SYNAPTIC INTERACTIONS BETWEEN CHOLINERGIC AND GABAERGIC SYSTEMS OF THE HIPPOCAMPUS

LEANNE M. FERRIGAN

A thesis presented for the degree of PhD

Division of Neuroscience and Biomedical Systems

Institute of Biomedical and Life Sciences

University of Glasgow

September

2004

ABSTRACT

γ-aminobutyric acid (GABA) and acetylcholine (ACh) are important regulators of hippocampal function. The hippocampus is governed by a diverse population of GABAergic local circuit neurones (interneurones) and moreover, receives a dual cholinergic and GABAergic input from the medial septal nucleus (MSN). This thesis describes electrophysiological, pharmacological and neuroanatomical approaches in rodent brain slice preparations to investigate the interaction between these cholinergic and GABAergic systems of the mammalian hippocampus.

Intracellular and patch clamp recordings were made from hippocampal pyramidal neurones in hippocampal and septo-hippocampal slice preparations. In the presence of ionotrophic glutamate and GABA receptor antagonists, electrical stimulation of afferent fibres within either the *stratum oriens* or MSN evoked an isolated slow excitatory postsynaptic potential (EPSP), with mean peak amplitudes of 6.109±0.54mV and 8.975±1.123mV and peak latencies of 10.14±2.743s and 10.264±0.95s respectively, in CA1 pyramidal neurones. These evoked slow depolarising synaptic responses were suppressed by the selective muscarinic acetylcholine receptor (mAChR) antagonist atropine (5μM) suggesting that they were mediated via activation of mAChRs.

In order to investigate whether GABAergic interneurones of the hippocampus are modulated by this cholinergic input, similar experiments were carried out in which

whole-cell current clamp recordings were made from hippocampal interneurones located within the *stratum oriens* and *stratum radiatum* of area CA1. In total, 42 out of 59 hippocampal interneurones responded to electrical stimulation within the *stratum oriens* with a general slow depolarisation of mean peak amplitude and peak latency of 7.091±0.521mV and 16.29±0.983s respectively. However, the underlying waveform of these slow depolarisations differed subtly and could be broadly classed as: 1. A slow depolarisation (n=20), similar to that seen in the pyramidal neurones (mean peak amplitude 8.207±0.852mV and peak latency 15.99±1.459s). 2. A "biphasic response" (n=10) where an initial fast hyerpolarisation (–9.011±1.646mV, 1.565±0.2076s) preceded a slower depolarisation (6.288±1.042mV, 19.14±1.954s). 3. A "fast plateau response" (n=10) where an initial fast depolarisation (9.841±1.175mV, 1.025±0.189s) merged with a slower depolarisation (9.076±0.878mV, 15.28±1.738s) and 4. an "oscillatory response" (n=2) where the membrane potential oscillated at a frequency of 1-2Hz. Pharmacological analysis showed that all types of evoked EPSPs in interneurones were sensitive to application of atropine (5μM, n=12) and thus were mediated via activation of mAChRs. A minority of interneurones (17 of 59) did not respond to electrical stimulation within the *stratum oriens* at all. This group was further investigated by bath application of the cholinergic agonist carbachol (10μM) in an attempt to pharmacologically activate mAChRs. Although some of these cells responded with a slow depolarisation (7 of 17) or an oscillatory response (2 of 17) a proportion of these cells remained entirely unresponsive (8 of 17) suggesting that a subpopulation of interneurones are

insensitive to cholinergic modulation, at least with respect to membrane depolarisation. Post-hoc neuroanatomical analysis of recorded cells revealed the majority of the interneurones in this study had a cell body location in either the *stratum oriens* or *stratum radiatum*. *Stratum radiatum* interneurones generally had a stellate-like appearance with dendrites radiating within the dendritic layer of the *stratum radiatum* and the *stratum lacunosum moleculare* (n=24). In contrast all *stratum oriens* cells were bipolar and displayed dendrites, which extended in a horizontal axis parallel with the *stratum oriens* (n=24). The recovery of axonal processes was generally insufficient for full identification of the cells in terms of efferent output selectivity. However, taken overall there was no apparent correlation between the location or dendritic morphology of individual interneurones and the nature of the cholinergic response evoked.

Further extracellular and patch clamp recordings were conducted to investigate the neuromodulatory role of nicotinic acetylcholine receptors (nAChR) within the hippocampus. Field excitatory postsynaptic potentials (fEPSPs) were recorded in both the CA1 and CA3 regions in response to stimulation of Schaffer-collateral and mossy fibre afferents respectively. The selective nAChR agonist, 1,1-dimethyl-4-phenyl-piperazinium iodine (DMPP, 30μM) resulted in a significant increase (30.84±5.98%, n=8, P=0.0078) in the fEPSP slope and amplitude at the mossy fibre-to-CA3 synapse but not at the Schaffer collateral-to-CA1 synapse (-16.99±7.04%, n=5, P=0.125). These data suggest that activation of nAChRs have a potentiating effect on glutamate transmission at the mossy fibre-to-CA3 synapse but not the Schaffer collateral-to-CA1 synapse.

In order to assess whether DMPP was acting pre- or postsynaptically, whole-cell voltage clamp recordings were performed in the presence of the voltage dependent Na^+ channel blocker tetrodotoxin (TTX, 1μM) in order to record miniature spontaneous EPSCs (mEPSCs). 30μM DMPP increased the frequency of mEPSCs from 0.24±0.08Hz to 0.78±0.13Hz (n=4, P=0.0326) but had no significant effect on amplitude (15.35±3.07pA to 12.06±1.5pA n=4, P=0.0473) of mEPSPs recorded in CA3 pyramidal neurones indicating a presynaptic locus of the nAChR responsible.

DMPP was also shown to increase the frequency of spontaneous IPSPs onto CA1 interneurones from 7.48±1.93Hz to 13.05±2.8Hz (n=6, P=0.0403) with no significant effect overall on amplitude (20.42±2.47pA to 23.41±3.04pA, n=6, P=0.0836) thus indicating a potentiation of GABA release within the CA1 region. This increase in frequency was TTX sensitive and therefore is likely to represent an action potential dependent effect, indicating a postsynaptic locus for the nAChR-mediated effect. In a proportion of CA1 interneurones (8 of 14) an inward current (mean amplitude 89.88±26.2pA) was recorded on application of DMPP, which was insensitive to TTX, indicating a direct activation of nAChRs on CA1 interneurones.

In conclusion it seems that the cholinergic modulation of the hippocampus is complex, with mAChR activation exciting pyramidal neurones and certain

interneurones whilst inhibiting other interneurones and suppressing glutamatergic transmission. Conversely, activation of nAChRs enhanced glutamate release at some synapses whilst exciting (inward current) certain interneurones.

DECLARATION

I declare that the work presented in this thesis is entirely my own, with the exception of the results presented in section 5.2.1 and figure 5.1, which were carried out by Shiva Roshan-Milani.

Signature:.....

Leanne M. Ferrigan

This work has not been presented in part or alone for any other degree course. Some of the work contained herein has been published in part: a list follows.

1. Roshan-Milani, S., **Ferrigan, L.**, Koshnood, M.J., Davies, C.H. and Cobb, S. (2003) Regulation of epileptiform activity in hippocampus by nicotinic acetylcholine receptor activation. *Epilepsy research* **56**, 51-65.

2. **Ferrigan, L.,** Roshan-Milani, S., Koshnood, M.J. and Cobb, S.R. (2002) Modulation of epileptiform activity in hippocampal slices by nicotinic acetylcholine receptor activation. *British Journal of Pharmacology*, 137, 134P.

3. Roshan-Milani, S., **Ferrigan,** L. Koshnood, M.J. and Cobb, S. (2002) Activation of nicotinic acetylcholine receptors modulate 4AP-induced epileptiform activity in rat hippocampal slices *FENS* (paris) **146**, 20.

4. **Ferrigan,** L., Khoshnood, M.J. and Cobb, S.R. (2003) Isolation of a slow cholinergic synaptic response in CA1 hippocampal interneurones. *British Neuroscience Association Abstracts* **17**, 37.04P.

5. **Ferrigan, L.,** Khoshnood, M.J. and Cobb, S.R. (2003) Cholinergic afferent-evoked slow synaptic responses in CA1 hippocampal interneurones. *Society for Neuroscience Abstracts* (New Orleans) 247.2.

6. **Ferrigan,** L., Roshan-Milani, S., McNair, K. and Cobb, S.R. (2004) An investigation into the pro-epileptogenic action of nicotinic acetylcholine receptor activation within hippocampal circuits. *Physiological Society Abstracts* 557P.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank my supervisor Dr Stuart Cobb for his constant support, guidance and patience over the past four years. Thank you for sharing your knowledge and expertise and providing a constant source of encouragement and inspiration.

Thank you to everyone who has worked in and frequented lab 313 over the years. To Shiva, Ed, Helen, Jane and Eion thank you all for your help, advice, encouragement and friendship. Special thanks to my constant companions Melissa, Kara and Andrew, thank you for making the past four years a lot of fun, for giving me some great laughs and good memories that I will never forget.

Thank you to my family, Mum, Dad, Shelia, Louise, Steve, Conor, Morven and Matthew I love you all and owe you so much for the support you have given me throughout the years.

I owe a special thanks to Marcus you are my best friend and a great person. Thank you for always believing in me.

ABBREVIATIONS

4-DAMP	*N,N*-dimethyl-4-piperindinyl diphenylacetate
4-AP	4-aminopyridine
5-HT	serotonin
α-BgTx	alpha bungarotoxin
AC	adenylate cyclase
ACh	acetylcholine
AChE	acetylcholinesterase
aCSF	artificial cerebrospinal fluid
AD	Alzheimer's Disease
AFDX 116	11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11,-dihydro-6H-pyrido[2,3b][1,4]benzodiazepin-6-one
AHP	after hyperpolarisation
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolpropinoic acid
ATP	adenosine triphosphate
BuChE	butyrylcholinesterase
Ca^{2+}	calcium
CACA	*cis*-4-aminocrotonic acid
cAMP	cyclic adenosine 5'-monophosphate
CB	calbindin
CCh	carbachol
CCK	cholecystokinin

CGP 40116	D-(E)-2-amino-4-methyl-5phosphono-3-pentanoic acid
CGP 55845A	3-*N*-[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl-*P*-benzyl-phosphinic acid
ChAT	choline acetyltransferase
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
CR	Calretinin
DAG	diacylglycerol
DC	direct current
DMPP	1,1-dimethyl-4-phenyl-piperazium iodine
EPSC	excitatory postsynaptic current
EPSP	excitatory postsynaptic potential
EPSPm	muscarinic acetylcholine receptor-mediated EPSP
fEPSP	field excitatory postsynaptic potential
GABA	γ-aminobutyric acid
GAD	glutamate decarboxylase
GAT	GABA transporters
G-protein	guanine nucleotide binding protein
I_A	fast inactivating A current
I_{AHP}	slow calcium activated K^+ current
$I_{K(LEAK)}$	leak K^+ current
I_M	M current
IP_3	inositol 1,3,5-triphosphate
IPSC	inhibitory postsynaptic current

IPSP	inhibitory postsynaptic potential
IS cell	Interneurone selective cell
K^+	potassium
mAChR	muscarinic acetylcholine receptor
mEPSC	miniature spontaneous excitatory postsynaptic current
mGluR	metabotrophic glutamate receptor
mIPSC	miniature spontaneous inhibitory postsynaptic current
MSN	medial septal nucleus
Na^+	sodium
nAChR	nicotinic acetylcholine receptor
NBQX	6-nitro-7-sulphamoybenzo[*f*]quinoxaline-2,3-dione
NMDA	*N*-methyl-D-aspartate
NPY	neuropeptide Y
PLC	Phospholipase C
PNS	peripheral nervous system
PV	parvalbumin
sADP	slow afterdepolarising potentials
S.E.M	standard error of the mean
SFA	spike frequency adaptation
sIPSC	spontaneous inhibitory postsynaptic currents
SLM	*stratum lacunosum moleculare*
SM	*stratum moleculare*
SO	*stratum oriens*
SOM	somatostatin

SP	*stratum pyramidale*
SR	*stratum radiatum*
TLE	temporal lobe epilepsy
TTX	tetrodotoxin
VDCC	voltage dependent calcium channels

CONTENTS

Abstract..... ii

Declaration.....vii

Acknowledgements.....ix

Abbreviations.....x

Contents.....xiv

1. INTRODUCTION.....1

1.1.General Introduction.....1

1.2. The cholinergic system.....2

1.2.1. Acetylcholine.....2

1.2.2. Cholinergic receptors.....2

1.2.2.1. Nicotinic acetylcholine receptors.....3

1.2.2.2. Muscarinic acetylcholine receptors.....6

1.3. The GABAergic system.....9

1.3.1. GABA.....9

1.3.2. GABAergic receptors.....10

1.3.2.1. $GABA_A$ receptors.....10

1.3.2.2. $GABA_B$ receptors.....12

1.3.2.3. $GABA_C$ receptors.....15

1.4. The Glutamatergic System.....16

1.4.1. Glutamate 16

1.4.2. Glutamatergic receptors 17

1.4.2.1. AMPA receptors 18

1.4.2.2. Kainate receptors 20

1.4.2.3. NMDA receptors 21

1.4.2.4. Metabotrophic glutamate receptors 24

1.5. The hippocampus 26

1.5.1. General 26

1.5.2. Anatomy 29

1.5.3. Cytology 32

1.5.3.1. Principal cells 32

1.5.3.2. GABAergic interneurones 37

1.6. The Septo-Hippocampal Projection 43

1.7. Cholinergic input 46

1.7.1. General 46

1.7.2. Actions of acetylcholine on hippocampal neurones 46

1.7.2.1. Cholinergic responses in Principal cells 46

1.7.2.1.1. Postsynaptic actions-mAChRs 47

1.7.2.1.2.Postsynaptic action-nAChRs 51

1.7.2.1.3.Presynaptic actions altering transmitter release 52

1.7.2.2. Cholinergic responses in Interneurones 54

1.7.2.2.1. Postsynaptic actions-mAChR 55

1.7.2.2.2. Postsynaptic actions-nAChR....58

1.7.2.2.3 Pre- and postsynaptic actions altering transmitter release....60

1.8. Summary....63

1.9. Aims....65

2. GENERAL METHODS....66

2.1. Slice Preparation....66

2.1.1. Hippocampal slices for traditional intracellular an extracellular recordings66

2.1.2. Hippocampal slices for patch clamp recordings....68

2.1.3. Septo-hippocampal slice preparation....69

2.2. Electrophysiology....71

2.2.1. Recording set-up....71

2.2.1.1. Types of Recording chambers....74

2.2.1.2. Recording & stimulation electrodes....76

2.2.2. Recording Configurations....78

2.2.2.1. Intracellular current clamp recordings....78

2.2.2.2. Extracellular field recordings....80

2.2.2.3. Patch clamp whole-cell recording....81

2.3. Data Display and Storage....85

2.4. Data Analysis....85

2.5. Biocytin/ Neurobiotin Labelling....86

2.6. Confocal Microscopy **87**

2.7. Drugs **88**

3. ISOLATION OF SLOW CHOLINERGIC RESPONSES IN CA1 PYRAMIDAL NEURONES BY STIMULATION OF CHOLINERGIC AFFERENTS **90**

3.1. Introduction **90**

3.2. Results **92**

3.2.1. Characterisation of CA1 pyramidal neurones 92

3.2.2. Fast amino acid synaptic transmission 95

3.2.3. Stimulation evoked slow EPSPs 97

3.2.4. Stimulation parameters 100

3.2.5. Carbachol evoked slow EPSPs 105

3.2.6. Muscarinic nature of the slow EPSP 105

3.2.7. Septo-hippocampal slices 109

3.3. Discussion **119**

3.3.1. The slow EPSPm 119

3.3.2. Integrity of the septo-hippocampal projection 124

3.4. Summary **126**

4. ISOLATION OF SLOW CHOLINERGIC RESPONSES IN CA1 GABAERGIC INTERNEURONES **127**

4.1. Introduction **127**

4.2. Results **129**

4.2.1. Verification of the septo-hippocampal projection 129

4.2.2. Characterisation and identification of cell types. 132

4.2.3. Evoked cholinergic responses 135

4.2.4. The interneurone response 141

4.2.5. Stimulation parameters 143

4.2.6. Carbachol. 149

4.2.7. Input resistance 150

4.2.8. Diversity of responses in interneurones 154

4.2.9. Location and morphology of interneurones 161

4.3. Discussion **165**

4.3.1. Verification of the septo-hippocampal projection 165

4.3.2. Evoked cholinergic responses 165

4.3.3. Diversity of the interneurone response 171

4.4. Summary **177**

5. INVESTIGATION OF THE ACTION OF NICOTINIC ACETYLCHOLINE RECEPTOR AGONISTS ON GLUTAMATE AND GABA RELEASE WITHIN THE HIPPOCAMPUS **178**

5.1. Introduction **178**

5.2. Results **180**

5.2.1. Proepileptogenic action of nAChR agonists in the hippocampus. 180

5.2.2. Effect of nAChR agonists on field EPSPs. 184

5.2.3. Effects of DMPP on Mini EPSCs. 190

5.2.4. Effects of DMPP on spontaneous GABA transmission 197

5.3. Discussion 210

5.3.1. Excitatory transmission 210

5.3.2. Inhibitory transmission 215

5.3.3. Physiological relevance 218

5.4. Summary 222

6. GENERAL DISCUSSION 223

6.1. Muscarinic acetylcholine receptor synaptic responses 223

6.2. Neuromodulatory role of nicotinic acetylcholine receptors 228

6.3. Summary 229

6.4. Future research 231

6.5. Therapeutic implications 231

7. REFERENCES 233

LIST OF TABLES AND FIGURES

Table 1.1 Types of dendritic inhibitory cells. 41

Table 2.1 Filling solutions and final d.c. resistance of recording electrodes. 77

Table 3.1 General properties of pyramidal neurones recorded in hippocampal slices. 94

Table 3.2 General properties of rat pyramidal neurones recorded from septo-hippocampal slices. 111

Table 3.3 General Properties of mouse pyramidal neurones recorded from the septohippocampal slice preparation.. 114

Table 4.1 Comparison of CA1 pyramidal and interneurone properties. 135

Table 4.2 Comparison of evoked depolarisations in CA1 interneurones and CA1 pyramidal neurones. 141

Table 4.3 Individual properties of each mAChR-mediated response type seen in CA1 interneurones. 160

Table 4.4 Distribution of muscarinic response types with stratum oriens and stratum radiatum of the CA1. 162

Table 5.1 Summary of the effect of DMPP on spontaneous and miniature currents. 208

Table 6.1 Summary of the cholinergic modulation of hippocampal systems. ...230

Figure 1.1 The Hippocampal Formation. 31

Figure 1.2 Pyramidal neurone structure 34

Figure 1.3 Schematic diagram of the trisynaptic circuit 36

Figure 2.1 Orientation for slicing 67

Figure 2.2 Orientation for septo-hippocampal slices 70

Figure 2.3 Flow chart of recording setup 72

Figure 2.4 Recording setup 73

Figure 2.5 Types of recording chambers. 75

Figure 2.6 Blow and seal Patch-clamp technique 84

Figure 3.1 Characterisation of CA1 pyramidal neurones 93

Figure 3.2 Inhibition of fast amino acid synaptic transmission in CA1 pyramidal neurones. 96

Figure 3.3 The Slow EPSP evoked by electrical stimulation within the *stratum oriens.* 98

Figure 3.4 Effect of the cholinesterase inhibitor eserine on the slow EPSP. 99

Figure 3.5 Consistent slow EPSPs can be evoked using a 5-minute stimulus interval. 101

Figure 3.6 Slow EPSPs evoked by delivery of a 20Hz train of stimuli within the *stratum oriens.* 102

Figure 3.7 Effect of stimulation intensity on the slow EPSP. 104

Figure 3.8 A comparison of the effects of carbachol and electrical stimulation within the stratum oriens. 106

Figure 3.9 Effect of the selective mAChR antagonist atropine on the slow EPSP. 107

Figure 3.10 The slow EPSP is inhibited by the selective mAChR antagonist atropine. 108

Figure 3.11 Effect of the selective mAChR antagonist pirenzepine on the slow EPSP 110

Figure 3.12 Isolation of a slow EPSP in CA1 pyramidal neurones using the septo-hippocampal slice preparation 113

Figure 3.13 Slow EPSPs evoked by electrical stimulation of the MSN in both rat and mouse slices 115

Figure 3.14 Atropine abolishes the slow EPSP evoked by MSN stimulation 117

Figure 3.15 Comparison of cholinergic responses evoked by MSN and *stratum oriens* stimulation 118

Figure 4.1 Structure of the septo-hippocampal (S-H) projection revealed by dual anticholine acetyltransferase (ChAT) and anti-parvalbumin immunolabelling. 131

Figure 4.2 Comparison of a CA1 pyramidal neurone and a CA1 interneurone. 133

Figure 4.3 Isolation of a slow excitatory postsynaptic potential in CA1 interneurones. 137

Figure 4.4 Quantification of the depolarisation recorded in CA1 hippocampal interneurones. 139

Figure 4.5 Isolation of cholinergic responses in CA1 pyramidal neurones in the whole-cell configuration. 140

Figure 4.6 mAChR-mediated responses in CA1 interneurones in the presence and absence of eserine. 144

Figure 4.7 Potentiation of the slow EPSP when evoked by a train of stimuli. .. 145

Figure 4.8 Potentiation of the slow EPSP on increasing stimulation intensity. . 147

Figure 4.9 Effect of stimulus frequency on evoked slow cholinergic responses in CA1 interneurones. 148
Figure 4.10 CA1 interneurone responses to carbachol application. 151
Figure 4.11 Assessment of input resistance changes during carbachol induced depolarisations. 152
Figure 4.12 Assessment of input resistance changes during stimulus-evoked depolarisations. 153
Figure 4.13 Diversity of the cholinergic response in CA1 interneurones. 155
Figure 4.14 Effect of atropine on both components of the "delayed" response. 157
Figure 4.15 Effect of atropine on both components of the "fast plateau" responses. 159
Figure 4.16 Example of filled interneurones with associated electrophysiology. 164
Figure 5.1 Pro epileptogenic action of nAChR agonists 183
Figure 5.2 Effect of DMPP on field excitatory postsynaptic potentials (fEPSP) evoked in the hippocampal slice preparation. 186
Figure 5.3 Effect of cholinergic ligands on fEPSP in the CA1 region of the hippocampus. 188
Figure 5.4 Spontaneous and miniature excitatory postsynaptic currents recorded from CA3 pyramidal neurones. 191
Figure 5.5 Effect of DMPP on miniature EPSC (mEPSCs) in CA3 pyramidal neurones. 193
Figure 5.6 Effect of DMPP on mEPSCs recorded from CA3 interneurones. 194
Figure 5.7 Effect of DMPP on mEPSCs recorded in CA1 pyramidal neurones. 195
Figure 5.8 Effect of DMPP on mEPSCs recorded in CA1 interneurones. 196

Figure 5.9 Effect of DMPP on spontaneous IPSCs (sIPSCs) in CA1 pyramidal neurones. 199

Figure 5.10 Effect of DMPP on sIPSCs in CA1 interneurones. 200

Figure 5.11 Spontaneous and miniature inhibitory postsynaptic currents recorded from CA1 interneurones. 202

Figure 5.12 Effect of DMPP on miniature IPSCs (mIPSCs) recorded in CA1 interneurones. 203

Figure 5.13 Effect of DMPP on mIPSCs recorded in CA1 pyramidal neurones. 205

Figure 5.14 Effect of DMPP on mIPSC recorded in CA3 interneurones 206

Figure 5.15 Effect of DMPP on mIPSCs recorded in CA3 pyramidal neurones. 207

Figure 5.16 Summary of nAChR location within the hippocampus. 209

Figure 6.1. Summary of evoked cholinergic responses in pyramidal neurones and interneurones in area CA1 of the hippocampus. 225

1. INTRODUCTION

1.1. General Introduction

Neurones are the building blocks of the central and peripheral nervous systems (CNS & PNS). In order for the nervous system to function effectively neurones must be able to communicate. Neurones communicate with each other at specialised junctions known as synapses where signalling takes place through chemical or direct electrical transmission in a process known as synaptic transmission. Signals transmitted from one neurone (presynaptic neurone) can lead to excitation or inhibition in the target neurone (postsynaptic neurone) through the modulation of a plethora of ion channels. Whether a neurone exerts an inhibitory or excitatory effect on the postsynaptic neurone depends on the nature of the neurotransmitter released from its terminal (presynaptic terminal) as well as the postsynaptic receptors present on the target cell.

The process of synaptic transmission between individual neurones is subject to modulation by other neurotransmitter systems by both pre- and postsynaptic mechanisms. This thesis aims to deal with the cholinergic modulation of synaptic transmission within the mammalian hippocampus, in particular the cholinergic modulation of GABAergic transmission. This introductory chapter will therefore serve to provide a brief overview of all relevant neurotransmitter systems. It will also introduce the hippocampus, summarising the major architecture, cell types

and cholinergic input, in addition to providing a detailed review of the present literature on the cholinergic modulation of hippocampal systems.

1.2. THE CHOLINERGIC SYSTEM

1.2.1. Acetylcholine

Acetylcholine was first described back in 1906 (Hunt & Taveau, 1906) and was the first substance shown to be a neurotransmitter (Dale, 1934; Dale *et al.*, 1936; Dale, 1938). Chemically it is composed of choline and an acetyl group, synthesised in the axon terminal by the enzyme choline acetyltransferase (ChAT) where it is subsequently taken up into vesicles for storage and release. It is released from presynaptic terminals to act on postsynaptic acetylcholine receptors generating an excitatory postsynaptic response that can be detected as excitatory voltage (EPSP) or current deflections (EPSC). The response is terminated when acetylcholine is broken down by the enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) back to choline and acetate.

1.2.2. Cholinergic receptors

Receptors for acetylcholine were originally classified by Dale (1914; 1938) as either nicotinic or muscarinic due to the separate actions of the plant alkaloids nicotine and muscarine. Each class of receptor is structurally and functionally distinct. Nicotinic acetylcholine receptors (nAChRs) belong to the superfamily of ligand-gated ion channel (ionotrophic) receptors, whereas muscarinic

acetylcholine receptors (mAChRs) are members of the guanine nucleotide-binding protein (G-protein) coupled (metabotrophic) receptor family.

1.2.2.1. Nicotinic acetylcholine receptors

nAChRs are the archetypal ligand-gated ion channel discovered over 90 years ago (Langley, 1907). They have since been subject to intense study and as a result have been purified, cloned and sequenced to elucidate their structure and function. nAChRs are present at both peripheral (neuromuscular junction and autonomic ganglia) and central synapses. In the periphery nAChRs modulate fast excitatory synaptic transmission at both the neuromuscular junction and autonomic ganglia whereas as in the CNS nAChR have been implicated in a more long-lasting neuromodulatory role in addition to their role in fast excitatory synaptic transmission (Wonnacott, 1997). nAChRs can now be further classified into three subfamilies (Clementi *et al.*, 2000a):

1) **Muscle nAChR** (neuromuscular junction).
2) **Neuronal nAChR- α-Bungarotoxin sensitive** (CNS).
3) **Neuronal nAChR- α-Bungarotoxin insensitive** (autonomic ganglia and CNS).

All members of the ligand-gated ion channel superfamily are believed to have a pentameric architecture and nAChRs are no exception (Cooper *et al.*, 1991; Unwin, 1995). Each receptor is made up of 5 separate subunits arranged within the plasma membrane to form a central aqueous cation channel (Unwin, 1995).

Agonist binding induces a conformational change, which leads to the direct opening of the ion channel and resultant ion (K^+ and Na^+) flux. Selective agonists for nAChRs include nicotine and DMPP with classical antagonists including the competitive antagonist tubocurarine known since Langley's original studies (1907) and the non-competitive antagonist mecamylamine.

Although most early work on the structure was conducted on the muscle, nAChR attention has now turned towards the neuronal types. Seventeen nAChRs subunit genes have now been cloned (Boulter *et al.*, 1986; Boulter *et al.*, 1987; Nef *et al.*, 1988; Couturier *et al.*, 1990a; Couturier *et al.*, 1990b; McGehee & Role, 1995; Sharples & Wonnacott, 2001). These genes encode for the seventeen nAChR subunits, α1-10, β1-4, γ, δ and ε, with two α1, one β1, δ and γ or ε making up the foetal and adult muscle types respectively (Mishina *et al.*, 1986). All remaining α and β subunits are expressed in numerous combinations to make up the large diversity of neuronal nAChRs subtypes. Subunit composition of the receptor is an important factor in conferring individual characteristics such as desensitisation rate, open and closure time, ion channel permeability and agonist and antagonist sensitivity (Deneris *et al.*, 1991; Alkondon & Albuquerque, 1993; Papke, 1993; McGehee & Role, 1995). The α-Bungarotoxin (α-BgTx) insensitive subfamily of nAChRs are composed of α2-6 subunits in a 2:3 stoichiometry with β2-4 subunits, whereas α7-9 subunits can form homomeric receptors which make up the α-BgTx sensitive subfamily (Couturier *et al.*, 1990a; Gerzanich *et al.*, 1994).

Choline, a precursor and one of the breakdown products of acetylcholine has recently been shown to be a selective agonist for the α7-containing subtype (Papke *et al.*, 1996; Alkondon *et al.*, 1997b). This receptor shows distinctive features such as high calcium permeability, high single channel conductance and rapid desensitisation kinetics (Seguela *et al.*, 1993). Selective antagonists for this receptor include αBgTx (Couturier *et al.*, 1990a) and methylcaconatine (Wonnacott *et al.*, 1993).

Binding studies with nAChR agonists have shown that nAChRs are located in numerous areas of the mammalian brain including cortical areas, the periaquaductal grey matter, the basal ganglia, the thalamus, the hippocampus, the cerebellum and the retina (Gotti *et al.*, 1997). Due to the large number of nAChR subunits, a large number of heteromeric subtypes may exist but the predominant forms expressed in the CNS are thought to include α4β2, α4α5β2, and α7, with α3β4 and α3β4α5 predominant in PNS (Clementi *et al.*, 2000b) . Use of the reverse transcription polymerase chain reaction (RT-PCR) technique has indicated the presence of α2, α3, α4, α5, α7, β2, β3 and β4 subunits within the hippocampal formation (Sudweeks &Yakel 2000, Jones *et al.*, 1999). Specific functional subtypes known to be expressed in the hippocampus include the α4β2 and α7 containing receptors (Alkondon & Albuquerque, 1993; Alkondon *et al.*, 1998; Alkondon *et al.*, 1999). These functional subtypes were identified using electrophysiological recording techniques in conjunction with a range of subtype selective antagonists. Various nAChR-mediated responses were recorded from

hippocampal neurones in both cultures and the acute slice preparation. The various nAChR-mediated responses were individually isolated by subtype selective antagonists such as αBgTx indicating the presence of α7-containing receptors and the selective antagonist dihydro-β-erythroidine indicating functional α4β2 receptors. Recent studies on the α7-containing subtype of nAChRs have shown that they are present at both pre- and postsynaptic sites at almost all synapses in the *stratum radiatum* and that they show the highest levels on the GABAergic interneurones (Fabian-Fine *et al.*, 2001; Kawai *et al.*, 2002)

1.2.2.2. Muscarinic acetylcholine receptors

mAChRs are G-protein coupled receptors in that they transduce their intracellular signal via coupling to guanine nucleotide-binding proteins (Hulme *et al.*, 1990). This superfamily of receptors has a characteristic structure consisting of one polypeptide chain formed into seven hydrophobic transmembrane-spanning domains joined by intracellular and extracellular loops. Agonist binding triggers a conformational change, which leads to the activation of specific G-proteins. The activated G-protein then stimulates or inhibits one of many effector systems. The ultimate outcome of agonist binding depends on the type of G-protein activated and the effector system targeted. Due to this additional step of G-protein activation mAChRs mediate slower neurotransmission than the nicotinic ligand-gated ion channels. Selective agonists for mAChRs include muscarine and pilocarpine, with the classical antagonist atropine.

Originally only two types of mAChR subtypes were defined based on the affinity for the antagonist pirenzepine (Hammer *et al.*, 1980). These were defined as M1 and M2. However molecular cloning techniques eventually lead to the identification of five distinct mAChR genes (m1, m2, m3, m4 and m5). These genes encode for 5 subtypes of mAChR termed M1-M5 (Kubo *et al.*, 1986; Bonner *et al.*, 1987; Bonner *et al.*, 1988). Clear pharmacological classification of mAChR subtypes has remained difficult to achieve due to limited selectivity of agonists, although certain antagonists can show some degree of selectivity, for example pirenzepine shows some selectivity for the M1 subtype, ADFX 116 shows some selectivity for the M2 subtype and *N,N*-dimethyl1-4-piperidinyl diphenylacetate (4-DAMP) can show selectivity for both M1 and M3 (Hammer *et al.*, 1980; Giachetti *et al.*, 1986; Hammer *et al.*, 1986; Doods *et al.*, 1987b). More recently toxins isolated from the green mamba snake venom have been shown to exhibit selectivity for certain mAChR subtypes including M1, M2 and M4 (Max *et al.*, 1991, 1993a; Max *et al.*, 1993b; Liang *et al.*, 1996; Carsi *et al.*, 1999). These toxins have proved useful for functional studies as subtype selective antagonists (Cuevas & Adams, 1997; Marino *et al.*, 1998).

In general M1, M3 and M5 mAChR subtypes predominantly couple to G_q/G_{11} class of G-protein leading to activation of the enzyme Phospholipase C (PLC) and thus stimulation of phosphoinositol hydrolysis to release the intracellular messengers inositol triphosphate (IP3) and diacylglycerol (DAG) (Bonner *et al.*, 1988; Peralta *et al.*, 1988; Hulme *et al.*, 1990; Felder, 1995). M2 and M4 mAChR subtypes predominantly couple to the G_i/G_o class of G-protein, which leads to the

inhibition of the enzyme adenylate cyclase (AC) and a decrease of the intracellular messenger cyclic adenosine 5' monophosphate (cAMP) (Peralta *et al.*, 1988; Hulme *et al.*, 1990; Felder, 1995). The ultimate outcome of mAChR activation depends on the cell type examined. However in excitable cells, such as muscle and neurones, mAChR activation can lead to the modulation of K^+ ion channels, voltage dependent calcium channels and non-selective cation currents via direct or indirect mechanisms (Caulfield, 1993).

All five subtypes of mAChR have been localised in the hippocampus, cortex, striatum, olfactory tubercle, thalamus and basal forebrain (Buckley *et al.*, 1988; Brann *et al.*, 1993). However mAChR proteins have differential cellular distributions within individual regions of the brain. Levey and colleagues (1995) used subtype specific antibodies to localise m1-m4 receptor proteins within the hippocampal formation by immunocytochemistry. These studies revealed that m1 and m3 receptor proteins were primarily expressed in pyramidal neurones and dentate granule cells and that m2 and m4 receptor proteins were primarily expressed in non-pyramidal neurones. m2 receptor proteins also showed a striking localisation in fibres and puncta surrounding pyramidal neurones (Levey *et al.*, 1995). A further immunohistochemical study showed that hippocampal interneurones show differential expression of m2 receptors, with the perisomatic inhibitory cells expressing m2 receptors only on their terminals and dendritic inhibitory cells expressing m2 receptors on their soma and dendrites (Hájos *et al.*, 1998). The study by Levey and Colleagues (1995) further investigated which mAChR receptor proteins were expressed by septo-hippocampal projection fibres.

192-igG immunotoxin lesions of the cholinergic septo-hippocampal projection produced a decrease in m2-immunoreactive fibres within the hippocampus and thus indicated that a subset of m2 receptors are presynaptically localised in cholinergic axons of the septo-hippocampal pathway (Levey *et al.*, 1995). Further immunocytochemical studies by Rouse and Colleagues in 1999 showed that the vesicular acetylcholine transporter (vAChT), a protein found exclusively in cholinergic synaptic vesicles co-localised with the m2 receptor protein. Electron microscopic examination of this tissue showed m2 receptors to be located presynaptically in cholinergic axon terminals throughout the hippocampal formation.(Rouse *et al.*, 1999).

1.3. THE GABAERGIC SYSTEM

1.3.1. GABA

The neutral amino acid γ-aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the adult CNS. GABA is synthesised from glutamate in neurones expressing the enzymes Glutamate Decarboxylase 65/67 (GAD). It is released from presynaptic terminals to act on postsynaptic GABA receptors generating an inhibitory postsynaptic response that can be detected as voltage (IPSP) or current (IPSC) deflections. Termination of action is due to GABA uptake into neurones and surrounding glial cells by GABA transporters (GAT1-3) and the subsequent breakdown of GABA by transaminase to form succinic acid semialdehyde (Cherubini & Conti, 2001).

1.3.2. GABAergic receptors

Early work with the neurotransmitter GABA indicated that it produced inhibitory hyperpolarising postsynaptic responses in neurones (Krnjevic & Schwartz, 1967), which could be blocked by the alkaloid bicuclline (Curtis *et al.*, 1970). During the 1970s Bowery and colleagues showed that GABA could also act to inhibit noradrenaline release in the heart (Bowery & Hudson, 1979; Bowery *et al.*, 1981). However this response was not blocked by bicuculline and ultimately lead to the classification of two pharmacologically distinct GABA receptors named $GABA_A$ and $GABA_B$ (Hill & Bowery, 1981). This novel $GABA_B$ receptor was also shown to be present in the brain (Bowery *et al.*, 1980; Bowery *et al.*, 1987) and was activated by the specific agonist baclofen (Bowery *et al.*, 1980; Hill & Bowery, 1981). However work with conformationally restricted analogues of GABA had identified a bicuculline insensitive receptor (Johnston *et al.*, 1975). These analogues were later shown not to effect binding of the $GABA_B$ agonist baclofen and as such a third subclass of GABA receptor was suggested and named $GABA_C$ (Drew *et al.*, 1984).

1.3.2.1. $GABA_A$ receptors

$GABA_A$ receptors mediate most of the fast inhibitory synaptic transmission in the CNS. They are responsible for the membrane hyperpolarisation corresponding to the fast IPSP seen on afferent stimulation and belong to the ligand-gated ion channel superfamily (Schofield *et al.*, 1987). As with all others members of this superfamily they have a pentameric architecture (Nayeem *et al.*, 1994). Five

subunits are arranged within the plasma membrane to form a channel that is mainly selective for chloride and bicarbonate ions (Bormann *et al.*, 1987). The channel is gated by GABA, muscimol and other synthetic agonists but can also be modulated, in terms of channel opening time and frequency, by benzodiazepines, barbiturates, neurosteriods and ethanol (Macdonald & Olsen, 1994; Johnston, 1996a) Classical antagonists are bicuculline and the channel blocking non-competitive antagonist picrotoxin (Akaike *et al.*, 1985).

The first two receptor subunits identified (α and β) were cloned by Schofield in 1987 and since then the number of known subunits has increased to six α (α1-6), three β (β1-3), four γ (γ1-4), and one δ, ε, θ, π (Pritchett *et al.*, 1989; Shivers *et al.*, 1989; Ymer *et al.*, 1989; Olsen & Tobin, 1990; Pritchett & Seeburg, 1990; Ymer *et al.*, 1990; Wisden *et al.*, 1991; Harvey *et al.*, 1993; Bonnert *et al.*, 1999; Martin & Dunn, 2002). Alternative splicing of individual subunit proteins increases diversity further (Whiting *et al.*, 1990; Bateson *et al.*, 1991). Despite the large variety of subunits only a limited number of combinations are thought to be expressed *in vivo* (McKernan & Whiting, 1996). Pritchett et al (1989) showed that at least one α, β and γ subunit is required to form a fully functional channel. The suggested stoichiometry of the receptor is a 2:2:1 arrangement (Chang *et al.*, 1996; Tretter *et al.*, 1997; Farrar *et al.*, 1999). Immunoprecipitation studies using $GABA_A$ subunit-selective antibodies in combination with western blot analysis and binding studies have shown that the most common formation is two α1 subunits in combination with two β2 subunits and a single γ2 subunit. This receptor subtype seems to show classical $GABA_A$ receptor pharmacology and

biophysical properties and accounts for an estimated 43% of $GABA_A$ receptor population in the adult brain (McKernan & Whiting, 1996). However alterations in subunit composition can alter both physiological and pharmacological characteristics of the receptor complex such as, channel open time, desensitisation kinetics, benzodiazepine recognition and neurosteriod efficacy (Pritchett *et al.*, 1989; Verdoorn *et al.*, 1990; Saxena & Macdonald, 1994; Adkins *et al.*, 2001; Rudolph *et al.*, 2001; Smith *et al.*, 2001).

$GABA_A$ receptors are present in most areas of the mammalian CNS, however expression of $GABA_A$ receptor subtypes shows regional, neuronal and synapse specificity (Fritschy & Brunig, 2003). A large diversity of $GABA_A$ receptor subunits are expressed within the hippocampus (Wisden *et al.*, 1992). The use of subunit-selective antibodies has allowed individual subtypes and subunit combination of these receptors to be uncovered. These include $\alpha1\beta2\gamma2$ found in hippocampal interneurones and pyramidal neurones, $\alpha2\beta2/3\gamma2$ in hippocampal pyramidal neurones, $\alpha4\beta\delta$ in hippocampal dentate gyrus cells and $\alpha5\beta3\gamma2$ in pyramidal neurones (McKernan & Whiting, 1996; Fritschy & Brunig, 2003).

1.3.2.2. $GABA_B$ receptors

$GABA_B$ receptors were cloned by Kaupmann and colleagues in (1997). These receptors are present at both pre- and postsynaptic sites mediating an inhibition of transmitter release and the prolonged membrane hyperpolarisation corresponding to the slow IPSP seen on stimulation of afferent fibres respectively (Dutar & Nicoll, 1988c, b). $GABA_B$ receptors belong to the G-protein coupled receptor

superfamily (Kuriyama & Ohmori, 1990; Kaupmann *et al.*, 1997). This family shows the characteristic structure of a seven transmembrane domain protein. However functional $GABA_B$ receptors are only formed after heterodimerisation of two of these seven transmembrane domain proteins known as $GABA_{(B1)}$ and $GABA_{(B2)}$ (Jones *et al.*, 1998; Kaupmann *et al.*, 1998; White *et al.*, 1998; Billinton *et al.*, 2000). They are activated by agonists GABA and (-) baclofen (Bowery *et al.*, 1980; Bowery *et al.*, 1981; Hill & Bowery, 1981) and antagonised by phaclofen, saclofen as well as a range of more recently developed selective and potent antagonists including CGP 55845A (3-*N*-[1-(S)-(3,4-dichlorophenyl)ethyl]amino-2-hydroxypropyl-*P*-benzyl-phosphinic acid) (Davies *et al.*, 1993).

The fact that a number of spliced variants have been identified for both $GABA_{(B1)}$ and $GABA_{(B2)}$ subunits adds to the diversity of these receptors. There are currently 5 known spliced variants for $GABA_{(B1)}$ named $GABA_{B1}$a-e and 3 known spliced variants for $GABA_{(B2)}$ named $GABA_{B2}$a-c. The precise spliced variant expressed can lead to differences in both regional and subcellular localisation of the receptor, agonist activation and transduction mechanisms (Billinton *et al.*, 2001). For example western blot analysis using spliced variant selective antisera on membranes prepared from several major brain areas indicate that $GABA_{B1}$a was the predominant spliced variant expressed within the olfactory bulb and striatum whereas $GABA_{B1}$b was predominant within the cerebral cortex, thalamus, cerebellum and medulla (Benke *et al.*, 1999). Further western blot analysis of various cellular subfractions demonstrated both $GABA_{B1}$a and $GABA_{B1}$b were

enriched within the synaptic plasma membrane fraction with only $GABA_{B1}a$ showing an enrichment in the postsynaptic densities indicating that $GABA_{B1}a$ and $GABA_{B1}b$ may be located post- and presynaptically respectively (Benke *et al.*, 1999).

$GABA_B$ receptors couple to the G-proteins of the G_i/G_o family, which can ultimately lead to an inhibition of adenylate cyclase, the opening of K^+ channels, responsible for the $GABA_B$-mediated slow IPSPs (Andrade *et al.*, 1986; Christie & North, 1988; Dutar & Nicoll, 1988b) and an inhibition of Ca^{2+} channels, which may be responsible for $GABA_B$-mediated inhibition of transmitter release. However, presynaptic inhibition of transmitter release within the hippocampus is not pertussis toxin sensitive and thus may be coupled to a different G-protein at the presynaptic site (Dutar & Nicoll, 1988c).

Immunocytochemical data has shown that both $GABA_B$ receptor proteins are present in the cerebellum, hippocampus, cortex, thalamus and basal ganglia (Margeta-Mitrovic *et al.*, 1999; Billinton *et al.*, 2000). Both $GABA_B$ receptor subunits show a similar distribution throughout the central nervous system although the $GABA_{(B2)}$ subunits are found exclusively in neurones (Clarke *et al.*, 2000). In the hippocampus $GABA_{(B1)}$ mRNA is shown to be expressed homogeneously throughout all areas of the hippocampus whereas $GABA_{(B2)}$ mRNA shows lower levels in the CA1 with a graded increase throughout towards the CA3 and dentate gyrus. (Clarke *et al.*, 2000). The $GABA_{(B1)}$ spliced variants $GABA_{(B1a)}$ and $GABA_{(B1b)}$ also show high and moderate levels of expression

within the hippocampus respectively (Benke *et al.*, 1999). It is interesting to note that $GABA_{(B1b)}$ receptors were rarely located at postsynaptic densities and therefore may be attributed to presynaptic or extrasynaptic sites (Benke *et al.*, 1999).

1.3.2.3. $GABA_C$ receptors

$GABA_C$ receptors are pharmacologically distinct from the $GABA_A$ and $GABA_B$ receptor subclasses in that they are insensitive to the $GABA_A$ antagonist bicuculline and the $GABA_B$ agonist baclofen (Drew *et al.*, 1984). They infact are thought to resemble a simpler version of the $GABA_A$ receptor in that they are ligand-gated chloride channels antagonised by picrotoxin but are not subject to modulation by benzodiazepines, barbiturates or neurosteriods (Polenzani *et al.*, 1991; Feigenspan *et al.*, 1993; Qian & Dowling, 1993, 1994; Johnston, 1996b). GABA is a more potent agonist at these receptors compared to $GABA_A$ receptors and the partially folded GABA analogue *cis*-4-aminocrotonic acid (CACA) acts as a selective agonist for this receptor subtype (Johnston *et al.*, 1975), with 1,2,5,6-tetrahydropyridine-4-yl methylphosphinic acid (TPMPA) acting as a selective antagonist (Murata *et al.*, 1996; Ragozzino *et al.*, 1996).

$GABA_C$ receptors are thought to be constructed from ρ subunits (ρ1-3) cloned in the 1990's (Cutting *et al.*, 1991; Cutting *et al.*, 1992; Wang *et al.*, 1994; Ogurusu *et al.*, 1995; Zhang *et al.*, 1995; Ogurusu & Shingai, 1996). These subunits show a high degree of sequence similarity to $GABA_A$ receptor subunits yet they can form

functional homomeric or heteromeric receptors with pharmacological properties similar to those exhibited by $GABA_C$ receptors. Differential expression of these subunits can again lead to diversity in the kinetics and pharmacology of receptors (Zhang *et al.*, 1995; Chebib *et al.*, 1998; Qian *et al.*, 1999; Zhang *et al.*, 2001). Recent studies have also shown that ρ subunits can co-assemble with $GABA_A$ receptor γ2 and α1 subunits to form heteromeric receptors with distinct pharmacology (Qian & Ripps, 1999; Pan *et al.*, 2000; Ekema *et al.*, 2002; Milligan *et al.*, 2004).

The ρ1, ρ2 and ρ3 subunits are expressed within the retina and throughout the mammalian CNS including the hippocampus (Enz *et al.*, 1995; Boue-Grabot *et al.*, 1998; Wegelius *et al.*, 1998; Enz & Cutting, 1999).

1.4. The Glutamatergic System

1.4.1. Glutamate

L-Glutamate is the major excitatory neurotransmitter within the adult CNS and was the first excitatory amino acid to be so recognised (Hayashi, 1952, 1954). Although it was initially difficult to accept that a common metabolite could have a specific neurotransmitter function it was subsequently shown that glutamate acted on specific postsynaptic receptors to elicit excitatory postsynaptic responses (EPSPs or EPSCs) (Curtis & Watkins, 1960, 1963) and strong evidence was provided to indicate that it could be stored in synaptic vesicles and released from

the presynaptic terminal by Ca^{2+} dependent exocytosis (Nicholls, 1989; Maycox *et al.*, 1990; Nicholls & Attwell, 1990). Together with the presence of glutamate uptake carriers in the plasma membrane of glial cells and neurones to terminate the postsynaptic action of glutamate (Nicholls & Attwell, 1990), glutamate successfully fulfilled the criteria for classification as a neurotransmitter.

1.4.2. Glutamatergic receptors

Glutamate receptors are found throughout the mammalian CNS. The first glutamate receptors to be characterised were cation-selective ligand-gated ion channels. These ionotrophic glutamate receptors are classified into three subtypes based on pharmacological and electrophysiological data (Johnston *et al.*, 1974; McCulloch *et al.*, 1974; Watkins & Evans, 1981).

1) **NMDA**

2) **AMPA**

3) **Kainate**

These three receptors were named after their selective agonists, *N*-Methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate (Watkins & Evans, 1981; Watkins *et al.*, 1991) and differ in activation and deactivation timecourses, desensitisation rates and ion permeability. Genes encoding subunits for all subtypes of ionotrophic glutamate receptors were cloned throughout the early 1990's (Hollmann *et al.*, 1989; Bettler *et al.*, 1990; Boulter *et al.*, 1990; Egebjerg *et al.*, 1991; Moriyoshi *et al.*, 1991; Werner *et al.*,

1991; Bettler *et al.*, 1992; Herb *et al.*, 1992; Monyer & Seeburg, 1992; Monyer *et al.*, 1992). Metabotrophic glutamate receptors belonging to the G-protein coupled superfamily have also been recognised (Sladeczek *et al.*, 1985; Sugiyama *et al.*, 1987) and subsequently cloned (Houamed *et al.*, 1991; Masu *et al.*, 1991; Tanabe *et al.*, 1992).

1.4.2.1. AMPA receptors

AMPA receptors (formerly known as quisqualate receptors) mediate the majority of fast excitatory neurotransmission in the CNS. In the hippocampus and neocortex they are usually responsible for the generation of the fast component of dual component EPSPs (Collingridge *et al.*, 1983). AMPA receptors are ligand gated cation-selective channels displaying fast kinetics of activation and inactivation (Trussell & Fischbach, 1989; Colquhoun *et al.*, 1992) permeable to both K^+ and Na^+ and generally show low calcium permeability (Lino *et al.*, 1990; Jonas & Sakmann, 1992). Most antagonists, including CNQX and NBQX, for these receptors are more commonly thought of as non-NMDA receptor antagonists as they are selective for both AMPA and kainate receptors. However more recently GYKI53655 has been shown to be selective for AMPA receptors alone (Paternain *et al.*, 1995).

Four genes (GluR1-4) encode for 4 AMPA receptor subunits (Hollmann *et al.*, 1989; Bettler *et al.*, 1990; Boulter *et al.*, 1990; Keinanen *et al.*, 1990), which assemble into both homomeric and heteromeric receptors. However alternative splicing leads to each subunit existing in two forms (spliced variants) known as

"flip" and "flop" (Sommer *et al.*, 1990). The expression of these two isoforms is developmentally regulated. Prenatal brains express only the "flip" form of each subunit, which persist throughout life, whereas the "flop" form is expressed postnatally and thus is co-expressed with the "flip" form (Monyer *et al.*, 1991). This differential expression is responsible for conferring differences in desensitisation kinetics (Colquhoun *et al.*, 1992). The GluR2 subunit is a particularly important AMPA subunit. An arginine residue (R) present in the second transmembrane domain of this subunit, introduced through RNA editing, is responsible for the characteristic low calcium permeability of AMPA receptors (Sommer *et al.*, 1991). However AMPA receptors lacking this subunit do exist and show significantly enhanced calcium permeability. These receptors have a discrete localisation within the CNS. In the hippocampus such GluR2 deficient receptors are preferentially expressed by interneurones where they are suggested to play an important role in the low threshold of spike generation (Geiger *et al.*, 1995).

Autoradiographic studies have shown AMPA receptors localised in numerous regions of the CNS including the hippocampus cortex, septum, striatum and cerebellum (Monaghan *et al.*, 1985; Young & Fagg, 1991). Individual AMPA receptor subunits show widespread but differential distribution within the CNS. Immunocytochemical studies have shown stronger reactivity of the GluR1 subunits in hippocampal interneurones than pyramidal neurones and the absence of GluR2 and GluR3 from all hippocampal interneurones with the exception of Calbindin containing subtypes (Geiger *et al.*, 1995; Leranth *et al.*, 1996). All

GluR1-4 subunits are absent from axons and presynaptic terminals (Leranth *et al.*, 1996).

1.4.2.2. Kainate receptors

Kainate glutamate receptors were originally identified based upon a high affinity for the agonist kainate. As kainate is also an agonist at AMPA receptors these receptors were sometimes known as high affinity kainate receptors. However with advances in cloning techniques they are now known to constitute an entirely separate group from AMPA receptors. Kainate receptors are ligand-gated ion channels present both pre- and postsynaptically within specific regions of the mammalian CNS. Although the demonstration of functional kainate receptors in the brain has proved difficult, use of the AMPA selective antagonist GYKI 53655 allowed postsynaptic kainate responses to be isolated in CA3 pyramidal neurones (Vignes & Collingridge, 1997) and the selective agonist ATPA allowed kainate receptors comprised of or containing the GluR5 subunit to be associated with presynaptic modulation of inhibitory GABA transmission within the hippocampus (Clarke *et al.*, 1997). Since the development of these selective agonists and antagonists a role for both pre- and postsynaptic kainate receptors in synaptic transmission has been proposed in many regions of the nervous system (Kullman, 2001; Huettner, 2003).

The first kainate receptor subunit gene cloned was for the GluR5 subunit (Bettler *et al.*, 1990). An additional four genes (GluR6, GluR7, KA1 and KA2) were subsequently cloned (Egebjerg *et al.*, 1991; Werner *et al.*, 1991; Bettler *et al.*,

1992; Herb *et al.*, 1992; Lomeli *et al.*, 1992) with the total number now standing at five genes known to encode kainate subunits, although alternative splicing and RNA editing can again increase the total number of subunit isoforms (Bettler *et al.*, 1990; Sommer *et al.*, 1991; Sommer *et al.*, 1992). GluR5-7 subunits can form homomeric receptors or pair with KA1 or KA2 subunits to form heteromeric receptors (Bettler *et al.*, 1990; Egebjerg *et al.*, 1991; Werner *et al.*, 1991; Herb *et al.*, 1992; Sommer *et al.*, 1992; Schiffer *et al.*, 1997) with subunit composition again conferring differing pharmacology (Alt *et al.*, 2004).

GluR5-7 subunit expression is generally more restricted than that of AMPA receptor subunits GluR1-4. However all subunits are expressed in the hippocampus although expression level varies according to regions. GluR5 and GluR7 subunits show lower levels of expression compared to GluR6, which shows high levels of expression particularly in CA3 pyramidal neurones (Bettler *et al.*, 1990; Egebjerg *et al.*, 1991; Lomeli *et al.*, 1992) KA1 expression is limited to CA3 pyramidal neurones and dentate gyrus granule cells whereas the KA2 subunit is expressed in all neurones in the CA1, CA3 and dentate gyrus (Werner *et al.*, 1991; Herb *et al.*, 1992).

1.4.2.3. NMDA receptors

The NMDA receptor is a very well characterised subtype of glutamate receptor widely distributed within the mammalian CNS (especially in the hippocampus). These receptors are responsible for the slower component of the dual component EPSP evoked in the hippocampus (Collingridge *et al.*, 1988) and can be

pharmacologically distinguished from other glutamate receptors by the competitive blockade by D-AP5 (Davies *et al.*, 1981). They bind both glutamate and NMDA, although activation of the channel requires glycine to bind as a co-agonist (Johnson & Ascher, 1987). Distinct biophysical properties of these ligand-gated ion channels include; a large single channel conductance, (Ascher *et al.*, 1988), slow kinetics (Lester *et al.*, 1990), high calcium permeability (Lino *et al.*, 1990), voltage sensitivity due to a voltage dependent channel block by magnesium ions (Mayer *et al.*, 1984; Nowak *et al.*, 1984) and modulatory sites for Zn^{2+}, and polyamines (Westbrook & Mayer, 1987; Ransom & Stec, 1988; Williams *et al.*, 1991)

The high calcium permeability of this receptor subtype is important in initiating intracellular processes including long-term metabolic (Collingridge & Singer, 1991) or structural changes. However excessive activity of this receptor could be detrimental leading to large calcium influx, excitotoxcity and ultimately cell death (Rothman & Olney, 1987). The Mg^{2-} block of this receptor is an important factor in controlling excessive activity of this receptor.

The first NMDA receptor subunit was cloned in 1991 (Moriyoshi *et al.*, 1991) and was named the NR1 subunit. These subunits could form homomeric channels with the hallmark features of NMDA receptors (Moriyoshi *et al.*, 1991). A total of 8 spliced variants have been reported for this NR1 subunit (Anantharam *et al.*, 1992; Durand *et al.*, 1992; Nakanishi *et al.*, 1992; Sugihara *et al.*, 1992; Yamazaki *et al.*, 1992; Hollmann *et al.*, 1993), which confer differences in pharmacology and

biophysical characteristics. An additional four NMDA receptor subunits were cloned in 1992 (Monyer & Seeburg, 1992; Monyer *et al.*, 1992) named NR2A-D. These receptor subunits cannot form functional NMDA receptors unless coexpressed with an NR1 subunit and are generally thought to have a modulatory role (Monyer *et al.*, 1992). Recently NR3 subunits have been cloned, NR3A subunits (originally known as NMDA-L) both rat (Sucher *et al.*, 1995) and human (Eriksson *et al.*, 2002), which are thought to be expressed during brain development and an NR3B subunit (Matsuda *et al.*, 2002). These subunits are again proposed to have a modulatory role with NR3A reducing conductance (Das *et al.*, 1998) and NR3B reducing calcium permeability (Matsuda *et al.*, 2002).

NR1 RNA is expressed in almost all neurones throughout the CNS and PNS with particularly high levels in the cerebellum, hippocampus and cerebral cortex (Moriyoshi *et al.*, 1991) although expression of spliced variants are differentially regulated. The level of NR1 subunit expression exceeds that of the other subunits combined (Goebel & Poosch, 1999). NR2 subunits show differential expression throughout the brain with the hippocampus showing higher levels of NR2A and NR2B compared to others (Goebel & Poosch, 1999), NR3A subunit expression is developmentally regulated peaking at postnatal day (P) 8 and decreasing from P12 to adulthood in the rat CNS and shows low levels of expression in the hippocampus (Goebel & Poosch, 1999; Wong *et al.*, 2002).

1.4.2.4. Metabotrophic glutamate receptors

Glutamate receptors that couple to effector systems through the action of G-protein were first discovered in the mid 1980's (Sladeczek *et al.*, 1985; Sugiyama *et al.*, 1987) and subsequently cloned in the early 1990's (Houamed *et al.*, 1991; Masu *et al.*, 1991; Abe *et al.*, 1992; Nakanishi, 1992; Tanabe *et al.*, 1992; Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Saugstad *et al.*, 1994). These metabotrophic glutamate receptors (mgluRs) are thought to be important in a number of important CNS functions including the regulation of synaptic transmission (Gereau & Conn, 1995), plasticity (Bortolotto & Collingridge, 1993; Fitzjohn *et al.*, 1998) and emergent network oscillations (Taylor *et al.*, 1995; Whittington *et al.*, 1995; Cobb *et al.*, 2000)

mGluRs possess the 7 transmembrane domain motif found in most G-protein coupled receptors but are larger than and show very little sequence homology to all other identified G-protein coupled receptors. These are therefore thought to comprise a new family of receptors (Conn & Pin, 1997; Pin *et al.*, 2003). They are now known to form a family of 8 subtypes known as $mgluR_1$-$mgluR_8$ with alternative splicing increasing diversity further (Pin *et al.*, 1992; Tanabe *et al.*, 1992; Minakami *et al.*, 1994). These subtypes are classified into three groups based on their amino acid sequence identity, signal transduction and pharmacology (Nakanishi, 1992).

