carbon monoxide in long-term potentiation in the hippocampus. **Learn Mem.** 5:467-480.

Zhuo M, Small SA, Kandel ER, Hawkins RD.(1993) Nitric oxide and carbon monoxide produce activity-dependent long-term synaptic enhancement in hippocampus. **Science.** 260:1946-1950.

Zucker RS. Calcium- and activity-dependent synaptic plasticity.(1999) **Curr Opin Neurobiol.** 9:305-313.