- Group I- $mgluR_1$ and $mgluR_5$ (spliced variants-$mgluR_{1a\text{-}d}$, $mgluR_{5a\text{-}b}$).
- Group II- $mgluR_2$ and $mgluR_3$.

- Group III-mgluR$_4$, mgluR$_6$, mgluR$_7$ and mgluR$_8$ (spliced variant mgluR$_{4a-b}$).

Group I mgluRs are activated by the selective agonist 3,5-dihydroxyphenylglycine (DHPG) (Ito *et al.*, 1992) with (R,S)-2-chloro-5-hydroxyphenylglycine (CHPG) selective for only mglu$_5$ receptors. These mgluRs couple to G_q to stimulate phospholipase C and phosphoinositol hydrolysis (Masu *et al.*, 1991; Abe *et al.*, 1992). Group II mgluRs are activated by the selective agonist 2R,4R-APDC (Schoepp *et al.*, 1995) and couple to G_i to inhibit adenylate cyclase and inhibit cAMP formation (Tanabe *et al.*, 1992). Group III mgluRs are activated by the selective agonist L-AP4 (Nakajima *et al.*, 1993; Saugstad *et al.*, 1994) and couple to G_i and inhibit adenylate cyclase (Nakajima *et al.*, 1993; Okamoto *et al.*, 1994; Saugstad *et al.*, 1994).

mGluRs can excite hippocampal pyramidal neurones via activation of a range of K^+ ion channels (Charpak *et al.*, 1990; Guerineau *et al.*, 1994) and nonselective cation conductances (Guerineau *et al.*, 1995) and can inhibit voltage dependent Ca^{2+} channels (Swartz & Bean, 1992).

All mGluR sunbunit RNAs are differentially but widely distributed within the brain. In the hippocampus mGluR$_1$ show low expression within the CA1 region whereas mGluR$_1$ and mGluR$_5$ show high expression within the CA3 pyramidal layer (Masu *et al.*, 1991; Abe *et al.*, 1992). More recent immunocytochemical studies showed that mGluR1b and c are absent in area CA1 and show strongest

expression in CA3 pyramidal neurones (Lujan *et al.*, 1996) and somatostatin containing interneurones are particularly enriched in the mGluR1a subtype (Baude *et al.*, 1993). mGluR4 subunits were shown to have a postsynaptic localisation on cell bodies and dendrites of pyramidal neurones and are also present at presynaptic sites (Bradley *et al.*, 1996) while mGluR7 were shown to have a presynaptic localisation at glutamatergic synapses (Bradley *et al.*, 1996; Shigemoto *et al.*, 1996). A recent comprehensive study for all subtypes mGluR1-8 in the hippocampus (Shigemoto *et al.*, 1997) reported group II and group III receptors to show a predominantly presynaptic localisation with group I receptors showing a predominantly postsynaptic localisation.

1.5. THE HIPPOCAMPUS

1.5.1. General

The hippocampus is a region of the temporal lobe known to play a central role in learning and memory formation since the 1950's. In 1954 a patient known as "HM" underwent a bilateral temporal-lobe resection, destroying the anterior two-thirds of the hippocampus and the hippocampal gyrus, to cure severe epilepsy. After the operation "HM" was reported to have "a very grave recent memory loss" (Scoville, 1954; Scoville & Milner, 1957) in that he could not remember any events subsequent to the resection and showed partial amnesia for the three years leading up to the operation. Since then many experimental and clinical studies have confirmed these observations showing that the hippocampus is involved in learning and memory processes in humans, monkeys and rats (O'Keefe & Nadel,

1978; Zola-Morgan *et al.*, 1986; Mumby *et al.*, 1992; Zola-Morgan *et al.*, 1992; Jaffard & Meunier, 1993; Jarrard, 1993; O'Keefe, 1993; Alvarez *et al.*, 1995; Mumby *et al.*, 1995; Rempel-Clower *et al.*, 1996). The cognitive map theory of O'Keefe highlighted the importance of the rodent hippocampus in forms of spatial learning (O'Keefe & Nadel, 1978; O'Keefe, 1993). Although more recently other studies in humans, primates and rats indicate an important role in various aspects of short-term memories and declarative memories (Eichenbaum, 1996; Rempel-Clower *et al.*, 1996; Jackson *et al.*, 1998; Hasselmo, 1999). The mechanisms underlying memory formation are complex but involve the generation of various network oscillatory states including theta and gamma rhythms coupled with changes in synaptic strength known as synaptic plasticity (Stewart & Fox, 1990; Bliss & Collingridge, 1993).

The hippocampus is an area of the brain that is important in the pathology relating to major neurological disorders such as Alzheimer's disease (AD) (Hyman *et al.*, 1990; Arriagada *et al.*, 1992) and temporal lobe epilepsy (Schwartzkroin, 1994; Dutar *et al.*, 1995). That the hippocampus is affected in AD is unsurprising given the foremost manifestation of AD as being memory impairment (Terry & Katzman, 1983). Numerous structural and chemical changes are present within the AD hippocampus including the presence of neurofibrillary tangles, neuritic plaques (Ball, 1978; Hyman *et al.*, 1990; Arriagada *et al.*, 1992) and neuronal loss (West *et al.*, 2000) leading ultimately to changes in major neurotransmitter systems. Of particular prominence is the cholinergic system. The "cholinergic hypothesis" highlights the significant loss of cholinergic activity in different areas

of the AD brain including the hippocampus (Bartus *et al.*, 1985; Kása *et al.*, 1997). This loss of cholinergic activity includes loss of cholinergic axons, reduced AChE and ChAT expression (Araujo *et al.*, 1988b; Kása *et al.*, 1997), consistent losses of nAChRs shown both *in vitro* and *in vivo* with specifically reduced levels of alpha 4, 3 and 7 subunit containing subtypes (Nordberg, 2001) and a loss of M2 subtype mAChRs (Araujo *et al.*, 1988b; Quirion *et al.*, 1989a; Quirion *et al.*, 1989b). Although numerous other neurotransmitter systems and hypotheses have been proposed to have a role in the development and progression of AD (Bowen *et al.*, 1983; Mann & Yates, 1986) the cholinergic system is most widely studied and relates to the work described in this thesis.

Before the hippocampal region was associated with the learning and memory process in the 1950's it was already known to play a significant role in the generation and/or spread of Temporal Lobe Epilepsy (TLE). The neurosurgical technique known as temporal-lobe resection was developed to treat this seizure syndrome. As the hippocampal formation dominates the region of excision, experience with this technique lead Scoville and Milner (1957) to their original conclusion that the hippocampus was involved in learning and memory but it also further implicated the hippocampus in generations of these seizures. Animal models of TLE alongside studies of human patients have consistently reported hippocampal involvement (McIntyre & Racine, 1986; Spencer *et al.*, 1993). The underlying cause of the epileptic seizures is thought to result from an excessive hyperexcitability of the principal cells within the hippocampal formation (Jefferys, 1993). Recently it has been suggested that cell damage particularly in the

populations of interneurones may lead to a loss of inhibition that may contribute to the hyperexcitability of the principal cell population (Dinocourt *et al.*, 2003; Kobayashi & Buckmaster, 2003).

1.5.2. Anatomy

The hippocampus is a limbic structure connected to cortical regions of the brain through the perforant pathway and numerous subcortical regions e.g. locus coeruleus, raphe nucleus and medial septum, through the fornix. These connections provide the hippocampus with environmental, motivational, emotional and autonomic information (Gulyas *et al.*, 1999a). This highly organised structure was first studied in detail by Ramon y Cajal in 1891 who divided it into four main regions named the Ammons horn or hippocampus proper, the dentate gyrus, the entorhinal cortex and the subicular complex (Raymon Y Cajal, 1891), which together form the hippocampal formation (Fig. 1.1). The Ammons horn can be further divided into *cornu ammon,* meaning rams horn, 1-4 (CA1-4) (Lorente de No, 1934), of which CA1 and CA3 are the major areas. The CA2 and CA4 regions are smaller in size and less defined, especially in rodent species.

The hippocampus proper and dentate gyrus are layered cortices consisting of three distinct layers of cells; a polymorphic layer, known as the *stratum oriens* (SO) or *hilus,* the principal cell layer, known as the *stratum pyramidale* (SP) or *stratum granulosum* (SG) and the molecular layers, known as the *stratum radiatum* (SR)

and *stratum lacunosum-moleculare* (S-LM) or *stratum moleculare* (SM) (Andersen *et al.*, 1971).

The entorhinal cortex provides the major sensory input to the hippocampus via fibres of the alvear and perforant pathways. Through its connections to regions such as the olfactory cortex it can send the hippocampus information on the surrounding environment, whereas the subicular complex provides the major source of efferent fibres from the hippocampus.

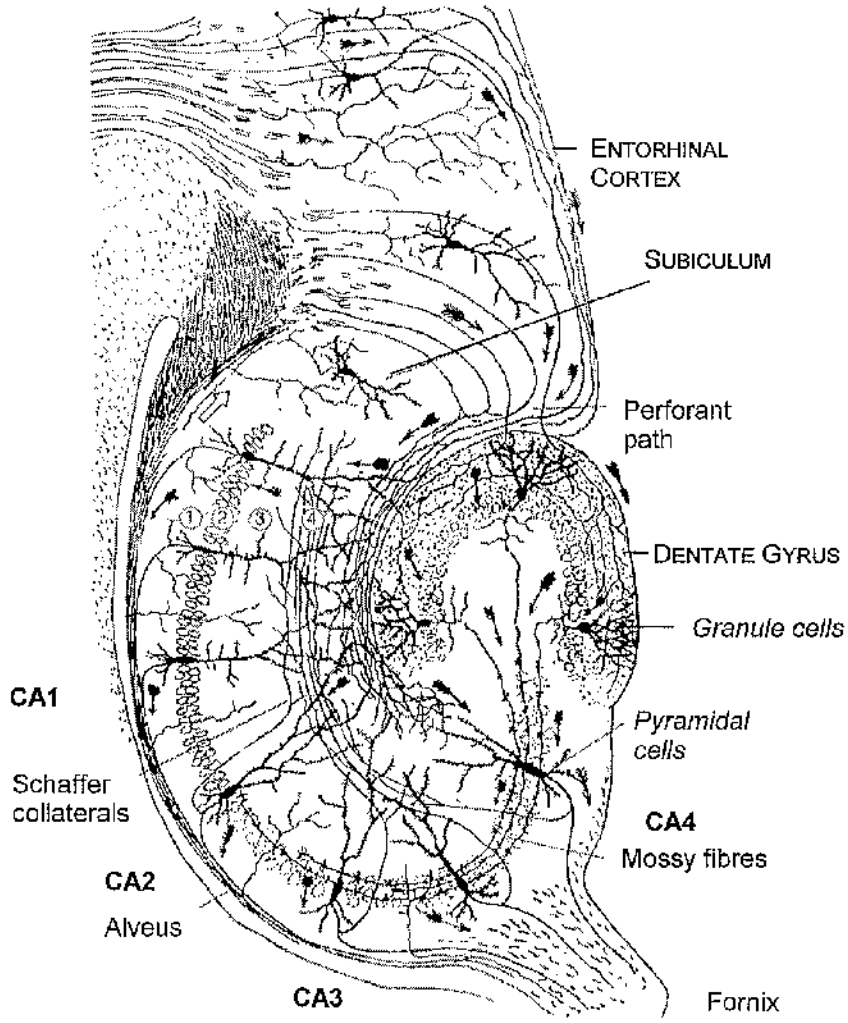


Figure 1.1 The Hippocampal Formation.

Diagram adapted from Brown and Zador (1990), original drawing by Raymon y Cajal. Each region of the hippocampal formation is indicated, dentate gyrus, entorhinal cortex, subiculum with the hippocampus proper separated into CA1-CA4 regions. Principal cell types are illustrated and the direction of the arrows represents the flow of information throughout the hippocampal formation. The circled numbers 1-4 indicate the different strata of the hippocampus; 1-*stratum oriens*, 2-*stratum pyramidale*, 3- *stratum radiatum*, 4- *stratum lacunosum-moleculare.*

1.5.3. Cytology

1.5.3.1. Principal cells

The major cell population within the hippocampus are the principal cells of which there are three types; pyramidal neurones, located within the hippocampus proper region; dentate granule cells and mossy cells both located within the dentate gyrus region (Fig. 1.1). These cells are all excitatory in nature and release glutamate as their major neurotransmitter.

The individual characteristics of hippocampal principal cells have been well documented since the studies of Raymon y Cajal and (1893) and Lorente de No (1934). Pyramidal neurones have large conical shaped cell bodies arranged in a thick layer to form the *stratum pyramidale*. Cell size and thus layer thickness decreases from CA3 to CA1. Each pyramidal neurone has one or two thick apical dendrites radially orientated in the molecular layers and numerous basal dendrites in the *stratum oriens* reaching the *alveus* (Fig. 1.2). The dendrites of pyramidal neurones are covered in spines and become longer and more slender as progress from CA3 to CA1 region. The branching pattern of dendrites also varies depending on location of the cell (Ishizuka *et al.*, 1995). CA3 pyramidal neurone axons usually emerge from the lower pole of cell body. The main axon generally projects outwith the hippocampus via the fimbria whilst a major collateral forms a side projection to the CA1 region. This is known as the Schaffer collateral projection and ramifies extensively within the *stratum oriens* and *stratum radiatum* of area CA1 (Schaffer, 1892). The CA1 pyramidal neurone axons

emerge adjacent to the apical dendrite and project to the subiculum and entorhinal cortex.

Granule cells are monopolar with smaller cell bodies arranged in a layer to form the *stratum granulosum.* These cells typically give rise to only two main radially orientated dendrites, these are confined to the molecular layer emitting several branches that are again covered in spines. Basal dendrites in this cell type are rare (Seress & Pokorny, 1981). Granule cell axons, known as mossy fibres give rise to numerous collaterals remaining in the *hilus,* and project close to the pyramidal cell layer innervating the most proximal apical dendritic region of CA3 pyramidal neurones (Claiborne *et al.*, 1986) forming the *stratum lucidum* (SL). Mossy cells are found in the hilar region of the dentate gyrus, they have spiny dendrites confined to the *hilus* and their axons innervate the molecular layer of the dentate gyrus (Amaral, 1978).

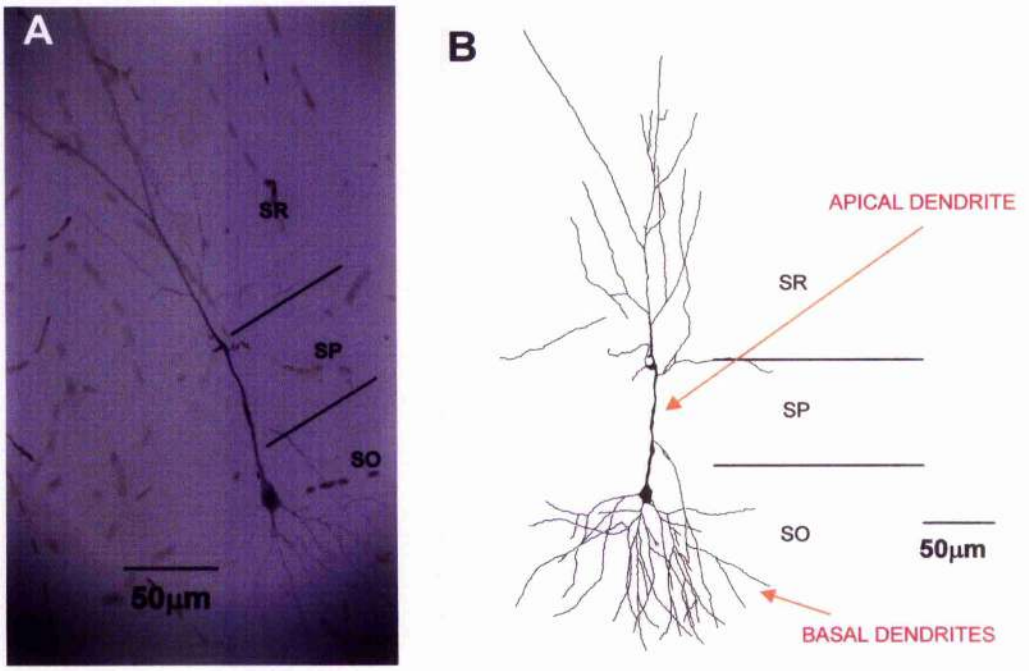


Figure 1.2 Pyramidal neurone structure

A. Light micrograph of a typical pyramidal neurone within the CA1 region of the hippocampus. B. Camera Lucida reconstruction of the same cell. Note the conical shape of the cell body, the thick apical dendrite extending through the *stratum radiatum* into the molecular layers and the numerous basal dendrites fanning out within the *stratum oriens*

The trisynaptic circuit can be used to show principal cell connectivity by outlining the three major synapses in the hippocampus (Andersen *et al.*, 1971):

I. Fibres originating in the superficial layers of the entorhinal cortex form the **perforant path** and synapse onto granule cells of the dentate gyrus (McNaughton, 1980).

II. Granule cells then send their axons, the **mossy fibres** to the CA3 region where they synapse on CA3 pyramidal neurones in the *stratum lucidium* (Blackstad & Kjaerheim, 1961).

III. The CA3 pyramidal neurones send their **Schaffer collateral** axons to the CA1 region where they synapse on CA1 pyramidal neurones.(Andersen *et al.*, 1971; Swanson *et al.*, 1978).

To complete the circuit CA1 pyramidal neurones send their axons back to the deep layers of the entorhinal cortex partially via the subicular complex. The major excitatory circuits are summarised in figure 1.3.

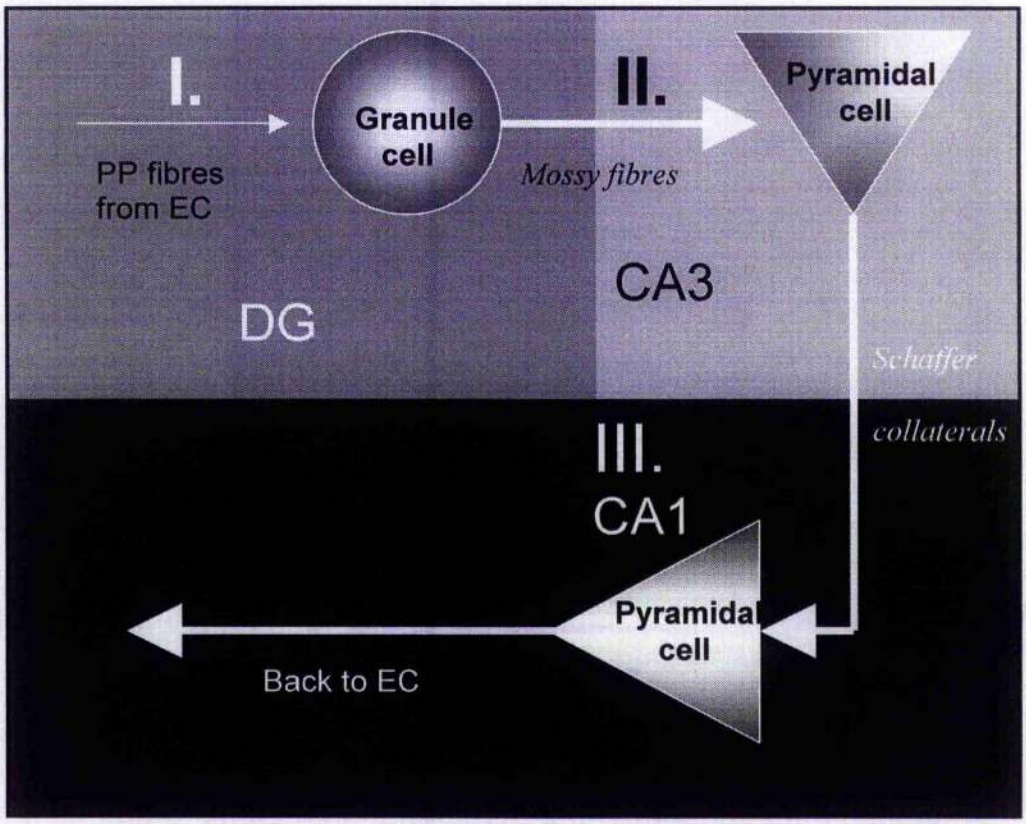


Figure 1.3 Schematic diagram of the trisynaptic circuit

Diagram illustrates the three major synapses (I to III) within the hippocampal formation. I. Fibres of the perforant path (PP) synapse onto dentate gyrus (DG) granule cells. II. The granule cells send their major axons *(mossy fibres)* to synapse onto the CA3 pyramidal neurones. III. The CA3 pyramidal neurones send their *schaffer collaterals* to synapse onto the CA1 pyramidal neurones.

1.5.3.2. GABAergic interneurones

Interneurones are classically thought of as local circuit neurones in that they do not project outside the region of the brain that their cell bodies are located. They can be excitatory or inhibitory and vary in their neurotransmitter content. However for the purpose of this thesis the word interneurone will be used to describe a GABAergic inhibitory cell of the hippocampus.

GABAergic interneurones make up 10-15% of the total cell population in the hippocampus (Woodson *et al.*, 1989; Aika *et al.*, 1994; Freund & Buzsaki, 1996). These cells are normally inhibitory in nature releasing GABA as their major neurotransmitter (Ribak *et al.*, 1978; Somogyi *et al.*, 1984; Kosaka *et al.*, 1985; Somogyi *et al.*, 1985). They share some common features such as excitatory and inhibitory inputs on their dendritic shafts, electrodense cytoplasm containing numerous mitochondria, soma containing large amounts of rough endoplasmic reticulum and invaginated nuclei containing intranuclear rods (Gulyas *et al.*, 1999b). However other than these basic features interneurones show a high degree of functional and structural diversity. They differ morphologically with respect to dendritic architecture and axonal targeting (Lorente de No, 1934; Somogyi *et al.*, 1983; Gulyas *et al.*, 1993a; Han *et al.*, 1993; Buhl *et al.*, 1994a; Freund & Buzsaki, 1996; Parra *et al.*, 1998), as well as neurochemically with respect to their expression of neuropeptides (Somogyi *et al.*, 1984; Kosaka *et al.*, 1985; Sloviter & Nilaver, 1987; Deller & Leranth, 1990) and calcium binding proteins (Kosaka *et al.*, 1987; Nitsch *et al.*, 1990; Gulyas *et al.*, 1991; Miettinen *et al.*, 1992). Interneurones can also be discriminated pharmacologically in terms of

neurotransmitter receptor expression (McBain & Dingledine, 1993; Hájos *et al.*, 1998; Parra *et al.*, 1998) and physiologically with regard to membrane properties, firing properties (Buhl *et al.*, 1994b; Buhl *et al.*, 1996; Parra *et al.*, 1998) and response to various neurotransmitters (Parra *et al.*, 1998). This overwhelming diversity leads to problems in a consistent classification system and suggests a large number of distinct subtypes of inhibitory cells must exist within the hippocampus (Maccaferri & Lacaille, 2003). Such wide variability has also lead to the suggestion that each hippocampal interneurone may be different from the next and that maybe no such classification system can be applied (Parra *et al.*, 1998). In order to provide an overview of hippocampal interneurones a brief summary of the major subtypes based on axonal targeting is given.

1. Perisomatic Interneurones

The first and best-characterised class of interneurone are the perisomatic interneurones so called because their postsynaptic target is the perisomatic region (somata, proximal dendrites and axon initial segment) of principal cells. This class of interneurone includes Chandelier (axo-axonic) cells and basket cells.

Axo-axonic cells have large cell bodies located within or adjacent to the principal cell layers, their (smooth beaded) radially orientated dendritic tree spans all layers (Buhl *et al.*, 1994b) and they contain the calcium binding protein parvalbumin (PV) (Kosaka *et al.*, 1987). They possess long myelinated axon trunks emitting unmyelinated collaterals which form synaptic contacts on axon initial segments of

principal cells (Somogyi *et al.*, 1983; Somogyi *et al.*, 1985; Gulyas *et al.*, 1993a; Buhl *et al.*, 1994a; Buhl *et al.*, 1994b).

Basket cells are not as homogenous and differ in their soma location, shape, axonal and dendritic arbors, receptor expression patterns and neurochemical content, containing either PV (Kosaka *et al.*, 1987; Sik *et al.*, 1995) or vasoactive intestinal polypeptide (VIP) (Acsady *et al.*, 1996a; Acsady *et al.*, 1996b) and cholecystokinin (CCK) (Harris *et al.*, 1985; Nunzi *et al.*, 1985). However all basket cells share the common feature that they form synaptic contacts on somata and proximal dendrites of principal cells (Buhl *et al.*, 1994a; Freund & Buzsaki, 1996).

In general perisomatic cells have large cell bodies located in the principal cell layers, numerous mitochondria and a radial dendritic tree spanning most, if not all layers of the hippocampus. They have long myelinated axon trunks emitting unmyelinated collaterals forming synaptic contacts on the perisomatic region of principal cells. The function of these interneurones is thought to be in controlling the output of principal cells (Miles *et al.*, 1996). They control the coherent activity of hippocampal networks (Freund, 2003) and have been shown directly to be effective in synchronising activity of principal cells (Cobb *et al.*, 1995).

2. Dendritic Inhibitory Interneurones

The second major class of interneurone form synaptic contacts on dendrites of principal cells and are therefore collectively known as dendritic inhibitory cells.

These cells show great variation in their soma location and their axonal and dendritic arbors, allowing further classification of dendritic inhibitory interneurones into at least 10 different subtypes. Table 1.1 will summarise some of the major types of dendritic inhibitory interneurones found in the hippocampus. In general these cells have horizontal or oblique dendritic trees with unmyelinated axons and contain the calcium binding protein calbindin D_{28K} (Gulyas & Freund, 1996). One of the most distinctive subtypes of dendritically targeting cells is the O-LM cell. These represent a discrete population of interneurones with horizontally orientated dendrites, soma within *stratum oriens* and whose axons typically extend to the *stratum lacunosum-moleculare* where they ramify extensively (Gulyas *et al.*, 1993a; Gulyas *et al.*, 1993b; McBain *et al.*, 1994; Sik *et al.*, 1995). They also express a number of distinct neurochemical markers including the neuropeptide somatostatin (Kohler & Chan-Palay, 1982) and the mGluR receptor (McBain *et al.*, 1994).

Subtype	Description	Markers	Refs
Bistratified:	➢ Soma located within or near SP or at SO/A border. ➢ Dendritic tree is spine-free, varicose and multipolar. Dendrites are radially orientated, occupying SO, SR and SP but not reaching SLM. ➢ Axon projects to SR and SO regions terminating on dendrites.	PV CB	1,2 & 3
Trilaminar: 1) Horizontal	➢ Large soma, similar in location to bistratified cells. ➢ Dendrites run horizontally at SO-A border. ➢ Axon in SR, SO and SP. ➢ Trilaminar cells also project outwith the hippocampus possibly to the medial septum.	CB	2& 3
2) Radial	➢ Soma location similar to bistratified cells. ➢ Dendritic tree similar to bistratified cell but often do reach SLM. ➢ Axon in SO, SR and SP.	CB	3
SLM-Projecting: 1) O-LM	➢ Oval soma located in SO. ➢ Horizontal dendrites in SO. ➢ Axon project to SLM region terminating on distal apical dendrites.	SOM and NPY	2,3,4 & 5
2) R-LM	➢ Pyramidal shaped soma located in SR. ➢ Dendritic tree spans SO and SR avoiding SLM. ➢ A single primary axon extends from an apical dendrite projecting to SLM.	SOM	6
3) P-LM	➢ Soma located in SP. ➢ Dendritic tree spans SO and SR rarely SLM ➢ Primary axon from apical dendrite projects to SLM.	SOM	6
Radiatum cells	➢ Soma in SP and SR ➢ Dendrites confined to SR. ➢ Axon forms sparse arbor extending throughout the SR.	CB	3& 4
LM cells	➢ Soma located in the SLM ➢ Dendrites predominantly horizontally orientated in SLM and SR may extend into SP or into SM of the dentate gyrus. ➢ Axon collaterals arborize in SLM or SLM/SR border.	CB	7,8,9 & 3

Table 1.1 Types of dendritic inhibitory cells.

Table summarises major features of distinct dendritic inhibitory interneurones including, soma location, dendritic arbour, axonal projection and neurochemical markers (if available). Abbreviations: SO-*stratum oriens*, SP-*stratum pyramidale*, SR-*stratum radiatum*, SLM-*stratum lacunosum-moleculare*, SM-*stratum moleculare*, A-alveus, PV-parvalbumin, CB-calbindin, SOM-somatostatin and NPY-neuropeptide Y. References: 1-(Buhl *et al.*, 1994a), 2-(Sik *et al.*, 1995), 3-(Freund & Buzsaki, 1996), 4-(Gulyas *et al.*, 1993a), 5-(McBain *et al.*, 1994), 6-(Oliva *et al.*, 2000), 7-(Kunkel *et al.*, 1988), 8-(Lacaille & Schwartzkroin, 1988), 9-(Williams *et al.*, 1994).

The function of dendritic inhibitory cells differs from perisomatic cells in that they are involved in controlling the efficacy and plasticity of excitatory inputs rather than the output of principal cells (Miles *et al.*, 1996; Freund, 2003).

3. Interneurone-selective interneurones.

The third and most recently defined class of interneurone form synaptic contacts only with other interneurones (Gulyas *et al.*, 1996). These interneurones are known as interneurone-selective cells (IS-cells). They differ in soma location, dendritic tree and can be divided into 3 subtypes (IS1-3) according to their connectivity and neurochemistry (Freund & Buzsaki, 1996). IS1-cells are most numerous in area CA1 but are found in other regions. They contain the calcium binding protein calretinin (CR) and contact dendritic inhibitory cells, other IS1-cells and VIP-containing basket cells (Acsady *et al.*, 1996b; Gulyas *et al.*, 1996). IS2-cells, unlike other IS-cells are only present in the hippocampus proper. Their cell bodies are located at the SR/SLM border and contain the neuropeptide VIP. These cells contact dendritic inhibitory cells and IS1-cells. IS3-cells also contain the neuropeptide VIP and contact dendritic inhibitory cells but are present in both dentate gyrus and hippocampus proper region (Acsady *et al.*, 1996a; Acsady *et al.*, 1996b). These IS cells in conjunction with an extrinsic input from GABAergic cells in the medial septal nucleus and input from interneurones which also innervate principal cells (Cobb *et al.*, 1997) are thought to exert GABAergic control over other interneurones of the hippocampus.

Principal cells along with interneurones, which are mainly GABAergic, although some cholinergic local neurones have been found, (Frotscher *et al.*, 1986; Frotscher *et al.*, 2000) make up the hippocampal neuronal network. It is proposed that patterned signalling of the principal cells, coupled with changes in synaptic plasticity, at the glutamatergic synapses, may underlie the processes of learning and memory (Stewart & Fox, 1990; Bliss & Collingridge, 1993). However the Interneurones of the hippocampus are extremely important in that they act to govern principal cell properties such as action potential generation, firing patterns and membrane potential oscillations, whilst also regulating other interneurones (Freund & Buzsaki, 1996; McBain & Fisahn, 2001). They may also have a direct role in regulating synaptic plasticity at glutamatergic synapse (Davies *et al.*, 1991). Overall they are considered to set the conditions for synaptic plasticity (Paulsen & Moser, 1998) and play an important role in the synchronisation of principal cell activity (Cobb *et al.*, 1995; Freund, 2003).

1.6. The Septo-Hippocampal Projection

The medial septal nucleus and diagonal band of Broca (MSN/DB) provides one of the major subcortical inputs to the hippocampus. Five morphologically distinct cell types have been distinguished in these nuclei (Jakab & Leranth, 1995). However, the two major populations are the cholinergic and GABAergic principle projection neurones. These neurones have large cell bodies (Brashear *et al.*, 1986) and project via the fimbria-fornix to terminate in all layers of the hippocampus. The septo-hippocampal projection was first described in early neuroanatomical

studies of the whole brain published in 1910 (Elliot Smith, 1910; Herrick, 1910). These studies were the first to note the existence of a "matrix of fibres" running from the septal area to the hippocampus. The existence of this projection was further established with immunohistochemical evidence showing the distribution of AChE within the rat brain (Lewis and Shute, 1967a; 1967b). These studies illustrated a cholinergic projection to the hippocampal formation arising from the MSN/DB complex, which was subsequently termed "the septal radiation". More recently monoclonal antibodies to the ACh synthesising enzyme, chAT, were used to study cholinergic structures within the hippocampus and showed chAT+ terminal fields organised in discrete bands and laminae. Lesions of the MSN/DB complex virtually abolished this chAT+ staining confirming that the majority of hippocampal cholinergic terminals originate from neurones of the MSN/DB complex (Matthews *et al.*, 1987). ACh release from septal neurone projection fibres within the hippocampus was shown *in vivo* by Smith in 1974. These experiments entailed placing modified cortical cups over the dorsal surface of the hippocampus in anaesthetised rabbits. The collected samples within the cups were subsequently assayed for ACh using the dorsal muscle of the leech. These experiments showed that electrical stimulation within the medial septum caused an increased release of ACh within the hippocampus and provided the first direct evidence for the existence of a cholinergic septo-hippocampal pathway (Smith, 1974). The GABAergic component of the septo-hippocampal projection was highlighted by Kohler and colleagues in 1984. This study used a combination of retrograde fluorescent tracing from the hippocampus and GAD-

immunohistochemistry to illustrate that a significant portion of the septohippocampal projection was GABAergic in nature. (Kohler *et al.*, 1984)

The cholinergic afferents are thin non-myelinated beaded fibres (Freund & Antal, 1988; Heimrich & Frotscher, 1993) making up 2/3 of all projections. These afferents contact both principal cells (pyramidal neurones, dentate granule cells and mossy cells) (Frotscher & Leranth, 1985; Deller *et al.*, 1999) and GABAergic interneurones (Leranth & Frotscher, 1987; Dougherty & Milner, 1999) synapsing on dendritic shafts, spines and cell bodies (Frotscher & Leranth, 1985). The GABAergic afferents are myelinated (Gartner *et al.*, 2001) and thus are thick in diameter with large varicose swellings (Freund & Antal, 1988). They make up only 1/3 of all projections. These GABAergic afferents contact only interneurones of the hippocampus (Freund & Antal, 1988). The MSN/DB therefore provides a major cholinergic and GABAergic input to the hippocampus.

Neurones in the MSN/DB show rhythmical activity, which is thought to be involved in the generation and patterning of hippocampal EEG activities such as theta rhythm and gamma oscillations (Vanderwolf, 1975; Stewart & Fox, 1990) recorded in behaving animals. These oscillatory states are essential for hippocampal function and are thought to underlie processes of learning and memory as the destruction or temporary inactivation of septal inputs can disrupt hippocampal theta rhythm ultimately impairing hippocampal dependent learning tasks (Winson, 1978; Mitchell *et al.*, 1982). Both the cholinergic and GABAergic components of the septo-hippocampal projection have been implicated in the

production of these oscillatory states (Stewart & Fox, 1990; Toth *et al.*, 1997; Fisahn *et al.*, 1998; Cobb *et al.*, 1999; Cobb *et al.*, 2000).

1.7. CHOLINERGIC INPUT

1.7.1. General

The cholinergic nature of the septo-hippocampal projection was first demonstrated by Lewis & Shute (1967a; 1967b) using histochemical detection of AChE and lesion studies. However the key evidence that ACh was a major neurotransmitter in this pathway came when stimulation of the MSN was shown to cause a direct increase in ACh release within the hippocampus (Smith, 1972; Rommelspacher & Kuhar, 1974; Smith, 1974; Dudar, 1975).

However septal afferents are not the only source of acetylcholine within the hippocampus, as intrinsic local cholinergic neurones have been described (Frotscher *et al.*, 1986; Matthews *et al.*, 1987; Frotscher *et al.*, 2000). It is suggested that these cells may be important in regulating hippocampal oscillatory states (Cobb *et al.*, 1999) although this remains to be conclusively established.

1.7.2. Actions of acetylcholine on hippocampal neurones

1.7.2.1. Cholinergic responses in Principal cells

1.7.2.1.1. Postsynaptic actions-mAChRs

Original observations that ACh excited mammalian cortical neurones (Krnjevic & Phillis, 1963; Spehlmann, 1963) showed that cells responded with an increase in their frequency of firing. This was some of the first direct evidence that ACh may act as a neurotransmitter within the mammalian CNS. The action of acetylcholine on hippocampal pyramidal neurones has since been widely investigated (reviewed below) and the response produced was reasonably uniform.

Extracellular studies showed that direct application of cholinergic agonists caused a slow excitation of hippocampal pyramidal neurones *in vivo* (Biscoe & Straughan, 1966; Bird & Aghajanian, 1976). Subsequent intracellular studies using the *in vitro* hippocampal slice preparation confirmed that application of cholinergic agonists, including the muscarinic selective agonists muscarine and pilocarpine, (Dingledine *et al.*, 1977; Benardo & Prince, 1981; Dodd *et al.*, 1981; Benardo & Prince, 1982; Haas, 1982; Segal, 1982; Cole & Nicoll, 1983, 1984a, b) produced a sustained membrane depolarisation of pyramidal neurones. Subsequent studies using direct electrical stimulation of cholinergic projection fibres in the *stratum oriens* (Cole & Nicoll, 1983, 1984a; Müller & Misgeld, 1986; Madison *et al.*, 1987; Segal, 1988) produced a similar period of sustained depolarisation. This event has been termed the slow excitatory postsynaptic potential (EPSPm), the m signifying the fact that the response could be attributed to the activation of mAChRs. This EPSPm was consistently accompanied by an increase in input resistance in both CA1 and CA3 pyramidal neurones (Cole & Nicoll, 1983, 1984a; Müller & Misgeld, 1986; Madison *et al.*, 1987; Segal, 1988).

Other reported features of hippocampal pyramidal neurones response to mAChR activation include; a depression of the slow afterhyperpolarisation potential (AHP) which follows action potentials (Benardo & Prince, 1982; Cole & Nicoll, 1983, 1984a). During a maintained depolarisation of a neurone at firing frequency the slow AHPs, which follow each successive action potential, summate due to the slow inactivation of the current involved (I_{AHP}). This summation results in a progressive increase in the duration between action potentials, a phenomenon known as spike frequency adaptation (SFA). As such any depression of the I_{AHP} ultimately results in a reduction of spike frequency adaptation, which has also been observed (Cole & Nicoll, 1983; Madison *et al.*, 1987; Segal, 1988). Indeed cholinergic agonists generally produce these effects at concentrations below that required to cause a substantial membrane potential depolarisation (Cole & Nicoll, 1983; Segal, 1988). In the absence of cholinergic agonists positive current injection results in repetitive action potential firing returning to resting levels on cessation of the current injection. However in the presence of cholinergic agonist slow afterdepolarising potentials (sADPs) and plateau potentials are observed after cessation of the evoked action potentials (Fraser & Macvicar, 1996).

These cholinergic responses could be enhanced by the acetylcholinesterase inhibitor physostigmine (Cole & Nicoll, 1984a; Segal, 1988) and all were completely abolished by the selective mAChR antagonist atropine (Cole & Nicoll, 1984a; Benson *et al.*, 1988; Dutar & Nicoll, 1988a; Segal, 1988; Fraser & Macvicar, 1996) confirming their muscarinic nature. The depolarisation (Benson

et al., 1988) and blockade of AHP was also antagonised by the M1 selective antagonist Pirenzepine (Dutar & Nicoll, 1988a; Segal, 1988). Although other studies (Müller & Misgeld, 1986; Pitler & Alger, 1990) have indicated that both M2 and M3 receptor subtypes may also be involved and that the receptor responsible may depend on the region studied, for example M2 receptors may contribute more in the CA3 region than the CA1 (Segal, 1988). Also earlier studies may have indicated a prominent role for the M1 subtype due to the lack of recognition of the M3 subtype (Pitler & Alger, 1990). These studies together with the selective mAChR agonist data described above suggests that these postsynaptic effects are mediated by numerous mAChRs present on hippocampal pyramidal neurones. This correlates well with autoradiographic and binding data showing that M1, M2 and M3 receptors are all expressed within the hippocampus (Spencer *et al.*, 1986; Doods *et al.*, 1987a; Levey *et al.*, 1995).

mAChR subtypes mediate these complex postsynaptic cholinergic responses (Müller & Misgeld, 1986; Benson *et al.*, 1988; Dutar & Nicoll, 1988a; Segal, 1988; Pitler & Alger, 1990) via modulation of various potassium conductances, calcium conductances and nonselective cation conductances (Colino & Halliwell, 1993; Haj-Dahmane & Andrade, 1999). The various potassium conductances include; the *M current* (I_M) (Halliwell & Adams, 1982; Madison *et al.*, 1987), the slow *Ca^+ activated K^+ current* (I_{AHP}) (Madison *et al.*, 1987), the *leak K^+ current* (I_{LEAK}) (Madison *et al.*, 1987; Benson *et al.*, 1988; Fraser & Macvicar, 1996) and the fast inactivating *A current* (I_A) (Nakajima *et al.*, 1986). The production of the slow EPSPm is thought to be the result of both the depression of the I_{LEAK}, hence

the associated increase in input resistance, and the activation of the non-selective cation conductance both presumably mediated via the activation of the M1 and/or M3 mAChR subtypes (Dutar & Nicoll, 1988a; Pitler & Alger, 1990). The mAChR-mediated depression of the I_{AHP} is responsible for the observed depression of the slow AHPs and ultimately for the observed reduction of SFA. This effect was initially suggested to be pirenzepine insensitive and thus mediated via activation of the M2 mAChR subtype (Müller & Misgeld, 1986). However subsequent studies by Dutar and Nicoll (1988a) showed that this effect was pirenzepine sensitive and was insensitive to the M2 preferring antagonist gallamine, suggesting an activation of M1 mAChRs. The slow ADPs and plateau potential reported by Fraser and MacVicar were suggested to result from activation of a Ca^{2+}-activated non-selective cation current and activation of high voltage activated Ca^{2+} channels.

A recent study by (Power & Sah, 2002) showed that activation of muscarinic receptors on CA1 hippocampal pyramidal neurones by synaptic stimulation of cholinergic afferents or application of muscarinic agonists evoked a calcium wave propagating from the apical dendrite to the soma where it invades the nucleus. These authors suggested that this rise in free calcium might initiate novel gene transcription and underlie the long-term effects of acetylcholine on learning and memory.

1.7.2.1.2.Postsynaptic action-nAChRs

The production of a nicotinic receptor mediated postsynaptic response in pyramidal neurones is the subject of an ongoing dispute.

Alkondon (1997a) reported that pyramidal neurones, recorded in the acute hippocampal slice preparation, responded to application of nicotinic agonists with an inward current that had slow kinetics of activation and inactivation. Hefft (1999) subsequently reported that afferent stimulation in the *stratum radiatum* could trigger currents generated by the activation of nicotinic receptors but attributed this to activation of intrinsic cholinergic neurones within the slice rather than stimulation of cholinergic fibres from the medial septum. However studies by four other groups (Jones & Yakel, 1997; Frazier *et al.*, 1998b; McQuiston & Madison, 1999c; Sudweeks & Yakel, 2000) generally disagree with these findings reporting no, or at least occasional and barely detectable, nAChR mediated responses to ACh application in the majority of pyramidal neurones.

The lack of a substantial postsynaptic nicotinic response in pyramidal neurones could be attributed to the receptor subunits they express. Receptor expression studies have found only very limited detection of any α nAChR subunits in pyramidal neurones (Sudweeks & Yakel, 2000). The most abundant α nAChR subunit expressed in pyramidal neurones is the α5 subunit, which requires the expression of other α and β subunits to form a functional channel (Ramirez-Latorre *et al.*, 1996). Although Sudweeks and Yakel did report a small yet detectable expression of α7 subunits within the pyramidal neurone population,

receptors containing this subunit are thought to be located presynaptically (Fabian-Fine *et al.*, 2001). Thus the major postsynaptic responses of pyramidal neurones to acetylcholine are mediated by mAChR receptors.

1.7.2.1.3.Presynaptic actions altering transmitter release

Acetylcholine acts on both presynaptic muscarinic and nicotinic ACh receptors to alter release of glutamate in many brain regions (McGehee *et al.*, 1995; Gray *et al.*, 1996; Qian & Saggau, 1997). The first evidence of a presynaptic effect of ACh in the hippocampus came from studies on evoked glutamatergic EPSPs in the CA1 region (Hounsgaard, 1978; Valentino & Dingledine, 1981; Rovira *et al.*, 1982; Rovira *et al.*, 1983).

Hounsgaard's (1978) initial experiments showed that iontophoretically applied acetylcholine decreased EPSP amplitude. Valentino and Dingledine (1981) reproduced these results and reported this effect to be muscarinic in nature as it was antagonised by the mAChR selective antagonist atropine. Rovira (1982; 1983) subsequently reported that EPSP amplitude was decreased by selective mAChR agonists, that septal stimulation produced a similar depression of the EPSP and that these effects could be reversed by selective mAChR antagonists. Further studies implicated the M2 receptor subtype (Dutar & Nicoll, 1988a; Marchi & Raiteri, 1989; Segal, 1989) and showed the reduction in EPSP amplitude to be due to a mAChR mediated inhibition of presynaptic voltage dependent calcium channels (VDCC) (Segal, 1989; Qian & Saggau, 1997; Fernández de Sevilla *et al.*, 2002). More recently Fernández de Sevilla and Buño

(2003) showed that stimulation of cholinergic fibres within the *stratum oriens* of the hippocampus produced a similar presynaptic inhibition of evoked EPSCs mediated by mAChRs. Thus synaptically released acetylcholine acts on presynaptic mAChRs (possibly of the M2 subtype) to inhibit VDCC and ultimately decrease glutamate release from the presynaptic terminal at the Schaffer collateral-to-CA1 synapse (Qian & Saggau, 1997; Fernández de Sevilla *et al.*, 2002; Fernández de Sevilla & Buño, 2003) and the associational-commisural-to-CA3 synapse (Vogt & Regehr, 2001).

The initial study by Rovira (Rovira *et al.*, 1982) also provided evidence that nAChR agonists had the opposite effect on glutamate transmission and increased the evoked EPSP amplitude. Studies on miniature EPSCs (mEPSCs) showed that activation of nAChRs increased the frequency of mEPSC recorded in CA3 pyramidal neurones, indicating an enhanced glutamate release at mossy-CA3 synapse (Gray *et al.*, 1996). This enhancement was proposed to be the result of increased calcium concentration in the presynaptic terminal due to direct activation of the calcium permeable α7 subunit containing nAChRs, rather than activation of voltage dependent calcium channels (Gray *et al.*, 1996; Radcliffe *et al.*, 1999). However a recent paper by Vogt & Regehr (2001) disagree with this hypothesis as when they repeated these experiments using Fura 2 as a ratiometric indicator, no detectable changes in presynaptic calcium levels were seen upon activating nAChRs.

In summary, presynaptic mAChRs and nAChRs have opposing effects on glutamate transmission. mAChR activation results in a decrease of glutamate release resulting in decreased glutamate transmission whereas nAChR activation results in an increase of glutamate release resulting in increased glutamate transmission.

1.7.2.2. Cholinergic responses in Interneurones

An interaction between the septo-hippocampal cholinergic input and the GABAergic interneurones of the hippocampus is suggested by a number of sources yet the physiology of this interaction has not been subject to a thorough investigation. The evidence suggesting that these two transmitter systems must interact comes from both anatomical, immunocytochemical and electrophysiological studies.

Anatomical studies showed that cholinergic afferents terminate on the surface of GABAergic interneurones where they form synaptic contacts (Frotscher & Leranth, 1985). Immunocytochemical studies have shown that mAChRs, m1 to m4, particularly m2, (Levey *et al.*, 1995; Hájos *et al.*, 1998) and nAChRs, including the calcium permeable α7-type (Freedman *et al.*, 1993; Fabian-Fine *et al.*, 2001; Kawai *et al.*, 2002), are present on the surface of interneurones. The study by Levey and colleagues (1995) showed m2 immunoreactivity in a network of neurones situated along the *stratum oriens*/alveus border in addition to m2 immunoreactivity forming a dense plexus of fibres and puncta in a basket-like formation within the pyramidal cells layer (Levey *et al.*, 1995). A more detailed

study by Hajos and colleagues showed that receptor distribution may vary depending on interneurone type (Hájos *et al.*, 1998). This study identified specific subtypes of interneurones expressing m2 receptors illustrating m2 immunoreactive somata located within the CA1 region at the *stratum oriens*/alveus border. These neurones were shown to have horizontal dendrites and axonal projections to the dendritic regions of pyramidal neurones. This study also reported the presence of m2-immunoreactive axon terminals within the *stratum pyramidale* surrounding the soma and axon initial segment of CA1 and CA3 pyramidal neurones in a basket like manner. Co-localisation of m2 receptor protein with GABA confirmed that the majority of m2 positive soma and terminals were GABAergic in nature (Hajos *et al.*, 1998). Thus it has been postulated that m2 receptors are expressed exclusively on the terminals of perisomatic inhibitory neurones (basket cells and axo-axonic cells) whereas they are expressed on the soma and axon initial segment of the dendritic inhibitory neurones (Hájos *et al.*, 1998). From these studies it appears likely that the interneurone circuits of the hippocampus are under a significant regulation by the cholinergic system.

1.7.2.2.1. Postsynaptic actions-mAChR

Numerous electrophysiological studies have shown that unlike principal cells, activation of mAChR (via agonist application) produces a variety of responses in interneurones. Initial studies on a very small number of interneurones (n=3) (Benardo & Prince, 1982) demonstrated that interneurones depolarised on application of ACh. Reece and Schwartzkroin (1991) recorded from two distinct types of interneurones (basket cells and LM cells) and showed that again

application of cholinergic agonists produced a depolarisation from resting membrane potential although the time course of this depolarisation varied. Atropine fully blocked these responses and the Na^+ channel blocker tetrodotoxin (TTX) had no effect thus providing the first direct evidence that cholinergic agonists directly excite hippocampal interneurones via the activation of muscarinic receptors.

Further evidence was provided in 1992 when Pitler and Alger (Pitler & Alger, 1992) recorded an increased frequency of IPSPs in pyramidal neurones in response to application of carbachol or synaptically released acetylcholine (due to electrical stimulation in the *stratum oriens*) indirectly showing an increase of GABA released onto pyramidal neurones and thus an excitation of interneurones mediated via activation of mAChRs. In 1993 Behrend & Ten Bruggencate also showed that acetylcholine receptor agonists excite GABAergic interneurones but showed a depression of GABA released from their terminals. This earlier work lead Hajos and colleagues (1998) to hypothesise from receptor expression studies that, ACh could decrease GABA release from interneurones expressing m2 receptors exclusively on their terminals and could increase GABA release from interneurones expressing m2 receptors on their soma and axon initial segment thereby switching inhibitory control of pyramidal neurones from predominantly somatic to predominantly dendritic sites.

The significance of such a switch in functional terms, has been discussed by Paulsen and Moser (1999). Briefly, these authors proposed a model for the role of

interneurones in mnemonic processing. They suggested that the hippocampal network alternates between information encoding ("read in") mode and information retrieval ("read out") mode and that the principal cells participate in each mode. However activity of specific sets of interneurones targeting specific domains of the principal cells may regulate these processing modes. For example during "read in" mode the principal cell axon may be inhibited by axo-axonic interneurones with dendrites disinhibited to receive excitatory inputs, whilst during "read out" mode the axon must be disinhibited to allow information to be transferred from the hippocampus to regions such as the neocortex.

By employing the whole cell patch clamp technique to record directly from visualised hippocampal interneurones, McQuiston and Madison (1999a) confirmed the varying nature of muscarinic response in interneurones. Pharmacological activation of mAChRs produced; 1) a depolarisation, 2) a hyperpolarisation, 3) a biphasic response or 4) no detectable change in membrane potential. Furthermore, these authors reported each form of response to be found in various anatomically distinct classes of interneurone but failed to find a relationship between interneurone type and the nature of the response. This same group also reported that, the AHP following a train of action potentials induced by application of a depolarising current could be replaced by a large and long-lasting ADP in the presence of muscarinic agonists. Again this response could not be attributed to a distinct interneurone subtype (McQuiston & Madison, 1999b).

These authors also attempted to uncover the ionic mechanisms underlying the various mAChR-mediated responses in CA1 interneurones and suggested that the depolarisation was a complex response involving the inhibition of a voltage independent leak K^+ current similar to that seen in the pyramidal neurones and the simultaneous activation of a cation current. The mAChR-mediated hyperpolarising currents showed similar properties to those of an inwardly rectifying K^+ channel and that the ADPs were produced by a novel ionic mechanism involving sodium influx (McQuiston & Madison, 1999b, a).

1.7.2.2.2. Postsynaptic actions-nAChR

Pharmacological activation of nAChRs is also reported to evoke a variety of responses in hippocampal interneurones. The majority of studies have concentrated on interneurones located within the *stratum radiatum* of the hippocampus and have typically shown a fast depolarisation mediated by α7-subunit containing receptors (Alkondon *et al.*, 1997a; Jones & Yakel, 1997; Frazier *et al.*, 1998b; McQuiston & Madison, 1999c). However McQuiston and Madison (1999c) reported a subset of interneurones within the *stratum oriens* that responded with the same α7 mediated fast depolarisation in combination with a depolarising response displaying slower kinetics. Alkondon and colleagues (1997) reported a similar fast and slow response in a small number of *stratum radiatum* interneurones but also reported *stratum radiatum* interneurones displaying only the slow response and subsequently attributed this slow response to the activation of α4β2 nAChR (Alkondon *et al.*, 1999). Sudweeks and Yakel (2000) showed fast responses in *stratum radiatum* interneurones and slow responses in *stratum oriens*

interneurones and attributed the kinetic differences to subunit composition of receptors expressed on each cell type, implicating the α2 subunit in *stratum oriens* interneurone responses, and the α7 and β2 subunits in *stratum radiatum* interneurone responses. Ji and Dani (2000) reported the predominant response of *stratum radiatum* interneurones was the fast current sensitive to the α7 subunit containing nAChR selective antagonist methyllcaconitine (MLA) although a small number responded with a slower current or a combination of both the fast and slow current. In a subsequent study by Buhler and Dunwiddie (2001) concentrating on *stratum oriens* interneurones, the authors showed most cells responded to ACh application with a fast α7 mediated response. In addition to interneurones which respond with a fast and/or slow response, a further population of interneurones, usually located near the pyramidal layer, showing no nicotinic response have also been observed (McQuiston & Madison, 1999c).

In summary, interneurones respond to nAChR activation with either a fast depolarisation/inward current mediated by α7 containing receptors, a slow depolarisation/inward current mediated by α4β2 and possibly α2 containing receptors or a combination of both response types. A further population of interneurones appear unresponsive to nAChR activation. However, relating these different responses to individual subtypes of interneurones on the basis of their soma/dendrite location and axonal projections is still not clear.

NAChR-mediated EPSCs produced by afferent stimulation of cholinergic fibres have been reported in two separate studies on *stratum radiatum* and *stratum lacunosum-moleculare* interneurones (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a). However the response was rather infrequent when compared to agonist application studies. Alkondon and Colleagues (1998) showed EPSCs remaining after addition of an antagonist cocktail, blocking NMDA and AMPA ionotrophic glutamate receptors, $GABA_A$ receptors, 5-HT3 receptors and ATP receptors, which could be blocked by α7-subunit selective antagonists in only 20% of interneurones recorded. Similarly Frazier and colleges (1998a) reported only 17 out of 125 interneurones (13.6%) with EPSCs remaining after addition of the same antagonist cocktail. Both authors proposed the lack of a consistent response was more likely due to difficulties in stimulating cholinergic afferent fibres within the hippocampal slice preparation rather than unresponsive interneurones. Other authors have been unable to reproduce these effects (McQuiston & Madison, 1999c). However, in another recent study, Buhler and Dunwiddie (2001) identified two *stratum oriens* interneurones responding to afferent stimulation with a fast α7 mediated response.

1.7.2.2.3 Pre- and postsynaptic actions altering transmitter release

GABA release from interneurones is reported to be modulated by the action of acetylcholine receptor agonists through presynaptic heteroreceptors.

Activation of mAChRs has been shown to decrease the frequency of spontaneous miniature IPSCs (mIPSCs) onto CA1 pyramidal neurones (Behrends &

TenBruggencate, 1993). Indicating a suppressant effect on the synaptic release mechanism at GABAergic terminals. M2 receptors have been located on the terminals of perisomatic interneurones (Hájos *et al.*, 1998) leading to the suggestion the activation of these receptors may mediate inhibition of GABA release. However activation of mAChR was also shown to increase both the frequency and amplitude of spontaneous IPSPs onto CA1 pyramidal neurones (Pitler & Alger, 1992; Behrends & TenBruggencate, 1993). This increase in GABA release was suggested to result from direct excitation of a subset of hippocampal interneurones expressing mAChRs on their soma and dendrites (Hájos *et al.*, 1998).

Activation of nAChRs also results in an increase of GABA release onto pyramidal neurones and other interneurones (Alkondon *et al.*, 1997a; Albuquerque *et al.*, 1998; Alkondon *et al.*, 1999; Hulo & Muller, 2001; Buhler & Dunwiddie, 2002). However, this effect is sensitive to TTX (Alkondon *et al.*, 1997a; Albuquerque *et al.*, 1998; Alkondon *et al.*, 1999; Hulo & Muller, 2001). Therefore, increase of GABA release is not due to the activation of presynaptic receptors but rather somatodendritic or preterminal nicotinic receptors leading to propagation of action potentials to the terminal region. Indeed, Buhler and Dunwiddie (2002) performed paired recordings from both hippocampal interneurones and pyramidal neurones and showed that application of ACh to the visually identified presynaptic interneurone could increase GABAergic IPSCs recorded in both a paired pyramidal neurone and a paired interneurone. If the cell body of the presynaptic interneurone was subsequently ruptured by advancing the pipette and returning it

to its original position application of ACh to this neurone could no longer increase IPSCs in the paired pyramidal neurone. These experiments therefore illustrated that an intact cell body is needed for the production of IPSCs in pyramidal neurones.

The overall effects of cholinergic modulation of hippocampal interneurones will depend on the receptors they express and their postsynaptic target. Pyramidal neurones could be inhibited via activation of receptors increasing GABA release from interneurones synapsing onto pyramidal neurones or could be disinhibited via activation of receptors 1) decreasing GABA release from interneurones synapsing onto pyramidal neurones or 2) increasing GABA release from interneurones synapsing onto other interneurones.

1.8. SUMMARY

Both GABA and ACh modulate the activity of individual principal cells as well as co-ordinating the activity of populations of principal cells and glutamatergic synaptic plasticity. Thus understanding how these neurotransmitters interact with each other is pivotal in understanding hippocampal function.

The evidence reviewed throughout this introduction gives reason to believe that an important interaction may take place between the cholinergic and GABAergic systems of the hippocampus. Due to the fact that receptor distribution varies across the subclasses of interneurones and additionally that numerous responses to ACh have been recorded from these cells it seems likely that may acetylcholine differentially modulate functionally distinct types of interneurone. This differential modulation of GABAergic transmission should be investigated further as it relates directly to the regulation of principal cell network dynamics and ultimately how information is processed through the hippocampus.

A good example differential regulation of distinct subtypes of interneurones is shown by the localisation of M2 mAChRs on dendritic targeting inhibitory cells compared to the localisation of M2 mAChRs on perisomatic inhibitory cells. ACh is proposed to act on M2 receptors expressed on the soma of dendritic targeting interneurones to enhance GABA release from these cells whereas it acts on M2 receptors expressed exclusively on the axon terminals of perisomatic targeting interneurones to suppress GABA release from these cells (Hájos *et al.*, 1998).

Therefore differential modulation of interneurones switches inhibition from predominantly perisomatic to predominantly dendritic in nature.

McQuiston and Madison (1999c) hypothesised that interneurones sensitive to nicotine are dendritic inhibitory interneurones whereas the majority of interneurones unresponsive to nicotine are of the perisomatic inhibitory cell class, again switching inhibition from perisomatic to dendritic.

Most previous studies have focused on cultured systems and upon pharmacological stimulation of ACh receptors. It was therefore hoped that further investigation focusing on physiological interactions between the cholinergic and GABAergic systems would reveal important insights into the regulation of interneurones, which may ultimately be exploited and lead to the production of selective drug treatments to target functionally distinct hippocampal microcircuits.

1.9. Aims

The aim of this thesis is to further investigate the interaction of cholinergic and GABAergic systems of the hippocampus. Specific questions to be addressed include;

1) Readdress the physiological action of the septo-hippocampal cholinergic projection on the principal cell population.
2) Assess the action of septo-hippocampal cholinergic afferents on hippocampal interneurones.
3) To assess how cholinergic modulation affects synaptic transmission in the hippocampus
4) To investigate how 1-3 affect network dynamics in the hippocampus.

2.GENERAL METHODS

2.1. SLICE PREPARATION

All Animals (rats and mice) were bred in house, housed in group cages under controlled environmental conditions (temperature 19-23°C and 12hr light/dark cycles). Animals had *ad libitum* access to food and water.

2.1.1. Hippocampal slices for traditional intracellular an extracellular recordings

Male and female Wistar rats (3-5weeks) were killed by cervical dislocation and decapitated in accordance with U.K. home office guidelines under the authority of the U.K. Animals (Scientific Procedures) Act, 1986.

An incision was made along the middle of the head using a scalpel and the skin separated to reveal the skull. Using sharp dissecting scissors the skull was cut down the midline from back to front and the bone folded to each side to expose the brain. The brain, separated from the spinal cord, was carefully removed with a small spatula and placed in a beaker of chilled (0-3°C), oxygenated (95% oxygen-5% carbon dioxide) artificial cerebrospinal fluid (aCSF; composition mM: NaCl, 124, KCl, 3, $NaHCO_3$, 26, NaH_2PO_4, 1.25, $MgSO_4$, 1, $CaCl_2$, 2, glucose, 10). 5% CO_2 was included to maintain a correct PH of 7.4. The brain minus the cerebellum

was subsequently hemisected and a surface trimmed parallel to the desired orientation was glued to the stage of a vibrating blade microtome (Leica VT1000, Milton Keynes, U.K.). Either horizontal (glued to top surface of brain) or parasagittal (glued to midline) orientation was chosen for slicing (Figure 2.1).

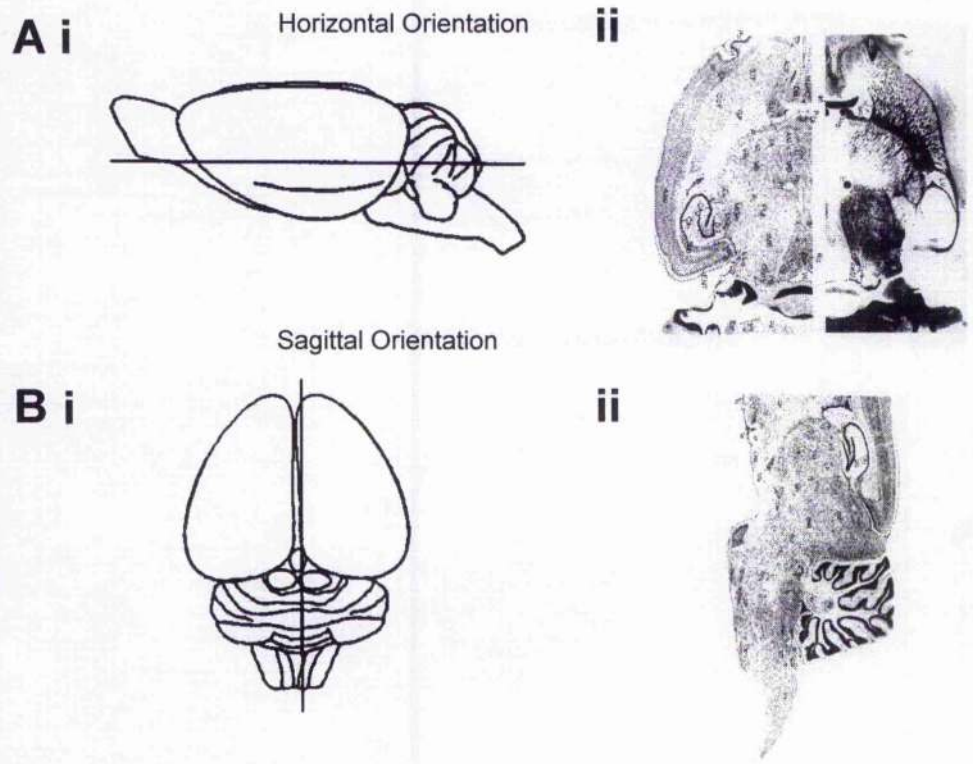


Figure 2.1 Orientation for slicing

Diagram illustrating orientation for slicing. Lines indicate the incisions made and the exposed surface of the brain, which was glued to the stage of the vibrating blade microtome. Slices were subsequently cut from the directly opposing surfaces. Ai. For preparation of slices in a horizontal orientation the top surface of the brain was trimmed in parallel to the stage surface and slices were cut from the bottom surface of the brain. Aii Photograph of a horizontal slice containing the hippocampal formation. Bi. For preparation of slices in a sagittal orientation the brain was sliced down the midline, the exposed surface was glued to the stage surface and slices were cut from the lateral surfaces. Bii Photograph of a sagittal slice containing the hippocampus.

400μm thick slices were cut on the vibrating blade microtome (Leica VT1000S). Throughout the slicing procedure the brain and slices were held in a chamber containing chilled aCSF. The slices were then transferred to a cold petri dish containing chilled aCSF where the hippocampal region of each slice was dissected free from surrounding brain tissue. Hippocampal slices were maintained in an interface incubation chamber with an oxygen-enriched atmosphere at room temperature. Following an incubation period of 1hour the slices were individually transferred to an interface recording chamber (see section 2.2.1.1) using a large bore suction pipette.

2.1.2. Hippocampal slices for patch clamp recordings

Male and female Wistar rats (P16-28) were terminally anaesthetised with an Intraperitoneal injection of Pentobarbitone Sodium (1000mg/kg). Following unconsciousness and lack of the paw pitch pain reflex, the abdomen and rib cage were opened and a transcardial perfusion was performed. A hypodermic needle (19G 1 ½) was inserted and fixed into the left ventricle and an incision made in the right atrium, the heart was then perfused with 20-60ml of chilled high sucrose aCSF (composition mM: NaCl, 87, KCl, 2.5, $NaHCO_3$, 25, NaH_2PO_4, 1.25, $MgSO_4$, 7, $CaCl_2$, 0.5, glucose, 11, sucrose, 75) in accordance with U.K. home office guidelines. Removal of the brain and preparation of 250μm slices followed an identical procedure to that stated above but in the presence of a high sucrose aCSF solution. Slices were transferred to a submerged holding chamber, heated to 37°C for 1 hour and the high sucrose aCSF was gradually (10% every 15mins) changed to normal aCSF over a period of two hours. Slices were stored in the

submerged style holding chamber at room temperature (19-24°C) until needed. They were then individually transferred to a submerged recording chamber (see section 2.2.1.1) using a large bore suction pipette as needed.

2.1.3. Septo-hippocampal slice preparation

Combined septo-hippocampal slices were prepared from male and female Wistar rats for use in electrophysiological recordings and confocal immunocytochemistry as previously described by Toth and colleagues (Toth *et al.*, 1997). Tissue used for electrophysiological experiments was prepared from 3-5 week old Wistar rats and Normal mice killed by cervical dislocation and subsequent decapitation in accordance with U.K home office guidelines under the authority of the U.K. Animals (Scientific Procedures) Act 1986. Tissue used for confocal immunocytochemistry experiments was prepared from 11 day old, 21 day old and 3 week old Wistar rats. These animals were terminally anaesthetised with an intraperitoneal injection of Pentobarbitone Sodium (1000mg/kg). Following unconsciousness and lack of the paw pitch pain reflex, the abdomen and rib cage were opened and a transcardial perfusion was performed as previously described above but with a fixative (4% paraformaldehyde in 0.1M phosphate buffer) replacing the aCSF.

The whole brain was removed as described above and subsequently hemisected down the mid-line separating the brain into two pieces (see figure 2.2, incision 1). Each half of the brain was taken separately and two subsequent incisions made. The first was a parasagittal cut made 2mm lateral from the mid-line extending

from the rostral pole of the brain to a site close to the start of the fimbria (see figures 2.2, incision 2). The second was made from this point at 120° with respect to the sagittal plane (see figure 2.2, incision 3). The surface of the brain exposed by these two excisions was flattened and glued onto the surface of the microtome stage thus straightening the septo-hippocampal projection and allowing 5-600μm thick slices containing the intact pathway to be cut on the vibrating blade microtome. These septo-hippocampal slices were either maintained in an incubation chamber and subsequently used for intracellular recording or frozen in 100% glycerol and later stained for confocal microscopy.

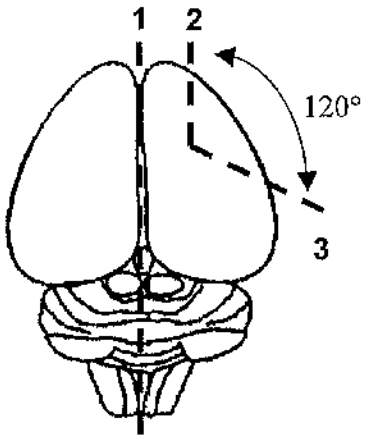


Figure 2.2 Orientation for septo-hippocampal slices

Diagram illustrates the preparation of septo-hippocampal slices. Dotted lines (2 and 3) indicate the incisions made to expose the surface of the brain, which was flattened and glued to the stage of the vibrating blade microtome. Slices containing the entire straightened septo-hippocampal pathway were subsequently cut from the directly opposing surface (indicated by line 1).

2.2. ELECTROPHYSIOLOGY

Three types of electrophysiological technique were employed throughout this thesis. Sharp electrode intracellular recordings were employed to record from hippocampal pyramidal neurones in chapter 3, the whole-cell patch clamp technique was employed to record from both CA1 and CA3 pyramidal neurones and interneurones in chapters 4 and 5 and standard extracellular recordings were employed in chapter 5. The recording setup, types of recording chambers, recording and stimulation electrodes and details of each recorded technique will be outlined in this section.

2.2.1. Recording set-up

The recording chambers, micromanipulators (mechanical model-Narishige, Japan & piezo electric driven model-Burleigh, USA), and upright microscope (BX50WI, Olympus, Japan) with fixed stage were mounted on a translation table. This itself was bolted to an anti-vibration table (Ealing, MA, USA). A steel Faraday cage, earthed to a ground common to the amplifier (Axoclamp-2B, Axon instruments, Union City, CA, USA), was placed around the recording system to isolate it from all extraneous electrical noise (Fig. 2.2). All other electrical equipment, signal processor (filter and DC amplifier -model 440, Brownlee Precision, CA, USA), digitiser (Digidata 1200 series, Axon instruments, Union City, CA, USA), humbug noise subtraction device (Quest Scientific, BC, Canada), PC, Digital tape (DAT) recorder (DTR-1404, bio-logic scientific instruments, Caix, France),

master 8 pulse generator (A.M.P.I, Israel) were secured in an equipment rack alongside the Axoclamp 2B current and voltage clamp amplifier (Fig. 2.3).

Amplification and filtering settings varied depending on the type of recording configuration employed. Details of these parameters for each configuration are provided in section 2.2.2.

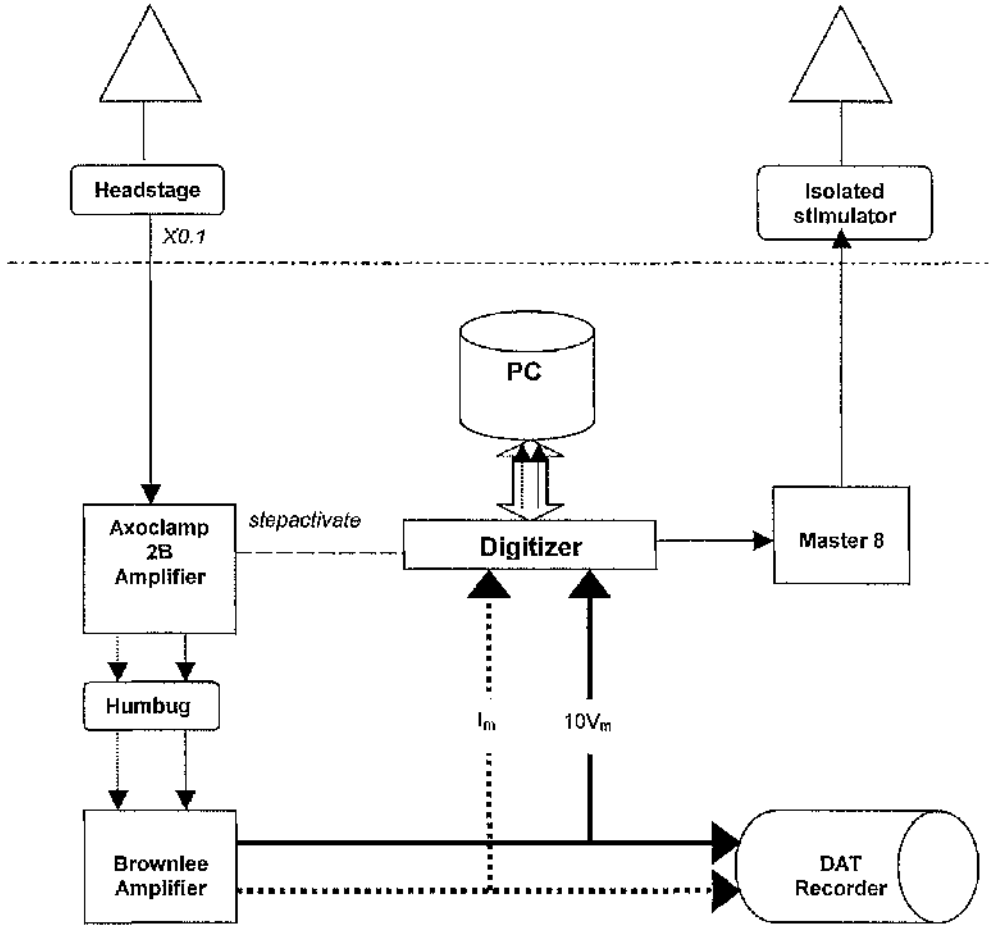


Figure 2.3 Flow chart of recording setup

Figure shows a flow chart of the apparatus used for electrophysiological recording. Direction of information transfer is represented by direction of arrows. The recording and stimulation electrode are indicated by the open triangles attached to the headstage and isolated stimulator respectively.

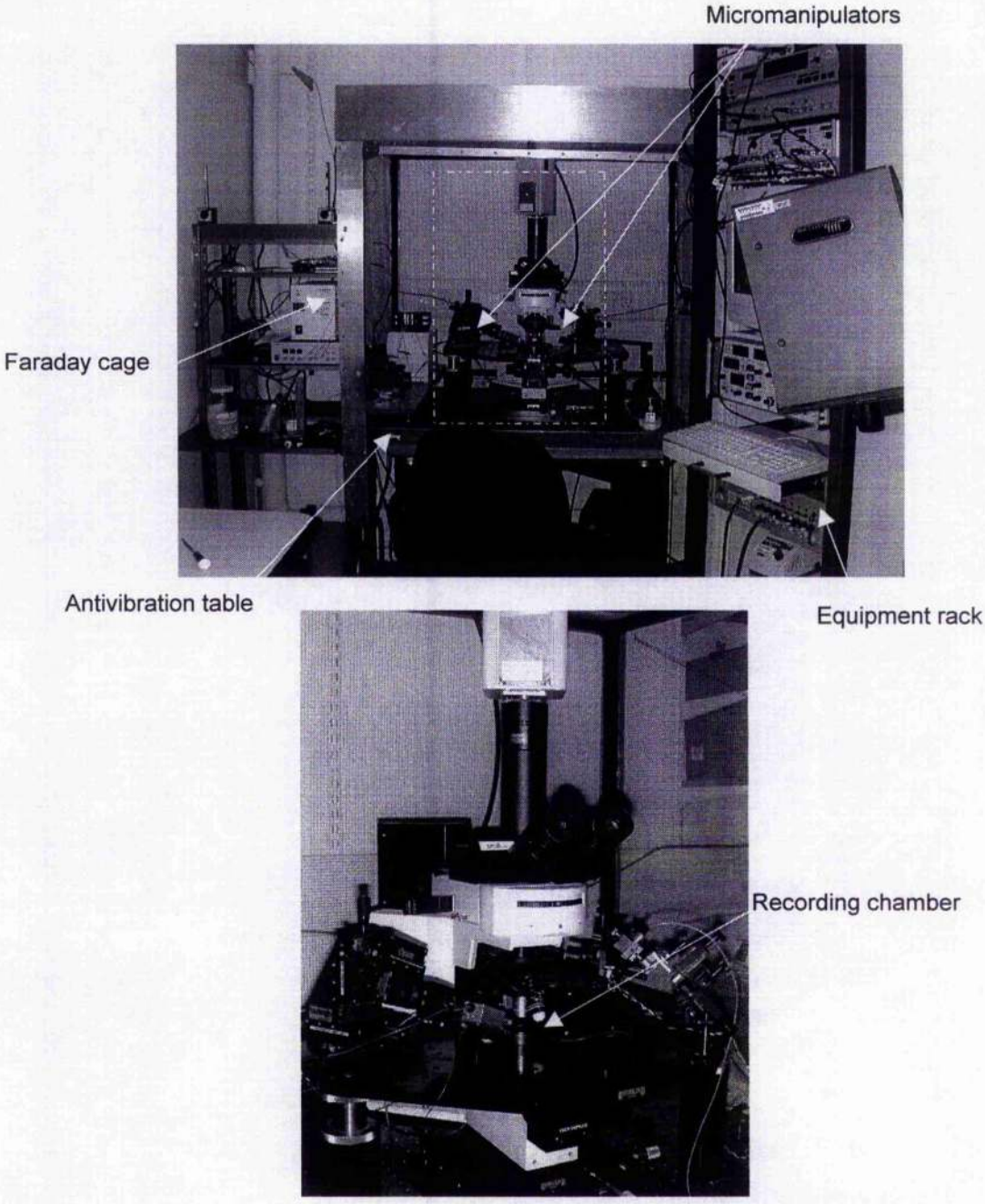


Figure 2.4 Recording setup

Photographic images illustrating manipulators and microscope secured on antivibration table inside faraday cage, with all other equipment secured in racks alongside. Close up illustrates recording chamber mounted under microscope.

2.2.1.1. Types of Recording chambers

For intracellular and extracellular recording configurations slices were transferred to an interface type chamber (Fig. 2.5A). This consisted of a central chamber surrounded by an outer water bath, filled with distilled water. The outer water bath was heated to approximately 37°c and bubbled with 95% O_2, 5% CO_2 to provide a humid oxygen enriched atmosphere. Oxygenated aCSF was pumped, by means of a peristaltic pump (Mini Pulse 3, Gilson, France), through the central chamber providing a constant flow rate of 1-3ml/min. The Hippocampal slices were placed on a nylon mesh suspended in the central chamber in order to be at the interface of warmed perfusing aCSF and the oxygen enriched atmosphere but not submerged.

All patch clamp and some extracellular recordings were performed in a submerged type of chamber (Fig. 2.5B) consisting of a small circular glass bottomed well (volume 1.5-2ml). The slices were submerged in the oxygenated perfusing aCSF (1-3ml/min) and maintained at a constant room temperature of 24°C. Slices were held in position on the bottom of the recording chamber by a platinum weighted grid of parallel nylon threads.

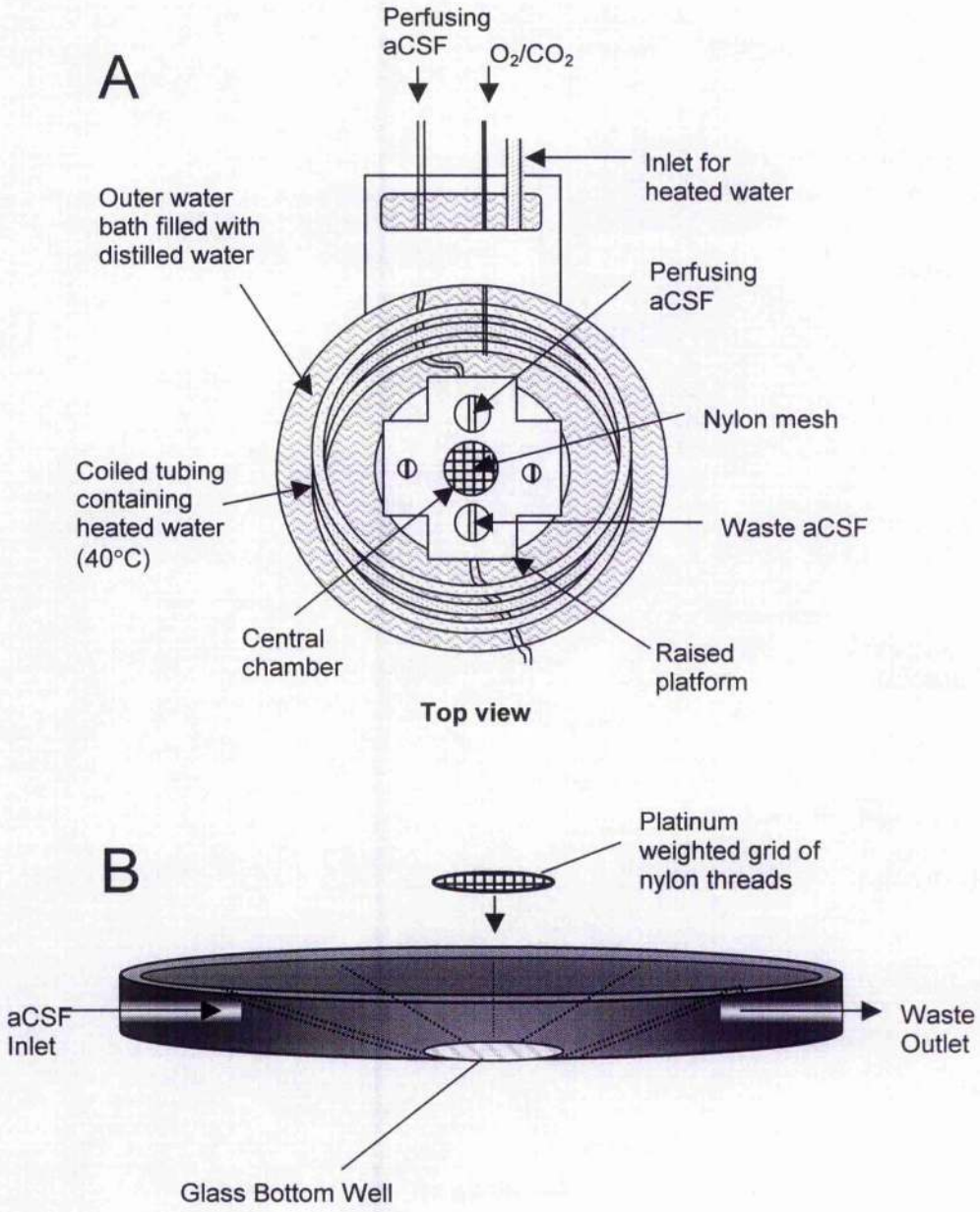


Figure 2.5 Types of recording chambers.

A. Diagram illustrating the interface type of recording chamber. Traditional sharp electrode intracellular and extracellular recordings were performed in this type of chamber. B. Diagram illustrating the submerged type of recording chamber. Both extracellular and patch clamp recordings were performed in this type of recording chamber

Hippocampal slices remained viable for up to 12 hours in the interface type of chamber and 6 hours for submerged type of chamber, although some deterioration was seen throughout the day, particularly in patch clamp recordings. At least a one-hour equilibration period was allowed before any electrophysiological recordings were obtained.

2.2.1.2. Recording & stimulation electrodes

Recording electrodes for intracellular and extracellular experiments were pulled in one stage using a Flaming Brown micropipette puller model P-87 (Sutter Instruments Co. USA) from standard walled (outer diameter: 1.2mm; inner diameter: 0.69mm) borosilicate glass capillaries (Harvard Apparatus, UK). Patch clamp electrodes were pulled in two stages using a vertical PP-830 type microelectrode puller (Narishige, Japan) from thin walled (outer diameter: 1.2mm; inner diameter: 0.94mm) borosilicate glass capillaries (Harvard Apparatus, UK). In the first stage the capillary was thinned over a length of approximately 7mm, the heating coil was then re-centred and the second stage started to pull and break the thinned section producing two separate microelectrodes. Electrode resistance and filling solutions varied according to the type of experiment performed. These are fully outlined in table 2.1.

	Filling Solutions	Electrode resistance (MΩ)
Intracellular	1.5M potassium methylsulphate	60-170
Extracellular	Standard aCSF	1-5
Patch Clamp	$KmeSO_4$-based intracellular solution	5-10
Patch Clamp	$CsCl^-$ based intracellular solution	5-10

Table 2.1 Filling solutions and final d.c. resistance of recording electrodes.

Table summarises the type of filling solution and final d.c. resistance of recording electrodes for all recording configurations employed.

Filled recording electrodes were mounted into electrode holders where their filling solution came into contact with silver chloride coated silver wire. The electrode holder was then inserted into a unity gain head stage (current gain 0.1: Axon instruments, CA, USA) and connected to the axoclamp 2B amplifier via the microelectrode 1 (ME1) port. A silver chloride bath reference electrode submerged in the recording chamber was also connected to the headstage.

Bipolar stimulation electrodes were constructed from two lengths of 0.05mm diameter Nickel 80% /chromium 20% wire (Advent Research Materials Ltd., England) twisted together and cut at the end. Stimuli, comprised of square wave pulses 20-200μs, 0-30mA fixed current intensity, were produced by constant current isolated stimulator boxes (Digitimer Ltd., England), which in turn were triggered by master-8 pulse generator (A.M.P.I., Israel).

All stimulating and recording electrodes were mounted on micromanipulators (Narishige, Japan and Burleigh, USA) to allow course and fine movement in all (*x*, *y* and *z*) directional planes.

2.2.2. Recording Configurations

2.2.2.1. Intracellular current clamp recordings

Intracellular recordings were performed in an interface-recording chamber. Recording electrodes were backfilled with 2% biocytin and 1.5M potassium methylsulphate (filtered using 0.2μm syringe filter) to achieve a final DC resistance between 60 – 170MΩ, which was measured before cell impalement in "bridge balance" mode by balancing the unwanted potential drop across the microelectrode.

Impalement of pyramidal neurones was achieved by advancing the recording electrode through the *stratum pyramidale* using a Narashige water hydraulic drive in two axes. Negative current pulses (0.1nA) were applied through the recording electrode so that any increase in voltage deflection gave an indication that the electrode tip was approaching a cell membrane. Subsequent application of an oscillatory current "buzz" was then used to facilitate penetration of the electrode tip. After impalement, the membrane potential (Vm) of the cell could be measured as the potential difference between the recording electrode and the bath reference electrode. Prior to searching for cells the microelectrode was positioned

extracellularly in the slice and the recorded voltage was set to zero. Immediately following the end of each experiment this reading was checked again and any correction in the recorded membrane potential was made.

Small (0.1 to 0.5nA) positive and negative current steps were applied and the resulting depolarising and hyperpolarising voltage responses recorded were analysed allowing measurement of numerous cell properties such as; cell input resistance, calculated from the membrane voltage response to the hyperpolarising current steps, action potential amplitude, and action potential duration measured at ½ maximum amplitude. These traces were also used to give a tentative identification of cell type. Putative pyramidal neurones were identified on the basis of physiological characteristics such as spike frequency adaptation and the presence of a slow afterhyperpolarisation following action potentials. Cell impalement and characterisation were routinely performed in normal aCSF, drugs were administered after a stable recording was obtained.

The bipolar stimulation electrodes were placed on the slice surface within the *stratum oriens* or directly within the medial septal nucleus (MSN) to stimulate cholinergic afferents known to be located within these areas (Frotscher & Leranth, 1985) while recording the effect on the membrane potential of the impaled pyramidal neurone. Stimuli (ranging between 0.01-12mA intensity and 0.02-0.2ms in duration) were delivered at a fixed intensity every 15s for ionotrophic glutamate and GABA receptor mediated responses and every 5 minutes for mAChR mediated responses.

Voltage and current signals recorded through the ME1 port were amplified using an Axoclamp 2B (headstage gain 0.1, amplifier gain 10) and further amplified (X10) using a Brownlee 440 signal processor giving a final signal amplification of X100. DC signals were low pass filtered at 5.0KHz, and 50Hz noise removed using a humbug (Quest scientific, BC, Canada) signal subtraction device. Output signals were digitised (500Hz to 2KHz) through a digidata 1200 interface (Axon Instruments Ltd.) connected to a PC.

2.2.2.2. Extracellular field recordings

These experiments were performed in both interface and submerged type recording chambers. Standard aCSF was used as the electrode filling solution to achieve a final DC resistance between 1-5MΩ. Extracellular field potential recordings were made in both the CA1 and CA3 region of the hippocampus. The 1-5MΩ recording electrodes were placed on the surface of the slice in the *stratum pyramidale*, stimuli were delivered every 15s via the bipolar stimulation electrode positioned within the *stratum radiatum* and *stratum lucidum* to activate Schaffer collaterals and mossy fibres respectively, resulting field excitatory postsynaptic potentials (fEPSP) were recorded with respect to the reference electrode. In experiments investigating the effect of drug on the slope of the field EPSP, the test fEPSP size was adjusted to 50% of the maximum.

Voltage signals were amplified using an Axoclamp 2B (headstage gain 0.1, amplifier gain 10) and further amplified (X100) using a Brownlee 440 signal

processor giving a final signal amplification of X1000 Signals were low pass filtered at 5.0KHz, and 50Hz noise removed using a humbug (Quest scientific) signal subtraction device. Output signal were digitised (500Hz) through a digidata 1200 interface (Axon Instruments Ltd.) connected to a PC.

2.2.2.3. Patch clamp whole-cell recording

Patch clamp experiments were performed in a submerged recording chamber. Electrodes were back filled with an intra-pipette solution, composition (mM): $KmeSO_4$, 122.5, KCl, 17.5, NaCl, 9, $MgCl_2$, 1, EGTA, 0.2, GTP, 0.3, ATP, 3 and 0.5% Neurobiotin or CsCl, 135, $MgCl_2$, 2, HEPES, 10 nd 0.5% Neurobiotin. (Filtered using 0.2µm syringe filter) to achieve a final DC resistance between 6-10MΩ. Whole-cell current and voltage clamp recordings were obtained from CA1 pyramidal neurones and interneurones under visual control using infrared differential interference contrast (DIC) optics and the blow and seal patch-clamp technique (Fig. 2.7).

Healthy target cells were chosen on a shiny, smooth appearance of the cell surface. The development of high resistance seal requires a clean plasma membrane surface and electrode tip, thus slight positive pressure was applied to the pipette interior while advancing through the slice towards the chosen target cell so that the pipette solution streamed outwards maintaining a clean electrode tip and cleaning the surface of the target cell. The electrode tip was slowly manoeuvred to the cell surface while recording voltage and current steps applied through the electrode. Once the electrode tip touched the cell membrane (as seen by slight

dimpling of the cell membrane) the positive pressure was immediately released to form a low resistance seal, characterised by a reduced current trace. At this point application of slight negative pressure could increase the seal resistance until a high resistance gigaohm seal was achieved characterised, by no current flow. In some cases a gigaohm seal developed spontaneously without the need for suction. Subsequent hyperpolarisation of the electrode to –70mV and application of short suction pulses ruptured the membrane patch without breaking the gigaohm seal to achieve the whole cell patch-clamp formation. Either voltage or current clamp configuration was chosen for the duration of the experiment.

In current clamp mode applications of small positive and negative current injections were again used for cell identification. Putative interneurones could be tentatively identified and distinguished from pyramidal neurones on the basis of their physiological characteristics, such as high spontaneous firing rates, large spike afterhyperpolarisations, weak spike frequency adaptation and high input resistances. Stimulation electrodes were placed on the surface of the slice within the *stratum oriens* to stimulate cholinergic afferents while recording from the patched CA1 pyramidal neurone or interneurone. Postsynaptic potentials were recorded as deflections in the membrane potential after stimulation.

In voltage clamp mode spontaneous and miniature excitatory and inhibitory postsynaptic currents (EPSC and IPSC) were recorded from both CA1 and CA3 pyramidal neurones and interneurones.

Voltage and current signals were amplified using an Axoclamp 2B (head stage gain 0.1, amplifier gain 10) and further amplified (X10 or X100) using a Brownlee 440 signal processor. DC signals were low pass filtered at 2.0KHz, and 50Hz noise removed using a humbug (Quest scientific) signal subtraction device. Output signals were digitised (500Hz to 2KHz) through a digidata 1200 interface (Axon Instruments Ltd.) connected to a PC.

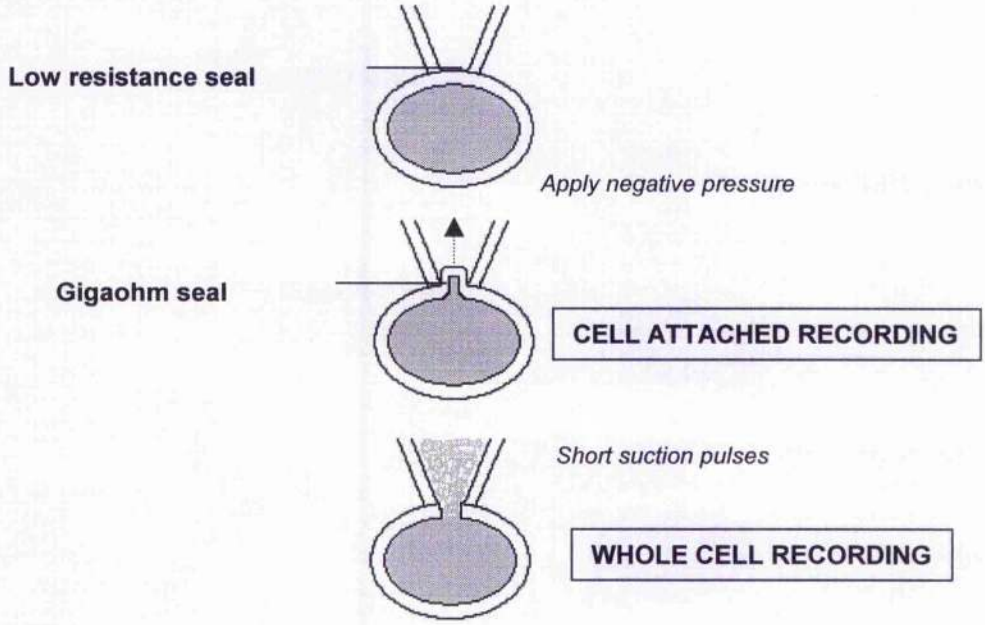


Figure 2.6 Blow and seal Patch-clamp technique

Diagram illustrating the formation of a high resistance gigaseal and subsequent steps to achieve the whole cell patch-clamp formation used for recording. Electrode tips were advanced through the hippocampal slice to the cell surface where positive pressure was released. This resulted in the formation of a low resistance seal between the electrode and cell membrane. Subsequent application of negative pressure increased seal resistance until a high resistance gigaohm seal was achieved resulting in the cell attached formation. After formation of the gigaohm seal application of short suction pulses ruptured the membrane patch to achieve the whole-cell recording formation.

2.3. Data Display and Storage

Data display and storage methods were the same for all types of recordings performed. All digitised data was captured, viewed and stored onto a PC hard disk using Clampex 8 software (Axon Instruments, CA, USA). The DAT recorder provided a DAT record backup copy and a source of high-resolution (44KHz) signal for further analysis.

2.4. Data Analysis

All data was analysed offline using the software packages Clampfit 8 (Axon Instruments, CA, USA) or Mini Analysis version 5.06.0028 (Synaposoft Inc. GA, USA). Data are presented as means ± standard error of the mean (S.E.M.). The n value refers to the number of times a particular experiment was performed, each in a different slice taken from a different rat.

Statistical analyses: Wilcoxon matched pairs test, Students t test, analysis of variants (ANOVAs) and subsequent post hoc tests (Bonferroini and Tukeys) were performed using Graph Pad Prism version 3.02 for windows (GraphPad Software, San Diego California, USA). The Kolmogorov-Smirnov two sample test for comparison of cumulative probability plots was performed directly on Mini Analysis (Synaptosoft Inc. GA, USA). All data was tested for normal distribution before any parametric tests were applied. Probability values of less than 0.05 were considered significant.

2.5. Biocytin/ Neurobiotin Labelling

The intracellular markers Biocytin (Sigma-Aldrich, Poole, UK) or Neurobiotin (Vector Laboratories, Peterborough, UK) were present in both patch and intracellular electrodes, application of depolarising current pulses allowed sufficient diffusion of these markers into the neurone under observation. On completion of recordings slices were fixed overnight in a fixative solution composed of 2.5% paraformaldehyde, 1.25% gluteraldehyde, 15% (v/v) picric acid in 0.1M phosphate buffer (PB; pH 7.4). After fixation slices were washed in 0.1M PB and infiltrated with 10% and 20% sucrose. Each slice was then snap-frozen, thawed in PB and washed for a further 20 minutes. The slices were embedded in 10% gelatin and fixed for a period of 2 hours before they were re-sectioned to 60μM thickness. The new 60μm sections were washed in PB containing 10% methanol and 3% H_2O_2 for 5 minutes to abolish background peroxidase activity.

After two 20 minute washes in 0.1M PB and three 10 minutes washes in phosphate buffer saline (PBS; 0.9% NaCl) the sections were incubated overnight at 4°C with a 1% avidin-biotinylated horseradish peroxidase complex (ABC kit, Vector Laboratories, Peterborough, UK).

Three 30 minute washes in PBS and two 20 minute washes in PB sufficiently removed excess ABC before developing the sections using the 3,3'-diaminobenzidine (DAB) substrate kit for peroxidase (Vector laboratories,

Peterborough, UK) with nickel intensification. Excess DAB was removed by three 20-minute washes in PB. The sections were then washed in distilled water for 20 minutes, floated onto glass slides and left to dry before being dehydrated in a series of ethanol solutions (50%, 70%, 90%, 100%, 2 minutes in each), 10 minutes in histoclear and finally cover slipped.

All cells were subsequently scrutinised under light microscopy to confirm their true location and to identify where possible the nature and extent of the dendritic and axonal arbourisation. Strongly labelled cells were selected for graphical reconstruction using a drawing tube attachment (camera lucida).

2.6. Confocal Microscopy

Septo-hippocampal brain slices for confocal analysis were prepared as described in section 2.1.3. Slices were washed thoroughly prior to overnight incubation with goat anti-ChAT (1:100 AB144P, Chemicon international) and mouse anti-parvalbumin (1:500, P-3088, Sigma-Aldrich, UK) in 0.3% triton X in PBS. After several washes in 0.1M phosphate buffer, sections underwent a further 3 hour incubation in fluorescent secondary antibodies in 0.3% triton X in PBS (donkey anti-goat Alex Fluor 488 at 1:500, Invitrogen Molecular Probes and anti-mouse Rhodamine at 1:100, Jackson ImmunoResearch Laboratories). Sections were finally washed 3 times before being mounted under a coverslip in Vectasheild mounting medium for fluorescence (Vector Laboratories, Peterbourgh, U.K).

Septo-hippocampal slices were visualised with a BioRad Radiance 2100 confocal laser scanning system equipped with a green (He-Ne) laser and a red diode laser. The excitation and emission wavelengths were 493nm and 519nm respectively for AlexoFlour 488 and 595nm and 61nm for Rhodamine.

Slices reacted for antibodies against ChAT and PV were visualised under brightfield in the first instance to locate the position of the medial septal nucleus and hippocampus and subsequently scrutinised under epifluorescence on a confocal microscope (BioRad Radiance 2100 confocal scanning system, BioRad, Hemmel Hempsted, UK). Z-series were scanned at 0.5μm z-separation. Confocal image stacks were viewed and analysed on Confocal Assistant version 4.02. Stacks were flattened and arranged to provide a complete image of the septo-hippocampal pathway.

2.7. DRUGS

All drugs were dissolved in deionised water and stored as frozen 1ml aliquots of stock solutions 100 to 1000 times' the final concentration. Drug application and final dilution was either via perfusion medium for a period no less than 15 minutes or pressure ejected directly onto the slices using a pico-injection system (Harvard Apparatus, UK) pressure pulse 3-8 PSI, 10msec in duration.

Atropine, Carbachol, Choline, Picrotoxin, Physostigmine (eserine) dimethylphenyl-piperanzinium (DMPP) all purchased from Sigma-Aldrich Company Ltd (Poole, England, UK); Bicuculline and 6-nitro-7sulphamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) purchased from tocris Cookson (Bristol, U.K.); Nicotine purchased from BDH chemicals Ltd (Poole, England, UK).

D-(*E*)-2-Amino-4-methyl-5-phosphono-3-pentanoic acid (CGP 40116), [1-(*S*)-3,4-dichlorophenyl)ethyl]amino-2-(*S*)-hydroxypropyl-p-benzyl-phosphoic acid (CGP 55845A) were gifts from Dr Kumlesh Dev, Novartis Pharmaceuticals, Basel, Switzerland.

3. ISOLATION OF SLOW CHOLINERGIC RESPONSES IN CA1 PYRAMIDAL NEURONES BY STIMULATION OF CHOLINERGIC AFFERENTS

3.1. INTRODUCTION

In recent years it has become evident that activation of muscarinic acetylcholine receptors (mAChRs) has a variety of effects on the passive and active properties of glutamatergic principal cells within the hippocampus. As mentioned in chapter one, the major responses of pyramidal neurones to mAChR activation include:

1) A slow depolarisation from resting membrane potential (EPSPm).
2) A depression of the slow afterhyperpolarisation (AHP) following a train of action potentials.
3) A blockade of spike frequency adaptation (SFA)
4) A blockade of the 'M current'. (Halliwell & Adams, 1982)
5) Production of afterdepolarisations (ADPs) and plateau potentials (PPs)
6) A calcium wave propagating from the apical dendrite to the soma.
7) Reduced glutamate release from presynaptic terminals of pyramidal neurones.

MAChRs present on both CA1 and CA3 hippocampal pyramidal neurones can be activated by application of the cholinergic agonists, acetylcholine (Benardo & Prince, 1981, 1982; Segal, 1982; Cole & Nicoll, 1983), carbachol (Haas, 1982; Cole & Nicoll, 1984a, b; Müller & Misgeld, 1986; Madison *et al.*, 1987; Benson *et al.*, 1988; Dutar & Nicoll, 1988a) muscarine (Cole & Nicoll, 1984b) and pilocarpine (Cole & Nicoll, 1984b) or by synaptically released acetylcholine on electrical stimulation of cholinergic afferents within the *stratum oriens* of the hippocampal slice preparation (Cole & Nicoll, 1983, 1984a; Müller & Misgeld, 1986; Madison *et al.*, 1987; Segal, 1988).

The aim of this initial results chapter was to further investigate the evoked cholinergic response in CA1 pyramidal neurones. A second aim was to verify the functional integrity of the septo-hippocampal cholinergic projection within the hippocampal slice preparation for future studies investigating cholinergic regulation of GABAergic interneurones as detailed in chapter four of this thesis.

3.2. RESULTS

3.2.1. Characterisation of CA1 pyramidal neurones

16 CA1 pyramidal neurones in the rat hippocampus were recorded initially using traditional sharp intracellular recording electrodes as described in chapter two. In order to fully characterise the impaled cell, positive and negative current injections were applied and the resultant depolarising and hyperpolarising voltage responses investigated (Fig. 3.1A). The resulting voltage traces were used to give a tentative indication of cell type prior to anatomical confirmation. As illustrated in figure 3.1 pyramidal neurones show characteristics such as spike frequency adaptation and small slow afterhyperpolarisations (AHPs). Throughout all experiments, cells were loaded with the intracellular marker biocytin and slices subsequently processed for light microscope evaluation to confirm the cells identity (Fig. 3.1B and C). Pyramidal neurones could be distinguished by their characteristic cone-shaped cell bodies, located within the *stratum pyramidale,* with basal dendrites extending into the *stratum oriens* and apical dendrites extending into the *stratum radiatum* and *lacunosum-moleculare* where they branched in a dichotomous manner. Their dendritic tree was characteristically covered with spines (Fig 3.1D).

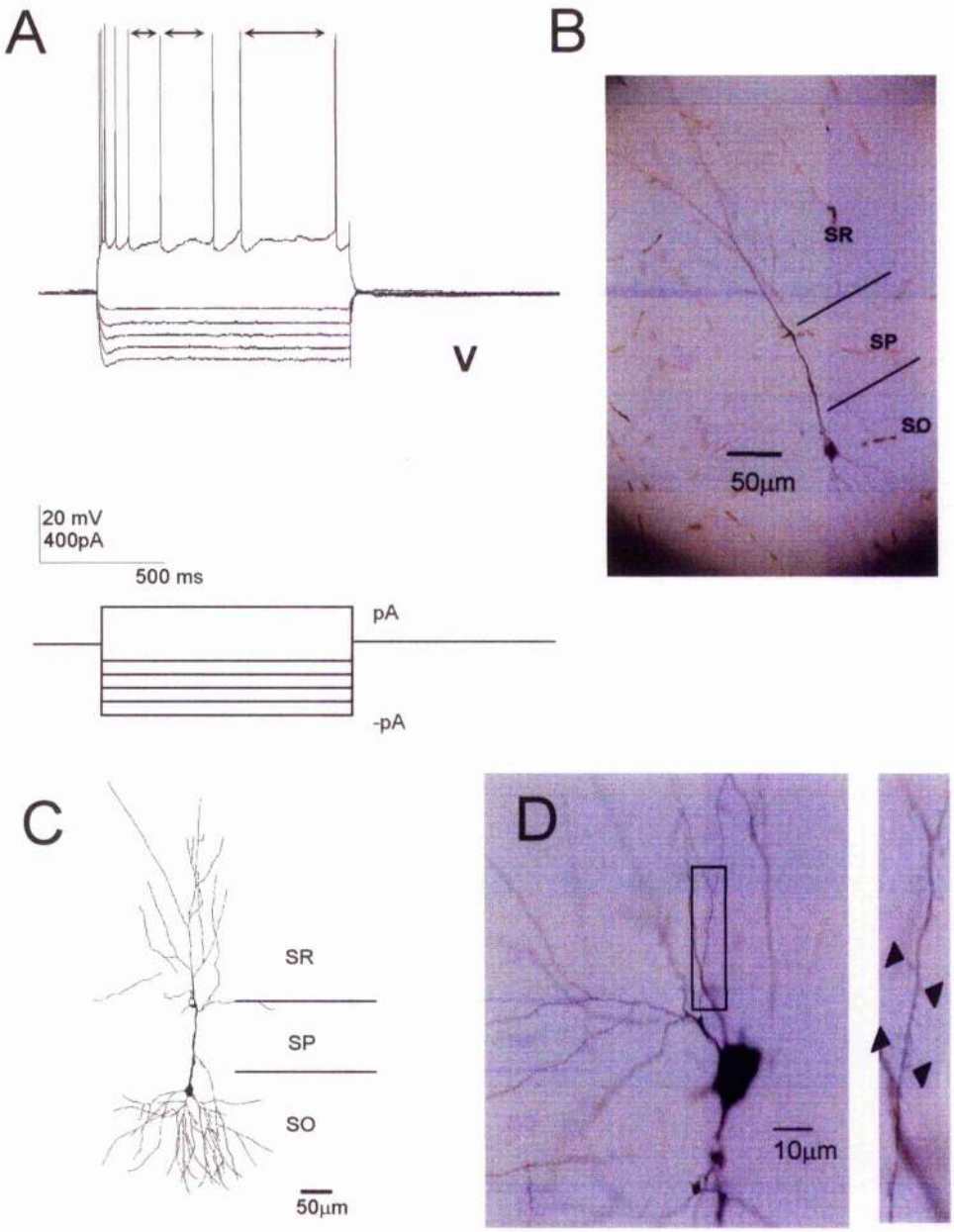


Figure 3.1 Characterisation of CA1 pyramidal neurones.

A. Representative voltage traces (upper trace) recorded from a CA1 pyramidal neurone in response to applied intracellular current injection (lower trace). Positive and negative current steps resulted in depolarising and hyperpolarizing voltage responses respectively. Note that this cell showed pronounced spike frequency adaptation (SFA), indicated by increasing arrow length between action potentials on the upper trace, and small slow afterhyperpolarisations (AHP) characteristic of a pyramidal neurone. B. Low power (x20) light micrograph of the corresponding CA1 pyramidal neurone stained by intracellular injection of biocytin. C. Camera Lucida reconstruction of the same cell revealed a bipolar neurone with basal dendrites fanning out in *stratum oriens* and primary apical dendrites extending through *stratum radiatum* and *lacunosum-moleculare* where it branched in a dichotomous manner. D. higher power (x 40) LM micrograph of another CA1 pyramidal neurone revealed characteristic dense spines on the dendritic surface.

Other properties such as resting membrane potential and input resistance were monitored throughout the experiment to give a complete characterisation and monitor the physiological status of each CA1 pyramidal neurone during the recorded period. Only neurones with a resting membrane potential of –50mV and lower were included in this study. The 16 pyramidal neurones recorded had a mean resting membrane potential of 60.73±2.93mV, mean input resistance of 50.59±4.16MΩ and mean action potential height and duration (measured at half maximal amplitude) of 69.41±2.03mV and 0.913±0.03ms respectively. This data is summarised in Table 3.1.

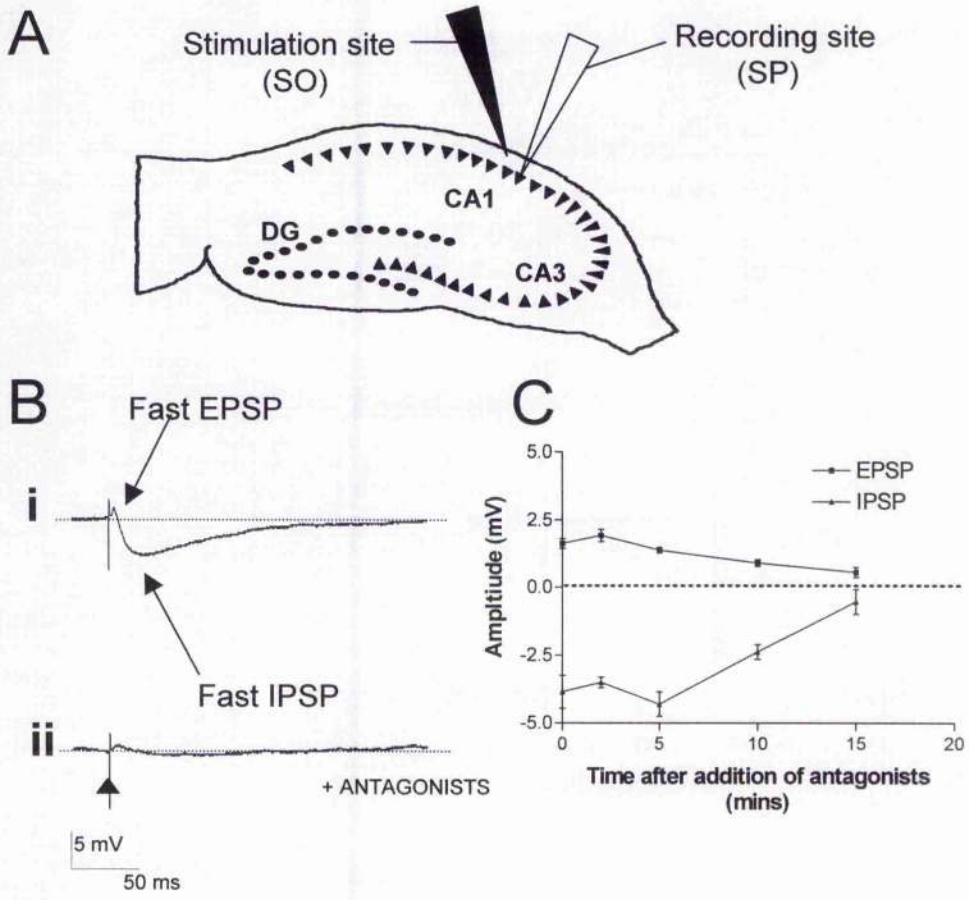
Resting membrane potential (mV)	**Cell input resistance (MΩ)**	**Action potential amplitude (mV)**	**Action potential duration (ms) (measured at ½ amplitude)**
-60.73±2.9	50.59±4.2	69.41±2.03	0.91±0.03

Table 3.1 General properties of pyramidal neurones recorded in hippocampal slices.

In order to monitor the physiological status of CA1 pyramidal neurones, four parameters were measured throughout the sharp-electrode intracellular recordings. This table shows mean values ±S.E.M. of each parameter calculated from all CA1 pyramidal neurones (n=16).

3.2.2. Fast amino acid synaptic transmission

Application of a low intensity (<200μA, 0.02ms) single stimulus within the *stratum oriens* (Fig 3.2A) produced a compound depolarising then hyperpolarising synaptic response in the impaled pyramidal neurone (Fig 3.2Bi). This compound EPSP-IPSP displayed fast kinetics consistent with the activation of glutamatergic and GABAergic postsynaptic receptors respectively. Both the depolarising and hyperpolarising evoked components were strongly suppressed by subsequent application of a cocktail of antagonists for amino acid receptors (n=16) (Fig. 3.2Bii and 3.2C). The antagonist cocktail consisted of NBQX, CGP40116, Picrotoxin and CGP55845A selective antagonists for AMPA, NMDA, $GABA_A$ and $GABA_B$ mediated responses respectively as outline in figure 3.2D.



D

Response	Transmitter	Receptor	Antagonist	Conc (μM)
EPSP	Glutamate	AMPA	NBQX	2
EPSP	Glutamate	NMDA	CGP40116	50
IPSP(slow)	GABA	$GABA_B$	CGP55845A	1
IPSP(fast)	GABA	$GABA_A$	Picrotoxin	50

Figure 3.2. Inhibition of fast amino acid synaptic transmission in CA1 pyramidal neurones.

A. Diagram illustrating the position of recording and stimulation electrodes within the hippocampal slice. B. Representative voltage traces recorded from a CA1 pyramidal neurone in response to a single stimulus (100μA, 0.02ms) delivered within the *stratum oriens*. Bi. Shows the response evoked in control medium (control) illustrating the production of a fast EPSP followed by an IPSP (trace is an average of 5 traces at 15s intervals). Bii. Illustrates the near complete blockade of this fast synaptic transmission following addition of NBQX (2μM), CGP40116 (50μM), picrotoxin (50μM) and CGP555845A (1μM). C. Point plots showing reduction in the mean amplitude of the EPSP and IPSP after addition of antagonist cocktail (each point is an average of 5 traces recorded at each time point for a single experiment). D. Table shows the components of the antagonist cocktail used to block all fast amino acid synaptic transmission including; the transmitter and receptor responsible for each response, the antagonist and the concentration applied to inhibit each response. In this and all subsequent figures, filled triangles (▲) below each voltage trace indicates the time point of afferent stimulation.

3.2.3. Stimulation evoked slow EPSPs

Having blocked fast amino acid transmission and in the continued presence of the amino acid receptor antagonist cocktail, subsequently increasing the stimulus intensity and duration (10-400μA, 0.2ms) uncovered a slow excitatory postsynaptic potential (EPSP) in 10 of 11 CA1 pyramidal neurones in which this was attempted. Such responses were similar to those previously reported (Cole & Nicoll, 1984a; Morton, 1998) in that they displayed a slow waveform with a mean peak amplitude of 5.078 ±1.82mV and mean time to peak of 10.07±2.972s. This slow EPSP could be suprathreshold for action potential generation, which resulted in an intense discharge of action potential firing.

Although clearly present this slow EPSP was very small in the majority of pyramidal neurones recorded, with a mean peak amplitude of 2.08±0.51mV and a mean time to peak of 14.18±4.084s (n=6). However in the presence of the acetylcholinesterase inhibitor eserine (1μM) this response could be significantly enhanced to a mean peak amplitude of 6.09±0.846mV and mean time to peak of 13.33±4.767s (P= 0.0208, n=6, Fig. 3.3 & 3.4). In order to obtain robust and consistent responses from all pyramidal neurones recorded all subsequent experiments were carried out in the presence of 1μM eserine.

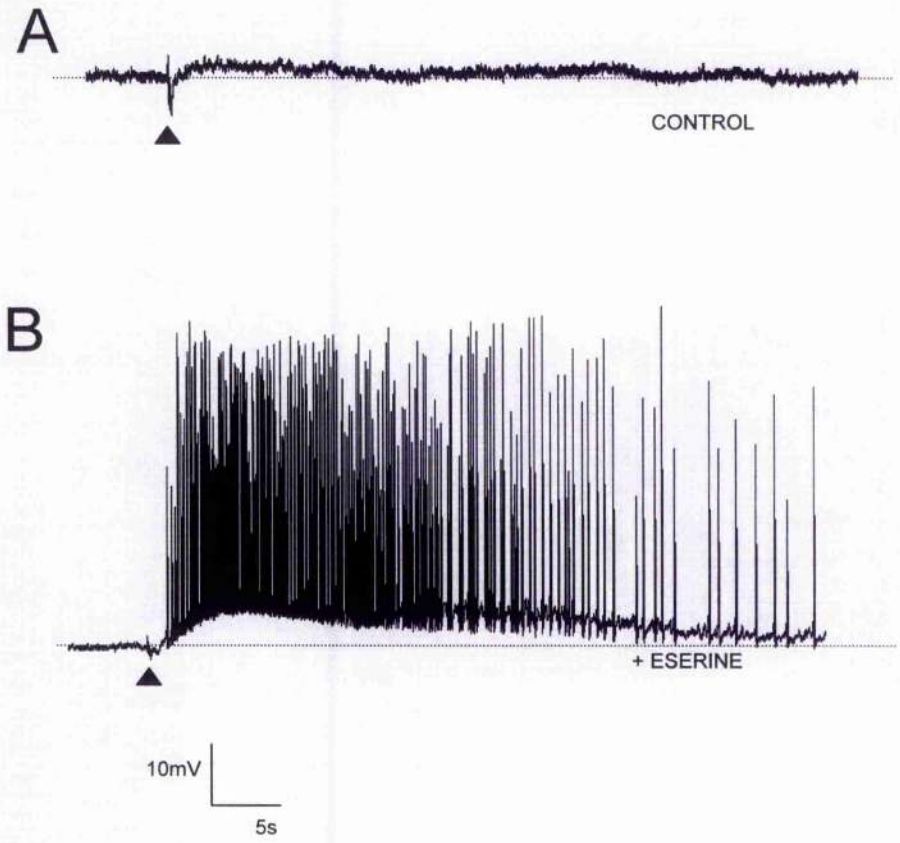


Figure 3.3. The slow EPSP evoked by electrical stimulation within the *stratum oriens*.

A. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 2.05mV, evoked by a train of stimuli (5 x 0.2ms,10μA pulses @ 20Hz) delivered within the *stratum oriens* when all fast synaptic transmission was blocked by the antagonist cocktail. B. Representative voltage trace recorded from the same pyramidal neurone showing a slow EPSP, with a peak amplitude of 6.75mV, evoked by the same stimulation parameters in the presence of the acetylcholinesterase inhibitor eserine (1μM). Note that the peak amplitude of depolarisation was enhanced in the presence of eserine by 229% in this example, and that the membrane potential was more likely to be depolarised to firing threshold. Unless otherwise stated all subsequent responses shown will be in the presence of 1μM eserine.

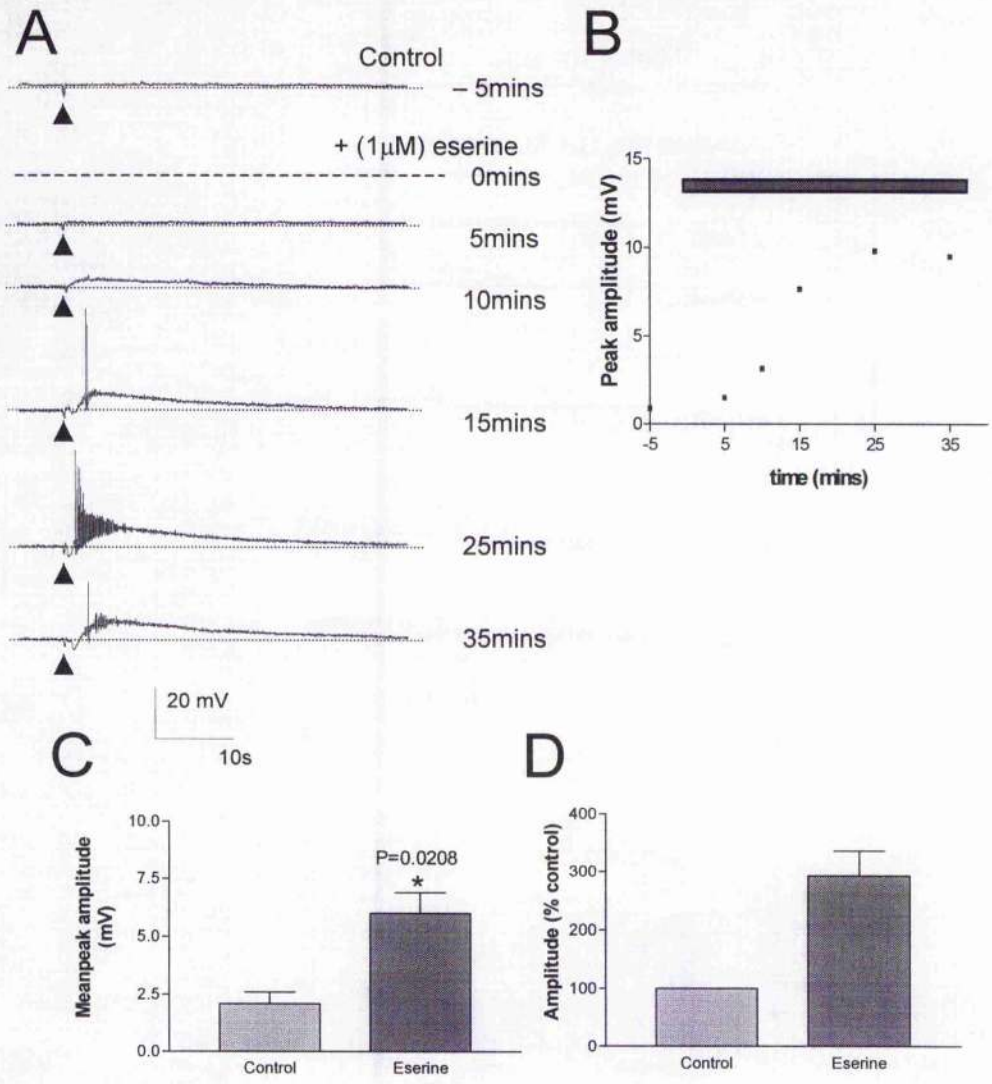


Figure 3.4. Effect of the cholinesterase inhibitor eserine on the slow EPSP.

A. Successive voltage traces recorded from a CA1 pyramidal neurone at 5-10 minute intervals illustrating the augmentation of the slow EPSP as 1μM eserine washes into the bath B. Point plot of the corresponding peak amplitudes of the successive EPSPs evoked every 5-10 minutes. The solid bar indicates duration of eserine application. Note that full augmentation of the slow EPSP was seen within 15-35 minutes after eserine application. C. Bar chart of pooled data from 4 individual pyramidal neurones illustrating the significant potentiation of the mean peak amplitude of the slow EPSP from 2.08±0.51mV to 6.09±0.846mV in the presence of eserine (n=4, P= 0.0208). D. Bar chart in which the pooled data for the peak amplitude of EPSPs recorded after 15-30 minutes of eserine application is expressed as a percentage of the mean value of peak amplitude prior to eserine application. Note that eserine application results in a 200% increase in mean peak amplitude. Values are expressed as a mean ± S.E.M, P value was calculated using a paired t-test after data passed test for normal distribution.

Overall 11 out of 12 pyramidal neurones responded to stimulation within the *stratum oriens* with a slow, often suprathreshold, depolarisation in the presence of eserine showing a mean peak amplitude of 6.109 ±0.54mV and a mean time to peak of 10.14±2.743s. The slow EPSP could be consistently evoked every 5 minutes with no significant rundown of the second response (n =6, P=0.609). This is shown graphically in figure 3.5, which compares consecutive responses evoked at 5-minute intervals.

3.2.4. Stimulation parameters

A consistent finding was that the amplitude and duration of the slow EPSP was dependent upon the precise stimulation parameters used. In particular, the stimulus amplitude (intensity), presumably reflecting the number of cholinergic fibres recruited, and the number of stimuli, presumably reflecting the amount or reliability of transmitter released at any given stimulated fibre, were critical determinants.

Figure 3.6A shows voltage traces from a representative pyramidal neurone where an initial single shock stimulus (10μA, 0.2ms) delivered within the *stratum oriens* evoked a slow EPSP with a peak amplitude of 4.3mV. Subsequent application of a brief train of 5 stimuli (10μA, 0.2ms) at a frequency of 20Hz dramatically increased the peak amplitude of depolarisation to 6.75mV and increased the firing duration and firing rate of the evoked slow response. This potentiation was seen in 5 of the 6 cells tested (Fig. 3.6B).

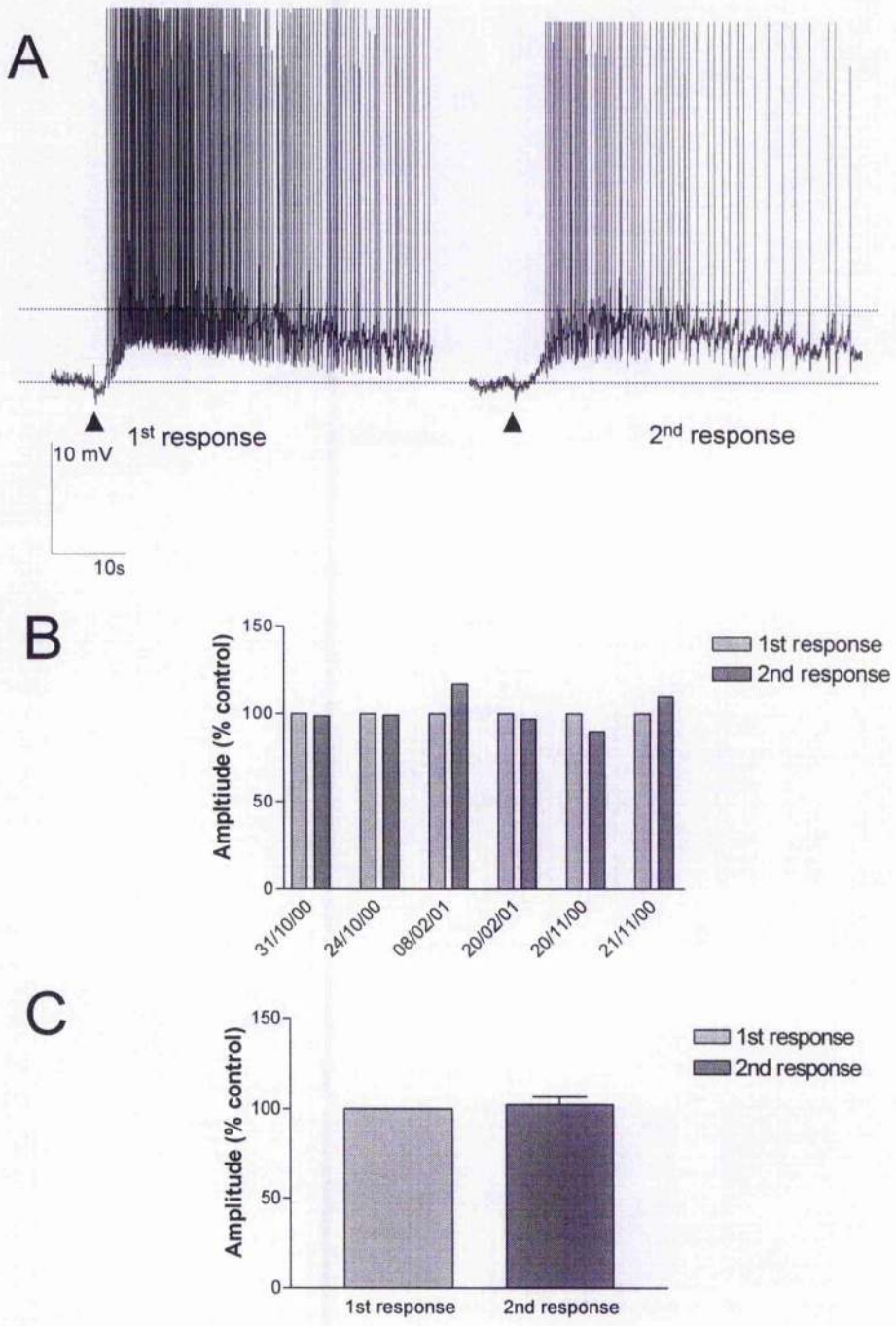


Figure 3.5. Consistent slow EPSPs can be evoked using a 5 minute stimulus interval.

A. Representative voltage traces recorded from a CA1 pyramidal neurone showing two successive slow EPSPs, of peak amplitude of 5.75mV and 6.75mV respectively, evoked by a train of stimuli (5 x 0.2ms, 10μA pulses @ 20Hz) delivered within the *stratum oriens* at an inter-stimulus interval of 5 minutes. Note that the second response shows no depression in peak amplitude, although a slight reduction in firing rate is apparent this was not a consistent finding in all cells. B. Bar chart showing no depression of the second synaptic response in 6 individual pyramidal cells when an inter-stimulus interval of 5 minutes was used. The second response is expressed as percentage of the peak amplitude of the first response (taken to be 100%). C. Bar chart of pooled data showing no significant depression of the second EPSP (n=6, P=0.609). P value was calculated from the raw data, using a paired t-test after data passed test for normal distribution.

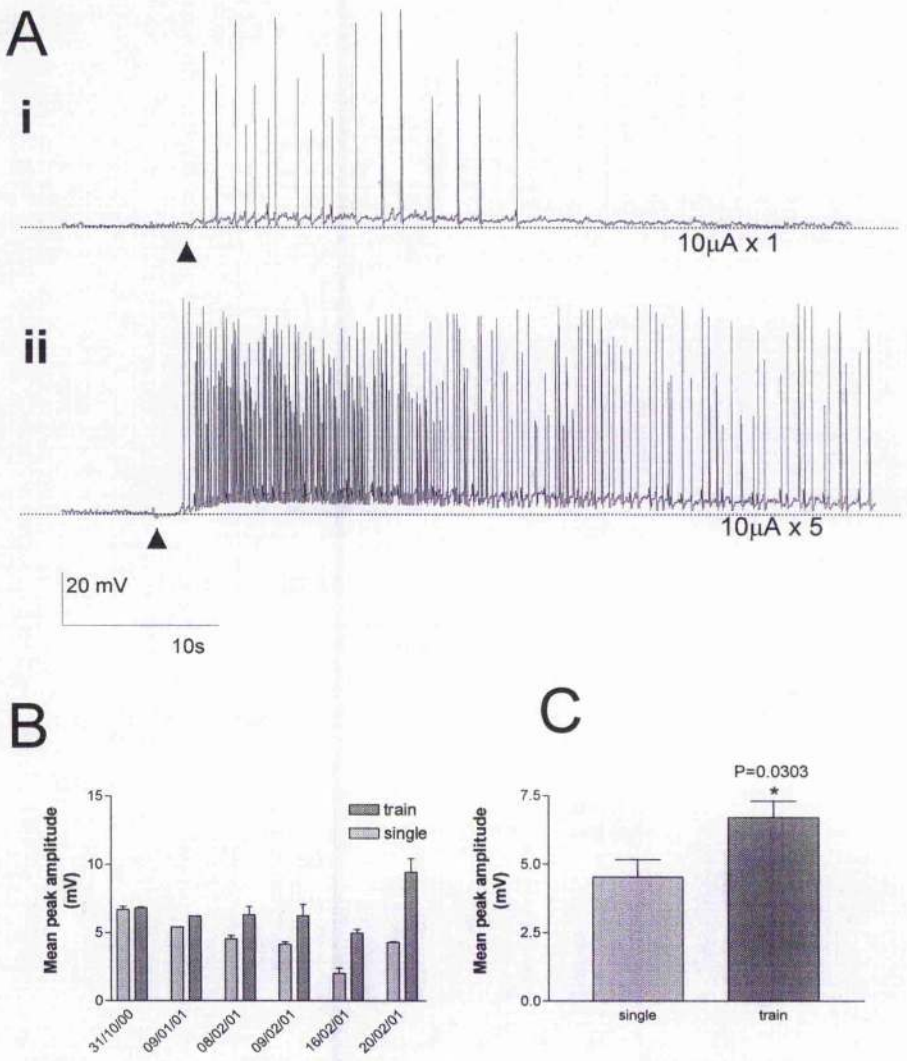


Figure 3.6. Slow EPSPs evoked by delivery of a 20Hz train of stimuli within the stratum oriens.

A, Representative voltage traces recorded from a CA1 pyramidal neurone showing a slow EPSP, of peak amplitude 4.3mV, evoked by a single 10μA (pulse duration 0.2ms) stimulus delivered within the *stratum oriens* (Ai) and a slow EPSP, of peak amplitude 6.75mV, evoked by a 20Hz train of 5 stimuli of the same intensity and duration (Aii). Note the increase in peak amplitude, firing duration and firing rate on application of a train of stimuli. B, Bar chart showing the mean peak amplitude of slow EPSPs evoked in 6 individual CA1 pyramidal neurones with a single stimulus and subsequently with a train of 5 stimuli. C, Bar chart of pooled data illustrating the significant increase in mean peak amplitude of the slow EPSP from 4.35±0.639mV when evoked with a single stimuli to 6.69±0.604mV when evoked by a train of stimuli (n = 6, P =0.0303). Values are expressed as a mean ± S.E.M., P value was calculated using a paired t-test, after data passed test for normal distribution. Unless otherwise stated all subsequent responses shown in this chapter were evoked using a train of 5 stimuli @ 20Hz and a pulse duration of 0.2ms.

Overall the mean peak amplitude of depolarisation significantly increased from 4.53±0.639 to 6.69±0.604mV on application of a train of stimuli (n=6, P=0.0303, Fig. 3.6C).

Increasing the stimulation intensity also increased the amplitude of the slow EPSP. This effect was apparent whether a single or train of stimuli were applied. Figure 3.7A shows voltage traces from a representative pyramidal neurone where a slow EPSP, with a peak amplitude of 4.3mV, was evoked with a single stimulus of 10μA (0.2ms duration). Subsequently increasing the stimulation intensity to 40μA increased the amplitude of the evoked slow EPSP to 5.65mV. Fig 3.7B shows a similar potentiation of the slow EPSP from 6.75mV to 9.4mV using a 20Hz train of 5 stimuli at 10μA and 40μA respectively.

In order to evoke robust and consistent slow EPSPs we routinely included the AChE inhibitor, eserine, in the aCSF and used a train of 5 stimuli at 20Hz every 5 minutes. The stimulation intensity needed to evoke a slow EPSP varied between cells, possibly due to the fact that the position of the stimulation electrode relative to the impaled cell and cholinergic afferents differed across slices.

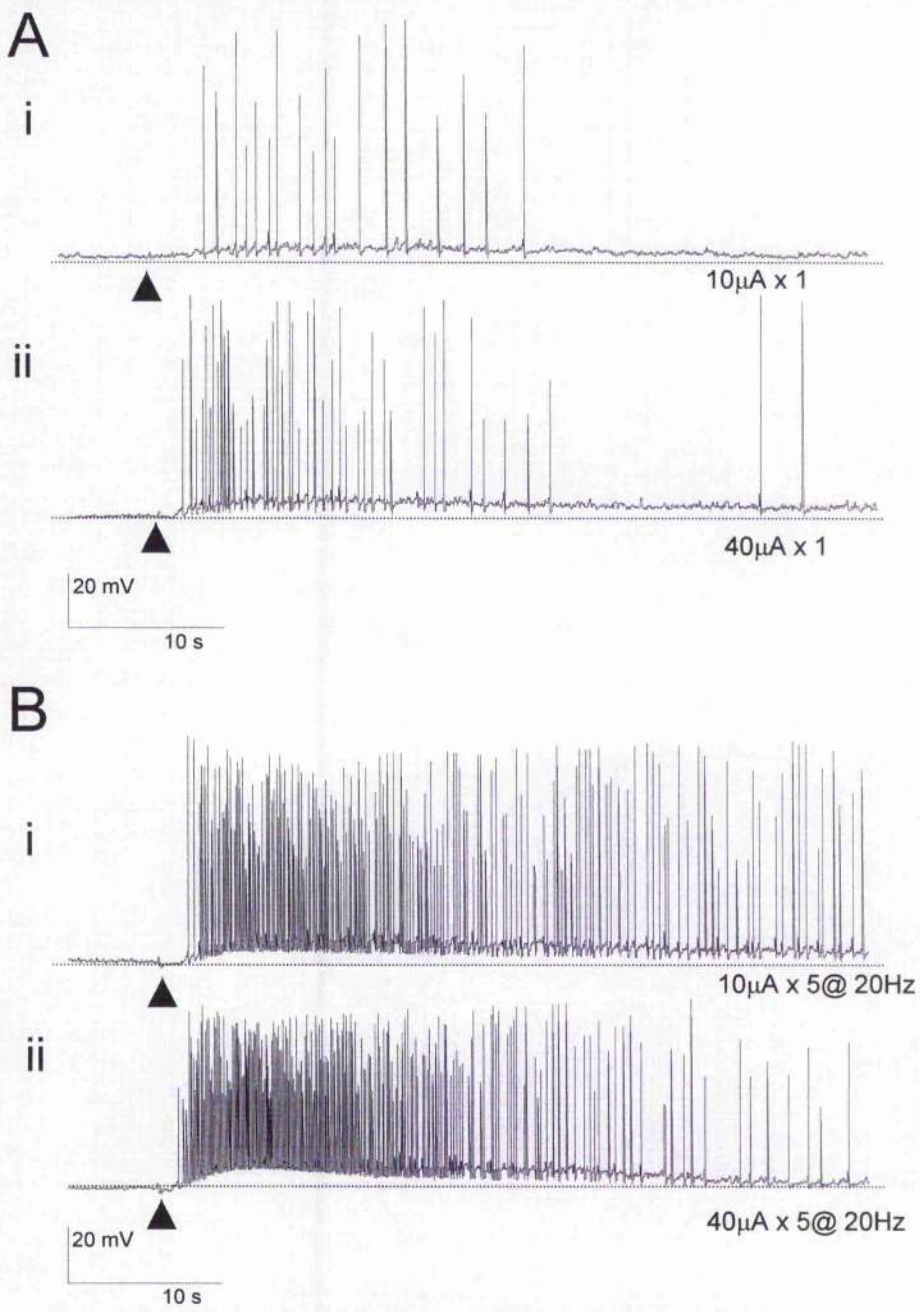


Figure 3.7. Effect of stimulation intensity on the slow EPSP

A, Representative voltage traces recorded from a single CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 4.3mV, evoked by a single 10μA (0.2ms duration) stimulus delivered within the *stratum oriens* (Ai) and a slow EPSP, with a peak amplitude of 5.65mV, evoked by a single 40μA (0.2ms duration) stimulus (Aii). B, Representative voltage traces from the same pyramidal neurone showing a slow EPSP with a peak amplitude of 6.75mV, evoked by a train of 10μA stimuli (5 x 0.2ms, 10μA @ 20Hz) delivered within the *stratum oriens* (Bi) and a slow EPSP, with a peak amplitude of 9.4mV, evoked by a train of 40μA stimuli (Bii). Note that increasing the stimulation intensity increased the peak amplitude of the slow EPSP whether a single or train of stimuli was used to evoke the initial response.

3.2.5. Carbachol evoked slow EPSPs

The effect of the acetylcholine analogue carbachol on the membrane potential of CA1 pyramidal neurones was compared with the effect of electrical stimulation of the *stratum oriens*. Rapid application of carbachol (10μM) by pressure ejection through a carbachol filled pipette tip placed close (~50μm) to the recording site elicited a slow depolarising response in 4 CA1 pyramidal neurones tested. This depolarising response displayed slow kinetics in a range similar to that of the previously described slow EPSP (mean peak amplitude 10.42±1.067mV, time to peak of 33.8±5.709s). As with evoked slow EPSPs direct carbachol application resulted in an intense discharge of action potential firing (Fig. 3.8).

3.2.6. Muscarinic nature of the slow EPSP

In order to establish the pharmacological nature of the slow depolarising synaptic responses, further studies were carried out using the selective mAChR antagonists atropine and pirenzepine.

Addition of atropine (5μM) significantly inhibited the mean peak amplitude of the evoked slow EPSP recorded in CA1 pyramidal neurones from 6.574±0.621mV to 0.866±0.252mV ($n=8$, $P<0.0001$) an inhibition of over 80% (Fig. 3.9 & 3.10).

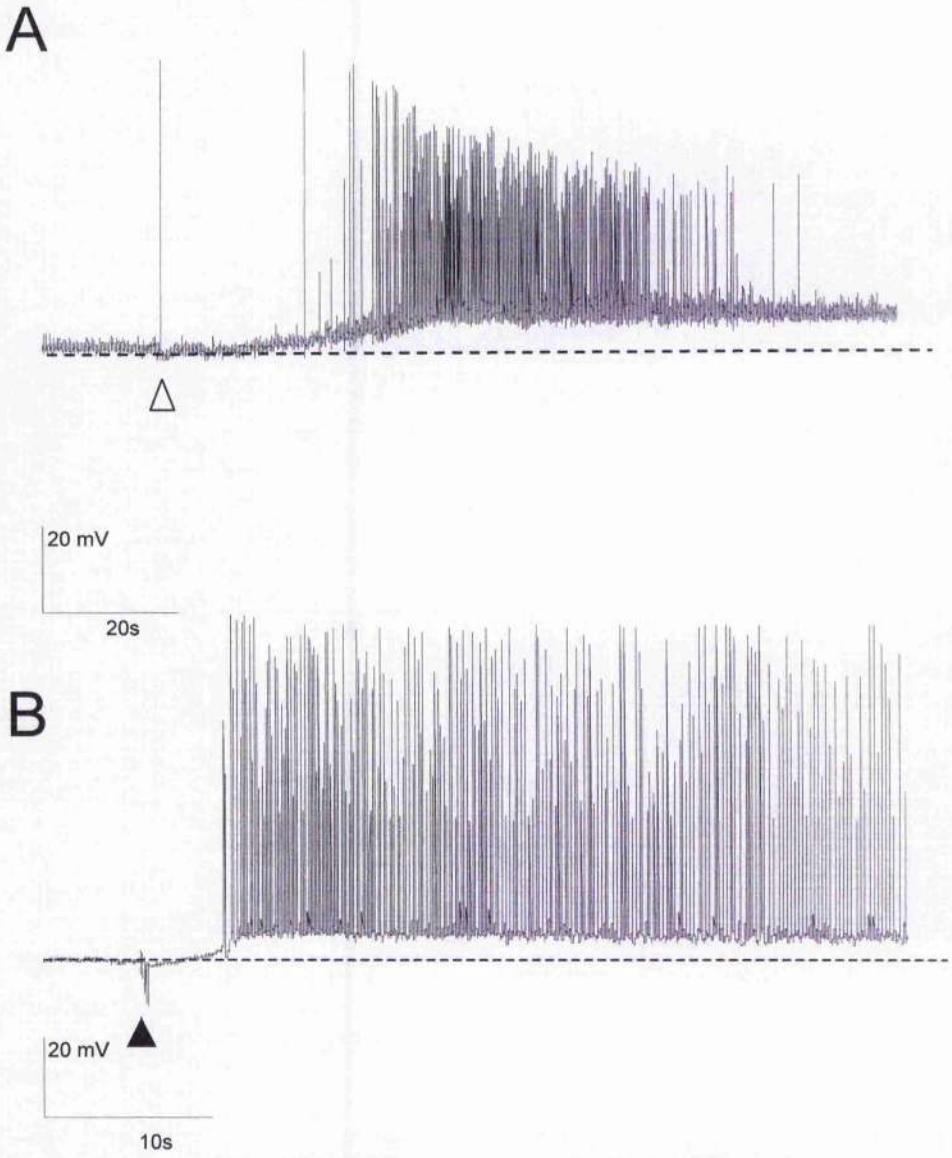


Figure 3.8 A comparison of the effects of carbachol and electrical stimulation within the *stratum oriens*.

A. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow depolarisation of the membrane potential, with a peak amplitude 13.2mV, induced by rapid application of carbachol (10μM). The point of carbachol application is indicated by the open triangle (△). This slow depolarisation in response to carbachol application was recorded in 4 pyramidal neurones, with a mean peak amplitude of 10.42±1.067mV and a mean time to peak amplitude of 33.8±5.709s. B. A representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 8.85mV, evoked by a train of stimuli (5 x 50μA, 0.2ms pulses @ 20 Hz) is shown for comparative purposes. Note that the carbachol induced depolarisation is similar in waveform to the slow EPSP evoked by electrical stimulation within the *stratum oriens* and that both protocols can induce repetitive action potential firing in CA1 pyramidal neurones. Values are expressed as a mean ± S.E.M.

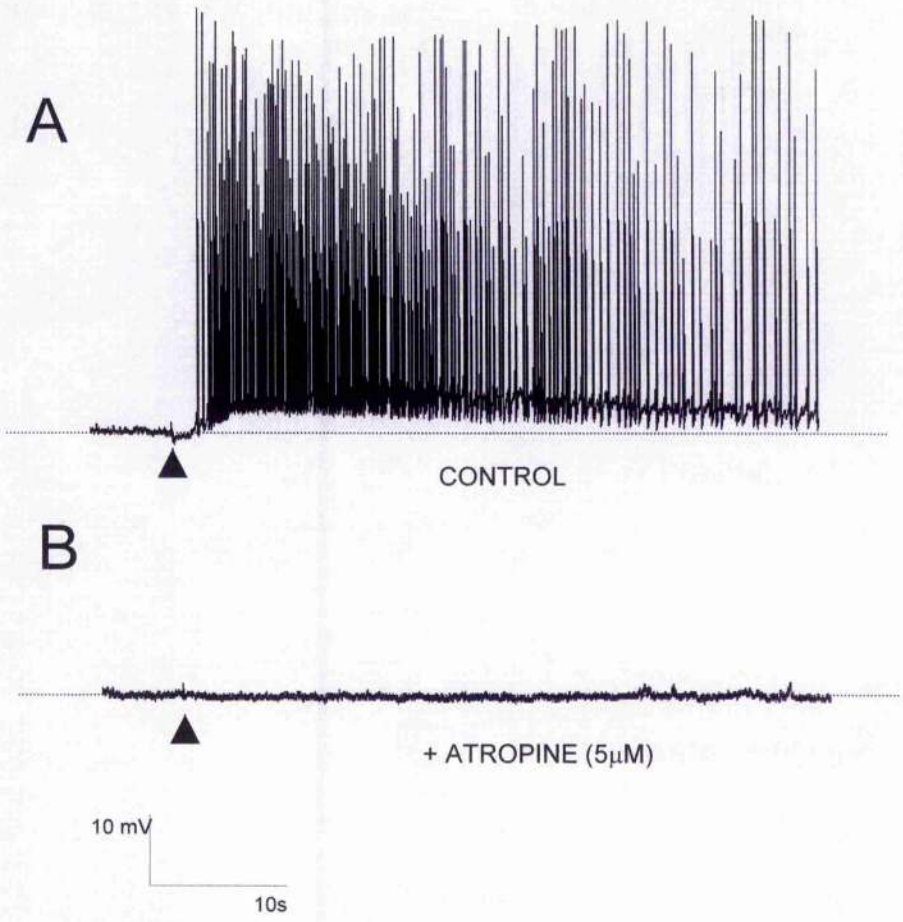


Figure 3.9. Effect of the selective mAChR antagonist atropine on the slow EPSP.

A. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 6.75mV, evoked by a train of stimuli (5 x10μA , 0.2ms @ 20Hz) delivered within the *stratum oriens*. B. Representative voltage trace from the same pyramidal neurone illustrating the effect of the selective mAChR anatgonist atropine (5μM) on the production of a slow EPSP (peak amplitude 0.4mV). Note that atropine inhibits the slow EPSP evoked by electrical stimulation within the *stratum oriens* by over 90% in this example.

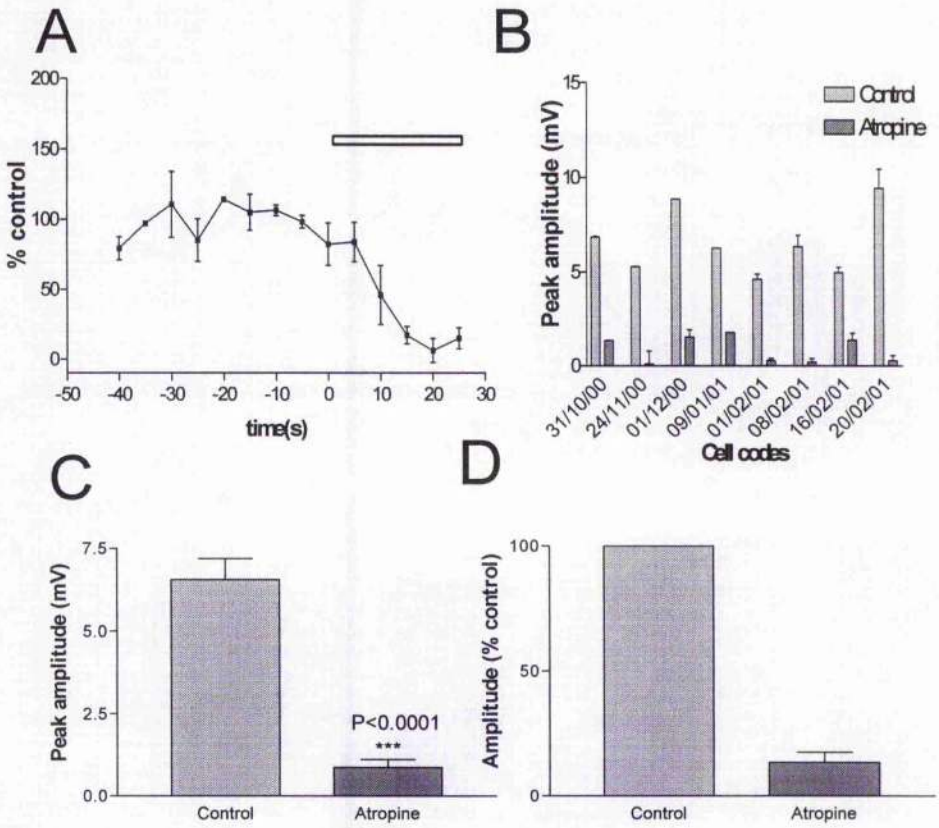


Figure 3.10. The slow EPSP is inhibited by the selective mAChR antagonist atropine

A. Point plot showing the time course of the atropine (5μM) effect as it washes into the bath. Each data point is a mean of the peak amplitude of the slow EPSP expressed as a percentage of its own control in 7 individual pyramidal neurones. Note that depression of the slow EPSP occurs within 15-30 minutes of atropine application (n=7, solid bar indicates period of atropine application). B. Bar chart illustrating the reduction of the mean peak amplitude of the slow EPSP in 8 individual CA1 pyramidal neurons. Control values are calculated as a mean of the peak amplitude of EPSPs recorded prior to atropine application, atropine values are calculated as a mean of the peak amplitude of EPSPs recorded 15-30 minutes after atropine application. C. Bar chart of pooled data for all 8 pyramidal neurones illustrating that atropine significantly depresses the mean peak amplitude of the slow EPSP from 6.574±0.621mV to 0.866±0.252mV (n=8, P<0.001). D. Bar chart in which the pooled data for the peak amplitude of the slow EPSPs recorded 15-30 minutes after atropine application is expressed as a percentage of the mean value of the control EPSPs recorded prior to atropine application. Note that atropine results in over an 80% reduction in mean peak amplitude. Values are expressed as a mean ± S.E.M., P values were calculated using a paired t-test, after data passed test for normal distribution.

In slices in which a cholinergic EPSP was repeatedly evoked every 5 minutes, addition of the selective mAChR antagonist atropine resulted in a gradual reduction of the response amplitude as the atropine washed into the bath and resulted in a near complete abolition of the evoked response within 15-30mins following the onset of application (Fig 3.10A).

The mean peak amplitude of the slow EPSP was also significantly reduced from 5.207±1.2mV to 0.7±0.49mV (n=4, P=0.0092, 86% inhibition) by the addition of the selective mAChR antagonist pirenzepine (Fig. 3.11) applied at a concentration that is reported to show selectivity towards the M1 subtype of mAChR (Hammer *et al.*, 1980). These experiments confirmed that the slow EPSP is principally mediated by mAChR activation and extends the work of Cole and Nicoll (1984a) by suggesting that the EPSPm is principally mediated through activation of the M1 mAChR subtype.

3.2.7. Septo-hippocampal slices

The aim of preparing septo-hippocampal slices was to produce acute slices, which would contain an intact septo-hippocampal projection allowing us to activate this functionally significant pathway and observe the postsynaptic responses in CA3 and CA1 pyramidal neurones of the hippocampus (Fig. 3.12A). In total, 9 pyramidal neurones were recorded in rat septo-hippocampal slices, their general properties are summarised in table 3.2.

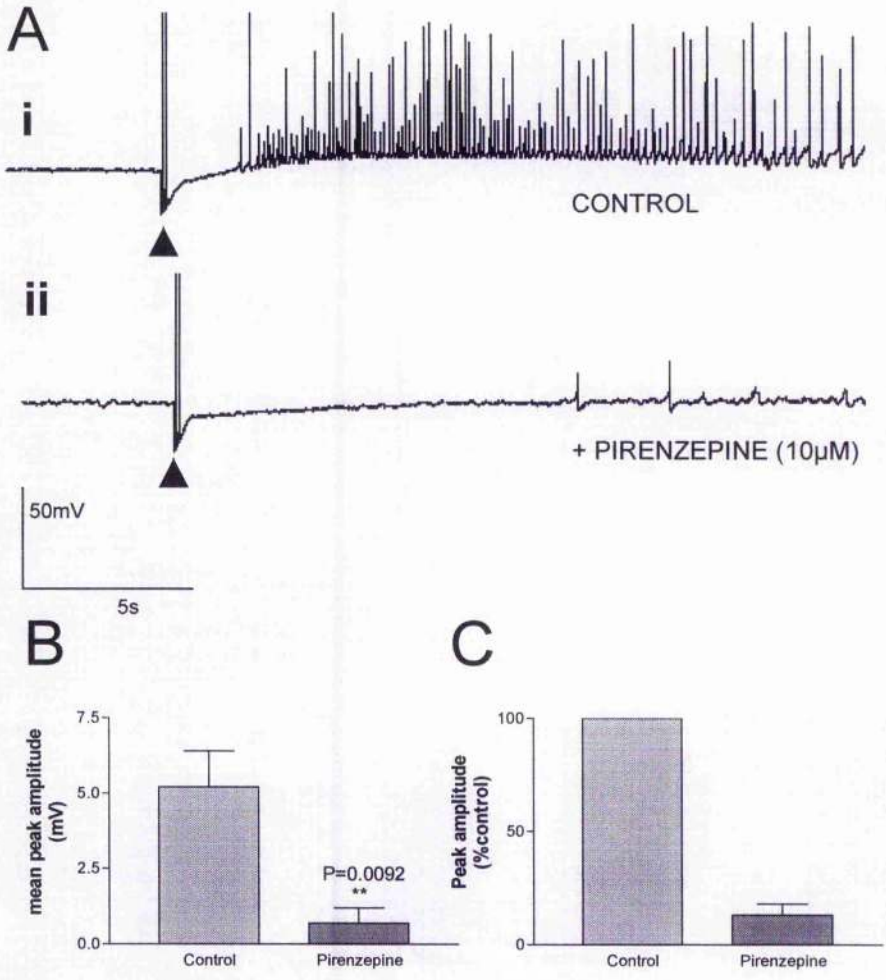


Figure 3.11. Effect of the selective mAChR antagonist pirenzepine on the slow EPSP.

A, Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 7.7mV, evoked by a train of stimuli (5 x 4mA, 0.2ms @ 20Hz) delivered within the *stratum oriens*. Aii. Representative voltage trace from the same pyramidal neurone illustrating the effect of the selective mAChR anatgonist pirenzepine (10μM) on the slow EPSP (peak amplitude 1.45mV). Note that pirenzepine depressed the peak amplitude of the slow EPSP evoked on electrical stimulation within the *stratum oriens* by 81% in this example B. Bar chart of pooled data for 4 pyramidal neurones illustrating the significant depression of the peak amplitude of the slow EPSP from 5.207±1.2mV to 0.7±0.49mV (P=0.0092). C. Bar chart in which the pooled data for the peak amplitude of the slow EPSP recorded after 15-30 minutes in the presence of pirenzepine is expressed as a percentage of the mean value of control EPSPs recorded prior to drug application (n=4). Note pirenzepine significantly depressed the slow EPSP by over 80%. Values are expressed as a mean ± S.E.M., P value was calculated using a paired t-test, after data passed test for normal distribution.

Resting membrane potential (mV)	Cell input resistance (MΩ)	Action potential amplitude (mV)	Action potential duration (ms) (measured at ½ amplitude
-58.27±2.54	73.11±3.04	71.8±5.2	1.45±0.1

Table 3.2 General properties of rat pyramidal neurones recorded from septo-hippocampal slices.

In order to monitor the physiological status of hippocampal pyramidal neurones, four parameters were measured throughout the sharp-electrode intracellular recordings. This table shows mean values ±S.E.M. of each parameter calculated from all pyramidal neurones recorded in the rat septo-hippocampal slice preparation (n=9).

Stimulation within the MSN elicited a slow EPSP in the 9 pyramidal neurones tested, with a mean peak amplitude of 10.32±1.651mV and a mean time to peak of 11.797±1.166s. This slow EPSP was similar in waveform to that seen in the hippocampal slice preparation (Fig 3.12B) and was similarly blocked by application of atropine (n=2, data not shown). This data confirmed that activation of cholinergic afferents, originating in the MSN, produce a slow cholinergic EPSP in hippocampal pyramidal neurones.

Figure 3.12B shows simultaneous recordings from two pyramidal neurones displaying slow EPSP in response to MSN stimulation. Note the striking synchronous timing of the responses with both cells firing the first spike at exactly the same time. This illustrates the tight interconnection of cells within the

hippocampal network and that release of ACh from septal cholinergic afferents may act to synchronise pyramidal neurone firing. In four of the recorded pyramidal neurones IPSPs could be seen throughout the evoked slow EPSP (fig 3.12C) suggesting some activation of a GABAergic input to the pyramidal neurones on stimulation within the MSN.

The preparation of septo-hippocampal slices from the rat brain proved difficult in that from visual inspection as well as by electrical stimulation it was clear that only a minority of slices contained the intact septo-hippocampal connection. This is consistent with previous reports that only a single slice with the intact connection is obtained from a single rat brain (Toth *et al.*, 1997). By preparing similar slices from smaller mouse brains the overall size and fragility of the slice was reduced increasing the likelihood that a single slice would include an intact functional septo-hippocampal projection and allowed easier implementation of the experimental protocol. In total 6 pyramidal neurones were recorded in mouse septohippocampal slices, properties of the cells are summarised below in table 3.3.

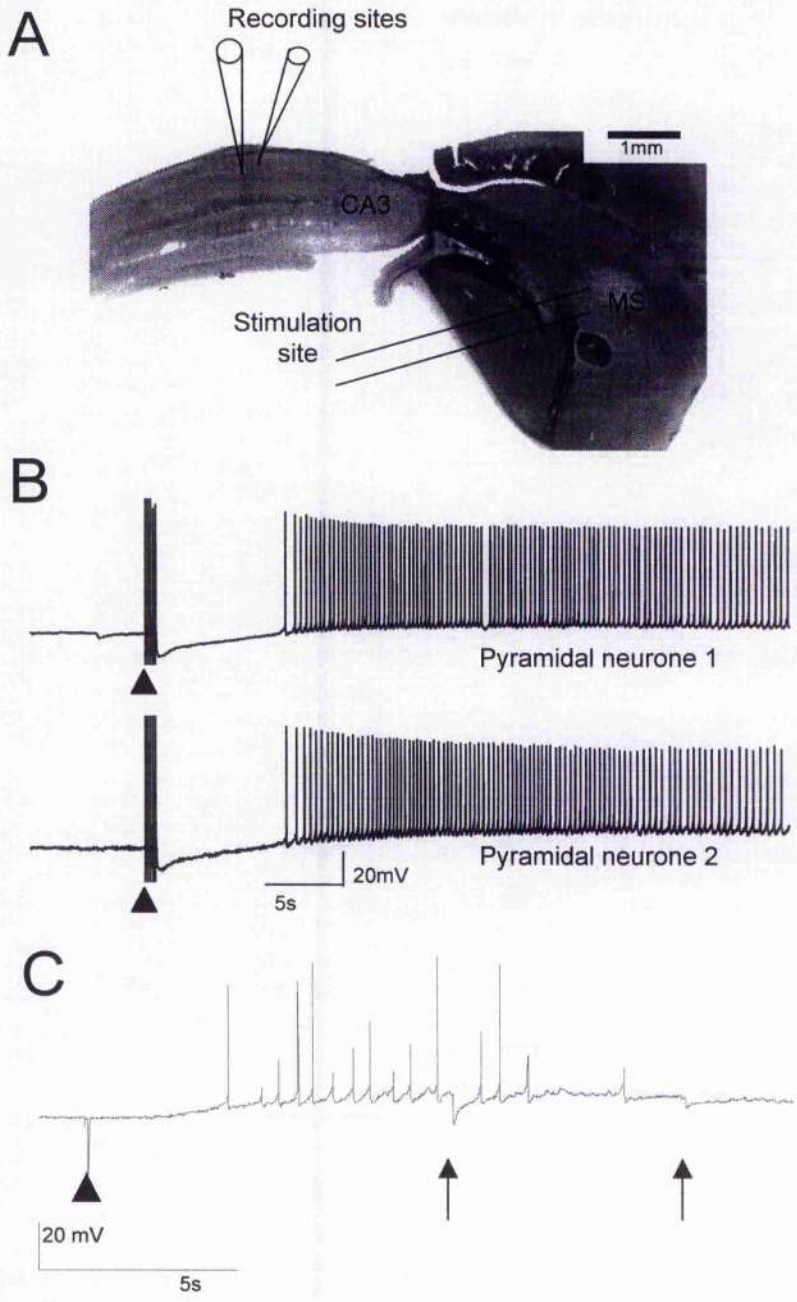


Figure 3.12 Isolation of a slow EPSP in CA1 pyramidal neurons using the septo-hippocampal slice preparation.

A. Low power micrograph of the septo-hippocampal slice preparation indicating the position of the stimulation electrode within the medial septal nucleus (MSN) and the position of recording electrodes within the hippocampus. B. Representative voltage traces from two simultaneously recorded pyramidal cells illustrating the production of a slow EPSP in both cells on electrical stimulation within the MSN. Note the peak amplitude, delay and time course of the response is very similar in the two postsynaptic neurons. C. Shows a representative voltage trace for a pyramidal neurone where IPSPs (indicated by arrows) are present throughout the slow EPSP.

Resting membrane potential (mV)	Cell input resistance (MΩ)	Action potential amplitude (mV)	Action potential duration (ms) measured at ½ amplitude
-63.5±2.68	101.33±12.02	70.55±2.45	0.9±0.03

Table 3.3 General Properties of mouse pyramidal neurones recorded from the septohippocampal slice preparation..

In order to monitor the physiological status of hippocampal pyramidal neurones, four parameters were measured throughout the sharp-electrode intracellular recordings. This table shows mean values ±S.E.M. of each parameter calculated from all pyramidal neurones recorded in the mouse septo-hippocampal slice preparation (n=6).

Figure 3.13A shows the production of a slow EPSP on stimulation of the MSN in both rat and mouse slices. The rat and mouse responses were very similar in appearance and a paired t-test indicated no significant difference in the mean peak amplitude of the slow EPSP recorded from the rat pyramidal neurones (10.32±1.65mV, n=9) compared to those recorded from the mouse pyramidal neurones (6.962±0.97mV, n=6, P=0.1495). As there was no significant difference between the rat and mouse responses data collected from both species was pooled and presented as one data set from this point forward (mean peak amplitude 8.975±1.123mV, mean time to peak 10.26±0.95, n=15).

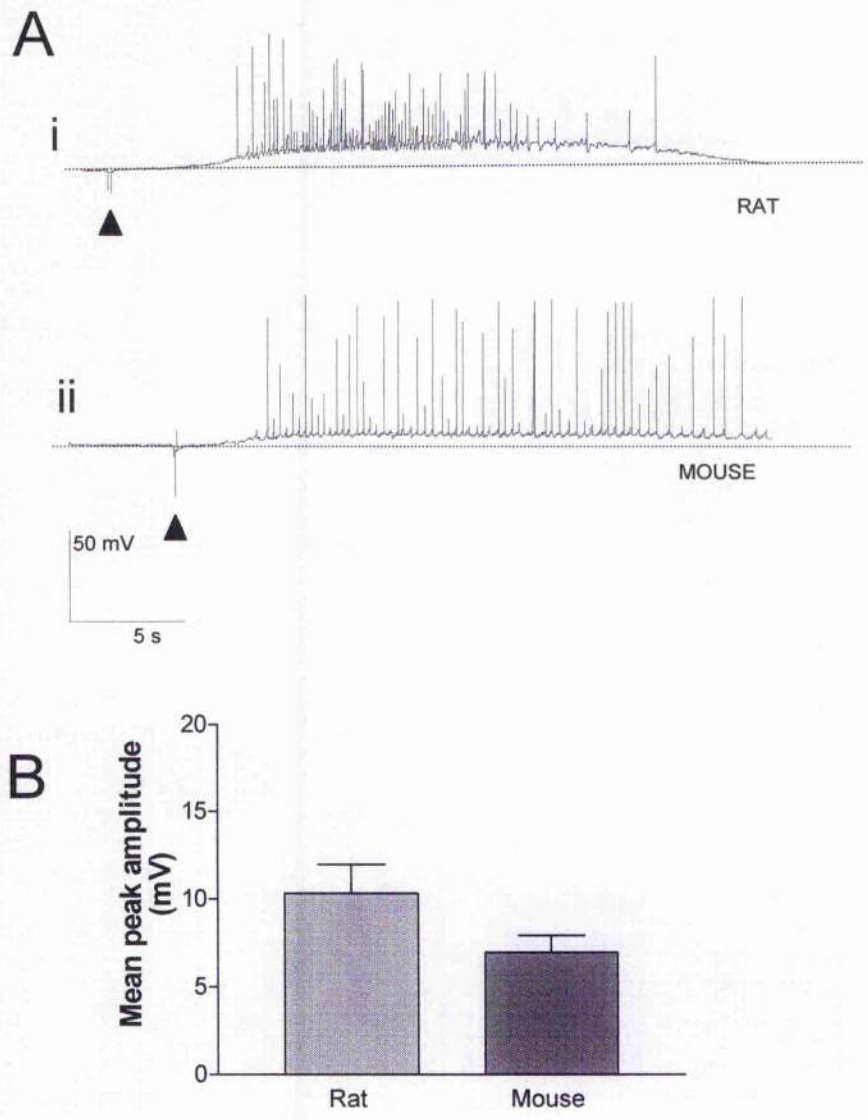


Figure 3.13 Slow EPSP evoked by electrical stimulation of the MSN in both rat and mouse slices.

A. Representative voltage traces recorded from CA1 pyramidal neurones showing slow EPSPs evoked by electrical stimulation within the MSN. Ai illustrates a slow EPSP, with a peak amplitude of 8.0mV evoked by a train of stimuli (5 x 5mA, 0.2ms @ 20Hz) in the rat septo-hippocampal slice. Aii Illustrates a slow EPSP, with a peak amplitude of 5.8mV, evoked by a train of stimuli (5 x 6mA, 0.2ms @20Hz) in the mouse septo-hippocampal slice (Aii). B. Bar chart of pooled data comparing the mean peak amplitude of the slow EPSP evoked by stimulation of the MSN in rat (10.32±1.65mV, n=9) and mouse (6.962±0.97mV, n=6) septo-hippocampal slices. An unpaired t-test indicated that there was no significant difference in mean peak amplitudes (P= 0.1495). Values are expressed as a mean ± S.E.M., P values were calculated using an unpaired t-test, after data had passed test for normal distribution.

In order to confirm the cholinergic nature of the slow EPSP evoked by electrical stimulation within the MSN of the septo-hippocampal slices, slices were perfused with the selective mAChR antagonist atropine (5μM). This concentration of atropine significantly inhibited the peak amplitude of the slow EPSP from 9.021±2.878 to 0.0344±0.087mV (n=5, P=0.0489, Fig. 3.14), an inhibition of over 96%. This data confirmed that the slow EPSP evoked by electrical stimulation within the MSN is mediated via activation of mAChRs.

Due to the difficulties in preparation of the septo-hippocampal slices and the generally low yield of slices, at best a single septo-hippocampal slice per experimental animal, we wished to asses whether there were qualitative or quantitative differences between the slow cholinergic EPSP obtained from stimulation of the cholinergic afferents within the MSN and direct stimulation of cholinergic fibres locally within the *stratum oriens* of the hippocampus.

Such a comparison, shown in figure 3.15, revealed a similar slow waveform showing no significant difference in amplitude or time course of slow EPSP. Slow EPSPs evoked on stimulation of the *stratum oriens* had a mean peak amplitude of 6.109±0.54mV (n=11) compared to 8.975±1.123mV on stimulation of the MSN (n=15, P=0.187), and a mean time to peak of 10.14±2.743s on *stratum oriens* stimulation compared to 10.264±0.95s on MSN stimulation (P=0.9620).

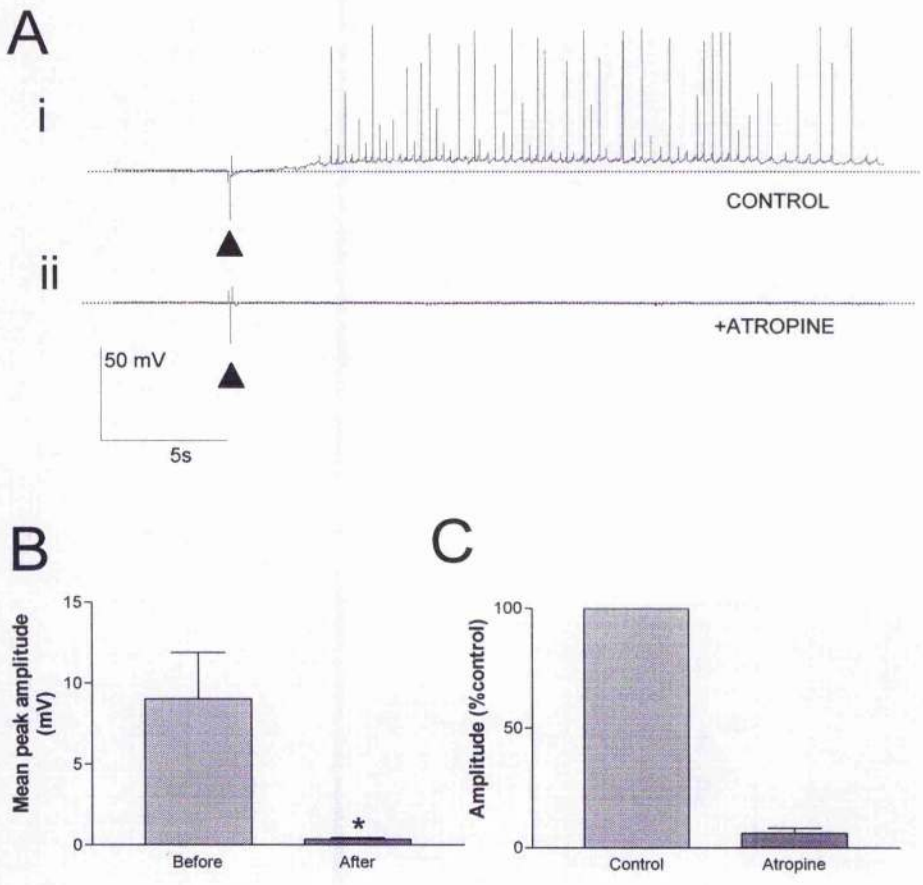


Figure 3.14 Atropine abolishes the slow EPSP evoked by MSN stimulation.

Ai. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, of peak amplitude 5.8mV, evoked by a train of stimuli (5 x 6mA, 0.2ms @20Hz) delivered within the MSN. Aii Representative voltage trace from the same pyramidal neurone illustrating the effect of atropine (5μM) on the production of the slow EPSP (peak amplitude 0.4mV). Note that atropine inhibits the slow EPSP evoked by electrical stimulation within the MSN. B. Bar chart of pooled data from 5 pyramidal neurones illustrating that atropine significantly depresses the mean peak amplitude of the slow EPSP from 9.021±2.878mV to 0.0344±0.087mV (n=5, P= 0.0410). C. Bar chart in which pooled data for the mean peak amplitude of the slow EPSPs recorded 15-30 minutes after atropine application is expressed as a percentage of the mean value of the control EPSPs recorded prior to atropine application. Note that atropine results in over an 90% reduction in mean peak amplitude. Values are expressed as a mean ± S.E.M., P values were calculated using a paired t-test, after data had passed test for normal distribution.

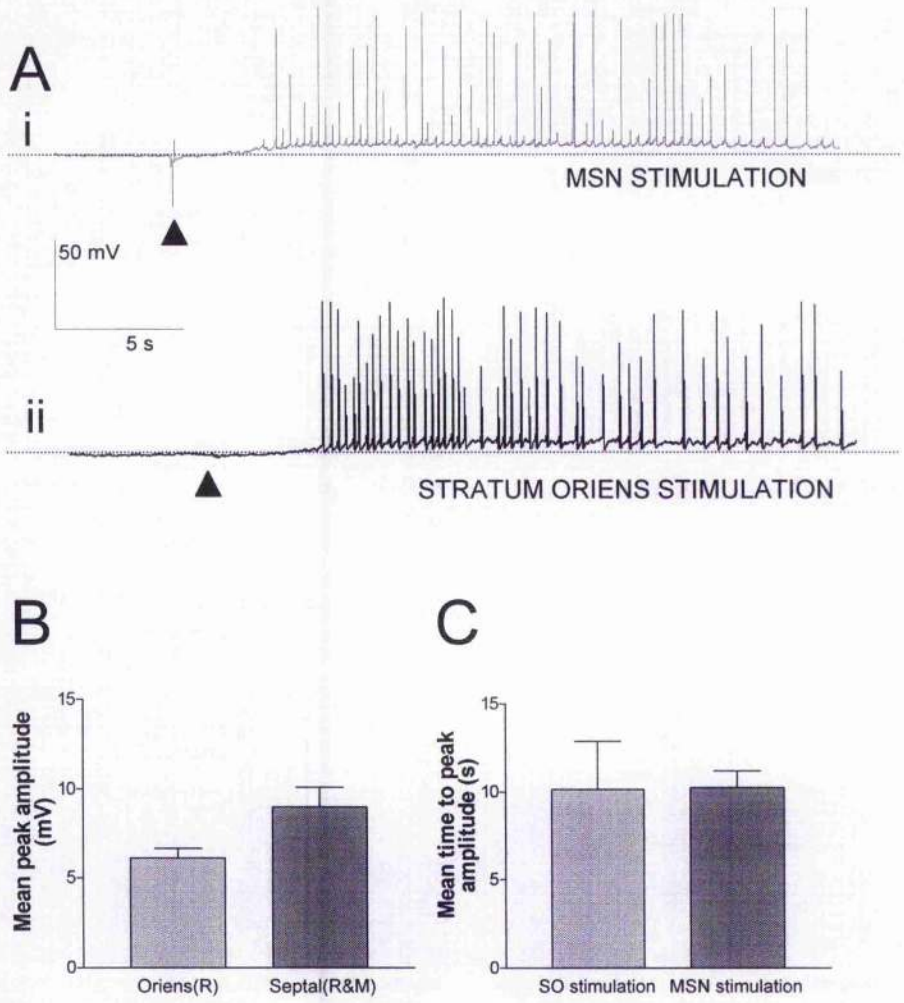


Figure 3.15 Comparison of cholinergic responses evoked by MSN and oriens stimulation

Ai. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 5.8mV, evoked by a train of stimuli (5 x 6mA, 0.2ms @ 20Hz) delivered within the MSN of the septo-hippocampal slice preparation. Aii. Representative voltage trace recorded from a CA1 pyramidal neurone showing a slow EPSP, with a peak amplitude of 6.3mV, evoked by a train of stimuli (5 x 10μA, 0.2ms @ 20Hz) within the *stratum oriens*. Note that stimulation within either area results in a slow EPSP with similar characteristic waveform irrespective of stimulation site. B. Bar chart of pooled data comparing mean peak amplitude of the slow EPSP evoked by *stratum oriens* (6.109±0.54mV, n=11) and MSN (8.975±1.123mV, n=15) stimulation. An unpaired t-test indicated no significant difference in peak amplitude of the response (P=0.0512). C. bar chart of pooled data comparing mean time to peak of the slow EPSP evoked by *stratum oriens* (10.14±2.743s) and MSN (10.26±0.95s) stimulation. An unpaired t-test indicated no significant difference in time to peak (P=0.9620). Values are expressed as a mean ± S.E.M., P values were calculated using an unpaired t-test after data had passed test for normal distribution.

This data indicates that stimulation within the *stratum oriens* region of the hippocampal slice is as robust and reliable a method for stimulating cholinergic fibres as direct stimulation within the MSN itself. It is also supportive of the fact that we activate the same fibres (albeit downstream) when we stimulate locally and that contamination by stimulation of other local fibres is not a confounding problem.

3.3. Discussion

3.3.1. The slow EPSPm

The results presented in this chapter build upon the original findings of Cole and Nicoll (1983) who first reported that electrical stimulation of intrinsic cholinergic fibres within the hippocampal slice resulted in a slow EPSP mediated by mAChRs (EPSPm) in hippocampal pyramidal neurones. Although a number of locations in the slice were chosen for the stimulation site Cole and Nicoll (1983) reported the strongest effects when stimulating within the *stratum oriens.*

The results presented here agree with these original findings showing that delivery of stimuli within the *stratum oriens* of the hippocampal slice preparation, in the presence of an antagonist cocktail, to block all fast amino acid transmission, consistently evoked a slow EPSP in CA1 pyramidal neurones. This slow EPSP was most likely due to the action of synaptically released acetylcholine as septo-

hippocampal cholinergic fibres are known to be present within the *stratum oriens* (Lewis & Shute, 1967b; Frotscher & Leranth, 1985). This observation was further supported by the subsequent data showing that the slow EPSP was enhanced in the presence of eserine, which acts to inhibit acetylcholinesterase (AChE) the enzyme that typically hydrolyses synaptically released ACh.

As the slow EPSP was more substantial and easier to evoke in the presence of the AChE inhibitor, eserine, this was routinely included in the perfusion medium. However, the fact that this slow EPSP could be uncovered in the absence of eserine provided evidence that the slow EPSP brought about by activation of cholinergic afferents is a physiologically relevant event and is not artificially produced due to the increased levels of ACh, which would not be achieved *in vivo*. However, it should be noted that more subtle electrophysiological changes have previously been reported at levels of afferent stimulation below that required to produce a membrane potential depolarisation. These include the reduction of the slow afterhyperpolarisation leading to a diminished level of spike frequency adaptation (Cole & Nicoll, 1983; Segal, 1988).

Application of a train of stimuli rather than a single stimulus enhanced the slow EPSP evoked by electrical stimulation. This result was expected and presumably reflects the accumulation of ACh following the repeated activation of cholinergic fibres. Moreover, it is known that putative cholinergic cells within the MSN which project to the hippocampus fire repetitively and in relation to the hippocampal theta rhythm *in vivo* (Brazhnik & Fox, 1999).

Increasing the stimulus intensity also increased the amplitude and duration of the slow EPSP. This agrees with previous studies (Segal, 1988; Morton & Davies, 1997; Morton *et al.*, 2001) and presumably reflects the fact that an increase in stimulation intensity would ultimately recruit more fibres and subsequently increase the amount of synaptically released acetylcholine.

Although not thoroughly investigated here the slow EPSP is known to show a significant refractory period in which responses are unable to be evoked or show diminished peak amplitudes if stimuli are delivered less than 5 minutes after prior stimulation (Morton, 1998). Due to these previously reported results we evoked subsequent slow EPSPs no less than 5 minutes after the previous response was recorded and as such observed no obvious depression of the second EPSP.

A similar slow depolarisation of the membrane potential could be produced by application of the cholinergic agonist carbachol. The similar nature of this depolarisation suggested that the response produced by electrical stimulation within the *stratum oriens* was due to activation of cholinergic receptors on pyramidal neurones. However as carbachol is a non-selective agonist at both nAChRs and mAChRs this did not allow determination of the receptor subtype responsible.

Application of the selective mAChR antagonist atropine substantially inhibited the slow EPSP produced by stimulation of cholinergic afferents. These data allowed

the slow EPSP to be attributed to activation of mAChRs present on hippocampal pyramidal neurones and as such to be defined as the EPSPm. These data support previous pharmacological and immunocytochemical studies, which have shown the expression of mAChRs on hippocampal principal cells (Benardo & Prince, 1981, 1982; Haas, 1982; Segal, 1982; Spencer *et al.*, 1986; Levey *et al.*, 1995) and confirms Cole and Nicolls (1983) original observation that the slow EPSP produced on stimulation of the *stratum oriens* is mediated by mAChRs.

A second mAChR antagonist pirenzepine also significantly inhibited the slow EPSP and thus may indicate a role of the M1 mAChR, as this agonist is known to show some selectivity for this mAChR subtype (Hammer *et al.*, 1980). This finding agrees with a number of previous studies, which reported a blockade of slow EPSP in the presence of pirenzepine (Müller & Misgeld, 1986; Dutar & Nicoll, 1988a; Segal, 1988; Pitler & Alger, 1990). Although these data suggest that a significant part of the slow EPSP is due to activation of the M1 mAChR subtype, pirenzepine's lack of complete selectivity for M1 and previous reports for a role of the M2 and M3 mAChR subtypes (Müller & Misgeld, 1986; Segal, 1988; Pitler & Alger, 1990) do not allow an exclusive role for the M1 mAChR subtype to be concluded.

The complexity and numerous components of the cholinergic response is produced by the depression of a variety of potassium currents and it is likely that this diversity is the result of an activation of different mAChR subtypes rather than the coupling of a single mAChR subtype with different ionophores. The

subtle effects such as depression of the slow AHP and resulting blockade of SFA are thought to be due to an inhibition of the slow calcium activated current (I_{AHP}) (Madison *et al.*, 1987), most likely mediated via activation of the M2 mAChR subtype (Müller & Misgeld, 1986; Segal, 1988), whereas the production of the slow EPSP is thought to be due to an inhibition of a K^+ leak current ($I_{K(LEAK)}$) (Madison *et al.*, 1987; Benson *et al.*, 1988), hence the previously reported associated increase in input resistance (Cole & Nicoll, 1983, 1984a; Morton, 1998), and activation of a non-selective cation current (Colino & Halliwell, 1993) presumably mediated via activation of the M1 (Müller & Misgeld, 1986; Benson *et al.*, 1988) and/or M3 mAChR subtypes (Pitler & Alger, 1990).

That the production of the slow EPSP was mainly due to activation of mAChRs and not nAChRs is consistent with other reports. Whilst pyramidal neurones do appear to express nAChR subunits (Sudweeks & Yakel, 2000), most electrophysiological studies in acute hippocampal slices suggest that activation of nAChRs does not produce any significant membrane response in pyramidal neurones. McQuiston and Madison (1999c) recently showed a barely detectable inward current on application of nAChR agonists in a minority of cells but this was in contrast to the overt membrane currents observed in hippocampal interneurones.

In general, the activation of mAChRs acts to excite hippocampal pyramidal neurones. This excitation can take the form of that seen here in production of the slow EPSP but also can take the form of plateau potentials. Although these events

arise through slightly different mechanism the ultimate outcome of either event is a sustained depolarisation of these neurones. This prolonged excitation of pyramidal neurones could be detrimental and if left unregulated may ultimately result in epileptogenesis. However the slow EPSPm is known to be regulated by other endogenous neuromodulators such as adenosine and GABA (Morton & Davies, 1997; Morton *et al.*, 2001). Adenosine acts to inhibit the slow EPSPm via activation of the A1 receptor subtype present at the presynaptic locus, presumably by an inhibition of ACh release. GABA also acts to inhibit the slow EPSPm via activation of $GABA_B$ receptors. These neuromodulators are active tonically and provide an important mechanism for controlling prolonged excitation in these cells.

3.3.2. Integrity of the septo-hippocampal projection

The primary source of ACh in the hippocampus is released from cholinergic afferents originating within the medial septal nucleus (MSN). Cell bodies within the MSN send their non-myelinated axons to the hippocampal formation where they ramify extensively. These fibres along with GABA afferents make up the septo-hippocampal projection. However, other sources of ACh do exist including intrinsic cholinergic interneurones (Frotscher *et al.*, 1986; Frotscher *et al.*, 2000) and other midbrain inputs including the basal nucleus of Meynert (Kitt *et al.*, 1982).

The aim of preparing septo-hippocampal slices was to produce acute slices, which would contain an intact septo-hippocampal projection allowing us to activate this functionally significant pathway and observe the responses in hippocampal pyramidal neurones. Similar stimulation protocols to those used in the hippocampal slice were carried out on the septo-hippocampal slice preparation. However with this preparation the stimuli could be directly applied to the MSN while recording the resulting membrane responses evoked in CA1 pyramidal neurones within the hippocampus. This protocol elicited a similar slow EPSP to that produced on *stratum oriens* stimulation.

Atropine again significantly inhibited the slow EPSP, this together with the lack of significant difference between responses evoked by *stratum oriens* stimulation and MSN stimulation confirms that the slow EPSP produced in pyramidal neurones on stimulation of the MSN is the same EPSPm produced when stimulation is applied within the *stratum oriens* of hippocampal slices. We suggest that stimulation within the *stratum oriens* presumably activates the same cholinergic afferent fibres as direct stimulation within the MSN. However this is only likely to be confirmed directly through lesioning experiments.

The observation that the mouse septo-hippocampal slice was easier to prepare in that it was more likely to encompass the whole septo-hippocampal projection indicates a promising model system for future studies. Indeed, an interesting approach would be to pharmacologically induce patterned oscillatory activities in the MSN and then assess the consequence of this upon hippocampal circuitry

dynamics. Authors in the past have been forced to use septo-hippocampal co-cultures (Gahwiler & Brown, 1985) which have many drawbacks including immature circuitry and a predisposition to epileptiform activity.

3.4. SUMMARY

These results show that electrical stimulation within the *stratum oriens* of the hippocampal slice preparation can activate cholinergic afferents originating from the MSN. Stimulation of these septo-hippocampal afferents results in a robust slow EPSP in pyramidal neurones, mediated by mAChRs. This directly confirms the notion that activation of the septo-hippocampal cholinergic system causes general excitation of the principal cell population in the hippocampus.

Evidence is also provided that the functional integrity of the septo-hippocampal cholinergic projection is maintained within the hippocampal slice preparation, despite the presynaptic axons being severed, as the same response is produced whether the cholinergic cell bodies are directly stimulated within the MSN or just the projection fibres within the *stratum oriens* of the hippocampus.

Due to the difficulties associated with the production of septo-hippocampal slices and the above evidence that the structural integrity of the cholinergic projection is maintained within the hippocampal slice preparation, it is therefore reasonable to use only the hippocampal slice preparation for all subsequent experiments.

4. ISOLATION OF SLOW CHOLINERGIC RESPONSES IN CA1 GABAERGIC INTERNEURONES

4.1. INTRODUCTION

A large body of evidence suggests that in addition to the principal cell population, hippocampal interneurones are also regulated by the cholinergic input from the medial septal nucleus (MSN). The production of cholinergic responses in hippocampal interneurones has previously been shown pharmacologically by the use of cholinergic agonists (Benardo & Prince, 1982; Reece & Schwartzkroin, 1991; Jones & Yakel, 1997; Frazier *et al.*, 1998b; Parra *et al.*, 1998; McQuiston & Madison, 1999c, a). However, much less is known about the action of physiologically released ACh upon the varied population of hippocampal interneurones.

As mentioned in chapter one, CA1 interneurones are known to express both nAChR and mAChRs (Freedman *et al.*, 1993; Levey *et al.*, 1995; Hájos *et al.*, 1998; Fabian-Fine *et al.*, 2001; Kawai *et al.*, 2002). A recent detailed study by McQuiston and Madison (1999a) reported that CA1 interneurones responded to mAChR agonists with one of four types of postsynaptic response

1) A depolarisation from resting membrane potential.
2) A hyperpolarisation from resting membrane potential.
3) A biphasic response consisting of the hyperpolarisation followed by the depolarisation.
4) No response.

mAChR agonists are also thought to act at presynaptic mAChRs to directly influence GABA release from presynaptic terminals. Previous studies have shown that mAChR activation leads to a decrease of miniature IPSP (mIPSC) frequency recorded in CA1 pyramidal neurones indicating an inhibition of GABA release from presynaptic terminals of interneurones (Behrends & TenBruggencate, 1993). Subsequent immunocytochemical studies showed that parvalbumin-containing basket cells and axo-axonic cells expressed M2 mAChRs exclusively on their terminals which may be responsible for this inhibition of GABA release (Hájos *et al.*, 1998).

In addition to these mAChR mediated responses, hippocampal interneurones also respond to nAChR agonists with a depolarisation from resting membrane potential. However these nAChR-mediated depolarisations vary in time course. The two major response types reported are a fast depolarisation mediated by α7 subunit containing receptors (Alkondon *et al.*, 1997b; Jones & Yakel, 1997; Frazier *et al.*, 1998b; McQuiston & Madison, 1999c) and a slower depolarisation mediated by α4β2 subunit containing nAChRs (Alkondon *et al.*, 1999).

Stimulation of cholinergic afferents within the *stratum oriens* has also provided some evidence of a synaptic nAChR response in CA1 interneurones consisting of an EPSC (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a) but the mAChR responses have not been investigated in this manner.

The aim of this chapter was to investigate and characterise the synaptically mediated effects of ACh release on CA1 interneurones evoked by stimulation of septo-hippocampal afferents within the hippocampal slice preparation, primarily focusing on eliciting the mAChR mediated responses that have been observed under direct application of ACh (McQuiston & Madison, 1999a).

4.2. Results

4.2.1. Verification of the septo-hippocampal projection

The whole-cell patch clamp technique on visually identified neurones was employed throughout this chapter in order to increase yield of successful recordings from the numerically sparse population of CA1 interneurones. As stated in chapter two, slices to be used for patch clamping were prepared from neonatal rats between 16 and 21 days old (P16-21). This change in age group was necessary due to the fact that slices prepared from younger animals have less connective tissue allowing easier visualisation and access to individual cells. However the septo-hippocampal cholinergic projection is not fully formed to adult level until at least postnatal day 14 (P14) (Matthews *et al.*, 1974; Milner *et al.*,

1983) with full adult expression of mAChRs present at P16 (Goldbach *et al.*, 1998). Therefore immunocytochemical studies, using antibodies directed towards the acetylcholine synthesising enzyme, choline acetyltransferase (ChAT) were initially carried out to ensure that the age of rat used in these experiments showed sufficient development of the septo-hippocampal cholinergic system.

Immunofluorescence labelling of a septo-hippocampal slice prepared from P16-21 Wistar rats revealed intensely labelled ChAT immunopositive neurones within the medial septal nucleus (MSN). The ChAT immunopositive neurones gave rise to a dense ChAT immunopositive axonal projection that coursed towards and innervated the hippocampal region of the slice. Within the hippocampal formation these ChAT immunopositive axons branched extensively, ramifying throughout all subfields where they formed a dense meshwork of varicosities. ChAT immunolabelling was present in all laminae but was particularly intense within the *stratum oriens* and *stratum radiatum.* Dual immunofluoresence with a second antibody directed toward the calcium binding protein parvalbumin (PV), a marker for a subpopulation of GABAergic interneurones (Kosaka *et al.*, 1987; Nitsch *et al.*, 1990; Gulyas *et al.*, 1991; Sik *et al.*, 1995), showed that PV immunopositive interneurones were present in both the MSN and the hippocampal formation. This dual labelling also suggested a strong cholinergic innervation of the hippocampal PV immunopositive cells with the appearance of putative sites of synaptic contact (Fig 4.1).

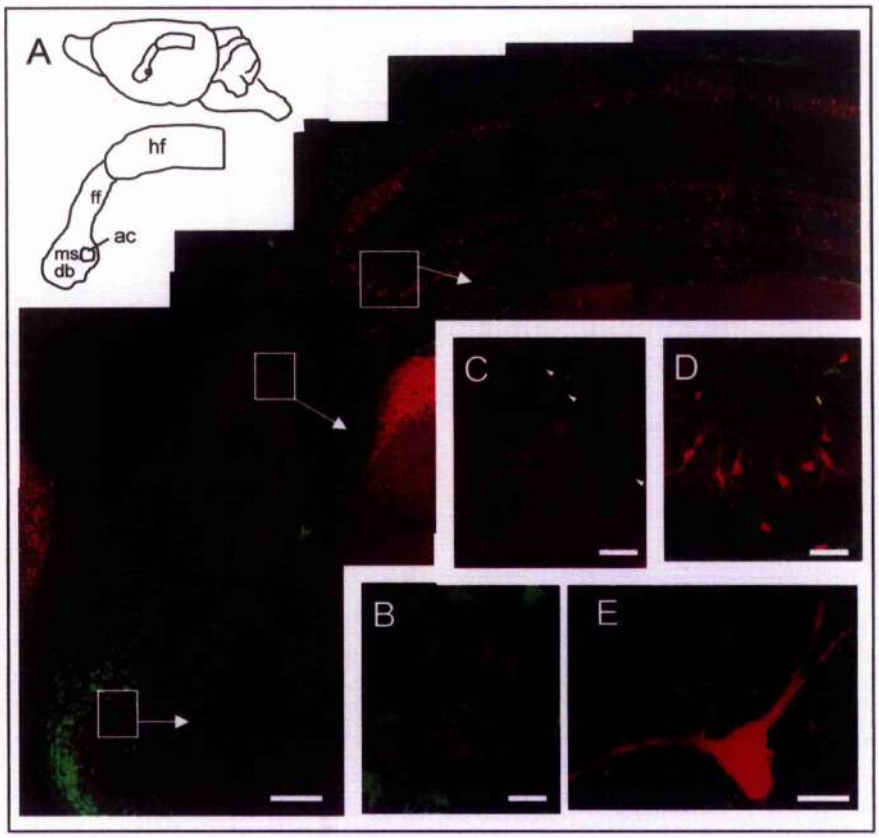


Figure 4.1 Structure of the septo-hippocampal (S-H) projection revealed by dual anti-choline acetyltransferase (ChAT) and anti-parvalbumin (PV) immunolabelling.

A. Low power micrograph montage showing S-H cholinergic projection in which ChAT-immunoreactive neurones are indicated in green and PV-immunoreactive profiles in red. Insets indicate relative position of structures. Section was cut to optimise S-H connectivity as described in methods. B. Higher power micrograph indicating the abundance of ChAT-immunoreactive and PV-immunoreactive cell bodies within the septal nucleus. C. Higher power micrograph indicating a dense projection of ChAT-immunoreactive fibres (arrowheads) coursing towards the hippocampal formation. Note also the presence of PV-immunoreactive somata within the pathway. D. Micrograph showing PV-immunopositive interneurones within the hippocampus together with ChAT-immunoreactive axon fibres which form boutons and ramify extensively within the hippocampus. E. High power micrograph indicating a PV-immunopositive neurone and putative sites of synaptic contact from ChAT immunoreactive fibres.

Abbreviations: hf, hippocampal formation; ff, fimbria-fornix; ms/db, medial septal nucleus / diagonal band complex; ac, anterior commissure. Scale bars: A, 250μm; B-C, 50μm;D, 100 μm; E, 10μm.

4.2.2. Characterisation and identification of cell types.

Hippocampal neurones were patch clamped under visual control and recorded in the whole-cell formation using $KmeSO_4$ based pipette solution as described in chapter two. Putative pyramidal neurones were easily distinguished from putative interneurones on the basis of their cell body location (pyramidal neurone somata being mainly restricted to the cell body layers) and biophysical membrane properties such as spike frequency adaptation and afterhyperpolarisations. Characterisation of the patched cells was identical in manner to that used in chapter three. Positive and negative current injections were applied to produce depolarising and hyperpolarising voltage responses respectively (Figure 4.2). The resulting voltage traces were used to give a tentative indication of cell type. Putative pyramidal neurones were again identified by the presence of a pronounced spike frequency adaptation (SFA) and small slow afterhyperpolarisations (AHPs) (Fig 4.2A) whereas GABAergic interneurones show little or no SFA and the presence of large fast AHPs (Fig 4.2B). During each individual recording the neurone was loaded with the intracellular marker Neurobiotin (Vector laboratories, U.K.), once again allowing the putative cell type to be confirmed by post hoc morphological light microscope examination (Fig 4.2 C).

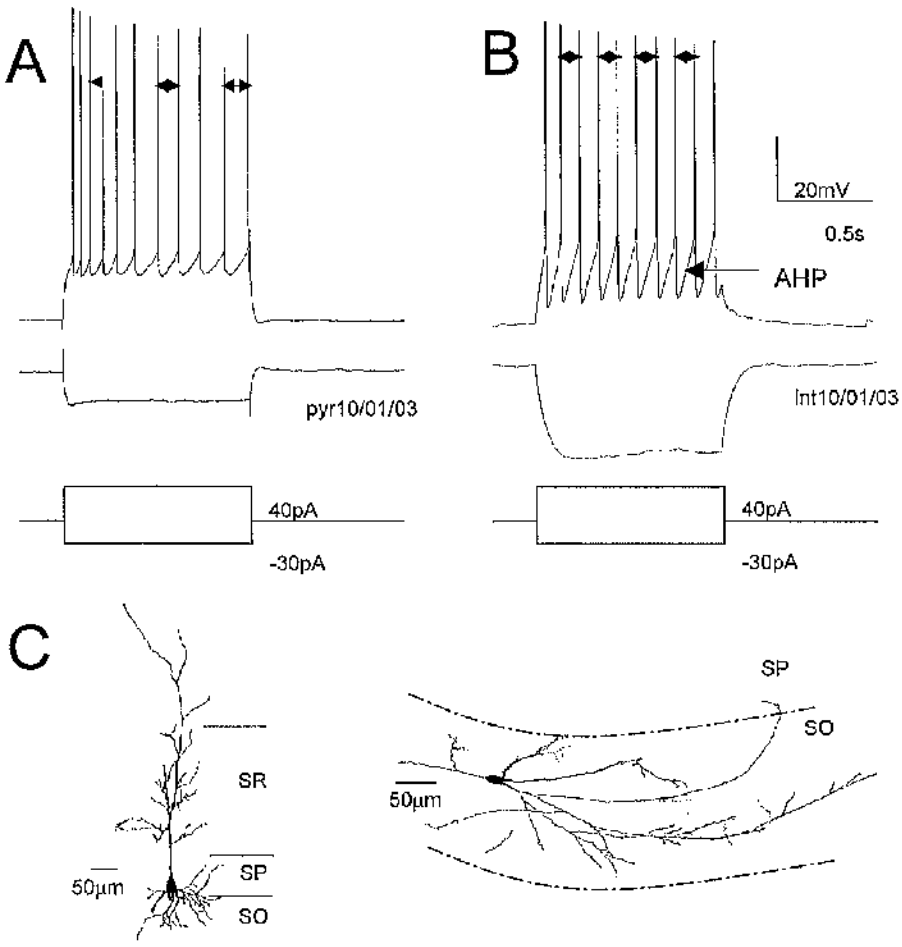


Figure 4.2 Comparison of a CA1 pyramidal neurone and a CA1 interneurone

Whole-cell patch clamp recordings from a CA1 pyramidal neurone and a CA1 interneurone A. Shows an example of a representative recording showing voltage traces (upper trace) from a CA1 pyramidal neurone in response to applied current injection (lower trace). Positive and negative current steps applied to a CA1 pyramidal neurone resulted in depolarising and hyperpolarising voltage responses respectively. Note the characteristic properties of a pyramidal neurone such as pronounced spike frequency adaptation (SFA, indicated by increasing arrow length) and small slow afterhyperpolarisations (AHP). B. Similar representative current and voltage traces recorded from a CA1 interneurone with somata located within the *stratum oriens*. Note this neurone shows little SFA and large fast AHPs (indicated by arrows) characteristic of hippocampal interneurones. Subsequent post hoc morphological LM examination was used where possible to confirm cell identity. C. Camera lucida partial reconstruction of the corresponding pyramidal neurone and *stratum oriens* interneurone. Note the conical shape of the CA1 pyramidal neurone soma located within the *stratum pyramidale*. The basal dendrites branch into the *stratum oriens* and the primary apical dendrites extending and branching through the *stratum radiatum* and *stratum lacunosum-moleculare*. The axons of CA1 pyramidal neurones (not shown) typically entered *stratum oriens* where they branched before running parallel with the alveus. The interneurone recorded in *stratum oriens* has a more rounded soma that is oval in shape located within the *stratum oriens* with dendrites running horizontally in both directions throughout the *stratum oriens*. The axon from this cell ascended into the *stratum radiatium* before becoming too faint to follow further.

Overall 59 CA1 interneurones with cell bodies located in both the *stratum oriens* and *stratum radiatum* of the hippocampus were recorded using the whole-cell patch clamp technique. These 59 interneurones had a mean resting membrane potential of 67.4±1.49mV, a mean input resistance of 738.8±43.11MΩ and a mean action potential height and duration of 68.7±1.787 and 2.3±0.1128 respectively. 9 CA1 pyramidal neurones were also recorded using the whole-cell patch clamp technique. These cells had a mean resting membrane potential of 74.5±2.502mV a mean input resistance of 265.8±35.64MΩ and a mean action potential height and duration of 80.45±4.577 and 2.3±0.1036 respectively. These properties were measured throughout each whole cell recording and could be used to further characterise neurone type and monitor the physiological status of each cell. In general, interneurones have a larger input resistance than pyramidal neurones and pyramidal neurones display characteristic overshooting action potentials. A full comparison of these cell properties is summarised in table 4.1.

	Resting membrane potential (mV) (with JP allowance)	Cell input resistance (MΩ)	Action potential amplitude (mV)	Action potential duration (ms) (measured at ½ amplitude)
CA1 Interneurones (n=59)	-67.2±1.41	738.8±43.11	68.7±1.787	2.3±0.1128
CA1 Pyramidal neurones (n=9)	-74.5±2.502	265.8±35.64	80.45±4.577	2.3±0.1036

Table 4.1 Comparison of CA1 pyramidal and interneurone properties.

In order to give a complete characterisation and monitor the physiological status of hippocampal pyramidal neurones and interneurones, resting membrane potential, cell input resistance and action potential amplitude and duration were recorded from all neurones throughout the whole-cell recordings. This table shows the mean values ± S.E.M. for the 59 CA1 interneurones and 9 CA1 pyramidal neurones recorded. Note the larger input resistance of the interneurones and the overshooting action potentials of the pyramidal neurones provided two additional discriminating factors.

4.2.3. Evoked cholinergic responses

Application of a low intensity (0.1-0.5mA, 0.02ms) single stimulus within the *stratum oriens* of the hippocampal slice produced a fast EPSP-IPSP complex in recorded CA1 interneurones consistent with the activation of glutamatergic and GABAergic afferents (n=12) (Fig. 4.3Ai). This fast synaptic transmission was inhibited by the addition of a cocktail of competitive antagonists for amino acid

receptors (Fig 4.3Aii). The antagonist cocktail consisted of NBQX (2μM), CGP40116 (50μM), CGP55845A (1μM) and Bicuculline (10μM) blocking AMPA, NMDA, $GABA_B$ and $GABA_A$ mediated responses respectively. The nature of evoked glutamatergic and GABAergic synaptic responses are well documented (Lacaille, 1991; Buhl *et al.*, 1994b; Sik *et al.*, 1995; Buhl *et al.*, 1996) and were thus not subjected to any further rigorous investigation.

Further studies were carried out to investigate and characterise evoked cholinergic responses from this cell population. Increasing the stimulus intensity and duration (1-20mA, mean 7.99±0.985mA, 0.2ms), in the continued presence of the amino acid antagonist cocktail, uncovered a slow depolarisation of the membrane potential, with a mean peak amplitude and time to peak of 7.0910±0.5212mV and 16.29±0.983s (Fig 4.3B) respectively in 42 of the 59 CA1 interneurones in which this was attempted. In order for a depolarisation to be included as a synaptically evoked response the change in membrane potential after afferent stimulation had to be greater than 2mV. Addition of the mAChR antagonist atropine (5μM) significantly inhibited the peak amplitude of the slow depolarisation recorded in CA1 interneurones from 8.262±0.8006 to 1.56±0.3648mV, an 81% inhibition ($P<0.0001$, $n=12$, Fig 4.3C).

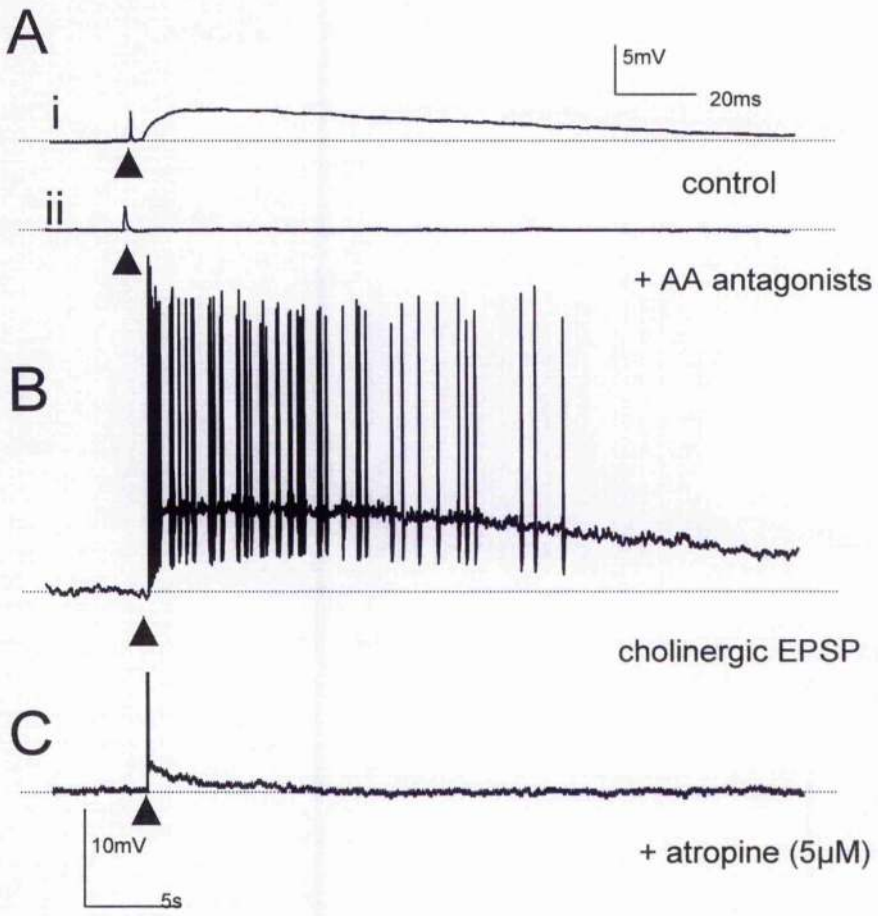


Figure 4.3. Isolation of a slow excitatory postsynaptic potential in CA1 interneurones.

A. Representative voltage traces recorded from a *stratum oriens* interneurone (SO interneurone) in the whole-cell patch clamp configuration. Ai. Illustrates a fast EPSP evoked in response to a single 0.5mA (0.02ms) electrical stimulation of afferents within the *stratum oriens*. Aii. Illustrates the complete blockade of this fast evoked synaptic response following the addition of NBQX (2μM), CGP40116 (50μM) and bicuculline (10 μM). B. Representative voltage trace illustrating that subsequently increasing the stimulation intensity and duration (1mA, 2ms) elicited a much more pronounced and prolonged depolarising response, with a peak amplitude of 9.1mV, similar to that described in CA1 pyramidal neurones. Slow depolarising responses were recorded in 42 out of 59 interneurones tested with a mean peak amplitude 7.86±0.533mV and a mean time to peak of 16.29±0.983s. C. Representative voltage trace showing the effect of the selective mAChR antagonist atropine (5μM) on the evoked slow depolarisation (peak amplitude 1.9mV). Note that atropine inhibits the peak amplitude of the slow depolarisation by 80% in this example. Application of atropine (5μM) significantly inhibited the peak amplitude of the slow depolarisation in 12 of 12 interneurones tested from 8.26±0.801mV to 1.56±0.365mV ($P<0.0001$). Values are expressed as a mean ± S.E.M., P values were calculated using a paired t-test after data passed test for normal distribution. Filled triangles (▲) below each voltage trace indicates the time point of afferent stimulation.

In order to fully characterise and quantify these depolarisations from CA1 interneurones four individual parameters were measured, as outlined in figure 4.4. These parameters were 1. Peak amplitude of depolarisation, measured in millivolts (mV) as the difference between the membrane potential before afferent stimulation and the peak membrane potential recorded after afferent stimulation. 2. Peak latency, measured in seconds (s) as the time taken to reach the peak amplitude of depolarisation from the point of afferent stimulation. 3. Time to first action potential, measured in seconds (s) as the time taken from point of afferent stimulation to the first action potential fired. 4. Number of action potentials fired throughout the recorded response.

Slow mAChR-mediated EPSPs of mean peak amplitude 7.797±1.751mV were also recorded in 8 out of 9 pyramidal neurones under similar conditions (Fig 4.5). These responses were analysed in a similar manner and the results are summarised in table 4.2. As the slow EPSPm recorded in CA1 pyramidal neurones was thoroughly investigated with traditional sharp intracellular recording in the previous chapter, the remainder of this chapter will concentrate only on the responses obtained from CA1 interneurones.

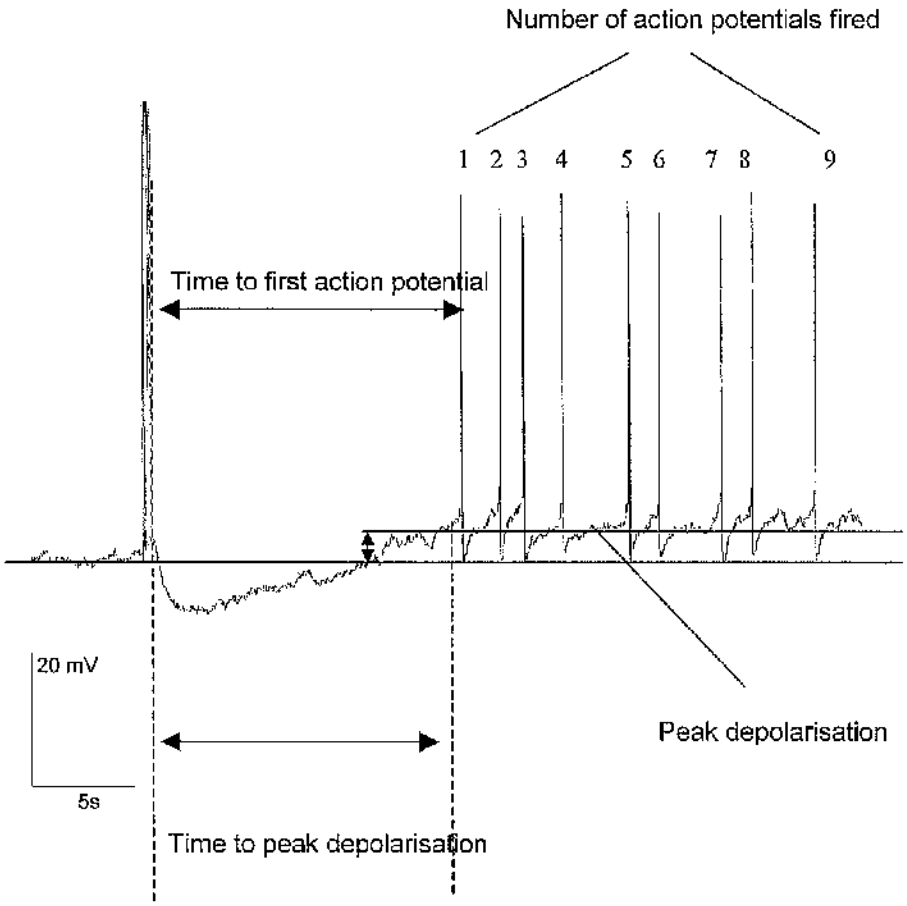


Figure 4.4 Quantification of the depolarisation recorded in CA1 hippocampal interneurones.

In order to provide quantitative evaluation of evoked cholinergic responses in CA1 interneurones, four parameters representing the magnitude and timecourse of the response were measured. **Peak amplitude** of depolarisation was defined as the difference between the membrane potential before afferent stimulation and the peak membrane potential after afferent stimulation (distance between solid lines) and expressed as a value in millivolts (mV). **Time-to-peak** depolarisation was defined as the interval from the end of the stimulation train to the first point of peak depolarisation (distance between dotted lines) and expressed as a value in seconds(s). **Time to first action potential** was defined as the interval from the end of the stimulation train (same time point used for time to peak depolarisation) to the first action potential fired and expressed as a value in seconds (s). The **number of action potentials fired** were simply counted.

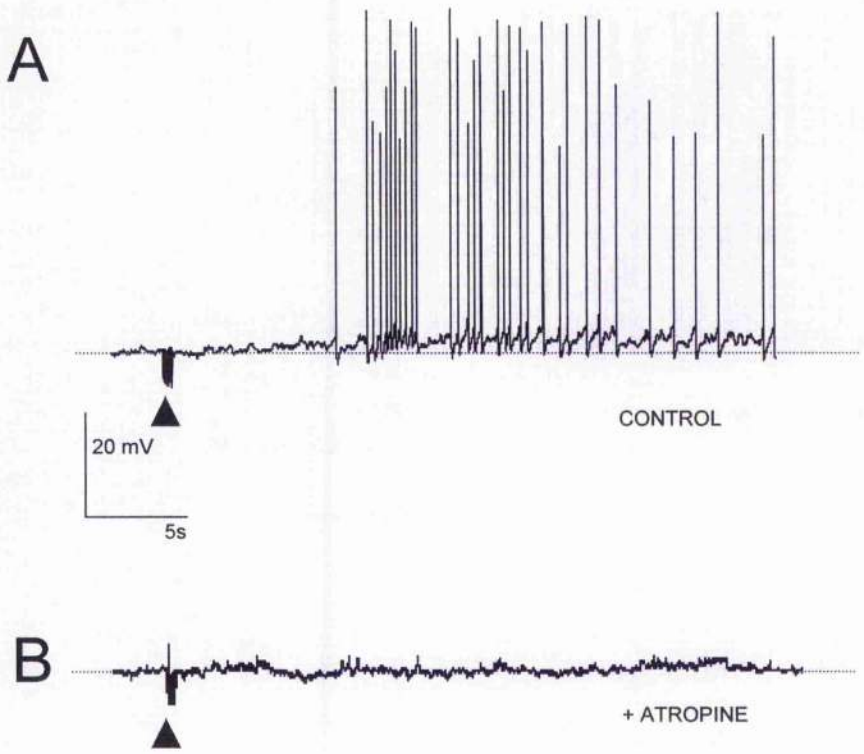


Figure 4.5 Isolation of cholinergic responses in CA1 pyramidal neurones recorded in the whole-cell configuration.

A. Representative voltage trace recorded from a CA1 pyramidal neurone in the whole-cell patch clamp configuration, showing a slow EPSP, with a peak amplitude of 4.7mV, evoked by a train of stimuli (5 x 3mA, 0.2ms @ 20Hz) delivered within the *stratum oriens*. A slow EPSP was recorded in 8 of 9 pyramidal neurones recorded with a mean peak amplitude of 7.797±1.751mV and a mean a time to peak of 17.55±2.24s. B. representative voltage trace from the same pyramidal neurone illustrating an 85% inhibition of the slow EPSP, to peak amplitude of 0.7mV, in the presence of mAChR antagonist atropine (5μM). Note that electrical stimulation within the stratum oriens elicits a mAChR-mediated response in CA1 pyramidal neurones recorded in the whole-cell configuration similar to that previously reported in chapter 3 using traditional sharp electrode recording. Values are expressed as a mean±S.E.M.

	Mean membrane potential(mV) at which response was evoked (with JP)	Peak amplitude of depolarisation (mV)	Time to peak amplitude (s)	Time to first action potential (s)	Number of action potential fired
CA1 interneurones (n=42)	-69.17±1.66 (range –96.3 to –52.0)	7.910±0.5212 (range 2.4-17.3)	16.29±0.9831 (range 4.5-29.54)	7.866±1.081 (range 0.08-22.97)	22.65±3.527 (range 0-87_
CA1 pyramidal neurones (n=8)	-69.1±2.69 (range –77.3 to –55.4)	7.797±1.751 (range 2.95-15.8)	17.55±2.24 (range 8.1-25.52)	5.863±0.789 (range 2.65-8.45)	37.5±7.420 (range 14-66)

Table 4.2 Comparison of evoked depolarisations in CA1 interneurones and CA1 pyramidal neurones.

This table shows the individual properties for a mAChR-mediated slow depolarisation in both CA1 interneurones and CA1 pyramidal neurones. Note the similarities in each individual property.

4.2.4. The interneurone response

Initially all experiments were conducted in the presence of the acetylcholinesterase inhibitor eserine (1μM) to increase the likelihood of evoking a response. In total 28 of the 59 interneurones investigated were recorded in this manner. In all, 19 of these 28 (68%) interneurones responded to electrical stimulation (3-20mA, mean 9.158±1.5mA, 0.2ms) within the *stratum oriens* with a slow depolarisation, with a mean peak amplitude of 7.33±0.0.7088mV, a mean peak latency of 17.1±1.566s, a mean time to first action potential of 8.751±1.778s and mean number of action

potentials fired of 21.21±5.742. The remaining 9 (32%) cells were unresponsive to this stimulation protocol (N.B. no overt membrane potential fluctuation >2mV).

However, to ensure physiological relevance to the *in vivo* state we carried out an additional 31 experiments in which eserine was absent from the bath at the start of the experiment. In total 19 out of these 31 (61%) cells responded to electrical stimulation (1-20mA, mean 7.184±1.305mA, 0.2ms) with a depolarisation from resting membrane potential with a mean peak amplitude of 7.298±0.66mV, a mean peak latency of 14.73±1.308s, a mean time to first action potential of 6.955±1.522s and mean number of action potentials fired of 18.54±3.380. The remaining 12 cells (39%) showed no response. Moreover, subsequent addition of 1μM eserine allowed a pronounced depolarising response to be uncovered in a further 4 cells with a mean peak amplitude 13.08±2.005mV, a mean time to peak amplitude of 19.78±3.323s, a mean time to first action potential of 7.913±3.142s and a mean number of action potentials fired of 49±16.08. The other 8 cells remained unresponsive even in the presence of eserine.

Overall, 23 CA1 interneurones responded to stimulation in presence of eserine with a mean peak amplitude of 8.329±0.806mV, a mean time to peak of 17.57±1.403s, a mean time to fist action potential of 8.598±1.535s and a mean number of action potentials fired of 26.04±5.785. 19 cells responded to stimulation in absence of eserine with a mean peak amplitude of 7.298±0.66mV, a mean time to peak of 14.73±1.308s, a mean time to first action potential of

6.955±1.522s and a mean number of action potentials fired of 18.54±3.380 and the remaining 17 cells were unresponsive even in the presence of eserine. Overall there was no significant difference between the corresponding parameters measured from the slow depolarisations recorded in the presence and absence of eserine. This is illustrated graphically in figure 4.6, which compares the mean of each parameter measured from slow depolarisations evoked in the presence and absence of eserine.

4.2.5. Stimulation parameters

As with the pyramidal neurones discussed in chapter three, the peak amplitude of the depolarisation was dependent upon the precise stimulation parameters used. Figure 4.7A shows a voltage trace from a representative CA1 interneurone. Initially a single stimulus (10mA, 2ms) delivered within the *stratum oriens* evoked a modest slow depolarisation of 5.15mV, subsequent application of a train of 5 stimuli (10mA, 2ms) at a frequency of 20Hz increased the peak amplitude of depolarisation by 140 % to 12.55mV. This was associated with a concomitant increase in the firing rate and the duration of the action potential discharge train. This potentiation was consistent in 7 of 7 cells tested (Fig 4.7B). Overall the mean peak amplitude of depolarisation was significantly increased from 4.964±1.28mV to 9.147±1.351mV (P=0.0192) on application of a train of stimuli (Fig. 4.7C).

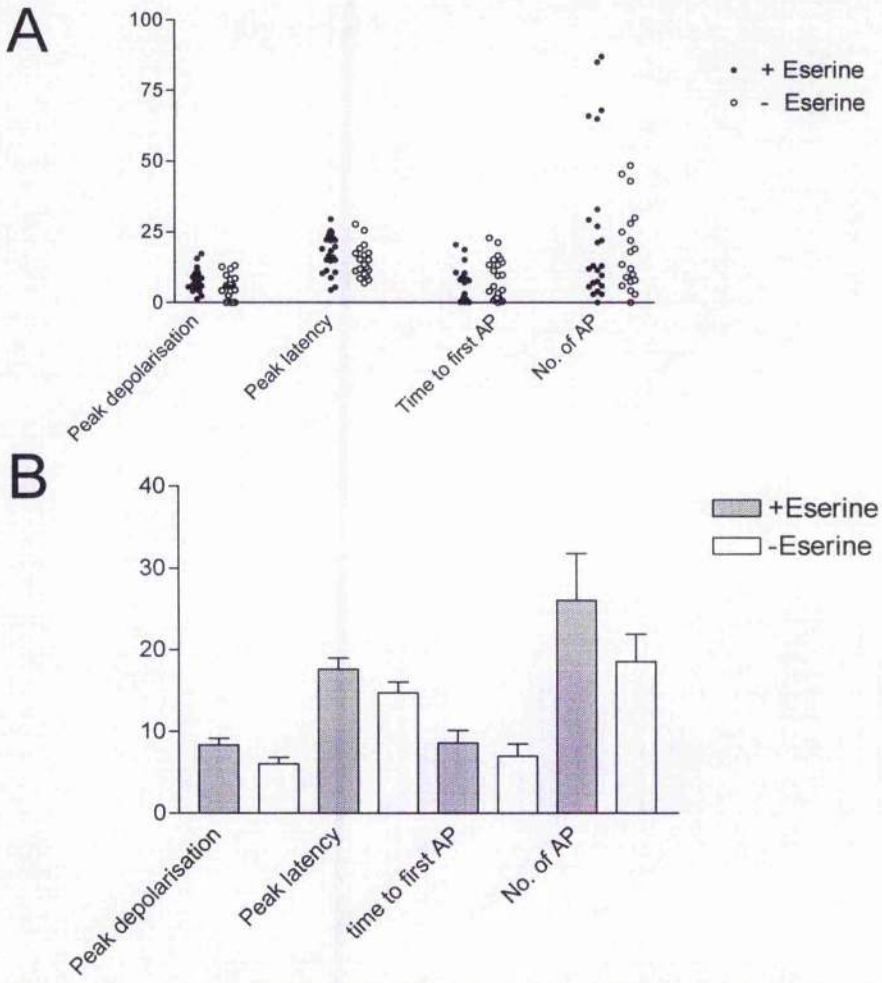


Figure 4.6 mAChR mediated responses in CA1 interneurones in the presence and absence of eserine

Initially all experiments were conducted in the presence of the cholinesterase inhibitor eserine (5μM) to increase the likelihood of evoking a cholinergic response. However to ensure physiological relevance to the natural *in vivo* state additional experiments were conducted where eserine was absent. A. Scatter plot showing each individual parameter measured (peak depolarisation, peak latency, time to first action potential and number of action potentials) to quantify the slow depolarisation recorded in each CA1 interneurone where eserine was present throughout the entire experiment (n=19) and the slow depolarisation recorded in each CA1 interneurone where eserine was absent (n=19). Note that responses recorded in the presence of eserine were similar in all parameters to responses recorded in the absence of eserine. B. Bar chart comparing the mean value of each parameter measured in the presence and absence of eserine. Unpaired t-tests indicated no significant difference between any recorded parameter of the slow depolarisation recorded in the presence and absence of eserine. However 4 CA1 interneurones that did not respond to stimulation within the stratum oriens in the absence of eserine subsequently responded in the presence of eserine. In total a slow depolarisation was recorded from 23 CA1 interneurones in the presence of eserine and 19 CA1 interneurones in the absence of eserine.

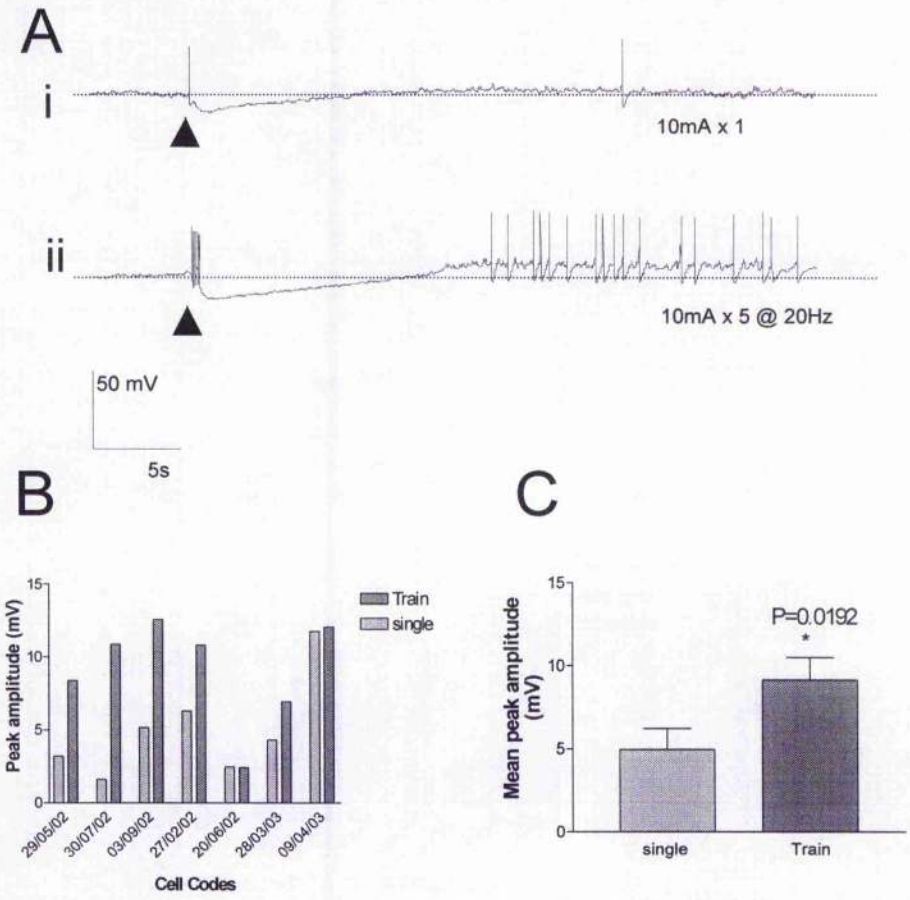


Figure 4.7. Potentiation of the slow EPSP when evoked by a train of stimuli.

A, Representative voltage traces recorded from a *stratum oriens* CA1 interneurone showing a slow depolarising response, with a peak amplitude of 5.15mV, evoked following delivery of a single stimulus (10mA, 0.2ms) within the *stratum oriens* (Ai) and the slow depolarising response, with a peak amplitude of 12.55mV, evoked by a train of 5 stimuli of the same intensity and duration delivered at 20Hz within the *stratum oriens* (Aii). Note the 140% increase in peak amplitude and the increase in action potential discharge on application of a train of stimuli. B. Bar chart showing the peak amplitude of a slow EPSP evoked in 7 individual CA1 interneurones firstly with a single stimulus and subsequently with a train of 5 stimuli. C. Bar chart of pooled data illustrating the significant increase in mean peak amplitude of the slow EPSP from 4.964±1.28mV when evoked with a single stimulus to 9.147±1.351mV when evoked with a train of stimuli (n = 7, P =0.0192). Values are expressed as a mean ± S.E.M., P values were calculated using a paired t-test, after data passed test for normal distribution. Unless otherwise stated all subsequent responses shown in this chapter were evoked using a train of 5 stimuli @ 20Hz and a pulse duration of 0.2ms.

All other parameters of the slow depolarisations evoked by a single stimulus and subsequent train of stimuli were also examined. The mean time to peak amplitude was unchanged whether a single or train of stimuli were used, 15.98±1.45s and 15.98±2.758s respectively. However, this parameter showed great variation between individual cells. The time to first AP was significantly decreased from 21.55±4.416 to 5.56±2.599s (P=0.0308) ultimately increasing the duration of firing and thus number of action potentials fired from 1.714±0.892 to 24.19±8.646 (P=0.0474).

Overall, all parameters of the slow depolarisation were enhanced with increasing stimulation intensity. Figure 4.8A shows a slow depolarisation evoked with a single stimulus of 0.5mA (pulse duration, 0.2ms) and subsequent enhanced responses in the same cell with increasing stimulus intensity (Fig 4.8B and C). Generally the peak amplitude of depolarisation and number of action potentials fired increased with increasing stimulation intensity. However it was noted that time to peak and the time to first action potential decreased with increasing stimulation intensity. These effects were consistent in 5 of 5 interneurones tested, however no pooled data can be shown as the stimulus intensity required to evoke the initial response varied considerably between cells.

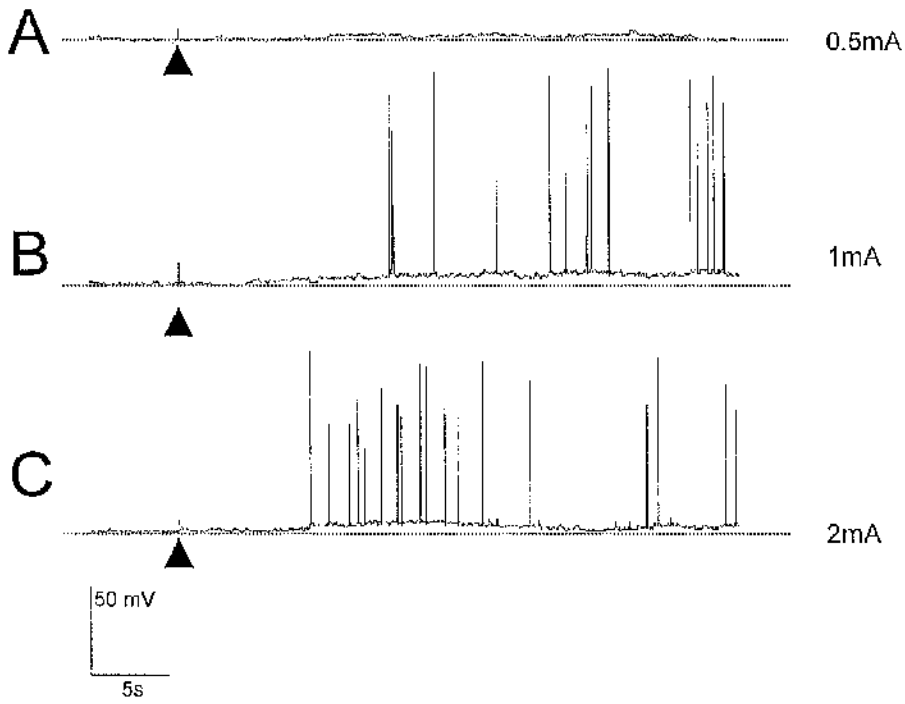


Figure 4.8 Potentiation of the slow EPSP on increasing stimulation intensity.

A. Representative voltage trace recorded from a *stratum oriens* CA1 interneuron showing a slow depolarisation, with a peak amplitude of 3.8mV and a time to peak of 28.7s, evoked by a single 0.5mA (0.2ms duration) stimulus delivered within the *stratum oriens*. B. Representative voltage trace from the same interneurone illustrating that subsequently increasing the stimulation intensity to 1mA increases the peak amplitude of response to 6.4mV with a time to peak of 19.4s and results in action potential discharge (14 action potentials fired with a time to first action potential of 13.3s). C. Representative voltage trace illustrating that increasing the stimulation intensity further to 2mA increases the peak amplitude to 7.9mV, decreases the time to peak amplitude to 12.3s and first action potential of 8.1s ultimately increasing the duration of firing and number of action potentials fired to 19.

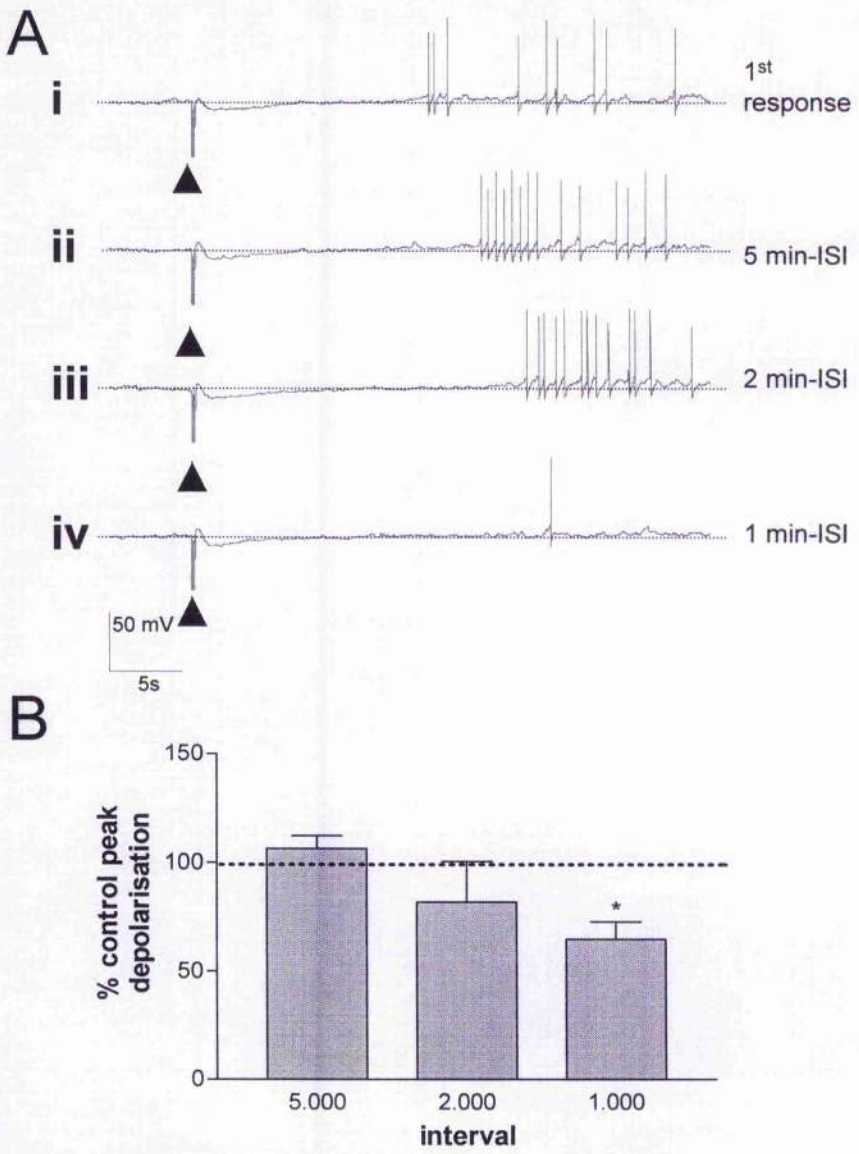


Figure 4.9 Effect of stimulus frequency on evoked slow cholinergic responses in CA1 interneurones.

A. Representative voltages traces recorded from a *stratum radiatium* CA1 interneurone illustrating that decreasing inter-stimulus interval (ISI) leads to diminished responses. Ai shows the initial slow depolarisation, with a peak amplitude of 10.45mV, evoked following the delivery of a train of stimuli (5 x 10mA, 0.2ms @ 20Hz) within the stratum oriens. Aii-iv. Shows subsequent slow depolarising responses evoked following the delivery of a train of stimuli (5 x 10mA,0.2ms @20Hz) within the *stratum oriens* after a 5-minute inter-stimulus interval (peak amplitude 11.35mV), a 2-minute inter-stimulus interval (peak amplitude 7.9mV) and a 1-minute inter-stimulus interval (peak amplitude 5.85mV). B. Bar chart of pooled data from 5 CA1 interneurones in which the mean peak amplitudes of the slow depolarisations elicited after 5, 2 and 1-minute inter-stimulus are expressed as a percentage of the mean peak amplitude of the initial response. A paired t-test indicated a significant decrement in response when the inter-stimulus interval was decreased from 5 minutes to 1 minute (n=5, P=0.0409). P values were calculated on the raw data using a one-way analysis of variants and a Tukey's post test after data passed test for normal distribution. As no obvious depression of the slow EPSP was apparent when using a 5 minutes inter-stimulus interval this was chosen as the inter-stimulus interval for all experiments.

Previous studies have shown that the inter-stimulus interval is also an important parameter in evoking consistent cholinergic responses in hippocampal neurones (Morton & Davies, 1997). Therefore a range of inter-stimulus intervals (1-5 minutes), were tested when evoking these responses in CA1 interneurones (n=5) (Fig 4.9A). This data showed that decreasing the inter-stimulus interval resulted in a decrease of the peak amplitude of the slow depolarisation. A 5-minute inter-stimulus interval was sufficient to allow no significant decrement in the mean peak amplitude of subsequent responses (Fig 4.9B). All other measured parameters of the slow depolarisation were also measured at decreased inter-stimulus intervals but did not show significant variation.

4.2.6. Carbachol.

Of the 59 CA1 interneurones recorded 17 (29%) did not respond to electrical afferent stimulation within the *stratum oriens* (Fig. 4.10A). Indeed the percentage change in membrane potential as measured 17s after stimulation, which corresponds to the average peak time in responsive interneurones, was less than 1% (-0.6±0.2%, n=17). In order to establish whether the lack of response was due to the electrical stimulation not resulting in the activation of cholinergic afferents or sufficient release of ACh we further tested these unresponsive interneurones with bath application of the acetylcholine analogue carbachol (10μM). In all, 7 of these 17 interneurones responded to application of carbachol with a slow depolarisation of the membrane potential broadly similar to that produced by electrical stimulation in the afferent-stimulation responsive population of

interneurones. The mean peak amplitude of depolarisation brought about by 10μM carbachol application was 11.10±1.089mV (Fig. 4.10B). A further 2 interneurones showed an oscillatory component to their depolarisation (Fig 4.10C). However the remaining 8 (14% overall) interneurones showed no response to either electrical stimulation or subsequent application of carbachol (Fig. 4.10D) suggesting that a small population of interneurones are truly unresponsive to activation of mAChRs.

4.2.7. Input resistance

In order to gauge the effect of cholinergic modulation on the passive membrane properties of interneurones, a short series of experiments were carried out in which the input resistance of interneurones was monitored throughout the slow depolarising response. The input resistance of each interneurone was calculated from the membrane voltage responses to small negative current steps (-0.02nA) recorded at the same membrane potential before and during both carbachol-induced slow depolarisations and stimulus-evoked slow depolarisations.

The input resistance before and during the carbachol-induced depolarisations were 701.8±128.9MΩ and 787.2±144.5MΩ respectively. This 13% increase in input resistance was not statistically significant (n=5, P=0.1002, Fig 4.11). The input resistance before and during the stimulus-evoked depolarisations were 758.7±49.82MΩ and 793.7±39.98MΩ (n=3, P=0.3019, Fig 4.12). This 5% increase in input resistance was again not significant. Thus both carbachol-

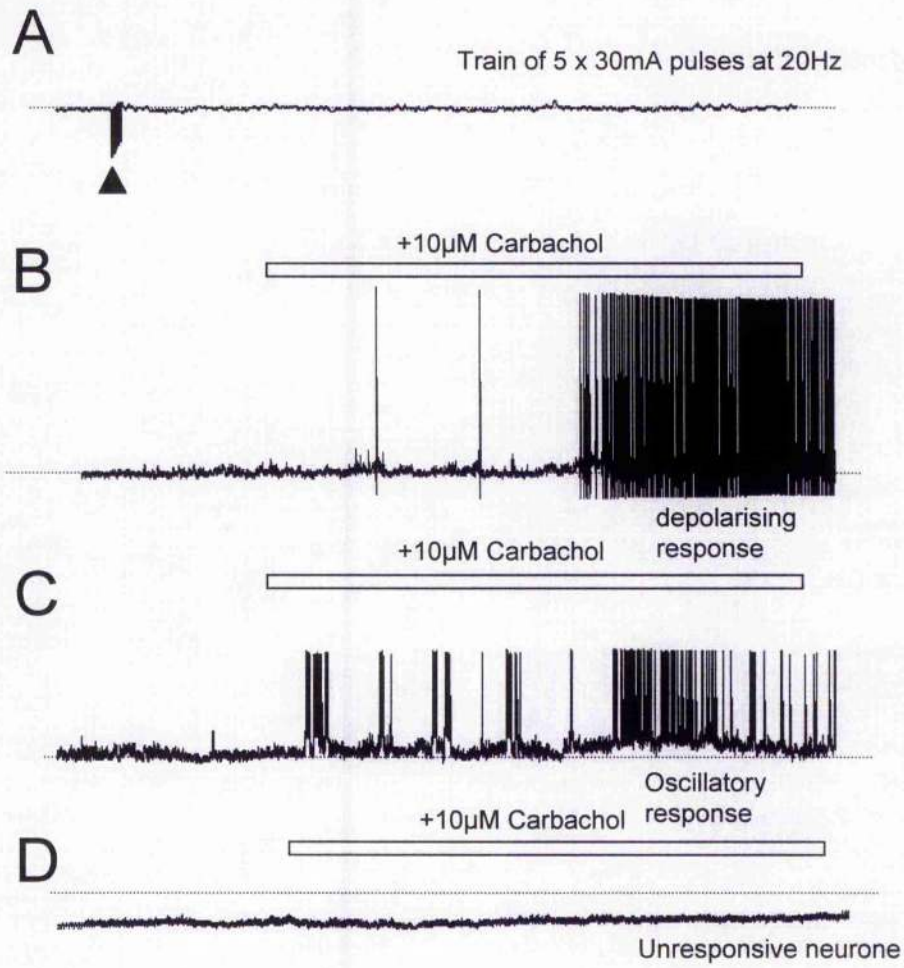


Figure 4.10. CA1 interneurone responses to carbachol application

In total 17 of 59 (29%) CA1 interneurones did not respond to electrical stimulation within the *stratum oriens* and thus were further tested with bath application of the cholinergic agonist carbachol. A. Representative voltage trace recorded from a *stratum radiatium* CA1 interneurone in which no depolarisation was apparent following a train of stimuli (5 x 30mA, 0.2ms @20Hz) delivered within the *stratum oriens*. B. Representative voltage trace from the same interneurone showing that subsequent bath application of carbachol (10μM) resulted in a slow sustained depolarisation, with a peak amplitude of 10.8mV, and intense action potential discharge. The period of carbachol application is indicated by the open bar. In total 7 of the 17 CA1 interneurones responded in this manner, with a mean peak amplitude of 11.10±1.089mV. C. Representative voltage trace from a *stratum oriens* CA1 interneuron (O-LM cell) showing an oscillatory component to the carbachol induced slow depolarisation. In total 2 of the 17 CA1 interneurones responded in this manner. D. Representative voltage trace from a *stratum oriens* CA1 interneurone showing no depolarisation of membrane potential in response to carbachol application. In total 8 of the 17 CA1 interneurones showed no overt change in membrane potential in response to both afferent stimulation and 10μM carbachol application. Values are expressed as a mean ± S.E.M.

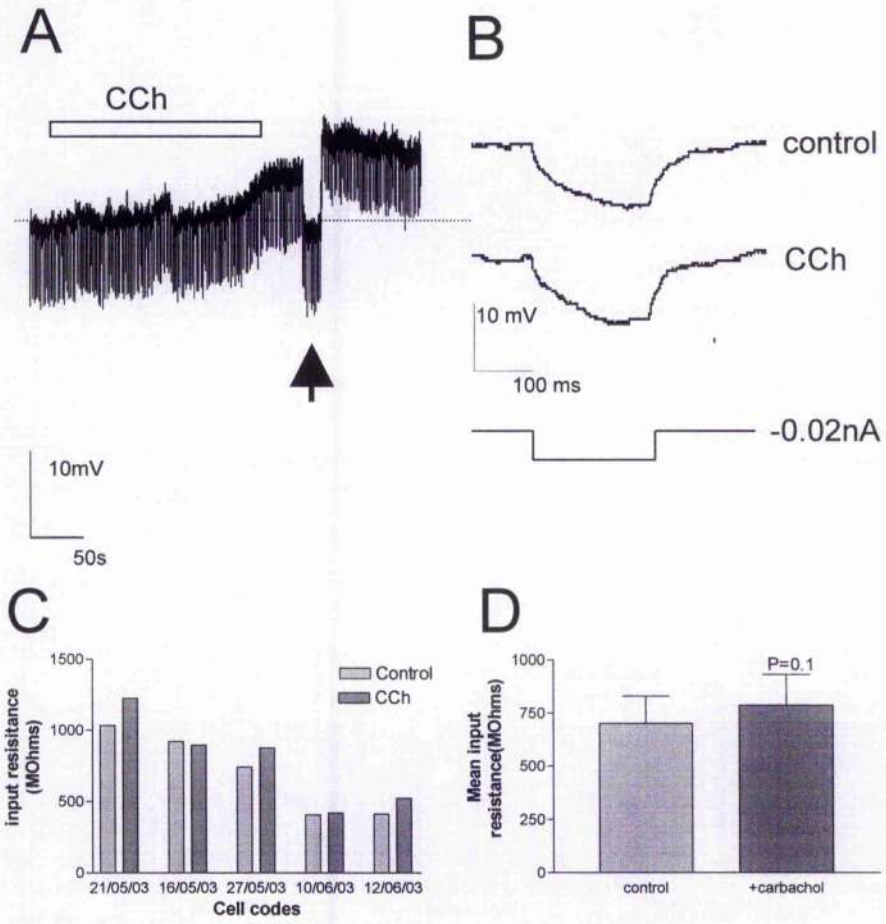


Figure 4.11 Assessment of input resistance changes during carbachol induced depolarisations.

A. Shows the effect of carbachol (CCh; 10μM) on the membrane potential and input resistance of a *stratum oriens* CA1 interneurone. The current injection was kept constant until the input resistance was measured during the CCh-induced depolarisation at the time point indicated by the arrow. The open bar above the trace indicates the period of CCh application. B. Shows a comparison of a hyperpolarising response in the control period and a hyperpolarising response after CCh application on an expanded time scale. The input resistance was measured from the change in membrane potential in response to hyperpolarising current step of –0.02nA applied for 0.2s every 2.0s. In this example the calculated input resistance was 405MΩ and 419MΩ before and during the CCh induced depolarisation respectively. C. Bar chart illustrating the change in input resistance associated with the CCh-induced depolarisation in 5 individual CA1 interneurones. D. Bar chart of pooled data showing no significant change in mean input resistance during the CCh-induced depolarisation (P=0.1, n=5) P values were calculated using a paired t-test after data passed test for normal distribution.

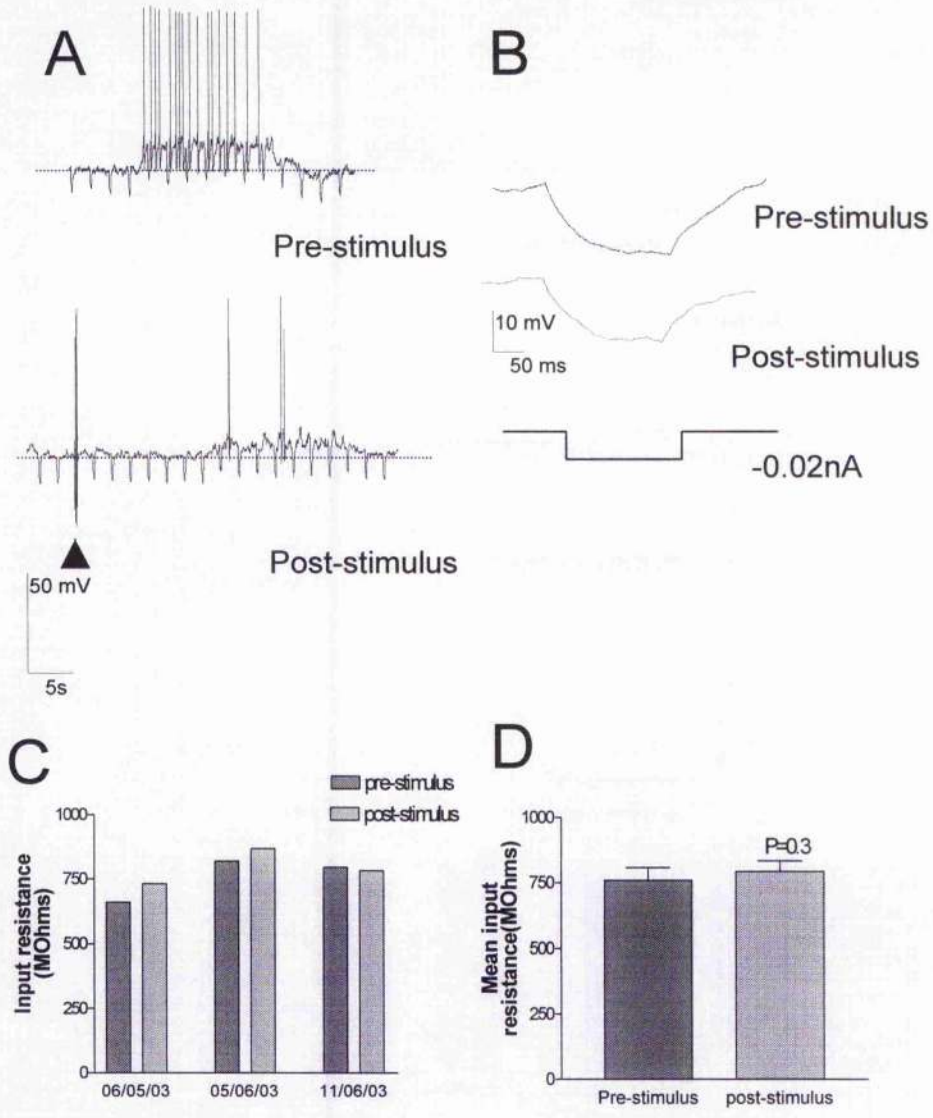


Figure 4.12. Assessment of input resistance changes during stimulus-evoked depolarisations

A. Representative voltage trace recorded from a *stratum radiatium* CA1 interneurone where the membrane potential was manually clamped at –50mV before a stimulus was applied so that the voltage response to hyperpolarising current steps could be compared with that evoked at the peak of the stimulus-evoked depolarisation (also at –50mV). The time of afferent stimulation is indicated by the filled triangle. B. Shows a comparison of these voltage responses on an expanded time scale. In this example the calculated input resistances were 820MΩ and 868MΩ pre- and post stimulation respectively. The current injected into the cell (-0.02nA) is shown below the voltage traces. The initial membrane potential of this interneurone was –55mV (without junction potential taken into consideration). C. Bar chart illustrating the change in input resistance associated with the stimulus evoked depolarisation in 3 individual CA1 interneurones. D. Bar chart of pooled data showing no significant change in input resistance during the stimulus-evoked depolarisation (P=0.3, n=3) P values were calculated using a paired t-test.

induced and stimulus-evoked slow depolarisations were not associated with any significant change in input resistance.

4.2.8. Diversity of responses in interneurones

By applying a similar protocol as used in chapter 3 to evoke mAChR responses in pyramidal neurones it was shown conclusively that broadly similar cholinergic responses may be evoked in CA1 interneurones. The mAChR-mediated response in pyramidal neurones was a uniform slow depolarisation from resting membrane potential. However, this was not found to be the case in CA1 interneurones. Although some form of consistent slow depolarisation was seen in 71% of all interneurones investigated, the pattern and time course of the depolarisation varied between cells. The majority of these responses could be separated and classified into 4 broad subtypes according to the specific waveform underlying the depolarising response, as illustrated in figure 4.13.

The most frequent type of response recorded was a simple slow depolarisation from resting membrane potential, often reaching threshold for action potential generation (Fig 4.13A). This type of response was seen in 34% of interneurones with a mean peak amplitude of 8.207±0.852mV, a mean time to peak of 15.99±1.459s, a mean time to first action potential of 8.851±1.232s and a mean number of action potentials fired of 20.34±5.430, n=20. These slow depolarisations could be inhibited by the addition of atropine (5μM, n=2).

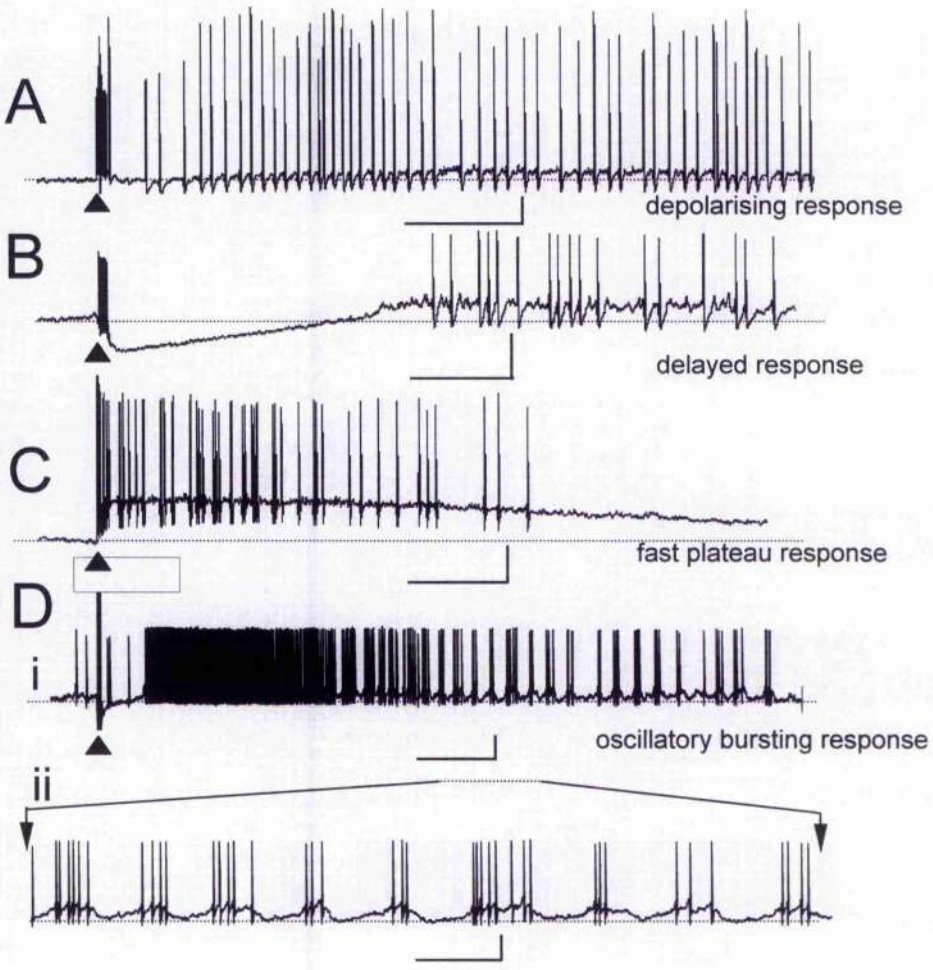


Figure 4.13 Diversity of the cholinergic response in CA1 interneurones

Although a consistent depolarisation from resting membrane potential was seen in 42 of 59 recorded, the patterns and time course of the response varied between interneurones. All of the above traces are representative cholinergic responses recorded from CA1 interneurones in response to electrical stimulation within the *stratum oriens*. The majority of responses could be classified into one of the above types A. A simple slow depolarisation of the membrane potential. B. A delayed response in which the depolarisation was preceded with a large hyperpolarisation. C Fast plateau depolarisation in which the slow depolarisation was preceded by a fast depolarisation. D. Oscillatory bursting response in which the cell initially discharges at high frequency before switching to an oscillatory mode in which the membrane potential displays. Scale bars: vertical 20mV , horizontal A-Di 5s, Dii, 1s

In a smaller proportion of interneurones (17%) a large hyperpolarisation of mean peak amplitude –9.011±1.646mV, a mean time to peak of 1.565±0.2076s and 8.74±1.232s in duration (n=10), preceded the slow depolarisation (Fig 4.13B). This lead to the latency to peak depolarisation and first action potential of the slow depolarisation to be slightly longer in these cells at 19.14±1.95s and 15.20±1.978s respectively, hence we termed this response type "delayed" (Fig 4.13B). Addition of atropine (5-10μM) significantly depressed the mean peak amplitude of both the hyperpolarisation and the slow depolarisation from –8.442±0.9115 to -6.73±1.25mV (P=0.0231) and from 8.124±1.318 to 1.379±0.437mV (P=0.0109) respectively. However the hyperpolarisation was inhibited to a lesser degree (21% inhibition) than the slow depolarisation (83% inhibition) (Fig. 4.14).

In (17%) of interneurones a fast depolarisation of mean peak amplitude 9.841±1.175mV and a mean time to peak of 1.025±0.189s was seen to precede the slow depolarisation (Fig 4.13C). These were termed "fast plateau" responses, as both responses seemed to merge to form a plateau. This merging effect made it very difficult to separate response types particularly when trying to establish the time to first action potential and number of action potentials for each response. These two properties were therefore combined for this response type. Ultimately the fast response decreased the time taken to first action potential and thus increased the number of action potentials fired. The addition of atropine (5-10μM) to these cells again significantly inhibited both the fast and slow depolarisation from 11.03±1.697mV to 6.17±1.348mV (P=0.0251) and from

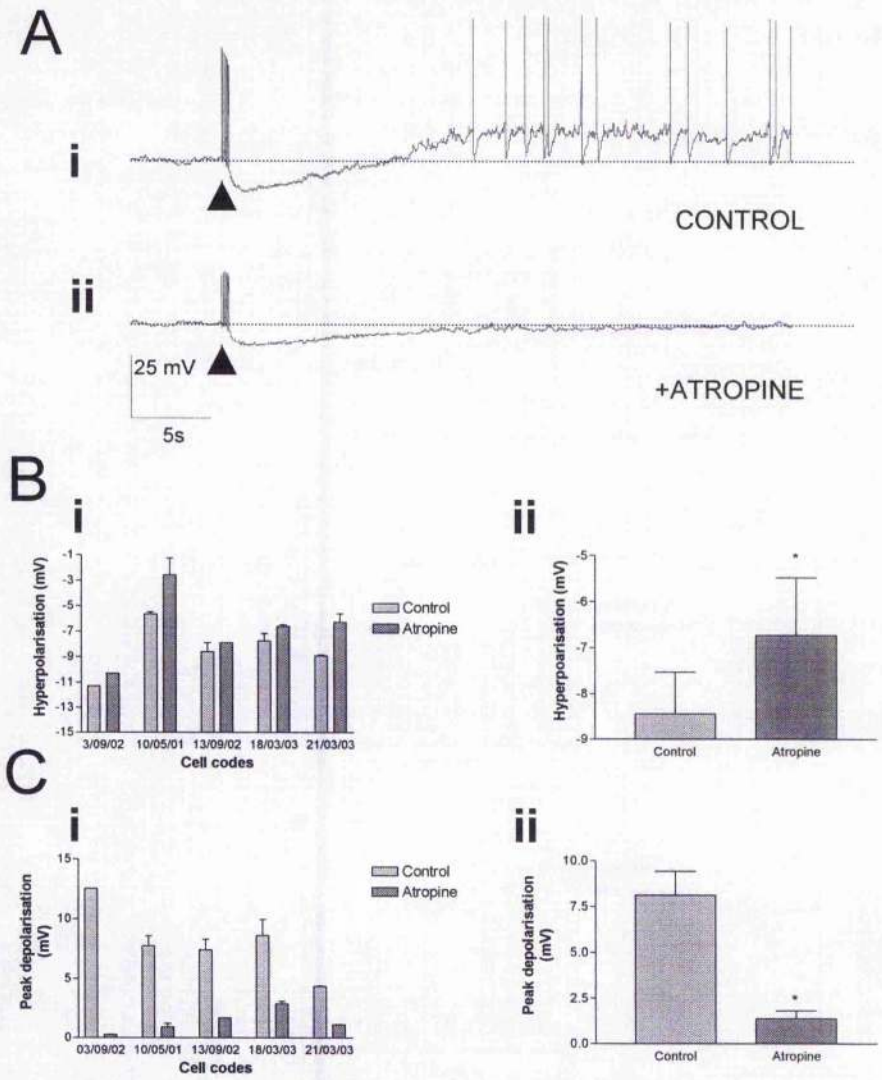


Figure 4.14 Effect of atropine on both components of the "delayed" response

Ai. Representative voltage trace recorded from a *stratum oriens* CA1 interneurone illustrating a "delayed" response evoked following delivery of a train of stimuli (5 x 10mA, 0.2ms @20Hz). This response consisted of a hyperpolarisation of peak amplitude -11.3mV, followed by a slow depolarisation of peak amplitude 12.55mV. Aii. subsequent addition of the mAChR antagonist atropine (10μM) depressed the peak amplitude of both the slow depolarisation (peak amplitude 0.25mV) and the preceding hyperpolarisation (peak amplitude -10mV). Note the hyperpolarisation is not completely inhibited by atropine. Bi. Bar chart showing the depression of the hyperpolarisation recorded in 5 individual CA1 interneurones. Bii. Bar chart of pooled data for all 5 individual interneurones illustrating the significant depression of the mean peak amplitude of the hyperpolarisation from –8.442±0.9115mV to –6.73±1.25mV (P=0.0231). Ci. Bar chart showing the depression of the slow depolarisation in the same 5 CA1 interneurones. Cii Bar chart of pooled data illustrating the significant depression of the mean maximum amplitude of depolarisation from 8.124±1.318mV to 1.379±0.437mV (P=0.0109). Values are expressed as mean ± S.E.M. P values were calculated using a paired t-test, after data passed tests for normal distribution.

9.536±1.052mV to 1.76±0.816 (P=0.003) respectively. Although the initial fast response was significantly reduced by atropine (44% inhibition), it was not completely abolished and thus allowed part of the initial fast response to be uncovered and isolated from the slow depolarisation (Fig 4.15). In a single experiment it was also observed that hyperpolarisation of the membrane potential by current injection abolished the slow depolarisation while increasing the fast depolarisation. These effects suggest that two specific conductances underlie the fast and slow components of the depolarisation. Detailed properties of each response type are summarised in table 4.3.

In a minority of cells (n=2/59, 3%) we observed an oscillatory depolarisation with phasic bursts of spikes with an interburst interval of 1-2Hz (Fig 4.13Di and ii). These responses were similar to the oscillatory responses noted on carbachol application (n=2/9) and were classed separately from the slow depolarisations. These cells did not display any differences from the other cells with respect to resting behaviours prior to stimulation.

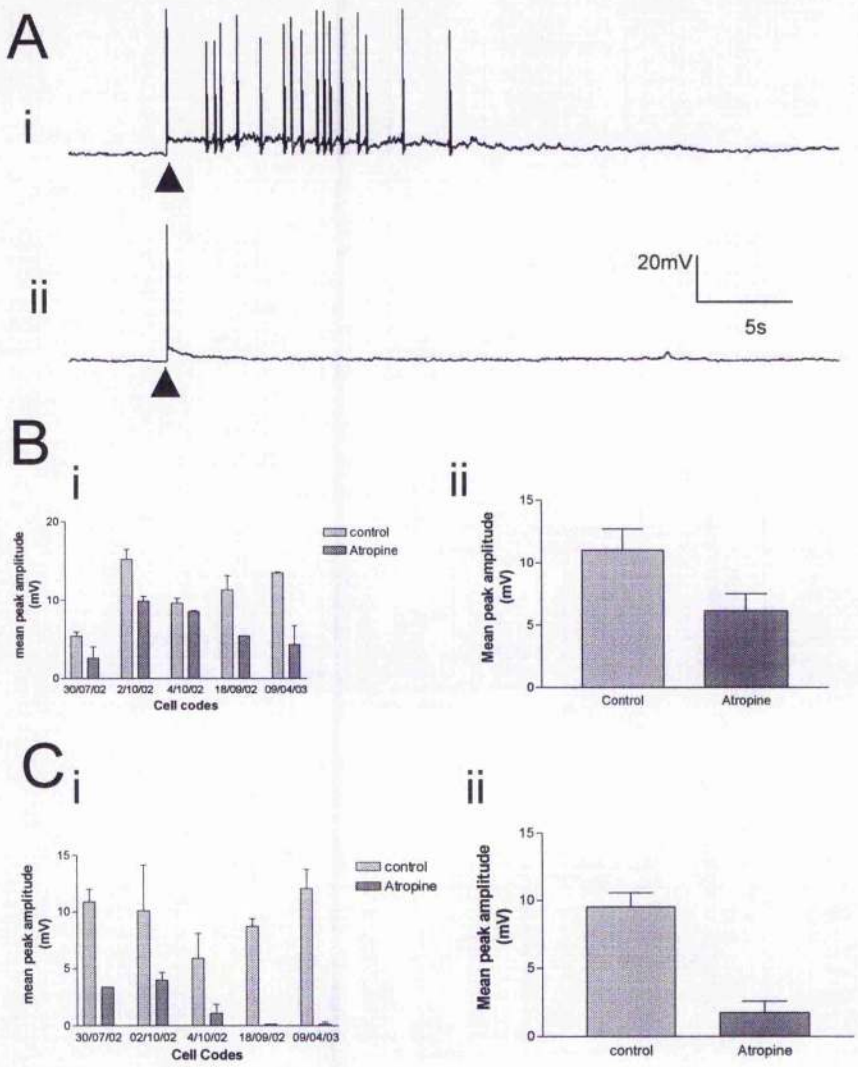


Figure 4.15 Effect of atropine on both components of the "fast plateau" responses.

Ai. Representative voltage trace recorded from a *stratum radiatium* CA1 interneurone illustrating a "fast plateau" response evoked following delivery of a single stimulus (30mA, 0.2ms). This response consisted of an initial fast depolarisation of peak amplitude 10.25mV, followed by a slower depolarisation, with a peak amplitude of 8.1mV. Aii. subsequent addition of the mAChR antagonist atropine (10μM) depressed the peak amplitude of both the fast depolarisation (peak amplitude 8.45 mV) and the slow depolarisation (peak amplitude 0.3mV). Note the fast depolarisation is not completely inhibited by atropine. Bi. Bar chart showing the depression of the fast depolarisation recorded in 5 individual CA1 interneurones. Bii. Bar chart of pooled data for all 5 individual interneurones illustrating the significant depression of the mean peak amplitude of the fast depolarisation from 11.03±1.697mV to 6.17±1.348mV (P=0.0251). Ci. Bar chart showing the depression of the slow depolarisation in the same 5 CA1 interneurones. Cii Bar chart of pooled data illustrating the significant depression of the mean maximum amplitude of depolarisation from 9.536±1.052mV to 1.76±0.816mV (P=0.003). Values are expressed as mean ± S.E.M. P values were calculated using a paired t-test, after data passed tests for normal distribution.

MAChR-mediated Response	PD (mV)	Time to PD (s)	Time to first AP (s)	No. of APs	(n)
Slow Depolarisation:	8.207±0.852	15.99±1.459	8.85±0.852	20.43±5.430	20
Delayed:					
Hyperpolarisation	-9.011±1.646	1.565±0.207	N/A	N/A	10
Slow Depolarisation	6.288±1.042	19.14±1.954	15.20±1.978	9.880±2.534	
Fast plateau:					
Fast depolarisation	9.841±1.175	1.025±0.189	0.687±0.169	39.80±6.324	10
Slow depolarisation	9.076±0.878	15.28±1.738			

Table 4.3 Individual properties of each mAChR-mediated response type seen in CA1 interneurones.

This table summarises the individual properties for each response type. Note that peak amplitude of the depolarisation for all slow depolarisations are similar (show no significant difference if analysed with a one-way analysis of variants. P>0.05) However Peak latency and time to first action potential are increased in the "delayed" slow depolarisation and time to first action potential is decreased in the "fast plateau" response leading ultimately to an increase in number of action potentials fired. Abbreviations: PD-peak amplitude of depolarisation, AP-action potential.

4.2.9. Location and morphology of interneurones

In order to determine whether CA1 interneurones that displayed a particular type of mAChR-mediated response could be categorised by their morphological subtype each interneurone was intracellularly labelled with neurobiotin. Post-hoc anatomical analysis of each interneurone showed that the majority of the interneurones in this study had a cell body location in either the *stratum oriens* or *stratum radiatum. Stratum radiatum* interneurones generally had a stellate-like appearance with dendrites radiating within the dendritic layer of the *stratum radiatum* and the *stratum lacunosum moleculare* (n=24, Fig 4.16). In contrast all *stratum oriens* cells were bipolar and displayed dendrites, which extended in a horizontal axis parallel with the *stratum oriens* (n=24). All types of mAChR-mediated responses were recorded in interneurones in both locations.

In the *stratum oriens* 38% of cells were unresponsive to synaptic stimulation, although 21% did respond to carbachol (17% with a slow depolarisation and 4% with an oscillatory response) therefore only 17% of *stratum oriens* cells were entirely unresponsive to mAChR activation. Of the cells that did respond to synaptic stimulation 29% responded with a depolarisation, 8% responded with a delayed response, 21% responded with a fast plateau response and 4% responded with an oscillatory response. In *stratum radiatum* only 17% of cells were unresponsive to synaptic stimulation, although 13% did respond to carbachol (9% with a slow depolarisation and 4% with an oscillation) therefore only 4% were entirely unresponsive to mAChR activation. Of the cells that did respond to

synaptic stimulation 33% responded with a depolarisation, 29% responded with a delayed response and 21% responded with a fast plateau response. This data is summarised in table 4.4.

Layer	Depolar.	Delayed	Fast	Oscill.	Carbachol	No response	Total
SO	29% (n=7)	8% (n=2)	21% (n=5)	4% (n=1)	21% (n=5)	17% (n=4)	100% (n=24)
SR	33% (n=8)	29% (n=7)	21% (n=5)	0% (n=0)	13% (n=3)	4% (n=1)	100% (n=24)

Table 4.4 Distribution of muscarinic response types with stratum oriens and stratum radiatum of the CA1.

Table shows percentage of CA1 interneurones with soma located within the *stratum oriens* (SO) and the *stratum radiatum* (SR) displaying each type of mAChR response. Note interneurones in both locations responded with all types of mAChR-mediated response although the slow depolarisation seemed to be the most frequent response in each layer.

One recorded interneurone showed soma location within the *stratum pyramidale* and responded to synaptic stimulation with an oscillatory response (1.5Hz). Thus soma location of CA1 interneurones showed no obvious correlation with type of mAChR-mediated response recorded.

Further classification of interneurones into subtypes with particular presumed functions, such as dendritic or perisomatic inhibition requires detailed analysis of the dendritic and axonal projections. Throughout this study problems with the intracellular labelling of the axonal projection did not allow further classification of the recorded interneurones. However, three CA1 interneurones with rounded somata located in the *stratum oriens*, horizontal dendrites extending through the *stratum oriens* and an axonal projection to the *stratum lacunosum-moleculare* characteristic of the O-LM subtype were identified. All three identified O-LM cells displayed a mAChR-mediated cholinergic response. One responded to electrical stimulation within the *stratum oriens* with a slow depolarisation (Fig 4.16), the others did not respond to electrical stimulation but did respond to carbachol application with either a slow depolarisation or oscillatory response.

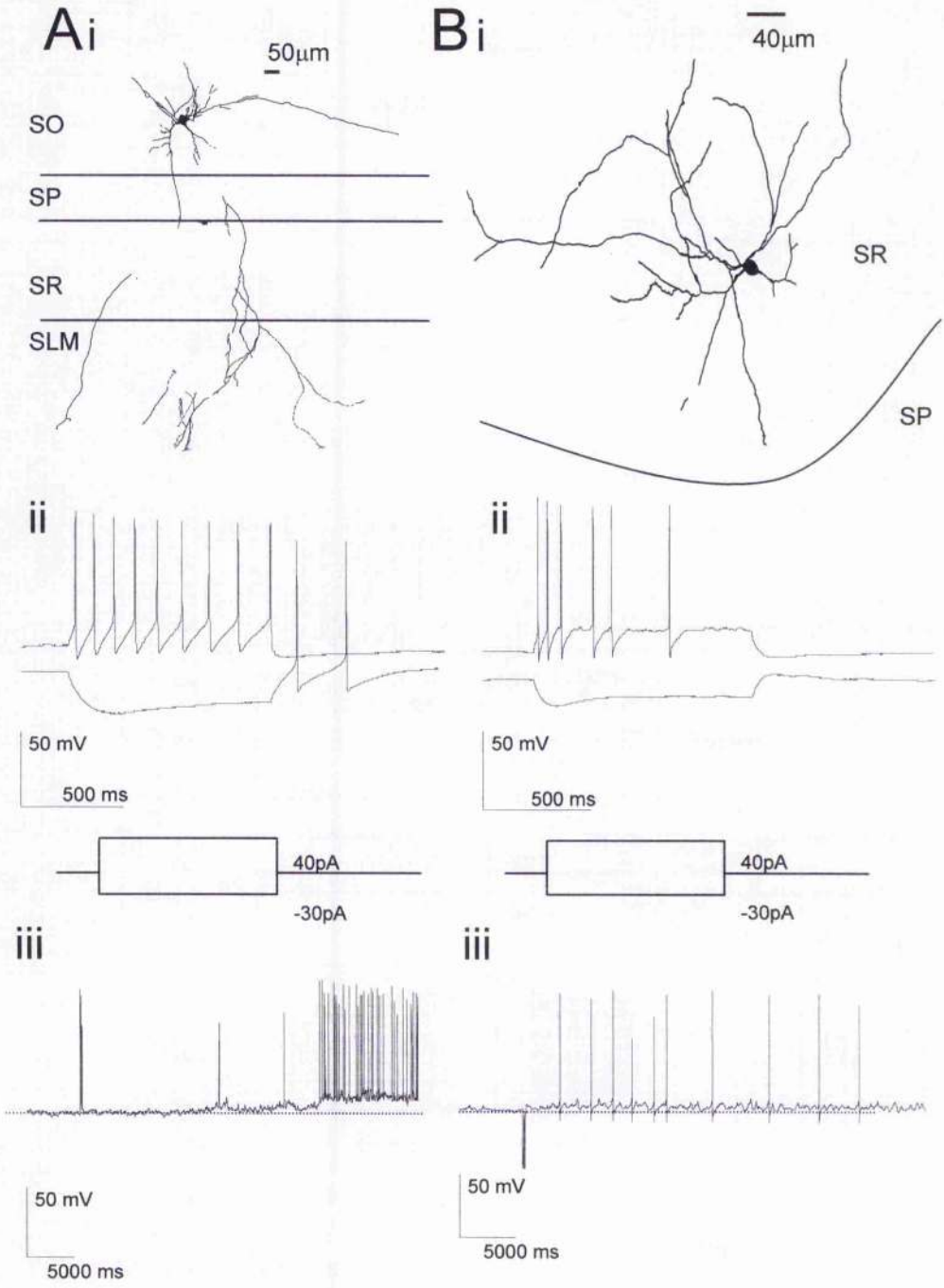


Figure 4.16. Example of filled interneurones with associated electrophysiology.

Ai. Camera lucida reconstruction of a representative O-LM cell. Aii Representative voltage traces (upper traces) recorded from the same cell in response to applied intracellular current injection (lower trace) illustrating the firing properties of this O-LM cell. Aiii. Typical cholinergic response from this O-LM cell. Bi. Camera lucida reconstruction of a representative *stratum radiatum* interneurone. Bii representative voltage traces (upper traces) recorded from the same cell in response to applied intracellular current injection (lower traces) illustrating the firing properties of this interneurone. Biii. Typical cholinergic response from this *stratum radiatum* interneurone.

4.3. DISCUSSION

4.3.1. Verification of the septo-hippocampal projection

It was apparent from our immunohistochemical staining of the septo-hippocampal projection that in the juvenile rats used in the electrophysiological experiments, the cholinergic septo-hippocampal projection was complete and that such cholinergic afferents ramified extensively within the hippocampus. Furthermore, the presence of multiple boutons in close apposition with PV-immunopositive interneurones strongly suggested that the functional connection was indeed intact. This was subsequently demonstrated conclusively using an electrophysiological approach to monitor the cellular (pyramidal and interneurone) response to cholinergic afferent stimulation in the juvenile tissues. Previous studies have reported that an adult pattern of theta rhythm is established by P15 or P16 (Leblanc & Bland, 1979; Lanfumey *et al.*, 1982). As theta rhythm is partly driven by the cholinergic input from the MSN (Stewart & Fox, 1990) this indicates that the pathway is functional at some level. This together with the findings presented in this chapter suggest that the septo-hippocampal cholinergic projection is sufficiently developed to be functional by postnatal day 16 in Wistar rats.

4.3.2. Evoked cholinergic responses

Electrical stimulation within the *stratum oriens* produced an atropine sensitive slow and sustained depolarisation from resting membrane potential in both CA1

interneurones and pyramidal neurones. All slow depolarisations seen in pyramidal neurones were recorded in the presence of the acetylcholinesterase inhibitor eserine and were similar in amplitude and waveform to the responses recorded in chapter three using sharp $KmeSO_4$ filled microelectrodes. Although the depolarisation seemed slightly slower in nature, reaching peak depolarisation at 17.55±2.24s compared to 10.14±2.73s. This may be due to the fact that patch clamp protocol was carried out at room temperature (thermostatically controlled at 26±2°C) rather than 37°C. This slow depolarisation was therefore concluded to be the same EPSPm investigated in chapter three. Previous studies investigating cholinergic responses have mainly focused on using sharp microelectrodes with the rational behind this choice of recording configuration being to minimise cell dialysis in order to prevent rundown of the G-protein coupled response. However, it is clear from these studies that use of the whole-cell patch clamp configuration with low resistance (~8MΩ) electrodes, which would inevitably cause some degree of dilution of intracellular millieu, nevertheless does not prevent the ability of the cell to display evoked mAChR-mediated responses similar to that seen in pyramidal neurones with sharp electrodes. Having confirmed that the whole-cell configuration was not adversely affecting the expression of evoked cholinergic responses in pyramidal neurones, this recording configuration was employed to investigate and characterise these responses in CA1 interneurones.

The atropine sensitive depolarisation observed in the CA1 interneurones is the first demonstration of a physiological synaptic mAChR-mediated response in these cells on electrical stimulation of cholinergic fibres within the *stratum oriens*.

71% of CA1 interneurones responded in this manner with the other 29% showing no overt depolarisation of the membrane potential.

Although initially responses were recorded in the presence of the cholinesterase inhibitor eserine, further experiments showed depolarisations of similar amplitude and duration could be evoked in the absence of eserine. This suggests that the response is a physiologically relevant event and is not an artefact of artificially increased levels of acetylcholine present within the slice by cholinesterase inhibition. Application of eserine was able to uncover slow depolarisations in interneurones, which seemed unresponsive in the absence of eserine. This is presumably due to an increase in the levels of released ACh by cholinesterase inhibition. It may therefore have been expected that all responses recorded in the presence of eserine would be of larger magnitude or require lower stimulation parameters to evoke than responses recorded in the absence of eserine, however this was not found to be the case. The fact that we did not add eserine to the perfusion medium when recording from cells which responded to afferent stimulation in the absence of eserine does not allow a direct analysis on the potentiating effect of eserine in CA1 interneurones as was carried out on the pyramidal neurones in chapter three. The reason that we did not investigate this effect was due to the fact that where possible responses were recorded in the most physiological state and not in artificially elevated levels of ACh as has been the case in the past when recording these responses in pyramidal neurones (Pitler & Alger, 1990).

Once again the synaptic response was dependent upon the stimulation parameters used. This was to be expected due to the similar protocol employed to evoke these responses. As mentioned in the previous chapter, trains of stimuli increased the amplitude of the synaptic response presumably due to the accumulation of acetylcholine following repeated activation of the cholinergic fibres and increasing stimulation intensity increased the response presumably by recruiting more fibres to release acetylcholine. It seems reasonable that both of these effects were apparent in both interneurones and pyramidal neurones as both cell types are innervated by the cholinergic fibres of the septo-hippocampal projection (Frotscher *et al.*, 1986), thus any protocol increasing or decreasing the release of ACh from these fibres would affect both cell types in a similar manner.

Earlier studies reported a fatigue of the cholinergic response in the pyramidal neurone population and found that an inter-stimulus interval length of 5-8 minutes was sufficient to avoid depression of the second evoked response (Morton & Davies, 1997). Here we report a similar effect in CA1 interneurones and show no significant depression of the slow depolarisation if an inter-stimulus interval of 5 minutes is adhered to. The fatigue of the slow depolarisation if evoked less than every 5 minutes may be due to presynaptic factors affecting the release of acetylcholine such as depletion of releasable ACh from terminals, or may also be due to postsynaptic factors such as desensitisation of mAChR receptors and depletion of Ca^{2+} from internal stores. Due to the long duration of the fatigue a presynaptic effect alone seems unlikely, it may be more likely that both pre- and postsynaptic factors contribute in some way.

In all, 17 of 59 (29%) of the CA1 interneurones recorded in this study were unresponsive to electrical stimulation within the *stratum oriens*. These cells were further tested with bath application of the cholinergic analogue carbachol. In total 9 of these unresponsive cells, a further 15% of the CA1 interneurones recorded, responded to carbachol application with either a slow depolarisation or an oscillatory response. Possible reasons for this lack of response to electrical stimulation within the *stratum oriens* and subsequent response to carbachol application may include problems in positioning of the stimulation electrode, in that too few fibres were activated and thus resulting in insufficient release of acetylcholine to evoke a postsynaptic response, or problems in slice preparation and maintenance in that, the septo-hippocampal cholinergic afferents may have degenerated after cutting of the slices or some factor within the slice is inhibiting ACh release. Morton and colleagues (Morton *et al.*, 1997; Morton & Davies, 1997; Morton *et al.*, 2001) showed that the EPSPm recorded in CA1 pyramidal neurones could be suppressed by the activation of presynaptic $GABA_B$ and A1 receptors to inhibit the release of ACh. As such high tonic levels of adenosine or GABA in slices may inhibit the production of a postsynaptic response, as $GABA_B$ receptor antagonists were included in the perfusion medium throughout the recordings a role of GABA can be discounted. However, no adenosine antagonists were present and as such a lack of response may be due to inhibition of ACh release by presynaptic A1 receptors. Pharmacological activation of mAChRs via application of carbachol would not be affected by any of the factors and thus would still elicit a response in interneurones expressing mAChR receptors. A

similar observation that synaptically evoked responses were indeed harder to evoke than responses evoked with agonist application was noted by two individual research groups (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a) when trying to elicit nAChR-mediated response in CA1 interneurones. These groups suggested the main reason behind this the lack of response was due to the precise positioning of their stimulation electrodes to activate cholinergic fibres.

In contrast to pyramidal neurones these results suggest that the mAChR-mediated depolarisations in interneurones were not accompanied by any significant change in input resistance. The result was the same for both carbachol-induced and stimulus-evoked depolarisations. These findings are in agreement with McQuiston and Madison (1999a) who also reported no change in input resistance during agonist-evoked depolarisations in CA1 interneurones. These authors suggested that the depolarisations were caused by the simultaneous inhibition of resting K^+ channels and the activation of a voltage independent nonselective cation current. Therefore the contribution of both channel types would cancel out the effect of the other on the input resistance of the cell, resulting in a depolarisation without reversal or any net change in input resistance. However the mAChR-mediated depolarisation in pyramidal neurones, due to depression of the *leak* K^+ current and activation of a non-selective cation conductance, is associated with an increase in input resistance. It seems more likely that no significant change in input resistance could be attributed to the fact that CA1 interneurones have a higher initial input resistance than pyramidal neurones, presumably in part due to a lower basal K^+ leak conductance, therefore if the slow depolarisations are a result of the inhibition

of the K^+ leak conductance as suggested this will not be apparent as a large change in input resistance and thus may be too small to show significance.

4.3.3. Diversity of the interneurone response.

As the interneurone population itself shows a large amount of diversity in terms of morphology, physiology and receptor expression it would be unreasonable to assume that all interneurones would respond to mAChR activation with a single uniform response. The results presented in this chapter show that CA1 interneurones show diversity in the characteristics of their evoked cholinergic responses and that they respond to mAChR activation in one of five ways.

1. **A slow depolarisation**, in which a slow, sustained depolarisation of the membrane potential is apparent.
2. **A "delayed" response**, in which a large hyperpolarisation precedes a slow, sustained depolarisation.
3. **A "fast plateau" response**, in which a fast depolarisation precedes a slow, sustained depolarisation with the responses merging to form a plateau.
4. **An oscillatory response,** in which oscillatory bursting was seen throughout the slow depolarisation.
5. **No response**, in which no detectable change in membrane potential was measured.

The results presented here show that the simple slow depolarisation of the membrane potential was the most frequent mAChR-mediated response to electrical stimulation of cholinergic fibres, recorded in 34% of CA1 interneurones. This finding is in agreement with previous studies carried out by McQuiston and Madison (1999a) who reported a depolarisation of membrane potential to be the most frequent response to application of mAChR agonists, in 48% of interneurones. Problems with the synaptic activation of cholinergic fibres most likely account for the lower percentage of cells showing this response type in this study. If the interneurones, which did not respond to electrical stimulation but did respond to carbachol application with a slow depolarisation, are included in the total of responsive cells the percentage is increased to a more comparable 46% of interneurones responding to mAChR activation with a slow depolarisation.

The mAChR-mediated response type termed "delayed" in this study was similar in nature to the "biphasic" response to mAChR agonists reported by McQuiston and Madison (1999a), in that it consisted of a large hyperpolarisation preceding a slower depolarisation. This study showed 17% of interneurones responding in this manner compared to 15% in the McQuiston study. However, in this study addition of the selective mAChR antagonist atropine, significantly reduced the initial hyperpolarisation but did not completely abolish it, as was shown to be the case for the biphasic responses reported in the McQuiston study. The remaining component of the hyperpolarisation may be due to action of another neurotransmitter such as noradrenaline or serotonin (5-HT) as both transmitter have been shown to elicit hyperpolarising responses in CA1 interneurones (Parra

et al., 1998). It may also be an AHP in response to direct antidromic activation of the cell axon or may simply reflect the competitive nature of the inhibition and that increasing the concentration of atropine applied may have decreased the response further, although this latter suggestion seems the most unlikely due to the relatively high concentration of atropine applied.

17% of the CA1 interneurones recorded in this study responded with the "fast plateau" type of response. This type of response resembled the plateau potentials recorded in CA1 pyramidal neurones in response to current injection in the presence of cholinergic agonist carbachol due to activation of a Ca^{2+}-activated nonselective cation conductance and high-voltage-activated Ca^{2+} channels (Fraser & Macvicar, 1996). However, although both the fast and slow components of this response were significantly inhibited by the selective mAChR antagonist atropine, the fast depolarisation was not completely abolished. A possible reason for this may be that the fast component was elicited by activation of a ligand gated ion channel such as the nAChR. Although the action of nAChR antagonists was not tested on this remaining response, as nicotinic responses have previously been reported in CA1 interneurones by two other groups (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a) it remains a possibility. If a portion of the fast component was due to nAChR activation, this may explain why McQusiton and Madison (1999a) did not report this type of response, as these authors routinely included nAChR antagonists in their aCSF when using the non-specific agonists ACh and carbachol to evoke mAChR-mediated responses.

In total, 3% of CA1 interneurones responded to electrical stimulation of cholinergic afferents with an oscillatory component to the depolarisation and an additional 3% of interneurones that were unresponsive to electrical stimulation but responsive to carbachol responded with an oscillatory component. McQuiston and Madison (1999a) also reported an interneurones responding with slow membrane oscillation but chose not to investigate it further as it was such an infrequent response. In this study, the slow oscillatory responses displayed a sinusoidal waveform with short trains of action potentials occurring at the top (most depolarised) peak of each cycle. This is similar to reported patterns of interneurone activity recorded *in vivo* (Ylinen *et al.*, 1995; Klausberger *et al.*, 2003). It is possible that such oscillatory activities recorded *in vivo* may be driven by cholinergic excitation.

Overall, only 14% of interneurones were entirely unresponsive to mAChR activation, this was comparable to the 21% reported by the McQuiston study. This suggests that a subpopulation of CA1 interneurones may not express mAChRs. However, application of mAChR agonists could produce effects in CA1 pyramidal neurones such as the reduction in SFA and AHPs at levels which did not produce a mAChR mediated membrane depolarisation (Cole & Nicoll, 1983; Segal, 1988). In addition a subsequent study by McQuiston and Madison (1999b) reported that the AHP following train of action potentials in response to injected current, could be replaced by a afterdepolarisation (ADP) on application of mAChR agonists. This effect was often accompanied by an effect on membrane potential but was seen in interneurones where no effect on resting membrane potential was apparent.

Thus it may be possible that the interneurones recorded in this study, which showed no membrane depolarisation may not be entirely unresponsive to mAChR activation, although future studies are required to fully address this possibility.

These results differed from the agonist studies carried out by McQuiston and Madison (1999a) in that we did not observe a hyperpolarisation of interneurone membrane potential alone without a subsequent depolarisation. However this response was only reported in 17% of interneurones and these authors performed a larger study recording from interneurones located in all strata of the CA1, including the *stratum pyramidale* and the *stratum lacunosum-moleculare*. Thus the subset of interneurones that respond with a hyperpolarisation may not have been recorded from due to the smaller sample size in this study.

The majority of interneurones recorded in this study showed cell body location in either the *stratum oriens* or *stratum radiatum*. Whilst the *stratum oriens* horizontal cells are indeed most likely to project to dendrites in the SLM, a proportion of cells located in the *stratum oriens* are known to project to the *stratum pyramidale* (Sik *et al.*, 1995; Buhler & Dunwiddie, 2001), whilst others may project back to the medial septal nucleus (Toth & Freund, 1992; Gulyas *et al.*, 2003). Interneurones with soma in the *stratum radiatum* may project to any region and often project to the *stratum pyramidale* (Vida *et al.*, 1998). Due to the lack of axonal staining in this study it is difficult to classify the recorded interneurones into any subclasses, such as dendritic inhibitory cells or perisomatic inhibitory cells. However, it can be reported that interneurones showing cell body

location in both the *stratum oriens* and *stratum radiatum* responded to mAChR activation with all types of mAChR-mediated response, including no response and thus concluded that cell body location cannot be used to predict mAChR mediated response type of CA1 interneurones.

Three of the recorded interneurones could be classified as the O-LM cell type, all of which were excited by mAChR activation. These findings are again in agreement with McQuiston and Madison (1999a) who reported that two O-LM cells responded to mAChR activation with a depolarisation. As these cells are known to express the neuropeptide somatostatin it is interesting to note that the immunocytochemical studies by Hajos (1998) showed only 15% of m2 expressing interneurones in the *stratum oriens* expressed this neuropeptide. All cells that did show co-expression of somatostatin and m2 showed relatively low levels of m2 receptor protein. However, the main finding of this immunocytochemical study reported that a subset of interneurones located within the *stratum oriens* with horizontal dendrites and an axonal projection to dendritic region of pyramidal neurones expressed M2 receptors on their soma and dendrites and postulated that these cells would be excited by muscarinic activation. The results presented in this chapter are consistent with the suggestion that *stratum oriens* interneurones are indeed excited by muscarinic activation, although this study makes no attempt to decipher the subtype of muscarinic receptor involved.

4.4. Summary

The results presented in this chapter provide the first direct evidence that CA1 interneurones respond to synaptically released ACh on electrical stimulation of septo-hippocampal cholinergic afferents with a mAChR-mediated depolarisation. In contrast to the mAChR-mediated slow depolarisation observed in CA1 pyramidal neurones, the evoked depolarisation in CA1 interneurones was not accompanied by any change in input resistance and showed greater heterogeneity in pattern and timecourse.

In summary, with respect to membrane depolarisation CA1 interneurones respond to mAChR activation in one of five ways including; 1, a slow depolarisation, 2. a "delayed response", 3. a "fast plateau response", 4. an oscillatory response, 5. no change. This variety of mAChR-mediated responses recorded from CA1 interneurones further reinforces the diversity and complexity of interneuronal function.

5. INVESTIGATION OF THE ACTION OF NICOTINIC ACETYLCHOLINE RECEPTOR AGONISTS ON GLUTAMATE AND GABA RELEASE WITHIN THE HIPPOCAMPUS

5.1. INTRODUCTION

Previous studies have shown that both mAChR and nAChR agonists can alter neurotransmitter release within the hippocampus (Gray *et al.*, 1996; Alkondon *et al.*, 1997a; Qian & Saggau, 1997). NAChRs in particular have been shown to modulate the release of a range of neurotransmitters in the central nervous system including glutamate (McGehee *et al.*, 1995; Gray *et al.*, 1996; Girod & Role, 2001; Liu *et al.*, 2003), GABA (Alkondon *et al.*, 1997a; Albuquerque *et al.*, 1998; Alkondon *et al.*, 1999; Zhu & Chiappinelli, 1999; Covernton & Lester, 2002), ACh (Araujo *et al.*, 1988a; McGehee *et al.*, 1995), dopamine (Rapier *et al.*, 1990; Zhou *et al.*, 2001) and noradrenaline (Clarke & Reuben, 1996; Sacaan *et al.*, 1996).

With respect to glutamatergic transmission nAChRs have previously been shown to enhance evoked EPSCs, evoked EPSPs and miniature EPSCs in numerous brain regions including the hippocampus (McGehee *et al.*, 1995; Gray *et al.*, 1996; Radcliffe & Dani, 1998; Chiodini *et al.*, 1999; Liu *et al.*, 2003) with such studies

indicating a predominantly presynaptic location of the nAChRs involved (McGehee *et al.*, 1995; Gray *et al.*, 1996). In contrast, studies investigating the modulation of GABAergic transmission within the hippocampus have indicated a predominantly action potential dependent mechanism (Alkondon *et al.*, 1997a; Alkondon *et al.*, 1999; Hulo & Muller, 2001). Such studies used electrophysiological recording techniques to record from hippocampal interneurones and pyramidal neurones and showed that application of ACh could increase both the frequency and amplitude of spontaneous GABAergic IPSPs recorded in both cell types. However this effect of ACh could be prevented by the application of the Na^+ channel blocker tetrodotoxin (TTX). These experiments therefore indicated that the effect is dependent upon propagation of action potentials from the cell soma to the axon terminal and not activation of presynaptic receptors situated at the axon terminal.

The neuromodulatory role of nAChRs is thought to be one of their prominent functions within the brain (Vizi & Lendvai, 1999) and could underlie numerous processes such as cholinergic involvement in learning and memory (Fujii *et al.*, 1999; Fujii *et al.*, 2000). In addition, there is significant evidence that nAChRs are important in a number of disease states including dementia, schizophrenia and epilepsy (James & Nordberg, 1995; Steinlein *et al.*, 1995; Freedman *et al.*, 1997; Phillips *et al.*, 1998; Freedman *et al.*, 2000; Loscher *et al.*, 2003). With regard to epilepsy, findings from this laboratory (see below Roshan-Milani *et al.*, 2003) using extracellular field recording techiniques within the acute hippocampal slice preparation have shown that nAChR agonists increase the frequency of 4-AP and

bicuculline induced epileptiform bursting activity. These results together with *in vivo* studies by Damaj and collegues showing nicotine induced seizures in mice (Damaj *et al.*, 1999) suggest that nAChR modulation of hippocampal synaptic transmission may be an important factor in epileptogenesis. It is unlikely that nAChRs are significantly affecting GABAergic transmission to promote this epileptiform activity as the frequency facilitation was observed in the presence of the $GABA_A$ receptor antagonist bicuculline (Roshan-Milani *et al.*, 2003). It may therefore be more likely that the frequency facilitation may reflect an enhancement of glutamate transmission.

The aims of this chapter were to investigate the effect of nicotinic agonists on both glutamate and GABA release from hippocampal pyramidal neurones and interneurones within the CA1 and CA3 regions of the hippocampus with a view to uncovering possible mechanisms responsible for the pro-epileptogenic action of nicotine.

5.2. RESULTS

5.2.1. Proepileptogenic action of nAChR agonists in the hippocampus.

Previous published data from our laboratory reported a range of nAChR agonists to exacerbate epileptiform activity in the rat hippocampus (Roshan-Milani *et al.*, 2003).

The extracellular recording configuration was used in order to measure synchronous neuronal discharges that form epileptiform activities. Experiments were performed on hippocampal slices prepared from 2-6 week old male Wistar rats. Slices were maintained in an interface-type of recording chamber with recording electrodes placed close to the *stratum pyramidale* within area CA3 (CA3c). Voltage recordings under control (drug free) conditions revealed the absence of any detectable spontaneous extracellular field events in all slices. Bath application of the potassium channel blocker 4-aminopyridine (4-AP, 20μM) resulted in the development of spontaneous epileptiform bursting activity apparent as spontaneous field potentials. These occurred every 2.6±0.1s (range 1.5-3.3s), a mean instantaneous frequency of 0.4±0.02Hz (n=37). Such activity was highly stable and lasted for the duration (up to many hours) of 4-AP perfusion. Upon subsequent co-application of the selective nAChR agonist 1,1-dimethyl-4-phenyl-piperazinium iodine (DMPP, 10-30μM, n=31/37) a sustained increase in burst frequency was produced (Fig. 5.1A and C), which was reversed upon DMPP washout. The mean maximal frequency potentiation induced by co-application of 30μM DMPP was 37 ± 5% (n=31, $P<0.05$, Fig 5.1 D). Further studies by laboratory co-workers (Roshan-Milani *et al.*, 2003) confirmed the nAChR-mediated nature of burst frequency potentiation as demonstrated by a reversal of the potentiation upon co-application of selective nAChR antagonists, including dihydro-β-erythroidine (20μM).

In order to examine whether nAChR activation modulates hippocampal network activity more widely, further studies were carried out to assess the action of nAChR activation upon network bursting brought about by bicuculline activation (reduced inhibition).

Bath application of the $GABA_A$ antagonist bicuculline (20μM) also induced epileptiform bursting within hippocampal slices. Such activity was apparent as spontaneous field potentials occurring every 3-33s, mean instantaneous frequency of 0.15±0.02Hz (n=30). Subsequent co-application of DMPP (30μM) again potentiated the burst frequency (Fig 5.1B and C). The mean maximal frequency potentiation of bicuculline (20μM)-induced bursting model was 248 ± 76% (n=14/14, $P<0.05$, Fig5.1 D).

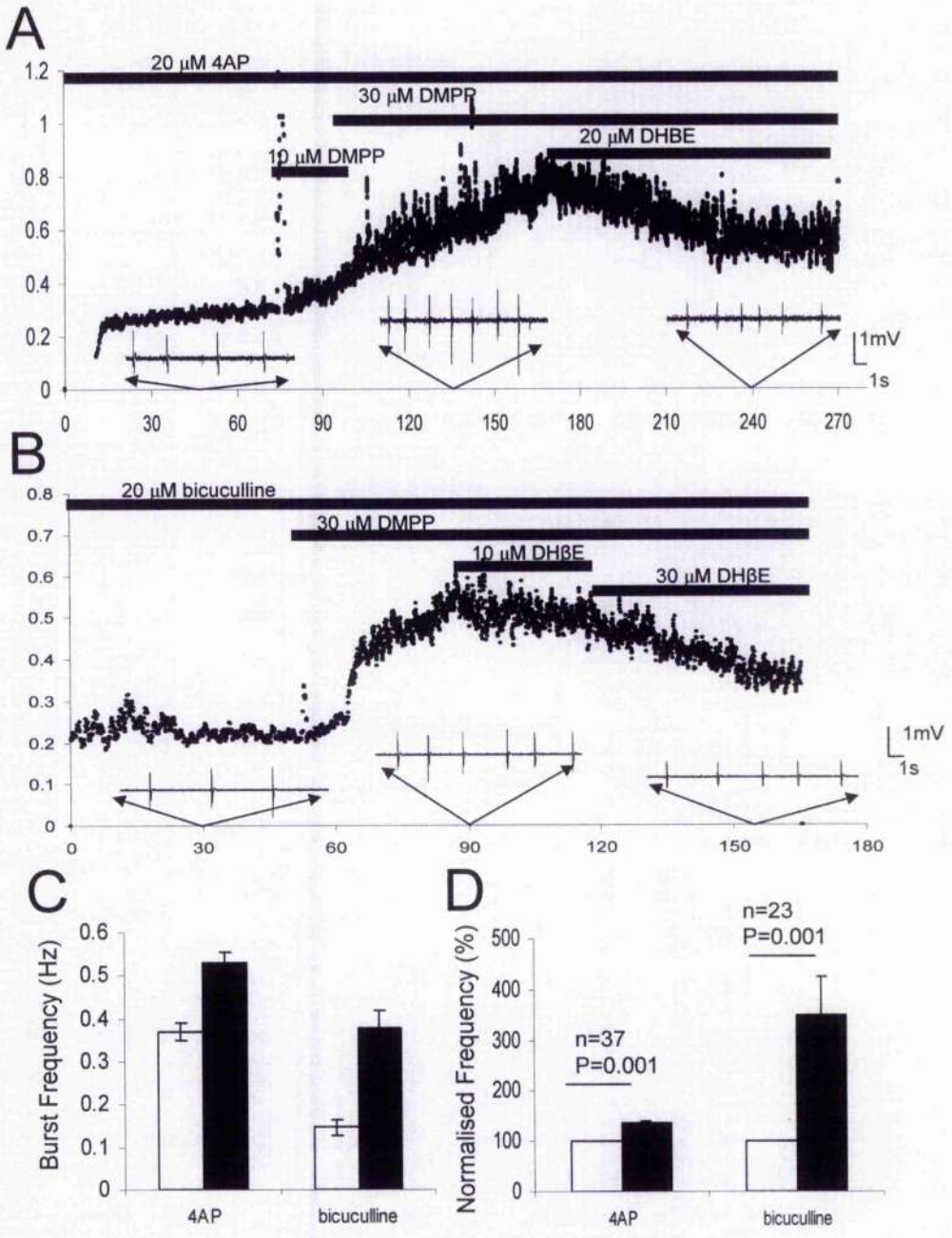


Figure 5.1 Pro-epileptogenic action of nAChR agonists

A. Scatter plot showing instantaneous burst frequency in response to continuous application of 20 μM 4AP (horizontal bar) for the duration of a representative experiment. Co-application of the selective nAChR agonist DMPP (10-30μM) (horizontal bar) resulted in an increase in burst frequency. This was reversed upon subsequent co-application of the selective nAChR antagonist Dihydro-β-erythroidine (DHβE, 20μM). Inset voltage traces show epileptiform bursting activity before and during DMPP application and on addition of DHβE. *B.* Similar scatter plot in which disinhibition of the slice following application of the $GABA_A$ receptor antagonist bicuculline (20 μM) produced a regular synchronised extracellular discharge. Application of the nAChR agonist DMPP (30 μM) resulted in an enhanced burst frequency that was reversed upon subsequent co-application of dihydro-β-erythroidine (30μM).Inset voltage traces show corresponding epileptiform bursting activity. *C.* Histogram summarising 10-30 μM DMPP-induced burst frequency potentiation in 4AP and bicuculline models. *D.* Similar histogram showing normalised frequency potentiation. Note the relative burst frequency enhancement is greatest for bicuculline.

Having firmly demonstrated that nAChR activation has a modulatory influence on network oscillatory states and in agreement with previous studies (Williams & Kauer, 1997; Cobb *et al.*, 1999) the subsequent experiments performed and reported in this chapter were carried out to uncover possible mechanisms by which nAChR agonists activation may modulate hippocampal circuits and synapses.

5.2.2. Effect of nAChR agonists on field EPSPs.

Initial extracellular recording experiments were carried out to investigate the action of nAChRs on evoked glutamatergic transmission. All extracellular experiments were carried out in hippocampal slices prepared from 3-5 week old Wistar rats. Initial studies were made in an interface-recording chamber with recording electrodes (1-5MΩ, filled with standard aCSF) placed on the surface of the hippocampal slice in *stratum pyramidale* of the CA1 and CA3 regions. Bipolar stimulation electrodes were positioned within the *stratum radiatum* of the CA1 and in the *stratum lucidum* of the CA3 to activate Schaffer collaterals and mossy fibres respectively.

Field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 region in response to stimulation of the Schaffer collaterals and in the CA3 region in response to stimulation of the mossy fibres. Stimuli were applied every 15s and stimulation intensity adjusted to evoke a fEPSP that was 50% of the test fEPSP maximum. The fEPSP showed fast kinetics characteristic of glutamatergic

transmission. The measured slope in the CA1 and CA3 were –0.41±0.064mV/ms and –0.8±0.16mV/ms respectively. Addition of the selective AMPA and NMDA antagonists, NBQX (2μM) and CGP40116 (50μM) completely abolished the fEPSPs confirming their glutamatergic nature (Fig 5.2A).

Bath application of the selective nAChR agonist DMPP (30μM) for a period of 25-30 minutes resulted in a gradual but sustained and reversible enhancement of glutamate afferent evoked field EPSP slope at the mossy fibre-to-CA3 synapse of the hippocampus (30.84±5.98%, P=0.0078, Wilcoxon matched pairs test, *n*=8) but not at the Schaffer collateral-to-CA1 synapse (-16.99±7.04, P=0.125, Wilcoxon matched pairs test, *n*=5, Fig 5.2). These data suggest that glutamate transmission was significantly enhanced by nAChR activation at the mossy fibre-to-CA3 synapse but not the Schaffer collateral-to-CA1 synapse. As no GABA receptor antagonists were present throughout these experiments the small reduction noted in the fEPSP slope recorded at the Schaffer Collateral-to-CA1 synapse may be due to an enhancement of GABA transmission, which may in turn reduce the glutamatergic fEPSP.

Further extracellular experiments, conducted in a submerged-type recording chamber, using nicotine and choline, a selective agonist of nAChRs containing the α7 subunit (Papke *et al.*, 1996), were carried out to confirm the lack of potentiation at the Schaffer collateral-to-CA1 synapse. fEPSPs were recorded in the same manner as before. In order to ensure that the lack of nicotinic response

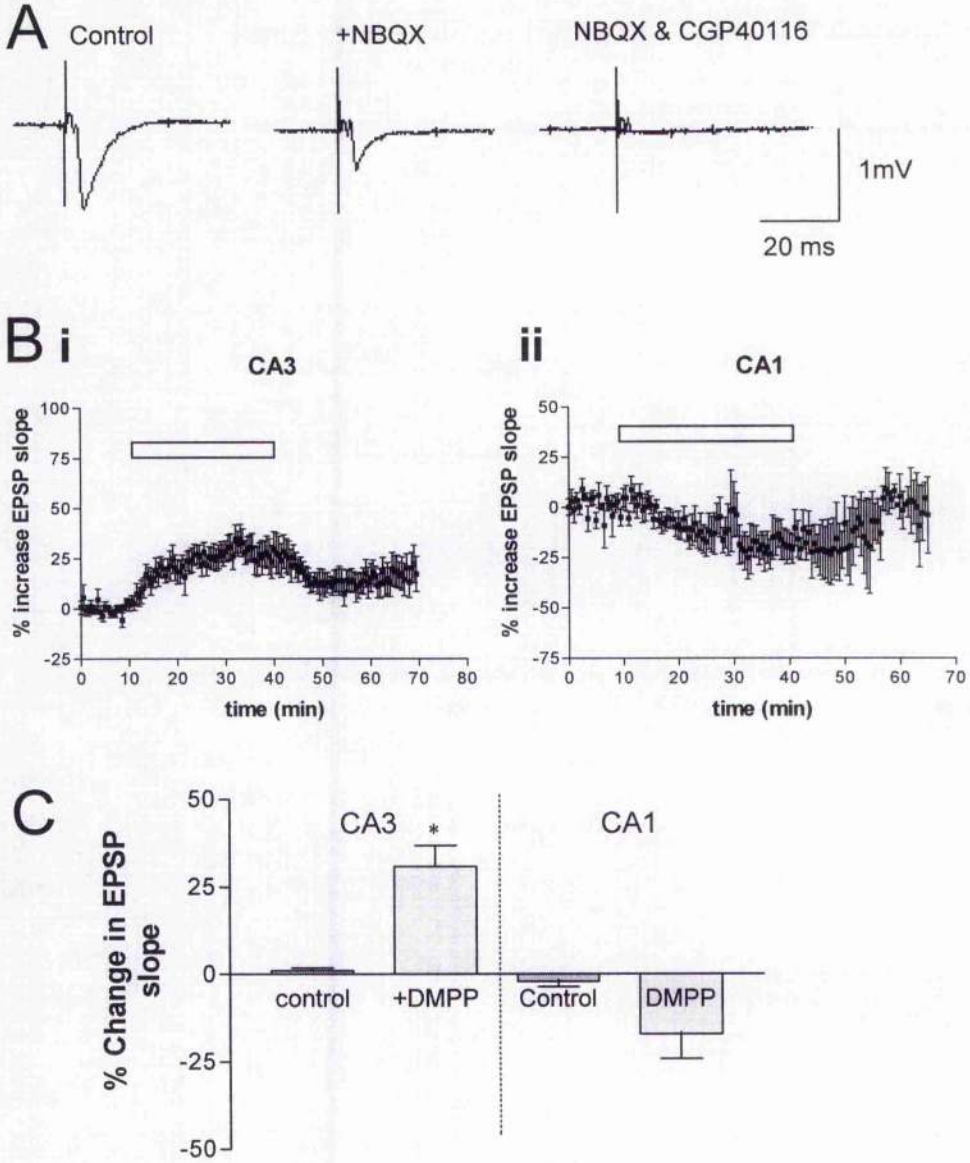


Figure 5.2. Effect of DMPP on field excitatory postsynaptic potentials (fEPSPs) evoked in the hippocampal slice preparation.

A. Representative voltage traces showing evoked fEPSP at the mossy fibre-to-CA3 synapse of the hippocampus, abolished by the addition of the AMPA and NMDA receptor antagonists NBQX (2μM) and CGP40116 (50μM) respectively Bi. Time-plot showing the evoked EPSP slope evoked at the MF-CA3 synapse upon application of the selective nicotinic agonist DMPP (30μM, horizontal bar, n=8) Each point is a mean of the mean fEPSPs recorded in 8 pyramidal neurones. Note the reversible increase in fEPSP slope in response to DMPP application. Bii Similar time-plot showing no consistent change of the evoked EPSP slope at the Schaffer collateral-to-CA1 synapse in response to DMPP application (n=5). C. Histogram summarising 30μM-induced change in fEPSP slope measured between 25-30 minutes. Wilcoxon-matched pair test indicated a significant increase in the CA3 region (P= 0.0078) but no significant change in the CA1 region (P=0.125).

was not due to desensitisation of the receptors that may be produced by a prolonged period of agonist application, a picospritzer injection system was instead employed so that pressure ejection of small amounts of the agonists could be made directly onto the area of study. The ejection electrode was placed close to the recording site (~50-100μm) and application of 3-6PSI for a 10msec duration ejected agonists directly onto the region of study. Agonists were applied prior to afferent stimulation every 15s for 150s (10 ejections separated by 15s intervals).

In initial control experiments aCSF was applied to the slice in the same manner to ensure that the injection protocol had no effect on the evoked responses. Picoinjection of aCSF consistently had no effect on the fEPSP slope (Fig 5.3Ai, n=8).

Four concentrations of nicotine (10μM, 20μM, 100μM and 1mM) and 1 concentration of choline (10mM) were used. The relatively high concentrations of agonists were selected, as with this method of agonist application in a submerged recording chamber a far smaller concentration of agonist will actually reach the tissue. No significant increase or decrease in the slope of the evoked fEPSPs was observed with any concentration of either agonist (Fig 5.3A-C). The fEPSP slope measured in the presence of 10μM, 20μM, 100μM and 1mM nicotine were 101.5±0.654% (n=4, P>0.05), 95.25±5.25% (n=4, P>0.05), 97.85±2.36% (n=4, P>0.05) and 93.83±2.173% (n=4, P>0.05) of the control fEPSP slope measured

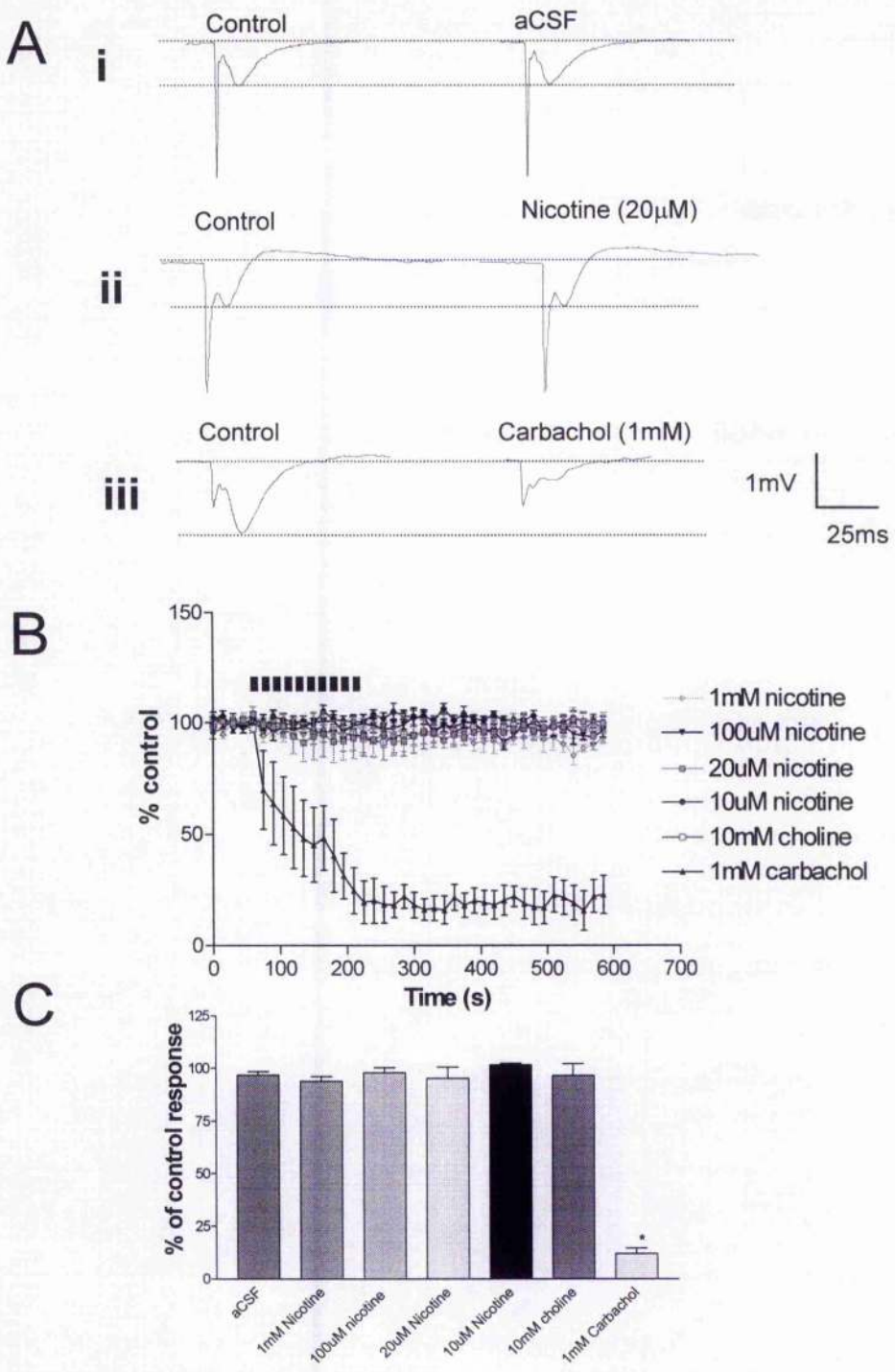


Figure 5.3 Effect of cholinergic ligands on fEPSPs in the CA1 region of the hippocampus.

A. Representative field recordings showing fEPSPs recorded in the CA1 *stratum radiatum* evoked every 15s by stimulation of Schaffer collaterals. i, aCSF picoinjected onto cells produced no change in fEPSP slope. ii, similar nicotine application produced no effect. iii, application of carbachol decreased fEPSP slope. B.Point plot of EPSP slope against time. EPSP slope is represented as a percentage of control (control value taken from initial 5 sweeps before drug application). Filled bars indicate period of drug application (picoinjection every 15s for 10 sweeps). C. Histogram summarising changes in EPSP slope. A one-way analysis of variants indicated that neither nicotine or choline produced a significant change of fEPSP. In contrast carbachol significantly decreased fEPSP slope to 11.88±2.66% of the control.

prior to drug application respectively. The field EPSP slope measured in the presence of 10mM choline was 96.38±5.736% (n=4, P>0.05).

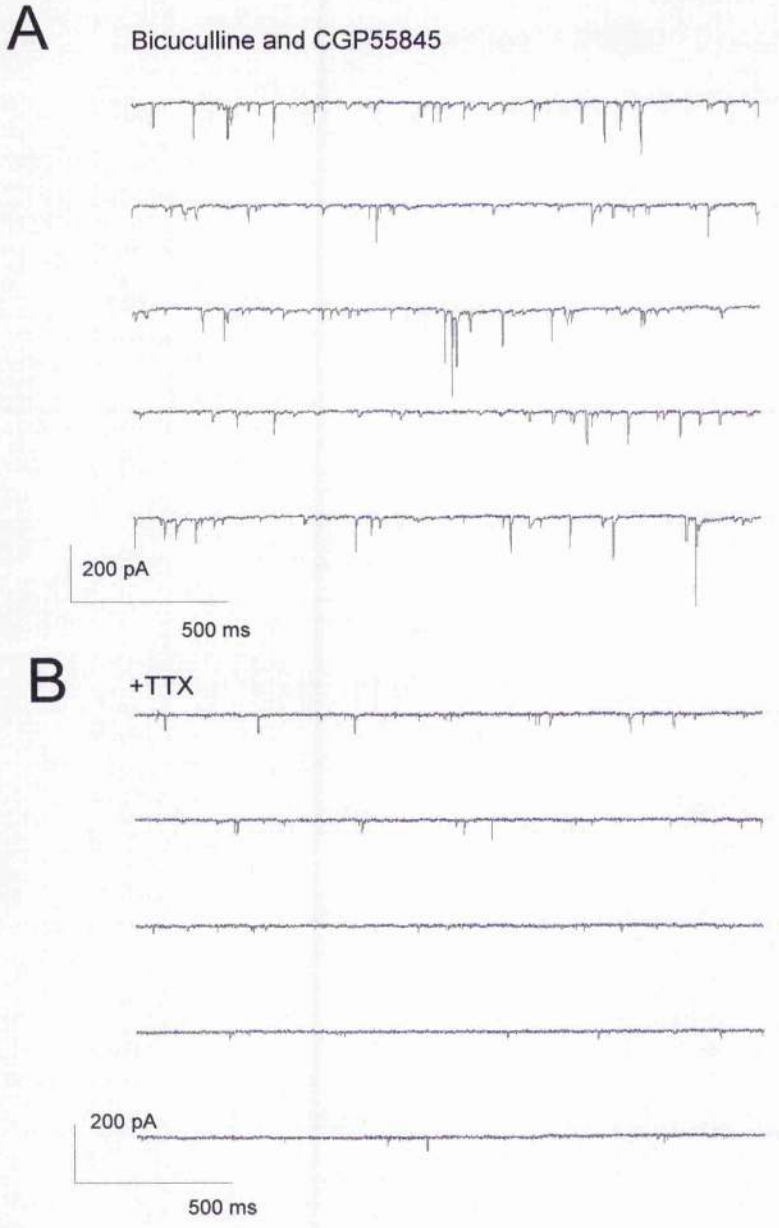
To check that the injection method was effective in the delivery of agonists, the acetylcholine analogue carbachol was applied in the same manner. Similar pressure ejection of carbachol (1mM) produced a rapid and significant (P<0.0001) reduction of the EPSC to 11.88±2.66% of the control slope measured prior to carbachol application (n=5, P<0.001%). This 88.22% reduction of the fEPSP is most likely due to activation of the mAChRs, which is widely reported to suppress evoked glutamatergic EPSPs at the Schaffer collateral-to-CA1 pyramidal neurone synapse (Rovira *et al.*, 1983; Hasselmo & Fehlau, 2001; Fernández de Sevilla *et al.*, 2002; Fernández de Sevilla & Buño, 2003) with a previous report by Qian & Saggau (1997) demonstrating an 85% inhibition of the fEPSP on application of carbachol mediated by mAChRs.

These results suggest that nAChR agonists have no effect on fEPSCs recorded at the Schaffer collateral-to-CA1 pyramidal neurone synapse and thus do not significantly alter glutamatergic transmission at this connection. Although an increase in GABA transmission at this synapse may have masked any effect on glutamate transmission, an increase in GABA transmission may account for the slight but not significant reduction in fEPSP slope in addition of DMPP.

5.2.3. Effects of DMPP on Mini EPSCs.

An increase in the fEPSP slope at the mossy fibre-to-CA3 synapse of the hippocampus indicated that glutamate transmission was enhanced in this region by nAChR agonists. In order to identify the location of the receptors involved in the enhancement of glutamate transmission we employed the whole-cell voltage clamp configuration using a CsCl based pipette solution, as described in chapter two, to record spontaneous miniature glutamatergic events on CA3 pyramidal neurones in the presence of GABA antagonists and the Na^+ channel blocker TTX.

Perfusion of the hippocampal slices with the $GABA_A$ receptor antagonist bicuculline (10μM) and the $GABA_B$ receptor antagonist CGP55845 (1μM) was used to block all GABA-receptor mediated events so that any spontaneous PSCs recorded in the CA3 pyramidal neurones were likely to be glutamatergic in nature (Fig. 5.4A). Spontaneous inward currents were recorded at a frequency and amplitude of 21.9±2.8Hz and 35.7±0.9pA respectively at a holding potential of –70mV (n=4). Subsequent perfusion of the hippocampal slices with the Na^+ channel blocker tetrodotoxin (TTX, 1μM, n=4)) allowed only miniature spontaneous EPSCs (mEPSC) to be recorded at a frequency and amplitude of 0.24±0.08Hz and 15.35±3.07pA respectively (Fig. 5.4B).



5.4 Spontaneous and miniature excitatory postsynaptic currents recorded from CA3 pyramidal neurones.

A. Shows a representative whole-cell voltage clamp recording from a CA3 pyramidal neurone. The $GABA_A$ and $GABA_B$ receptor antagonists bicuculline (10μM) and CGP55845 (1μM) were included in the perfusion medium to ensure that only spontaneous glutamatergic EPSCs were recorded. Spontaneous events occurred at a mean frequency and amplitude of 21.9±2.8Hz and 35.7±0.901pA respectively. B. On addition of the voltage operated Na^+ channel blocker TTX (1μM) all action potential dependant events were abolished leaving only miniature spontaneous EPSCs (mEPSCs). These mEPSCs occurred at a mean frequency and amplitude of 0.24±0.08Hz and 15.3±3.07pA respectively (n=4).

Under these conditions and after a period of control recording of 5-10mins, bath application of DMPP (30μM) for a period of 5 minutes resulted in a significant increase in mEPSC frequency from 0.24±0.08Hz to 0.78±0.13Hz (n=4, P=0.0326) but not amplitude from 15.35±3.07pA to 12.60±1.50pA (n=4, P=0.4738) in CA3 pyramidal neurones (Fig 5.5).

A similar experimental protocol showed 30μM DMPP to have no significant effect on either mEPSC frequency (1.52±0.7Hz to 1.06±0.34Hz, n=4, P=0.7840) or amplitude (14.11±2.72pA to 11.38±0.94Hz, n=4, P=0.1942) in CA3 interneurones recorded with the *stratum oriens* and *stratum radiatum* (Fig 5.6).

A similar protocol was again employed to test the effect of DMPP on mEPSCs from both CA1 pyramidal neurones and interneurones. DMPP had no significant effect on the frequency (0.44±0.16Hz to 0.58±0.39Hz, n=4, P=0.5808) or amplitude (9.03±0.58pA to 7.99±0.19Hz, n=4, P=0.1580) of mEPSCs recorded in CA1 pyramidal neurones (Fig 5.7) and had no significant effect on frequency (0.85±0.26Hz to 1.1±0.35Hz, n=4, P=0.0961) or amplitude (10.37±1.16pA to 11.52±1.41pA, n=4, P=0.4707) of mEPSCs recorded in CA1 interneurones (Fig 5.8). This lack of effect on mEPSC in CA1 region was consistent with the earlier data showing no effect of DMPP on the fEPSP in this region.

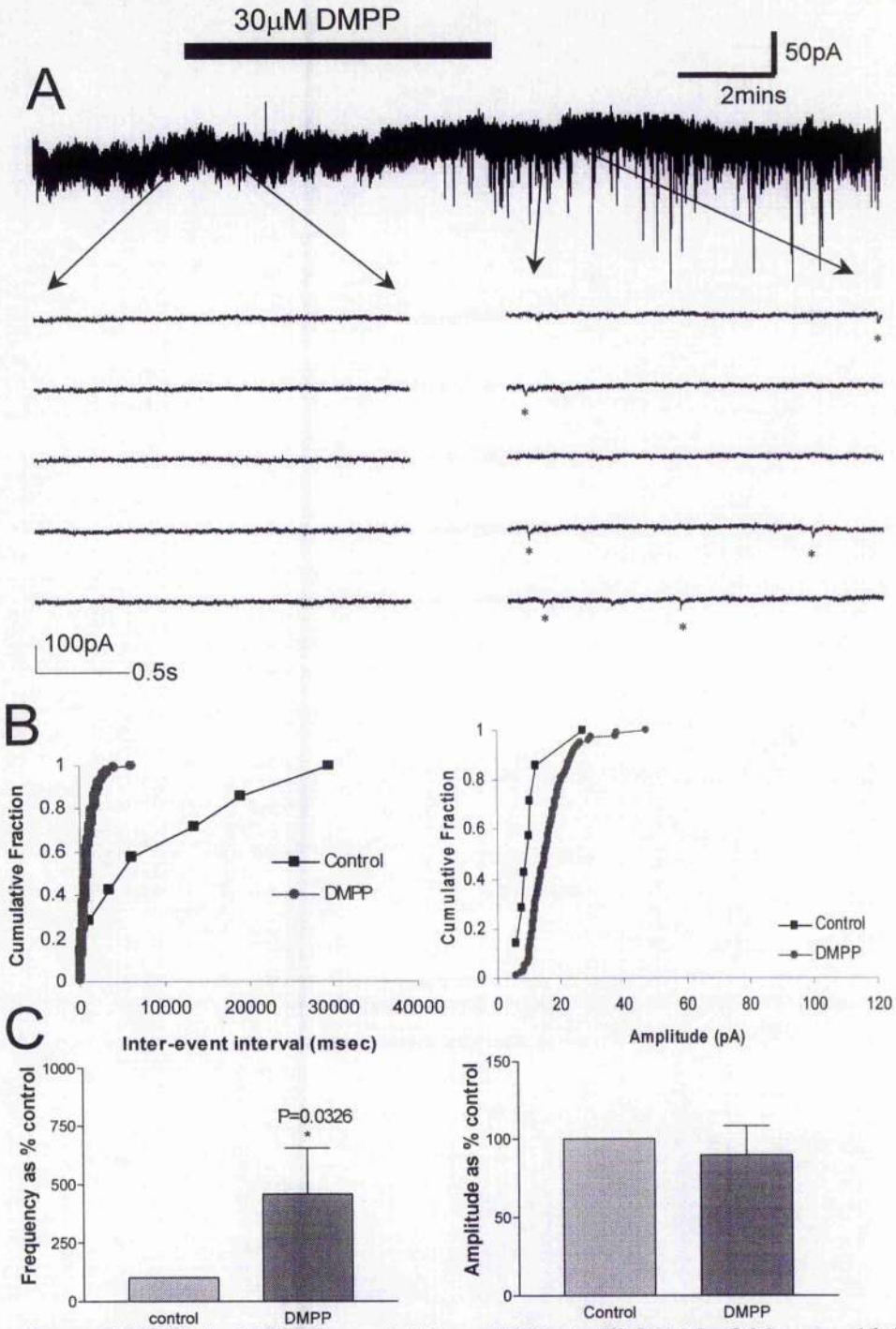


Figure 5.5 Effect of DMPP on miniature EPSCs (mEPSCs) in CA3 pyramidal neurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mEPSCs in a CA3 pyramidal neurone. The neurone was clamped at –70mV in aCSF with bicuculline (10μM), CGP55845 (1μM) and TTX (1μM). Note the increase in frequency of mEPSCs (inward currents indicated by ∗) in response to 30μM DMPP application. The delay in onset of the appearance of mEPSCs reflects the time taken for the drug to reach the bath and equilibrate therein. B. Representative cumulative inter-event interval and amplitude distributions created from mEPSCs before (*filled squares*) and during (*filled circles*) 30 μM DMPP application. Kolmogorov-smirnov tests indicated a significant increase in frequency but not amplitude distributions. C. Histograms summarising the effect of DMPP on mean mEPSC frequency and amplitude (n=4) expressed as a percentage of control. Note the 300% increase in the frequency of mEPSCs. A paired t-test performed on raw data indicated a significant increase mEPSC frequency (P=0.0326) but not amplitude (P=0.474).

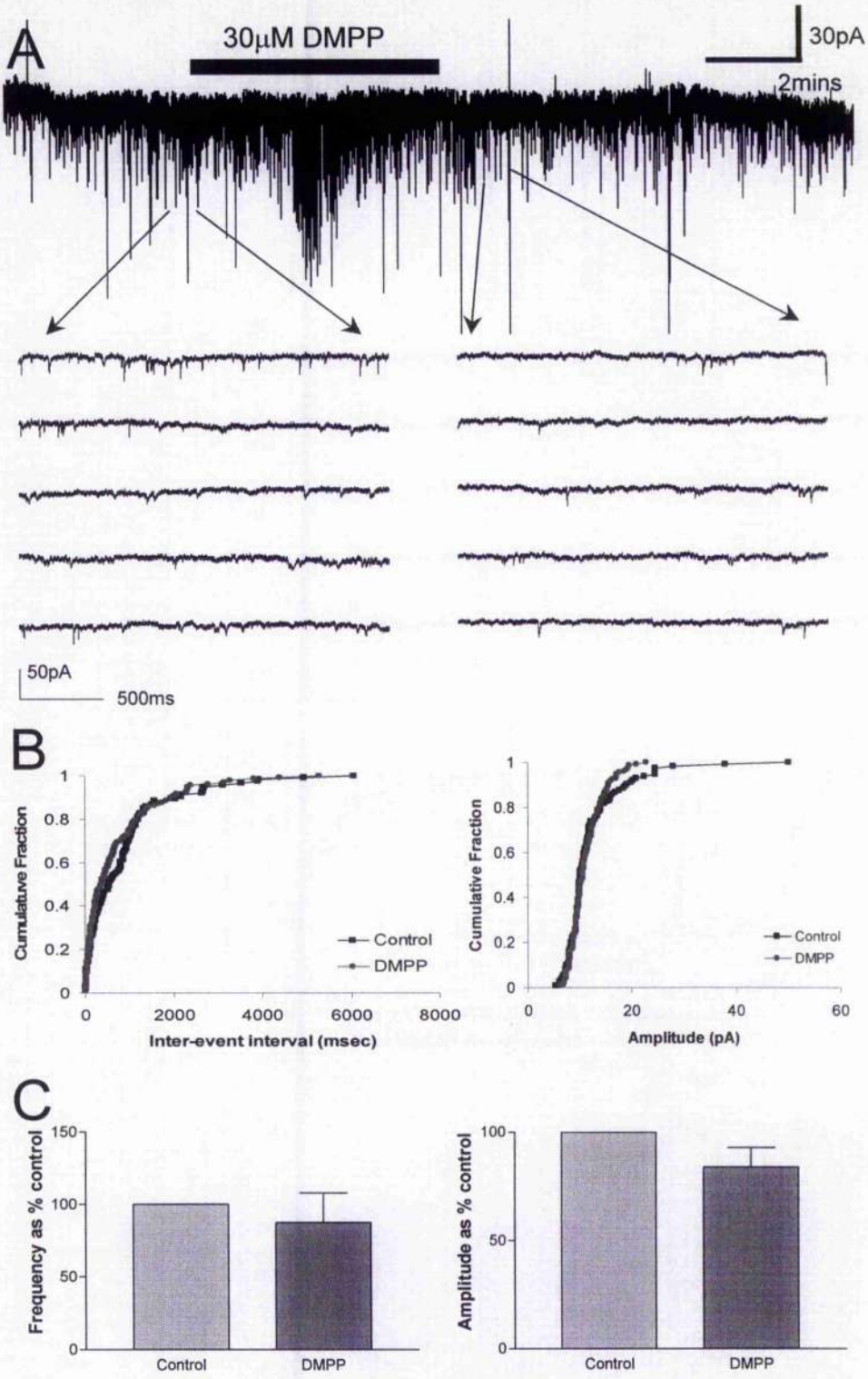


Figure 5.6 Effect of DMPP on mEPSCs recorded from CA3 interneurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mEPSCs recorded in a CA3 *stratum oriens* interneurone.The neurone was clamped at –70mV in aCSF with bicuculline (10μM), CGP55845 (1μM) and TTX (1μM). B. Representative cumulative inter-event interval distributions and amplitude distributions created from mEPSCs before *(filled squares)* and during *(filled circles)* 30μM DMPP application. The Kolmogorov-Smirnov test indicated no significant difference in cumulative distributions. C. Histograms summarising the effect of 30μM DMPP on mean mEPSC frequency and amplitude (n=4) expressed as a percentage of the mean control value. A paired t-test on raw data indicated no significant change in either mean frequency (P=0.3781) or amplitude (p=0.2311) following DMPP application.

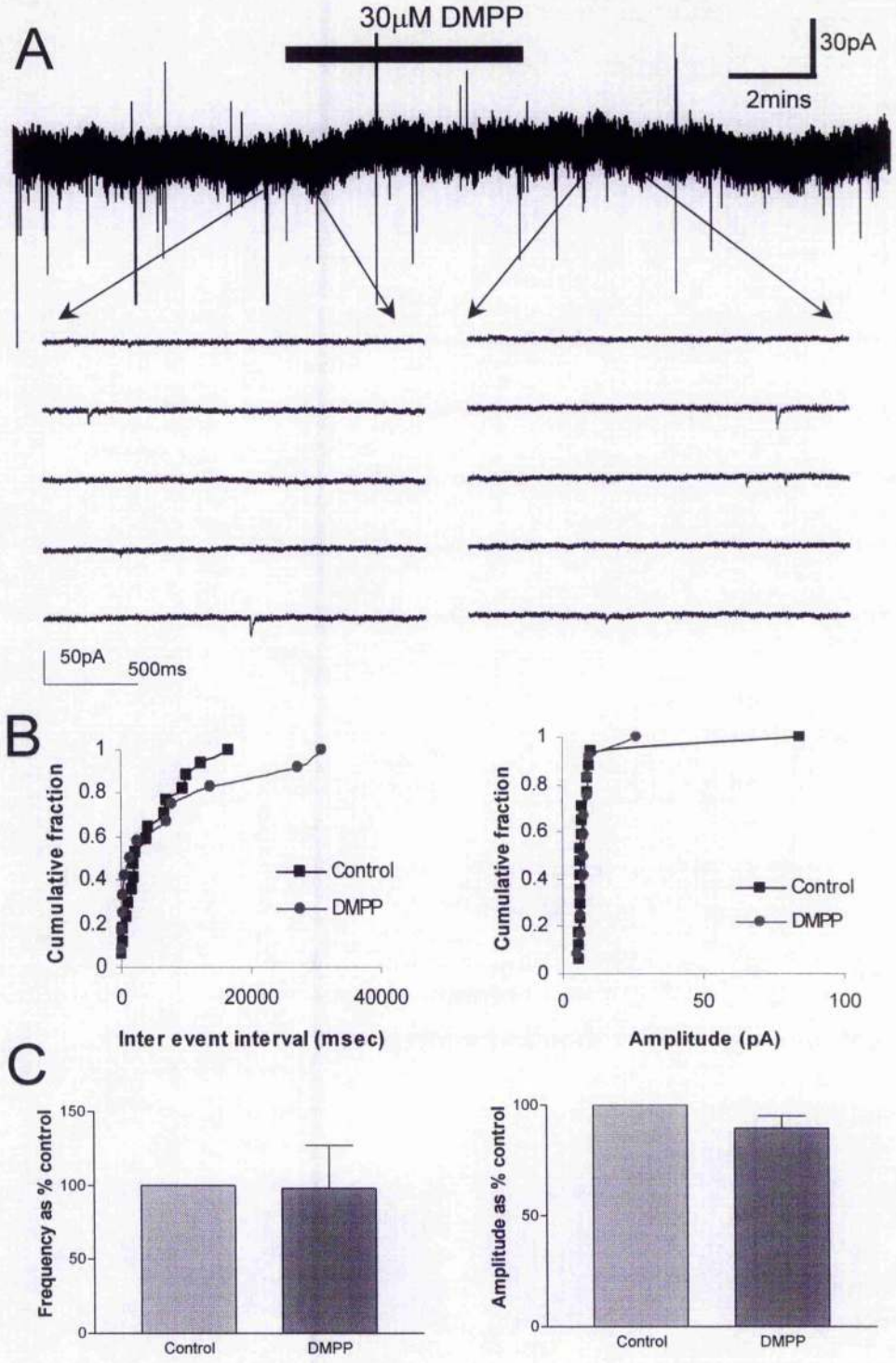


Figure 5.7 Effect of DMPP on mEPSCs recorded in CA1 pyramidal neurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mEPSCs recorded from a CA1 pyramidal neurone. The neurone was clamped at –70mV in aCSF, bicuculline (10μM), CGP55845 (1μM) and TTX (1μM). B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from mEPSCs before *(filled squares)* and during *(filled circles)* 30μM DMPP application. A Kolmogorov-Smirnov test indicated no significant difference between cumulative distributions. C. Histograms summarising the effect of 30μM DMPP on mean mEPSC frequency and amplitude (n=4) expressed as a percentage of the mean control value. A paired t-test performed on raw data indicated no significant change in either frequency (P=0.5808) or amplitude (p=0.1580) following DMPP application.

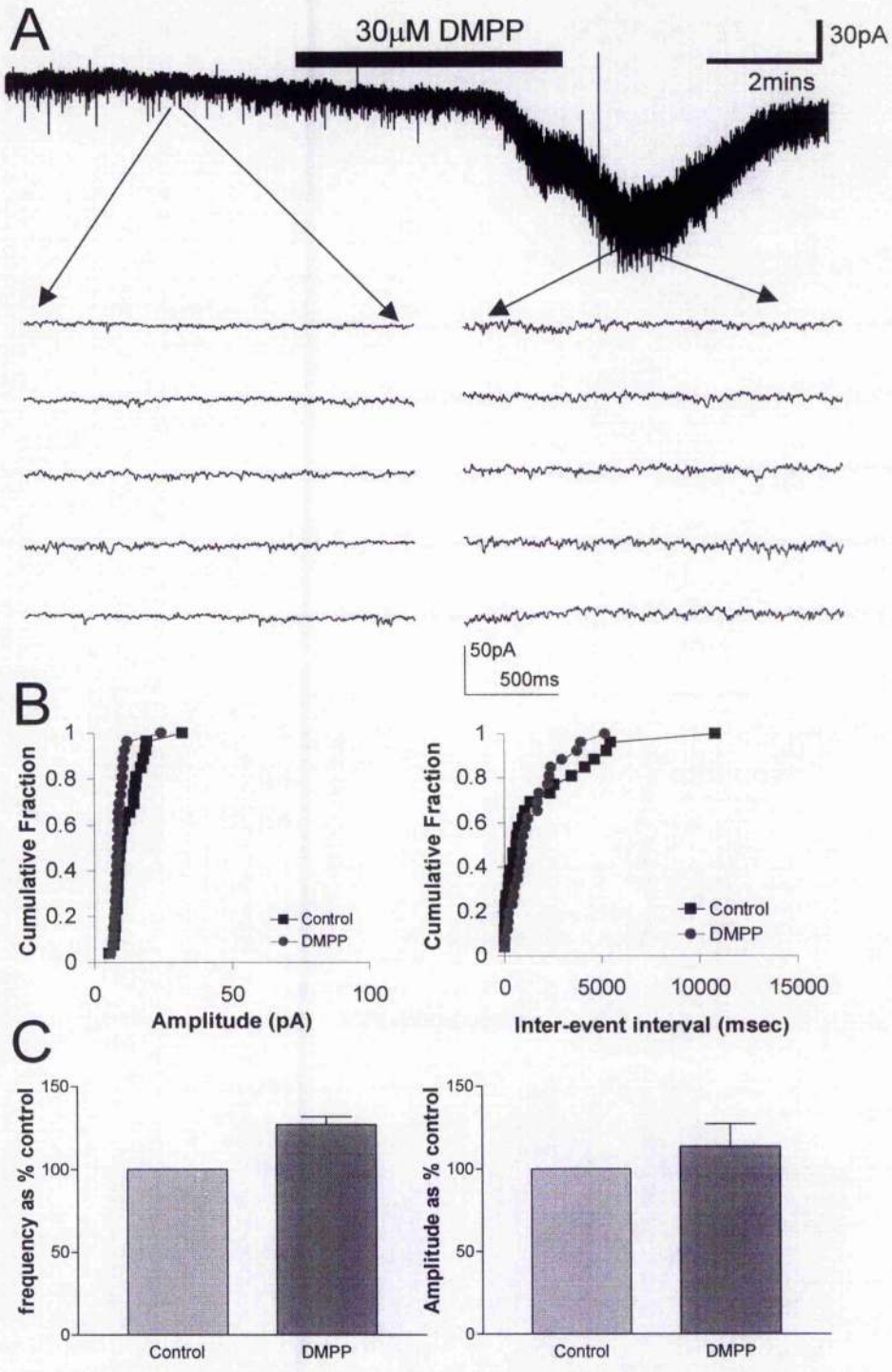


Figure 5.8 Effect of DMPP on mEPSCs recorded in CA1 Interneurones

A. Representative whole-cell voltage clamp recordings trace and expanded raster plots illustrating mEPSCs in a CA1 *stratum oriens* interneurone. The neurones was clamped at –70mV in aCSF with bicuculline (10μM), CGP55845 (1μM) and TTX (1μM). B Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from mEPSCs before *(filled squares)* and during *(filled circles)* 30μM DMPP application. The Kolmogorov-Smirnov test indicated no significant difference between cumulative distributions. C. Histograms summarising the effect of 30μM DMPP on mean mEPSC frequency and amplitude (n=4) expressed as a percentage of the mean control value. A paired t-test indicated no significant change in either mEPSC frequency (P=0.0961) or amplitude (p=0.4707) following DMPP application.

However, DMPP did produce an inward current (mean amplitude of 70.67±31.21pA) in 3 out of 4 CA1 interneurones all recorded within the *stratum oriens*. This inward current was activated in the presence of TTX suggesting a direct activation of nAChR present on the interneurone surface.

5.2.4. Effects of DMPP on spontaneous GABA transmission

As application of DMPP produced a slight but not significant decrease in the fEPSP evoked at the Schaffer collateral-to-CA1 synapse and produced inward currents in CA1 interneurones it seemed likely that although DMPP may have no effect on glutamatergic transmission within this region, it may be able to modulate GABA transmission.

To investigate the effect of nAChR agonists on GABA transmission within area CA1, we next investigated the action of DMPP (30μM) on spontaneous IPSC events recorded in both CA1 pyramidal neurones and CA1 interneurones.

CA1 pyramidal neurones were recorded under the whole-cell voltage clamp configuration and in the presence of the AMPA receptor antagonist NBQX (2μM) and the NMDA receptor antagonist CGP40116 (50μM). In a period of control recording of 5-10mins spontaneous IPSCs (sIPSC) were recorded with an average frequency and amplitude of 5.04±2.0Hz and 29.26±5.98pA respectively. These values were consistent with sIPSCs recorded in CA1 pyramidal neurones in

previous studies (Hájos & Mody, 1997). Subsequent bath application of DMPP (30μM) had no significant effect on sIPSC frequency (5.1±1.86Hz, P=0.8292, n=4) or amplitude (29.42±8.4pA, P=0.9565, n=4) (Fig 5.9).

A similar protocol was carried out for recording from CA1 interneurones with cell bodies in either the *stratum oriens* or *stratum radiatum*. sIPSCs were recorded at an average frequency and amplitude of 7.48±1.93Hz and 20.42±2.47pA respectively. Bath application of 30μM DMPP resulted in a significant increase in frequency to 13.05±2.8Hz (P=0.0403, n=6) but had no significant effect on the mean amplitude (23.41±3.04, P=0.0836, n=6) (Fig. 5.10), although in some individual experiments a significant increase in amplitude was noted.

In CA1 interneurones DMPP also elicited an inward current (mean amplitude 131.7±63.13pA) in 3 of 6 (50%) interneurones recorded (Fig 5.10 A). Two of the interneurones responding with an inward current showed cell body location in the *stratum radiatum* and the other showed cell body location in the *stratum oriens*. No such inward currents were apparent in any of the CA1 pyramidal neurone recordings (n=4).

These changes in sIPSC frequency indicate that nAChR activation results in an increase of GABA release onto CA1 interneurones but not CA1 pyramidal neurones. In addition activation of nAChRs can produce an inward current in a subset of CA1 interneurones.

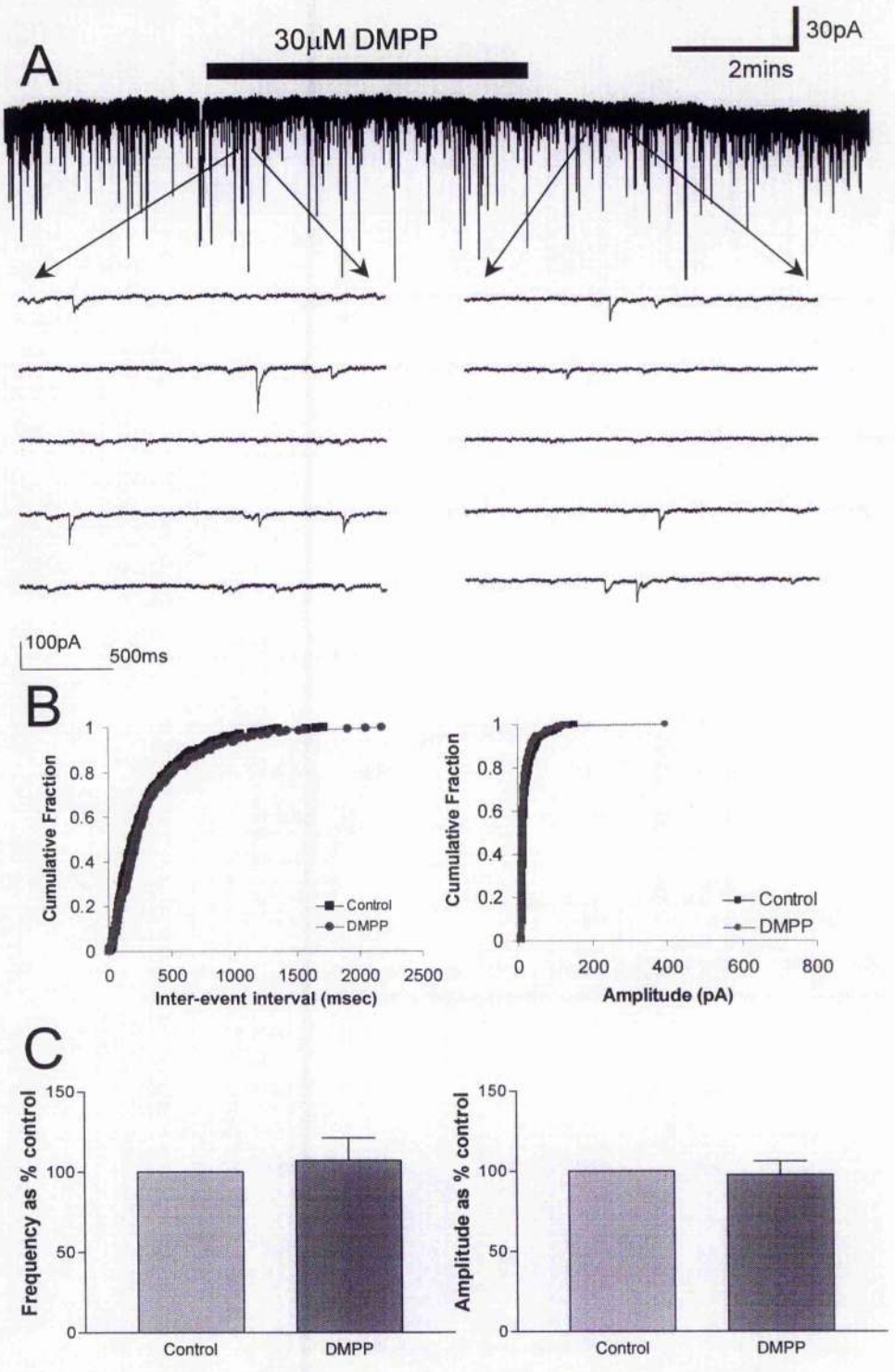


Figure 5.9. Effect of DMPP on spontaneous IPSCs (sIPSCs) in CA1 Pyramidal neurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating sIPSCs from a CA1 pyramidal cell. The neurone was clamped at –70mV in aCSF with NBQX (2μM) and CGP40116 (50μM) B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from sIPSCs before *(filled squares)* and during *(filled circles)* 30μM DMPP application. K-S test indicated no significant difference between cumulative distributions. C. Histograms summarising the effect of 30μM DMPP on mean sIPSC frequency and amplitude (n=4). A paired t-test performed on the raw data indicated a no significant change in sIPSC frequency (P=0.8292) or IPSC amplitude (P=0.9565) following DMPP application.

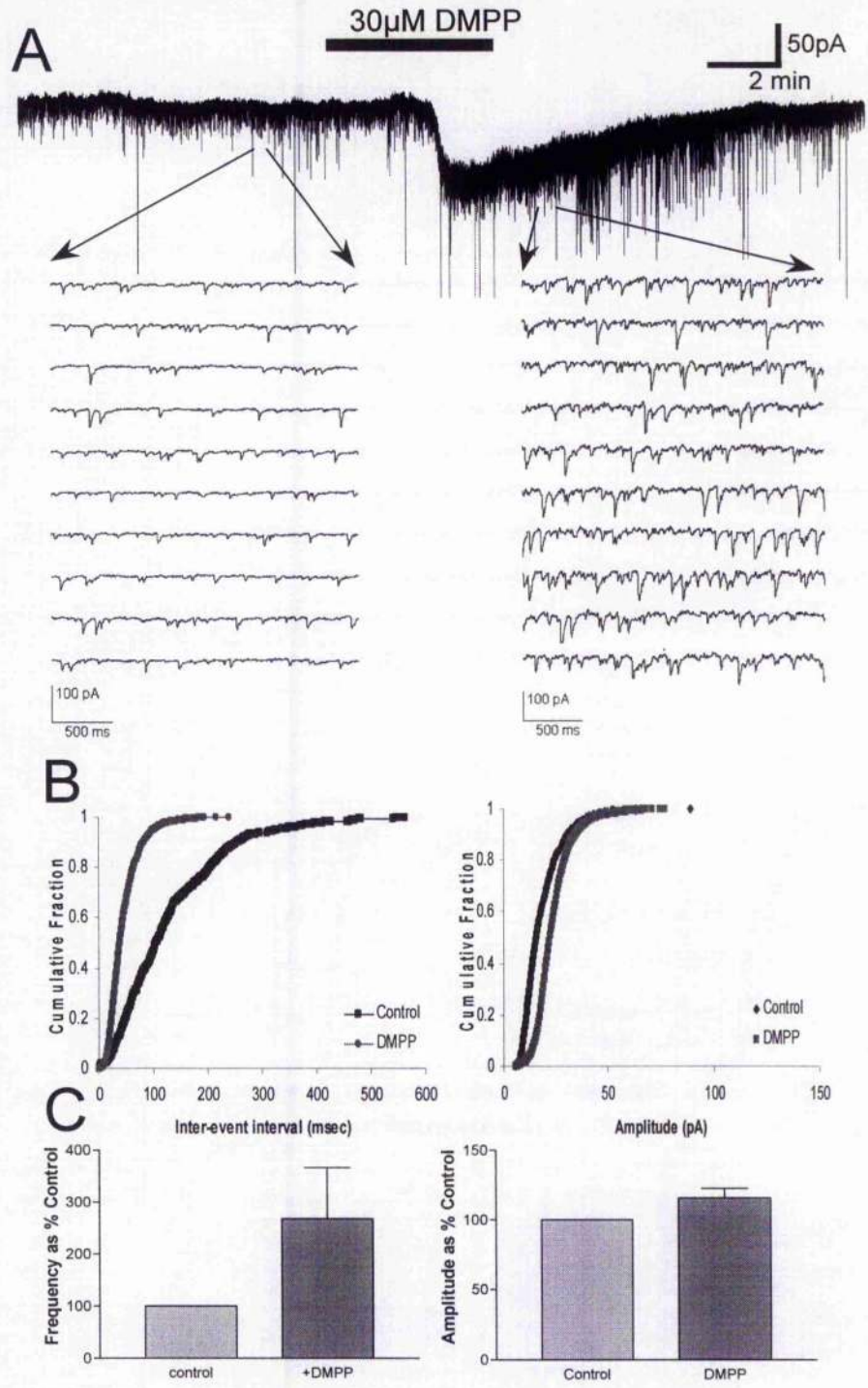


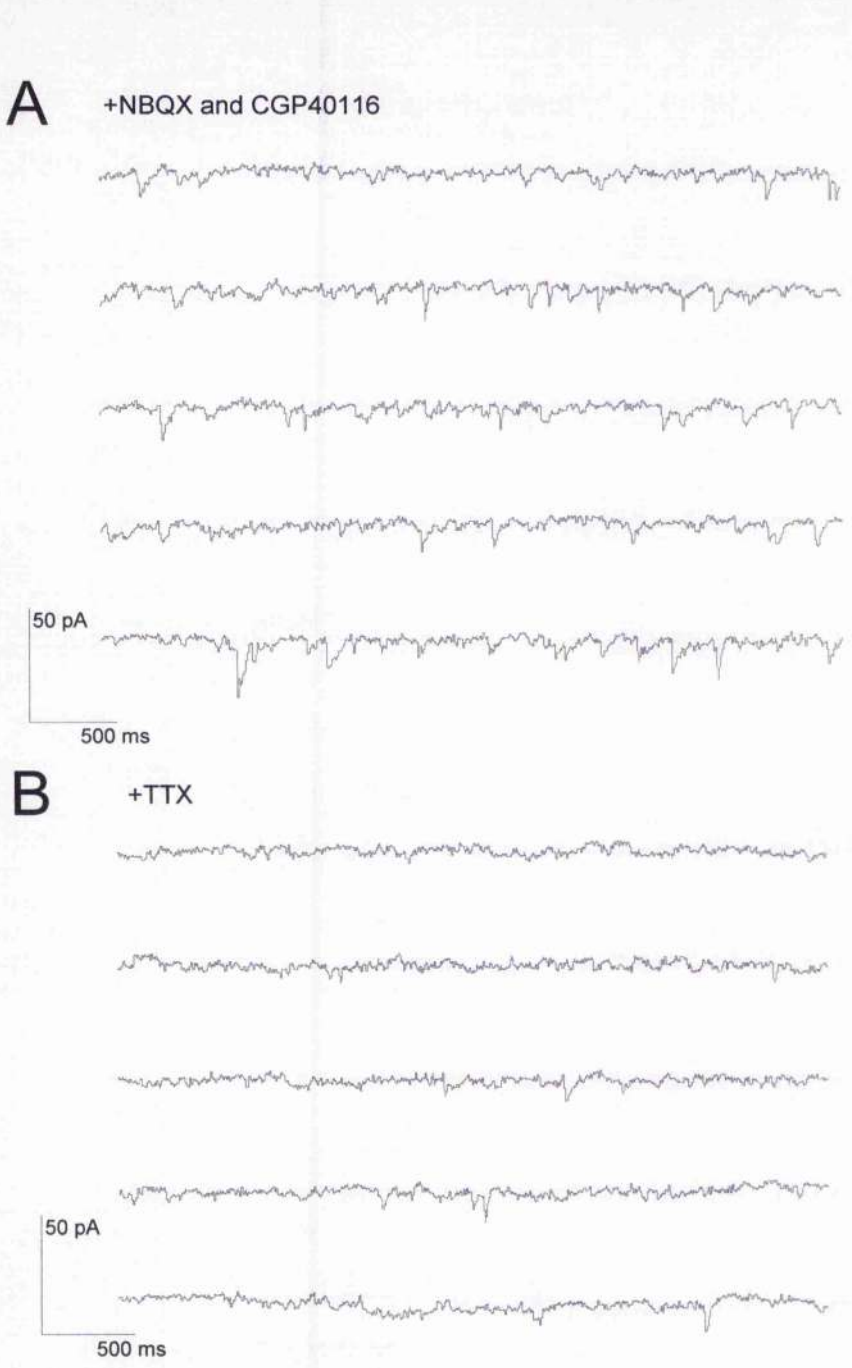
Figure 5.10. Effect of DMPP on sIPSCs in CA1 interneurones

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating sIPSCs from a CA1 *stratum oriens* interneurone. Neurone was clamped at –70mV in aCSF with NBQX (2μM) and CGP40116 (50μM). Note the inward current and increase in mEPSC frequency in response to DMPP application B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from sIPSCs before *(filled squares)* and during *(filled circles)* 30μM DMPP application. K-S tests indicated a significant increase in both frequency and amplitude in this example. C. Histograms summarising the effect of DMPP on mean sIPSC frequency and amplitude (n=7). A paired t-test performed on the raw data indicated a significant increase in sIPSC frequency (P=0.0403) and no change in IPSC amplitude (P=0.0836) following DMPP application.

5.2.5. Effect of DMPP on mini IPSCs

In order to investigate whether this increase in GABAergic transmission was due to pre- or postsynaptic mechanisms, studies were carried out on mIPSCs in this region. In studies to investigate the effect of nAChR activation on miniature IPSCs (mIPSCs) slices were bathed in the AMPA receptor antagonist NBQX (2μM), the NMDA receptor antagonist CGP40116 (50μM) and the Na^+ channel blocker TTX (1μM) to eliminate all fast glutamate events and action potential dependent IPSCs so that mIPSCs could be uncovered (Fig 5.11).

Recordings from visually identified interneurones within the *stratum oriens* and *stratum radiatum* of area CA1 revealed mIPSCs with a mean frequency and amplitude of 0.46±0.0914Hz and 20.18±2.83pA respectively (n=4). Subsequent bath application of 30μM DMPP had no significant effect on either frequency (0.504±0.086Hz, P=0.395, n=4) or amplitude (20.87±2.09, P=0.6994, n=4) of mIPSCs (Fig 5.12). As before DMPP resulted in an inward current in 2 out of 4 (50%) of CA1 interneurones (mean amplitude 56.0±6.0pA). As inward currents produced on DMPP application were apparent even in the presence of TTX (Fig 5.12A), these data suggest a direct action of nAChR activation on a subpopulation of CA1 interneurones. Thus it seems that the observed increase of sIPSC onto CA1 interneurones is most likely due to a direct activation of somatodendritic or preterminal nAChRs increasing the action potential dependent increase in GABA release and not a presynaptic modulatory effect.



5.11 Spontaneous and miniature inhibitory postsynaptic currents recorded from CA1 interneurones

A. Shows a representative voltage clamp recording from a CA1 interneurone. The AMPA and NMDA receptor antagonists NBQX (1μM) and CGP40116 (50μM) were included in the perfusion medium to ensure that only spontaneous GABAergic IPSCs were recorded. These spontaneous events were recorded at a frequency and amplitude of 7.4Hz and 20.42pA respectively (n=6). B. On addition of the voltage operated Na^+ channel blocker TTX all action potential dependant events were abolished and only miniature spontaneous IPSCs (mIPSCs) were recorded. These mIPSCs were recorded at a frequency and amplitude of 0.46Hz and 20.18pA respectively (n=4).

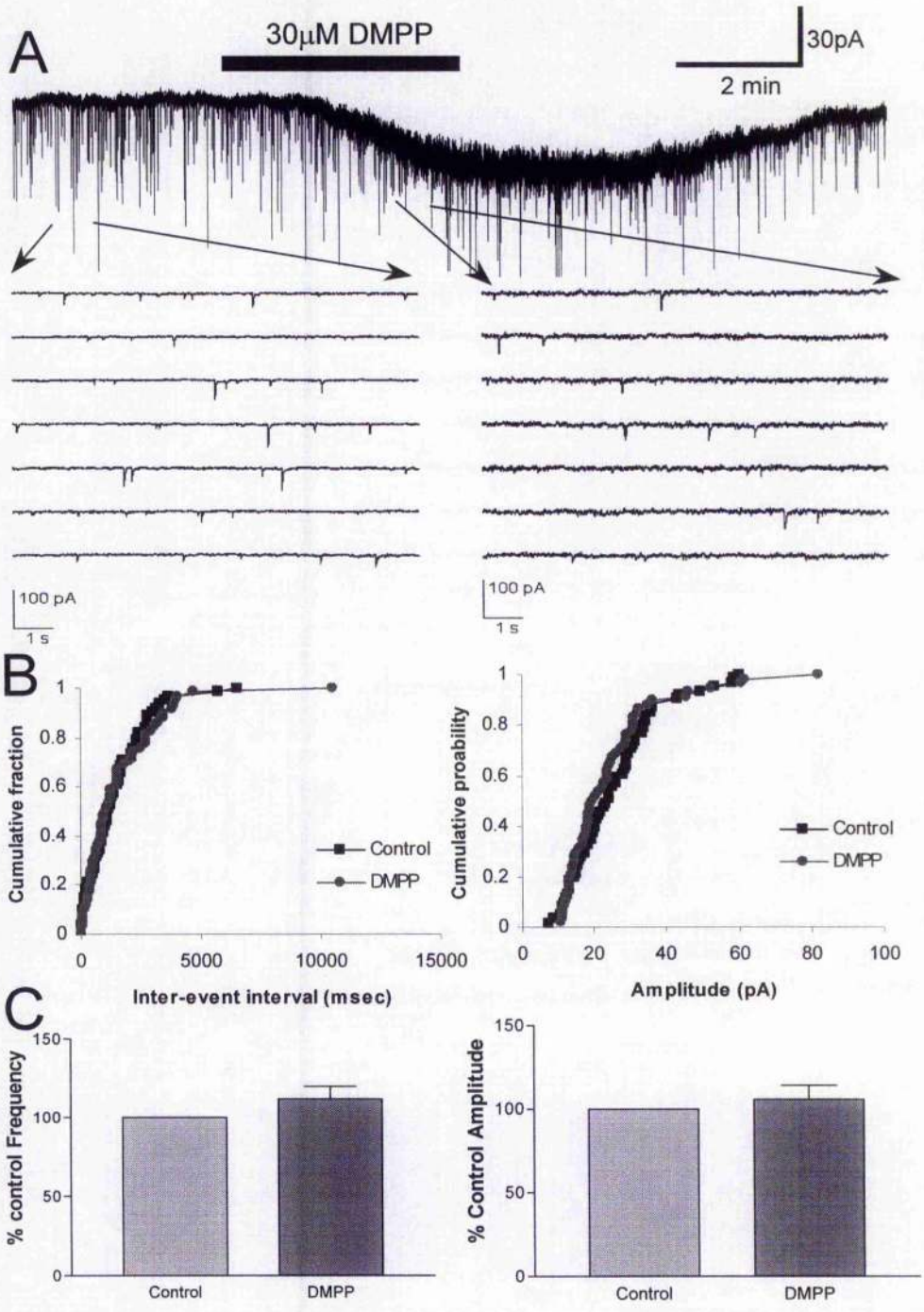


Figure 5.12. Effect of DMPP on miniature IPSCs (mIPSCs) recorded in CA1 interneurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mIPSCs in a CA1 *stratum oriens* interneurone. The neurone was clamped at –70mV in aCSF with NBQX (2μM), CGP40116 (50μM) and TTX (1μM). Note the inward current evoked by 30μM DMPP application. B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from mIPSCs before (*filled squares*) and during (*filled circles)* 30μM DMPP application. A K-S test indicated no significant difference in cumulative distributions following DMPP application. C. Histograms summarising the effect of 30μM DMPP on mean mIPSC frequency and amplitude (n=4) expressed as a percentage of the mean control value. A paired t-test indicated a no significant difference in IPSC frequency (P=0.3695) and mIPSC amplitude (P=0.6994) following DMPP application.

A similar protocol was adopted to investigate whether nAChR activation affects mIPSCs recorded in CA1 pyramidal neurones (fig. 5.13). During a 5-10min control period mIPSCs were recorded at a frequency and amplitude of 2.8±2.2Hz and 28.42±4.2pA respectively. Bath application of DMPP had no effect on the frequency (1.99±1.4Hz, P=0.578, n=4) or amplitude (28.59±2.9pA, P=0.979, n=4). Again no inward currents were apparent in any of the CA1 pyramidal neurone recordings (n=4).

A similar protocol was again employed to test the effects of DMPP on mIPSCs recorded from both CA3 pyramidal neurones and CA3 interneurones. DMPP had no significant effect on mIPSC frequency (0.5±0.09Hz to 0.6±0.15Hz, P=0.425, n=4) recorded in CA3 pyramidal neurones (fig. 5.14) and had no effect on mIPSC frequency (0.83±0.34Hz to 0.95±0.44Hz, P=0.368, n=4) or amplitude (27.28±4.9pA to 22.21±2.65pA, P=0.199, n=4) in CA3 interneurones (fig. 5.15).

The overall effect of DMPP on spontaneous and miniature EPSCs and IPSCs are summarised in table 5.1 and the most likely location of nAChRs indicated by these results are summarised in figure 5.16.

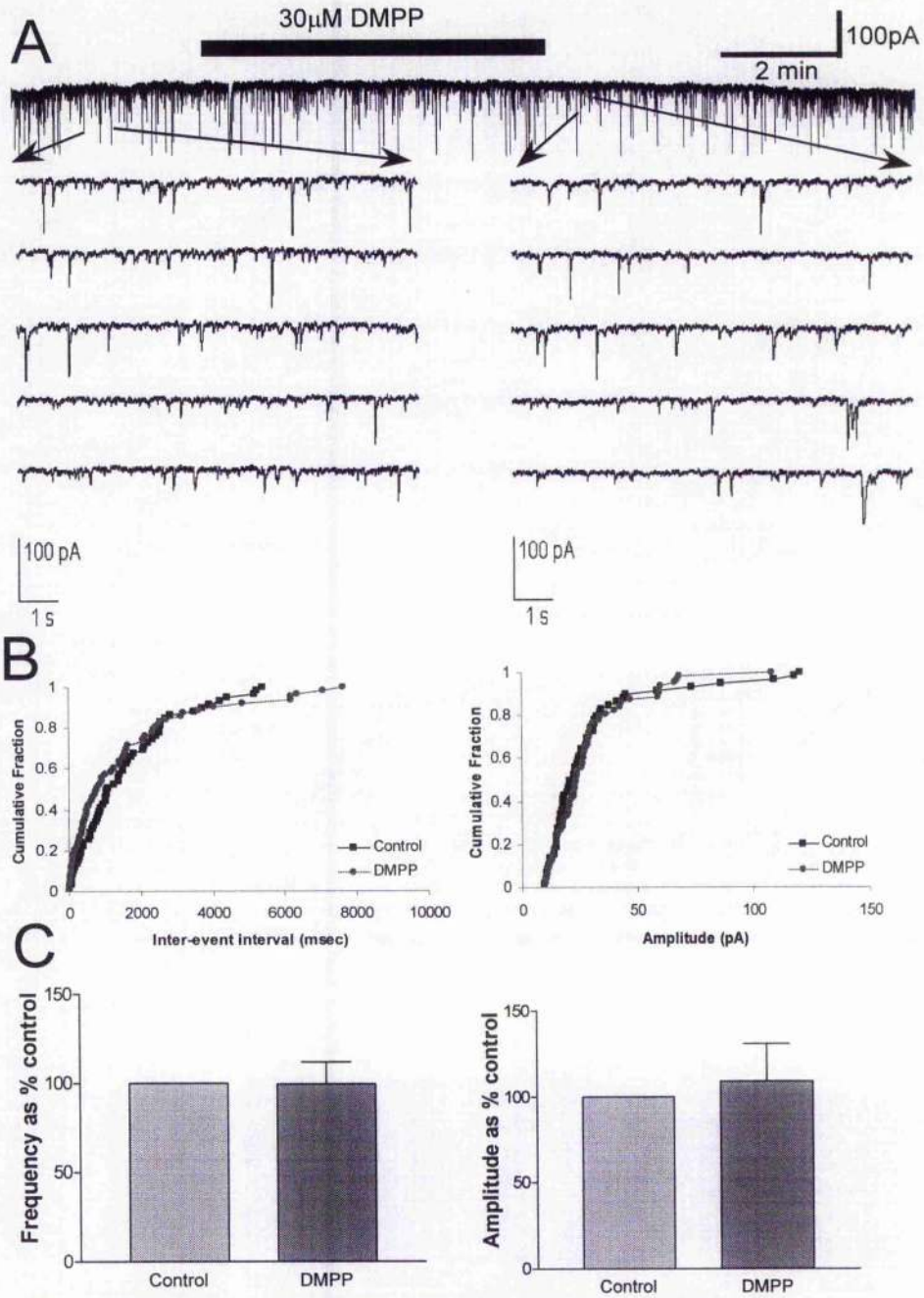


Figure 5.13 Effect of DMPP on mIPSCs recorded in CA1 pyramidal neurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mIPSCs recorded in a CA1 pyramidal neurone. Neurones clamped at –70mV in aCSF with NBQX (2μM) and CGP40116 (50μM) and TTX (1μM) B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from mIPSCs before *(filled squares)* and during *(filled circles)* DMPP application. K-S tests indicated no significant difference in cumulative distributions. C. Histograms summarising the effect of DMPP on mean mIPSC frequency and amplitude (n=4). A paired t-test indicated a no change in IPSC frequency (P=0.5783) and no change in mIPSC amplitude (P=0.9785) following DMPP application.

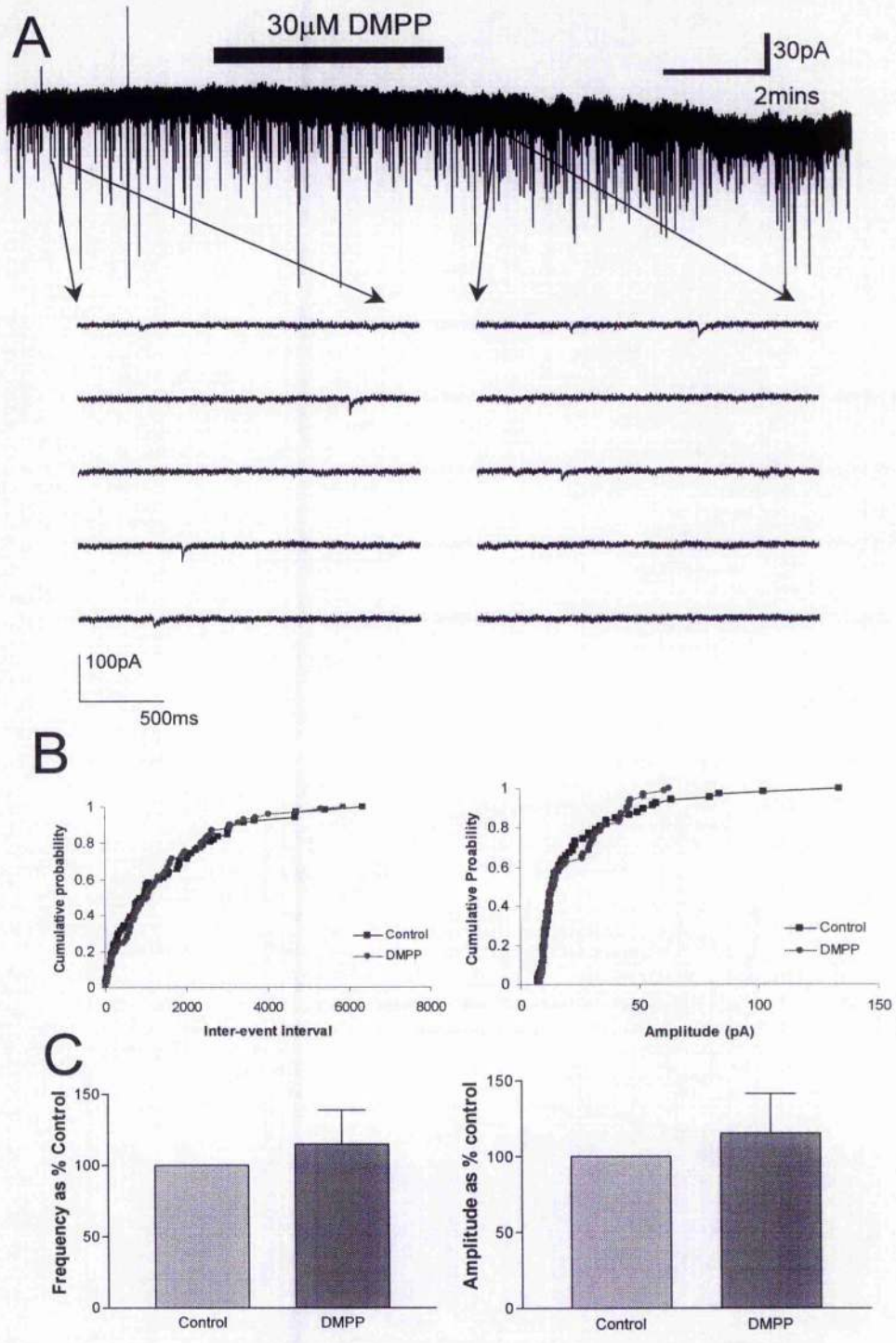


Figure 5.14 Effect of DMPP on mIPSCs recorded in CA3 interneurones

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mIPSCs recorded in a CA3 *stratum oriens* interneurone. Neurone clamped at –70mV in aCSF with NBQX (2μM) and CGP40116 (50μM) and TTX (1μM) B. Representative cumulative inter-event interval distributions and cumulative amplitude distributions created from mIPSCs before (*filled squares*) and during (*filled circles*) DMPP application. C. Histograms summarising the effect of DMPP on mean mIPSC frequency and amplitude (n=4) expressed as a percentage of the mean control value. A paired t-test indicated a no change in IPSC frequency (P=0.4248) and no change in mIPSC amplitude (P=0.5837) following DMPP application.

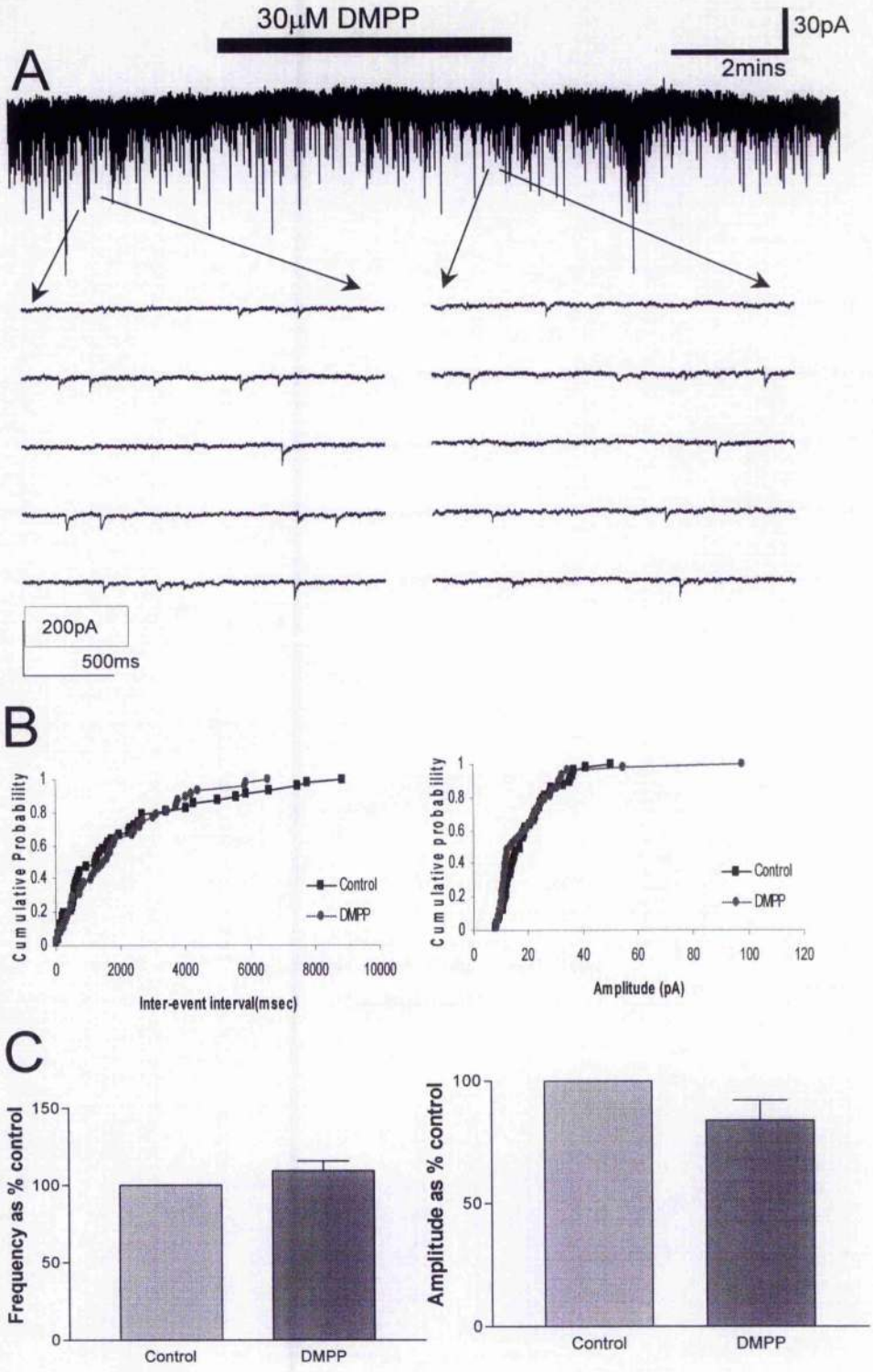


Figure 5.15 Effect of DMPP on mIPSCs recorded from CA3 pyramidal neurones.

A. Representative whole-cell voltage clamp recording trace and expanded raster plots illustrating mIPSCs recorded in a CA3 pyramidal neurone clamped at –70mV in aCSF with NBQX (2μM) and CGP40116 (50μM) and TTX (1μM). B. Representative cumulative inter-event interval distributions created from mIPSCs before *(filled squares)* and during *(filled circles)* DMPP application. K-S tests indicated no significant difference in cumulative distributions. C. Histograms summarising the effect of DMPP on mean mIPSC frequency and amplitude (n=4)expressed as a percentage of the mean control value. A paired t-test indicated a no change in IPSC frequency (P=0.3680) and no change in mIPSC amplitude (P=0.1989) following DMPP application.

Cell Type	MEPSC		sIPSC		MIPSC		Inward current
	Freq	*Amp*	*Freq*	*Amp*	*Freq*	*Amp*	
CA3 pyramidal neurones	**increase**	No change	N/A	N/A	No change	No change	No
CA3 interneurones	No change	No change	N/A	N/A	No change	No change	No
CA1 pyramidal neurones	No change	No change	No change	No change	No change	No change	No
CA1 interneurones	No change	No change	**increase**	No change?	No change	No change	**Yes**

Table 5.1 Summary of the effect of DMPP on spontaneous and miniature currents.

Table summarises the effect of DMPP on glutamatergic and GABAergic transmission in the hippocampus. DMPP increased the frequency but not amplitude of miniature EPSCs (mEPSCs) in CA3 pyramidal neurones but had no effect on mEPSCs recorded in CA3 interneurones, CA1 pyramidal neurones and CA1 interneurones. DMPP increased the frequency of spontaneous IPSCs (sIPSCs) recorded in CA1 interneurones but not CA1 pyramidal neurones, CA3 interneurones or CA3 interneurones. DMPP had no effect on miniature IPSCs (mIPSCs) recorded in any of the aforementioned cell types and consistently produced an inward current in CA1 interneurones.

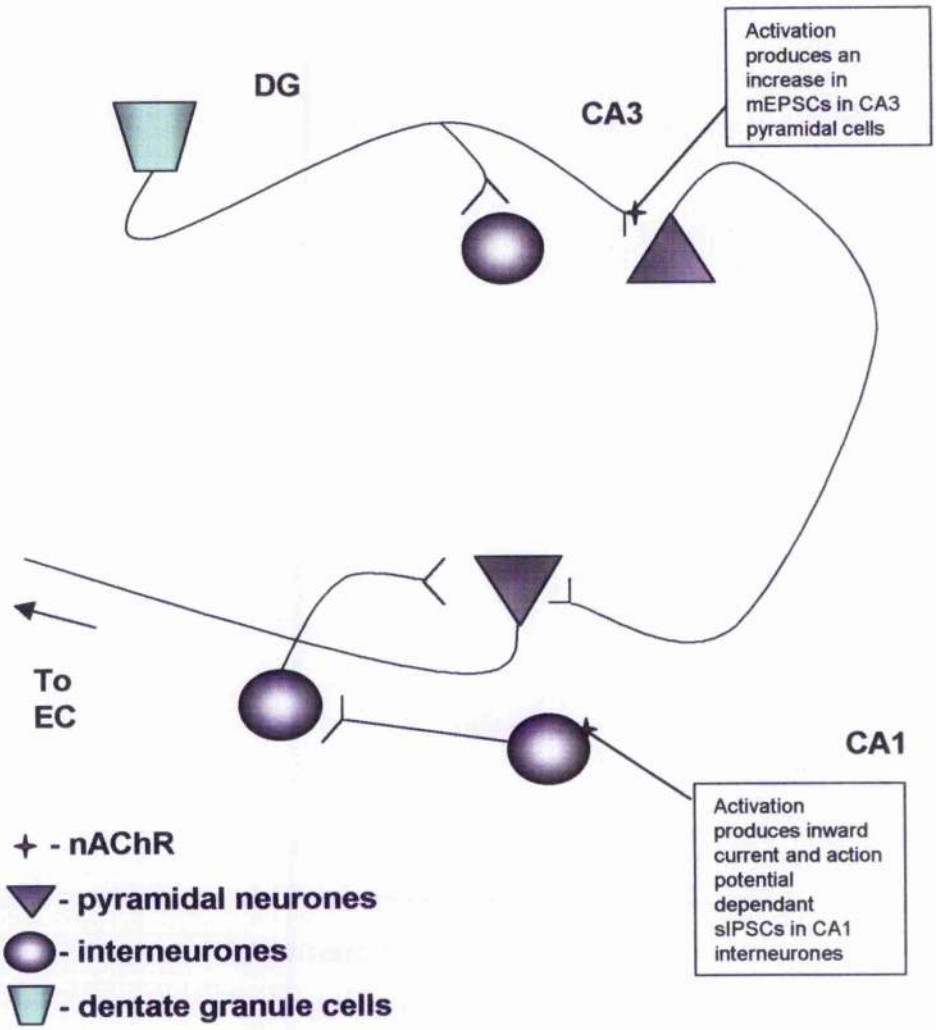


Figure 5.16 Summary of nAChR location within the hippocampus.

Diagram summarising the location of nAChRs as indicated by the results presented within this chapter. DMPP induced an increase in the frequency of miniature EPSCs recorded in CA3 pyramidal neurones but not CA3 interneurones indicating that nAChRs are present presynaptically on the mossy fibre terminals synapsing onto CA3 pyramidal neurones but not CA3 interneurones. DMPP also produced an increase in spontaneous IPSPs in CA1 interneurones but not pyramidal neurones. This was shown to be a TTX sensitive effect and therefore indicates the presence of nAChRs postsynaptically on interneurones innervating other CA1 interneurones but not CA1 pyramidal neurones.

5.3. Discussion

Taken together, the results presented in the chapter suggest that nAChRs present on the mossy fibre terminals of dentate granule cells and interneurones can regulate both glutamatergic and GABAergic circuits. However it is apparent that differences in such regulation exist within different regions and cell types.

5.3.1. Excitatory transmission

The results presented in this chapter report a significant 30.84±5.98% increase in evoked field EPSP slope on addition of the nAChR agonist DMPP at the mossy fibre-to-CA3 synapse indicating an enhancement of glutamate transmission within this pathway. This reported increase is small in comparison to other studies where nAChR agonists have been shown to enhance evoked glutamate transmission. In another limbic system pathway, at synapses between medial habenula nucleus (MHN) and the interpeduncular nucleus (IPN) the evoked EPSC (eEPSC) more than doubles in response to nicotine application (McGehee *et al.*, 1995). In autaptic synapses or synapses between two coupled cultured hippocampal neurones eEPSC amplitude was enhanced by 46% in response to nicotine application (Radcliffe & Dani, 1998). The larger effect in these different preparations may be due to differences in receptor expression patterns. Hippocampal cultures are known to show an increased expression of nAChRs compared to slice preparations (Sudweeks & Yakel, 2000) .

Further studies were carried out to assess whether this enhancement may be due to actions at somatodendritic, preterminal or presynaptic receptors (i.e. an increase in amount of glutamate released) or on the postsynaptic site (i.e. an increase in postsynaptic detection of glutamate). Generally modulation of transmitter release by presynaptic nAChRs is insensitive to blockade by the Na^+ channel blocker tetrodotoxin (TTX) whereas modulation of transmitter release by preterminal or somatodendritic receptors depends on the propagation of action potentials and will be therefore sensitive to TTX (Clarke & Reuben, 1996; Gray *et al.*, 1996; Wonnacott, 1997). In the case of TTX insensitive effects measuring changes in frequency and amplitude can discriminate between an increase in transmitter release via presynaptic receptors and an increase in the postsynaptic detection of transmitter. Increases in frequency but not amplitude indicate an increase in the rate of transmitter release and not postsynaptic detection (McGehee *et al.*, 1995; Gray *et al.*, 1996).

In the case of nAChRs it has been reported that activation increases frequency not amplitude of mEPSCs in numerous brain regions including the CA3 pyramidal neurones of the hippocampus (McGehee *et al.*, 1995; Gray *et al.*, 1996; Girod & Role, 2001).

The data provided here, that DMPP indeed increases mEPSC frequency but not amplitude in CA3 pyramidal neurones confirms that hippocampal glutamate transmission is enhanced via a presynaptic mechanism. This finding is in agreement with previous studies in CA3 pyramidal neurones (Gray *et al.*, 1996).

These authors also reported an increase in intracellular Ca^{2+} in mossy terminals in response to nicotine application and proposed that activation of presynaptic nAChRs on mossy terminals could allow a large influx of Ca^{2+} that may contribute to the enhanced glutamate release onto CA3 pyramidal neurones. Together these findings suggest that the increase in fEPSPs recorded at the mossy fibre-to-CA3 pyramidal neurone synapse may be mediated by activation of presynaptic nAChRs, which open to allow a large Ca^{2+} into the presynaptic terminal and thus increase release of glutamate. However a recent study by Vogt and Regher (2001) disagreed with Gray's results showing no increase in Ca^{2+} within the mossy terminal on application of nAChR agonists.

However, no increase in glutamate released onto CA3 interneurones was noted. This may be due to the fact that mossy fibres innervate principal cells and interneurones via anatomically distinct synapses. The CA3 interneurones are innervated by small en passant or filopodial mossy fibre synapses rather than the large mossy terminals which innervate the CA3 pyramidal neurones (Acsady *et al.*, 1998). It therefore seems likely that nAChRs are not expressed at these mossy-fibre-to CA3 interneurone synapses.

DMPP had no significant effect on evoked fEPSPs at the Schaffer collateral-to-CA1 pyramidal neurone synapse within area CA1 of the hippocampus. One possible explanation for this lack of effect could be desensitisation of the nAChRs, although this is unlikely as a significant effect was apparent at the mossy fibre-to-CA3 synapse using the same concentrations of agonists and application

protocol. However, desensitisation is particularly prominent for α7 containing nAChRs and these receptors have been strongly implicated in this modulatory role. In order to minimise the extent of desensitisation we carried out additional experiments within the CA1 region using rapid pressure application of agonists. Application of nicotine in this manner had no effect on the fEPSP at Schaffer collateral-to-CA1 pyramidal neurone synapse, although this paradigm was not used to investigate the mossy fibre-to-CA3 synapse. The nAChR agonist choline was also tested, as receptor desensitisation is much less pronounced with this agonist than nicotine (Alkondon *et al.*, 1997b) but again failed to record any effect on the fEPSP.

The lack of any effect on fEPSP slope at the Schaffer collateral-to-CA1 synapse reported here disagrees with a previous study by Chiodini and colleagues (1999), which reported a significant increase in fEPSP slope of up to 54%. However, these authors reported a similar increase of fEPSP slope on application of nAChR antagonists and therefore postulated that the excitatory effects of nicotine at the Schaffer collateral-to-CA1 synapse could be mediated through receptor desensitisation rather than activation of nAChRs on CA3 pyramidal neurones. For example desensitisation of nAChRs expressed on interneurones or axonal fibres could enhance CA3 pyramidal neurone excitation by a reduction of interneurones firing or modulation of action potential propagation in the axons. The concentration of DMPP used in our experiments may not have induced such a desensitisation and it is most likely that nicotine and choline applied by pressure

injection did not induce a receptor desensitisation and therefore may explain why no change in fEPSP was observed.

These authors also reported an increase and decrease of GABA transmission within the CA1 at low and high concentrations of nicotine respectively. Therefore another possibility for the lack of effect on fEPSPs evoked at the Schaffer collateral-to-CA1 synapse in this study may be that glutamate transmission is enhanced but is masked by a concominant enhancement of GABAergic transmission. This seems a more likely possibility due to the slight, albeit non-significant, decrease in the fEPSPs. An inhibition of glutamatergic EPSPs by activation of $GABA_B$ receptors has been shown previously at this synapse (Wu & Saggau, 1995; Hasselmo & Fehlau, 2001).

As the investigation of mEPSCs in CA1 neurones in the presence of GABA-receptor antagonists and the Na^+ channel blocker TTX, showed no change in the frequency or amplitude of mEPSC recorded in CA1 pyramidal neurones or CA1 interneurones. These results suggest that there was no enhancement of glutamate transmission onto CA1 pyramidal neurones, at least via a presynaptic effect that may have been masked by an increase in GABAergic transmission. These results disagree with findings (Gray *et al.*, 1996; Liu *et al.*, 2003) that reported an increase in mEPSC frequency on CA1 pyramidal neurones. Gray and colleagues (1996) reported an increase of mEPSCs onto CA1 pyramidal neurones but did not show any data, claiming the mEPSCs were smaller and harder to analyse. Moreover, the lack of effect on glutamate transmission in the CA1 region is

supported by a study by Alkondon and colleagues (1997a) who stated that nAChR activation is linked to the release of GABA but not glutamate in the CA1 region of the hippocampus.

5.3.2. Inhibitory transmission

An investigation into the regulation of GABA transmission within area CA1 showed that application of DMPP elicited an inward current and increased the frequency of spontaneous IPSCs in CA1 hippocampal interneurones. These results indicate that nAChR agonists can depolarise CA1 interneurones and increase GABA release within the CA1 region of the hippocampus agreeing with numerous previous studies (Alkondon *et al.*, 1997a; Jones & Yakel, 1997; Frazier *et al.*, 1998b; Alkondon *et al.*, 1999; McQuiston & Madison, 1999c; Alkondon *et al.*, 2000a; Alkondon *et al.*, 2000b; Ji & Dani, 2000; Sudweeks & Yakel, 2000; Alkondon & Albuquerque, 2001; Buhler & Dunwiddie, 2001).

An inward current in CA1 interneurones could be elicited by either nAChR agonists acting directly on nAChRs known to be expressed on the interneurone surface (Freedman *et al.*, 1993; Fabian-Fine *et al.*, 2001; Kawai *et al.*, 2002) or by nAChR exciting CA1 pyramidal neurones, which in turn would release glutamate to act on glutamate receptors present on the interneurone surface and thus potentiate interneurone activity and GABA release through a postsynaptic mechanism. However in the case of these experiments the latter proposal can be discounted due to that fact that glutamate receptor antagonists were present in the

perfusion medium throughout all experiments and additionally that no inward current was noted in pyramidal neurones on application of DMPP. Thus it seems most likely that the inward current represents a direct effect of nAChR agonists on the CA1 interneurone under study. Further experiments carried out in the presence of TTX showed that the inward current was insensitive to TTX further supporting the suggestion that it is the result of direct activation of nAChR on the interneurone surface. These results are in agreement with previous studies, which have reported inward currents in CA1 interneurones in response to pharmacological activation of nAChRs by rapid application range of agonists including ACh, nicotine, DMPP and choline (Alkondon *et al.*, 1997a; Jones & Yakel, 1997; Frazier *et al.*, 1998b; Alkondon *et al.*, 1999; McQuiston & Madison, 1999c; Ji & Dani, 2000; Sudweeks & Yakel, 2000; Buhler & Dunwiddie, 2001). A recent study showed application of nicotine for a period of 1-5mins, similar to the agonist application protocol performed in this study, elicited slowly desensitising inward currents in *stratum lacunosum moleculare* CA1 interneurones (Alkondon *et al.*, 2000b). The inward currents reported here show similar range in amplitude to those previously reported. The lack of inward current in CA1 pyramidal neurones agrees with most previous studies (Jones & Yakel, 1997; Frazier *et al.*, 1998b; McQuiston & Madison, 1999c; Sudweeks & Yakel, 2000)

Previous studies indicate an inward current in dendritic inhibitory interneurones and not perisomatic interneurones (McQuiston & Madison, 1999c). As only 50% of our cells responded with an inward current it may be that we have recorded

from both cells types and that the 50% of cells that did respond were dendritic and the 50 % that showed no inward current were perisomatic.

An increase in the frequency of sIPSCs recorded in CA1 interneurones in response to DMPP application showed that nAChR activation increased GABA release onto CA1 interneurones presumably due to an increase in GABAergic interneurone activity. The fact that no increase in mIPSC frequency was recorded in the presence of the Na^+ channel blocker TTX illustrates that the increased release of GABA from CA1 interneurones is a TTX-sensitive effect. This finding indicates that GABA release from CA1 interneurones is action potential dependent and thus is due to the activation of nAChRs present on preterminal or somatodendritic sites. These results are in agreement with previous studies by Alkondon and colleagues and Hulo and Muller who have also reported that activation of nAChRs on CA1 interneurones facilitates an action potential-dependent release of GABA (Alkondon *et al.*, 1997a; Albuquerque *et al.*, 1998; Alkondon *et al.*, 1999; Hulo & Muller, 2001).

That the frequency and amplitude of the sIPSC and mIPSCs in CA1 pyramidal neurones remain unchanged by DMPP suggests that nAChRs activated to increase the release of GABA are most likely present on the interneurone-selective (IS) class of interneurones as activation of these cells would increase GABA transmission onto other CA1 interneurones but not CA1 pyramidal neurones.

These findings disagree in part with some work by Alkondon and colleagues (1997) in that these authors report nAChR agonists to increase sIPSC frequency and amplitude in both CA1 interneurones and CA1 pyramidal neurones indicating an action potential dependent increase of GABA release from interneurones synapsing onto both other CA1 interneurones and CA1 pyramidal neurones. Hulo and Muller 2001 also reported an increase of sIPSC frequency and amplitude in CA1 pyramidal neurones that was sensitive to TTX. These differences may be representative of the small sample size in this study.

5.3.3. Physiological relevance

The results presented in this chapter show that application of nAChR agonists enhanced the strength of glutamate transmission at the mossy fibre-CA3 synapse resulting in an increased excitation of CA3 pyramidal neurones as well as activating the IS class of CA1 interneurones to release GABA onto other interneurones. In this section it will be discussed how these effects may relate to the pro-epileptogenic action of nicotine and specifically how these actions may result in the increase of burst frequency reported by Roshan-Milanin and colleagues (2003).

The mechanisms underlying the generation of epileptiform activities in the hippocampus is thought to arise from a hyperexcitability within the principal cell population (Jefferys, 1993). This hyperexcitability may be the result of either an

increased excitatory drive (especially through recurrent connections) and/or decreased inhibition to these cells. The effects reported in this chapter are;

1) An increase of excitatory input onto pyramidal neurones via increased glutamate released from the mossy fibre terminals.
2) An inhibition of interneurones via an increase in the frequency of inhibitory GABAergic events.

These effects may lead to a hyperexcitability of the principal cell population and thus have a pro-epileptogenic effect. However, how would these effects of nAChR activation on glutamatergic and GABAergic transmission lead to the observed increase in burst frequency seen in previous studies (Roshan-Milani *et al.*, 2003)?

With regard to the apparent direct enhancement of evoked glutamatergic transmission (Fig 5.2B), Staley and colleagues have proposed that:

"The rate of glutamate release (R_{Glu}) is the product of the number of releasable vesicles (N_R) multiplied by the probability of release (P_R)." *(Staley et al., 1998)*

$$R_{Glu} = N_R \, x \, P_R$$

By using the above equation the following possibilities exist:

1) Burst termination is due to depletion of glutamate from recurrent collaterals (if N_R=0 then R_{Glu} =0). If DMPP increased the amount of glutamate released then depletion should occur more rapidly, thus decreasing the duration of the burst and allowing the next burst to come quicker. However, Staley (1998) stated that burst length does not predict the time interval until next burst, but length of interval preceding a burst is correlated with burst length.

2) The most likely explanation is that DMPP increases the probability of glutamate release (as agents that increase probability of release will increase burst frequency). Stately (1998) stated that "if probability of release is increased then the inter-burst interval decreases therefore resulting in an increase of frequency, which will cause a decrease of burst length". This explanation fits with the original finding that nAChR agonists increase the burst frequency while decreasing overall burst length (Roshan-Milani *et al.*, 2003).

When an action potential reaches a presynaptic terminal voltage dependent calcium channels (VDCC) mediate an increase in intracellular calcium. This does not ensure transmitter release from the presynaptic terminal but only increases probability of release. If DMPP acts to open presynaptic nAChR channels, which are permeable to Ca^{2+} (Seguela *et al.*, 1993), this may increase intracellular Ca^{2+} just prior to the arrival of an action potential that may be able to increase the probability of release in a similar manner to that seen with paired pulse facilitation. Another possibility may be that Ca^{2+} entry through nAChRs may

promote Ca^{2+} dependent Ca^{2+} release from intracellular stores resulting in an increase of transmitter release.(Engelman & Macdermot, 2004).

All inputs onto a neurone, whether excitatory or inhibitory, summate to decide if a cell will fire an action potential and increase the probability of transmitter release. With regard to the increased inhibition of CA1 interneurones, DMPP may infact lead to an overall disinhibition of pyramidal neurones. This decrease in inhibitory inputs onto pyramidal neurones may result in an increase in the probability of that cell to be able to release glutamate. Thus a similar situation may arise where the probability of glutamate release is increased resulting in a decrease of inter-burst interval and an increase of burst frequency. If this mechanism had a prominent role in epileptogenesis a decrease in sIPSCs recorded in CA1 pyramidal neurones would have been expected, however this was not the case. These observations, taken together with the fact that epileptic burst frequency was increased by nAChR agonists in the bicuculline model (Roshan-Milani *et al.*, 2003) suggest that it is unlikely that nAChRs are significantly affecting GABAergic circuits to promote epileptiform activity.

Therefore the proepileptogenic action of nAChR agonists may be mediated mainly through glutamatergic mechanisms.

5.4. SUMMARY

The results presented in this chapter show that selective nAChR agonists increase both glutamatergic and GABAergic transmission within the hippocampus. Glutamatergic transmission is increased via activation of presynaptic nAChRs whereas GABAergic transmission is increased through an action potential dependent mechanism. The observed alteration in glutamate transmission seems to be confined to the CA3 region whereas the alteration in GABA transmission is confined to the CA1 region (see table 5.1 & fig 5.16). However, it is possible that these effects may be capable of increasing the probability of glutamate release within the hippocampus as a whole and as such may play some role in the proepileptogenic action of nAChR agonists within the hippocampus.

6. GENERAL DISCUSSION

The main aim of this thesis was to further investigate the physiological interactions of the cholinergic and GABAergic systems within the septo-hippocampal axis. This was achieved by conducting two parallel approaches. The first investigated mAChR-mediated synaptic responses in both the principal cells and GABAergic interneurones in response to physiological activation of cholinergic afferents. The second study investigated the neuromodulatory role of nAChRs in hippocampal cells and circuits using nAChR selective pharmacological agents.

6.1. MUSCARINIC ACETYLCHOLINE RECEPTOR SYNAPTIC RESPONSES

Chapter 3 confirmed the presence of the mAChR-mediated slow EPSP in hippocampal pyramidal neurones. That this was a reasonably uniform response from all pyramidal neurones was most likely due to the fact that pyramidal neurones represent a relatively homogenous population of cells. The use of septo-hippocampal slices provided the first direct evidence, in vitro, that physiological activation of cholinergic afferents from the medial septal nucleus produces a slow depolarisation in hippocampal pyramidal neurones. In some cases IPSPs were noted throughout the slow depolarisation. This observation along with previous pharmacological studies (McQuiston & Madison, 1999b, a) suggested that mAChR activation also excites GABAergic interneurones within the

hippocampus. This initial results chapter also confirmed that stimulation within the *stratum oriens* was an effective and sufficient method to stimulate cholinergic afferent fibres within the hippocampal slice preparation. Whilst the septal origins of such fibres cannot be guaranteed, previous lesion studies in which slices were prepared from fimbria/fornix transected animals suggest that the overwhelming majority of fibres originate from this structure (Cobb *et al.*, 1999). These results allowed us to proceed with this proposed method to stimulate these cholinergic afferents with a view to eliciting mAChR-mediated synaptic responses in the CA1 interneurone population (chapter 4).

Chapter 4 provided the first direct evidence that stimulation of cholinergic afferents elicit a slow depolarisation in CA1 GABAergic interneurones. In contrast to the response observed in pyramidal neurones, which was a stereotyped slow depolarising response, evoked cholinergic responses in interneurones showed great heterogeneity with respect to the resultant underlying waveforms and discharge patterns. A variety of mAChR-mediated responses were elicited in CA1 interneurones including a slow depolarisation, a delayed response where an initial hyperpolarisation preceded a slower depolarisation, a fast plateau response where a fast and slow depolarisation were present, an oscillatory response and finally, interneurones which did not respond to cholinergic afferent stimulation at all. All of these response types, with the obvious exception of the non-response, were attributed to activation of mAChR receptors. As such, this study extends current knowledge of mAChR mediated synaptic transmission within the hippocampus and is summarised in figure 6.1.

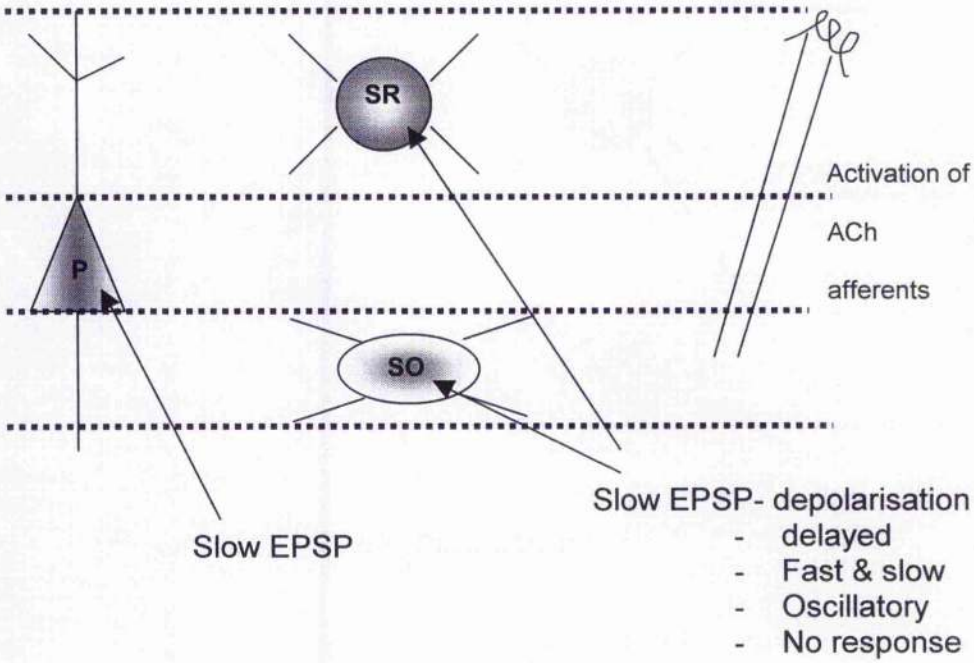


Figure 6.1. Summary of evoked cholinergic responses in pyramidal neurones and interneurones in area CA1 of the hippocampus.

CA1 pyramidal neurones respond to stimulation of cholinergic afferents located within the *stratum oriens* with a stereotyped slow EPSP whereas the interneurones of area CA1 respond with a range of EPSPs including a slow depolarisation, a delayed response, a fast plateau response, an oscillatory response or finally no response.

The fact that the interneurone population shows diversity in their mAChR mediated response rather than the uniform response observed in pyramidal neurones provides a further level of diversity to the interneuronal population and suggests further complexity to interneuronal function and their control over the principal cell population. However, a more detailed anatomical study of the responsive interneurones may provide a clearer view, although previous studies have failed in this respect (Parra *et al.*, 1998; McQuiston & Madison, 1999a).

Although one type of response contained a fast component that was not fully abolished by selective mAChR antagonists, the precise identity of the fast component could not be established. One likely possibility is that this may have been due to released ACh activating nAChRs as previously reported (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a). However, in the previous studies which have shown the production of a nAChR-mediated response in CA1 interneurones (Alkondon *et al.*, 1998; Frazier *et al.*, 1998a; Buhler & Dunwiddie, 2001), the responses were evoked via a single stimulation within the *stratum oriens*. Buhler and Dunwiddie (2001) showed that these synaptic responses were inhibited if a train of stimulation at 10Hz was used. This is in contrast to the mAChR-mediated responses evoked in this study, which were instead potentiated upon delivery of a train of stimuli. This may suggest that differential signalling from the MSN may be able to evoke a predominantly nAChR mediated or a predominantly mAChR mediated response, depending upon the precise input pattern. When septal cholinergic neurones fire at relatively low rates nAChR-mediated responses will

predominate, whereas when the septal cholinergic neurones fire at higher rate a mAChR-mediated response may predominate.

As both mAChR and nAChR activation has been shown to be involved in generation and patterning of network activities in the hippocampus and that addition of $GABA_A$ receptor antagonists abolished hippocampal theta-mode activity (Cobb *et al.*, 1999), the activation of mAChRs and nAChRs on hippocampal interneurones by ACh released from cholinergic afferents from the medial septum may mediate down stream phasic inhibition of pyramidal neurones contributing to the generation and maintenance of hippocampal oscillatory states.

Many septal neurones including putative cholinergic neurones fire in vivo at theta (4-12Hz) frequencies (Stewart & Fox, 1990; Brazhnik & Fox, 1999). The rhythmical activity of septal cholinergic neurones may be able to preferentially activate mAChRs on hippocampal pyramidal neurones and interneurones to initiate and sustain hippocampal oscillatory states. However, there is no direct evidence that the septal activity must be phasic and not tonic in order to induce hippocampal oscillatory states. The role for nAChR may be more complicated depending on the receptor subtypes involved.

6.2. NEUROMODULATORY ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS

The second study (chapter 5) concentrated on pharmacological activation of nAChRs in both pyramidal neurones and interneurones. The main focus was to look at the neuromodulatory role of these receptors rather than their role in direct ACh mediated fast synaptic transmission. Chapter 5 provided a detailed analysis of the effects of nAChR activation in modulating both glutamatergic and GABAergic synaptic transmission. The results confirmed that nAChRs are able to modulate the release of both neurotransmitters but showed that this modulation may be both regional and cell specific.

The data presented in this thesis shows that activation of presynaptic nAChR increased the frequency of mEPSPs onto CA3 pyramidal neurones but not CA3 interneurones, indicating an increase of glutamate release onto CA3 pyramidal neurones but not CA3 interneurones. Thus nAChR activation acts to produce an overall excitation in the CA3 region. However, in the CA1 region the outcome seems more complicated in that nAChR activation seems to have no effect on evoked fEPSPs or mEPSCs measured in CA1 pyramidal neurones but activated an inward current and increased the frequency of sIPSPs in CA1 interneurones. Analysis of the TTX sensitivity of these effects suggested that nAChR agonists appeared to act on somatic, dendritic or preterminal nAChRs located on CA1 interneurones to produce an inward current and thus excite certain CA1 interneurones to produce an action potential dependent release of GABA from these cells. The ultimate outcome of this nAChR activation will depend on the

postsynaptic target of the interneurone under study. As we have no detailed post-hoc neuroanatomical data for these results we are unable to conclude which interneurone subtype responded with an inward current and which subtype did not. However, as nAChR activation seems to produce an increase of GABAergic IPSPs onto other CA1 interneurones but not pyramidal neurones this indicates that nAChR activation may preferentially excite the interneurone selective (IS) cells (Acsady et al., 1996a; Acsady et al., 1996b; Gulyas et al., 1996) and evoke an action potential dependent increase in release of GABA onto other hippocampal interneurones. Although we did not observe any concomitant decrease in IPSCs recorded in pyramidal neurones as might be expected from such a disinhibitory mechanism, it is likely that the pyramidal neurone terminating interneurones may be silent in brain slices and that such activity would only be seen in a dynamically active network.

6.3. SUMMARY

The seemingly complex actions of the cholinergic system within the hippocampus is summarised in table 6.1. Cholinergic effects demonstrated throughout this thesis together with complimentary observations/citations are indicated in order to help build up a more complete picture of the way by which ACh modulates hippocampal circuits

	MAChR	NAChR
Pyramidal neurones:		
Excitability-	$\uparrow$ [1],	$\leftrightarrow$ [3,4]
Glutamatergic transmission-	$\downarrow$ [3]	$\uparrow$ [3] or $\leftrightarrow$ [3]
Interneurones:		
Excitability-	$\uparrow$ [2] $\downarrow$ [5] $\leftrightarrow$ [2]	$\uparrow$ [3,4] or $\leftrightarrow$ [3,6]
GABAergic transmission-	$\downarrow$ [7]	$\uparrow$ [3]

Table 6.1 Summary of the cholinergic modulation of hippocampal systems.

Table summarises the effects of mAChR and nAChR activation on both pyramidal neurone and interneurone excitability and the effect on glutamatergic and GABAergic transmission within the hippocampus. Activation of mAChRs can lead an excitation of pyramidal neurones whilst at the same time resulting in a direct inhibition of glutamate transmission. Activation of nAChRs has no effect on the excitability of pyramidal neurones but can facilitate glutamate transmission at certain synapses. Activation of mAChRs can produce a variety of responses in hippocampal interneurones ranging from excitation to no effect to hyperpolarisation. mAChR activation also leads to an inhibition of GABAergic transmission. Activation of nAChRs can lead to an increase or no change in the excitability of hippocampal interneurones. nAChR activation also appears to increase GABAergic transmission at some synapses.**1**. Effect confirmed in chapter three, **2**. Effect confirmed in chapter four, **3**, Effect confirmed in chapter five, **4**. Frazier et al (1998b), **5**. McQuiston and Madision (1999a), **6**. McQuiston and Madison (1999c), **7**. Behrends and TenBruggencate (1993).

6.4. FUTURE RESEARCH

This thesis has highlighted the complexity of interactions between the cholinergic and GABAergic systems and their ultimate influence over the principal cell population. However, a number of important questions remain unanswered including:

- What precise types of interneurone are differentially modulated by ACh?
- How does such differential modulation effect somatic vs dendritic inhibition and ultimately hippocampal network dynamics?
- Are mAChR-mediated and nAChR-mediated responses differentially recruited depending upon the firing pattern of cholinergic afferents?
- How do intrinsic cholinergic neurones (Frotscher *et al.*, 1986; Frotscher *et al.*, 2000) modulate hippocampal circuits?
- How does hippocampal activity feed back to affect septal activity?

6.5. THERAPEUTIC IMPLICATIONS

As the hippocampus is a prominent region of the brain affected by numerous neurological disorders (Hyman *et al.*, 1990; Schwartzkroin, 1994) Further advances in understanding how different neurotransmitters interact within this structure will ultimately aid designing therapeutic strategies to combat dysfunction in such interactions.

In Alzheimer's disease in which there is a dramatic loss of cholinergic function (Kása *et al.*, 1997), greater knowledge of how ACh normally regulates hippocampal function might aid the development of targeted and rational attempts to compensate for such imbalance.

The hippocampus is also one of the most seizure prone structures in the brain (Schwartzkroin, 1994) and we have demonstrated that nAChR activation has proepileptogenic actions in this structure (Roshan-Milani *et al.*, 2003). Again, the nAChR specific pharmacology of this response will perhaps aid in determining future strategies to influence network pathology unbalances such as occurs in epilepsy. Indeed, the very specific cholinergic modulation of certain circuits might present a more selective scope for intervention than the currently used antiepileptic drugs which cause a rather general and non-selective suppression of neuronal activity resulting in unwanted side effects.

7.REFERENCES

ABE, T., SUGIHARA, H., NAWA, H., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1992). Molecular characterisation of a novel metabotrophic glutamate receptor mGluR5 coupled to inositol phosphate/Ca^{2+} signal transduction. *Journal of Biological Chemistry* **267,** 13361-13368.

ACSADY, L., ARABADZISZ, D. & FREUND, T. F. (1996a). Correlated morphological and neurochemical features identify different subsets of VIP-immunoreactive interneurons in the rat hippocampus. *Neuroscience* **73,** 299-315.

ACSADY, L., GORCS, T. J. & FREUND, T. F. (1996b). Different populations of VIP-immunoreactive interneurones are specialized to control pyramidal cells or interneurones in the hippocampus. *Neuroscience* **73,** 317-334.

ACSADY, L., KAMONDI, A., SIK, A., FREUND, T. F. & BUZSAKI, G. (1998). GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *Journal of Neuroscience* **18,** 3386-3403.

ADKINS, C. E., PILIAI, G. V., KERBY, J., BONNERT, T. P., HALDON, C., MCKERNAN, R. M., GONZALEZ, J. E., OADES, K., WHITING, P. J. & SIMPSON, P. B. (2001). alpha(4)beta(3)delta GABA(A) receptors characterised by fluorescence resonance energy transfer-derived measurements of membrane potential. *Journal of Biological Chemistry* **276,** 38934-38939.

AIKA, Y., REN, J. Q., KOSAKA, K. & KOSAKA, T. (1994). Quantitative analysis of GABA-like-immunoreactive and paravalbumin-containing neurons in the CA1 region of the rat hippocampus using a stereological method, the disector. *Experimental Brain Research* **99,** 267-276.

AKAIKE, N., HATTORI, K., OOMURA, Y. & CARPENTER, D. O. (1985). Bicuculline and picrotoxinin block γ-aminobutyric acid-gated Cl^- conductance by different mechanisms. *Experientia* **41,** 70-71.

ALBUQUERQUE, E. X., PEREIRA, E. F. R., BRAGA, M. F. M. & ALKONDON, M. (1998). Contribution of nicotinic receptors to the function of synapses in the central nervous system: The action of choline as a selective agonist of α7 receptors. *Journal of Physiology, Paris* **92,** 309-316.

ALKONDON, M. & ALBUQUERQUE, E. X. (1993). Diversity of nicotinic acetylcholine receptors in rat hippocampal neurones. I. Pharmacological and functional evidence for distinct structural subtypes. *The Journal of Pharmacology and Experimental Therapeutics* **265,** 1455-1473.

ALKONDON, M. & ALBUQUERQUE, E. X. (2001). Nicotinic acetylcholine receptor α7 and α4β2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *Journal of Neurophysiology* **86,** 3043-3055.

ALKONDON, M., BRAGA, M. F. M., PEREIRA, E. F. R., MAELICKE, A. & ALBUQUERQUE, E. X. (2000a). α7 nicotinic acetylcholine receptors and modulation of gabaergic synaptic transmission in the hippocampus. *European Journal of Pharmacology* **393,** 59-67.

ALKONDON, M., PEREIRA, E. F. R. & ALBUQUERQUE, E. X. (1998). α–Bungarotoxin and methyllycaconitine-sensitive nicotinic receptors mediate fast synaptic transmission in interneurons of rat hippocampal slices. *Brain Research* **810,** 257-263.

ALKONDON, M., PEREIRA, E. F. R., ALMEIDA, L. E. F., RANDALL, W. R. & ALBUQUERQUE, E. X. (2000b). Nicotine at concentrations found in cigarette smokers activates and desensitizes nicotinic acetylcholine receptors in CA1 interneurons of rat hippocampus. *Neuropharmacology* **39,** 2726-2739.

ALKONDON, M., PEREIRA, E. F. R., BARBOSA, C. T. F. & ALBUQUERQUE, E. X. (1997a). Neuronal nicotinic Acetylcholine receptor activation modulates γ-aminobutyric acid release from CA1 neurons of rat hippocampal slices. *Journal of Pharmacology and Experimental Therapeutics* **283,** 1396-1411.

ALKONDON, M., PEREIRA, E. F. R., CORTES, W. S., MAELICKE, A. & ALBUQUERQUE, E. X. (1997b). Choline is a selective agonist of alpha 7 nicotinic acetylcholine receptors in the rat brain neurons. *European Journal of Neuroscience* **9,** 2734-2742.

ALKONDON, M., PEREIRA, E. F. R., EISENBERG, H. M. & ALBUQUERQUE, E. X. (1999). Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. *The Journal of Neuroscience* **19,** 2693-2705.

ALT, A., WEISS, B., OGDEN, A. M., KNAUSS, J. L., OLER, J., HO, K., LARGE, T. H. & BEAKMAN, D. (2004). Pharmacological characterisation of glutamate agonists and antagonists at recombinant human homomeric and heteromeric kainate receptors in vitro. *Neuropharmacology* **46,** 793-806.

ALVAREZ, P., ZOLA-MORGAN, S. & SQUIRE, L. R. (1995). Damage limited to the hippocampal region produces long-lasting memory impairment in monkeys. *Journal of Neuroscience* **15,** 3796-3807.

AMARAL, D. G. (1978). A Golgi study of cell types in the hilar region of the hippocampus in the rat. *Journal of Comparative Neurology* **182,** 851-914.

ANANTHARAM, V., PANCHAL, R. G. & WILSON, A. (1992). Combinatorial RNA splicing alters the surface charge on the NMDA receptor. *FEBS Letters* **305,** 27-30.

ANDERSEN, P., BLISS, T. V. P. & SKREDE, K. K. R. (1971). Lamellar organisation of hippocampal excitatory pathways. *Experimental Brain Research* **13,** 222-238.

ANDRADE, R., MALENKA, R. C. & NICOLL, R. A. (1986). A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. *Science* **234,** 1261-1265.

ARAUJO, D., LAPCHAK, P. A., COLLIER, B. & QUIRION, R. (1988a). Characterisation of N-[3H]methylcarbamylcholine binding sites and effect of N-methylcarbamylcholine on acetylcholine release in rat brain. *Journal of Neurochemistry* **51,** 292-299.

ARAUJO, D., LAPCHAK, P. A., ROBITAILLE, Y., GAUTHIER, S. & QUIRION, R. (1988b). Differential alteration of various cholinergic markers in cortical and subcortical regions of human brain in Alzheimer's disease. *Journal of Neurochemistry* **50**.

ARRIAGADA, P. V., GROWDON, J. H., HEDLEY-WHYTE, T. & HYMAN, B. T. (1992). Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. *Neurology* **42,** 631-639.

ASCHER, P., BREGESTOVSKI, P. & NOWAK, L. (1988). N-metyl-D-aspartate-activated channels of mouse central neurones in magnesium free solutions. *Journal of Physiology* **399,** 207-226.

BALL, M. J. (1978). Topographical distribution of neurofibrillary tangles and granulovacuolar degeneration in hippocampal cortex of ageing and demented patients: a quantitative study. *Acta Neuropathologica* **42,** 73-80.

BARTUS, R. T., DEAN, R. L., PONTECORVO, M. J. & FLICKER, C. (1985). The cholinergic hypothesis: a historical overview, current perspective, and future directions. *Annals of the New York Academy of Sciences* **444,** 332-358.

BATESON, A. L., LASHAM, A. & DARLISON, M. G. (1991). γ-Aminobutyric acid$_A$ receptor heterogeneity is increased by alternative splicing of a novel β-subunit gene transcript. *Journal of Neurochemistry* **56,** 1437-1442.

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

BEHRENDS, J. C. & TENBRUGGENCATE. (1993). Cholinergic modulation of synaptic inhibition in the guinea pig hippocampus in vitro: excitation of GABAergic interneurons and inhibition of GABA release. *Journal of Neurophysiology* **69,** 626-629.

BENARDO, L. S. & PRINCE, D. A. (1981). Acetylcholine induced modulation of hippocampal pyramidal neurons. *Brain Research* **211,** 227-234.

BENARDO, L. S. & PRINCE, D. A. (1982). Cholinergic excitation of mammalian hippocampal pyramidal cells. *Brain Research* **249,** 315-331.

BENKE, D., HOMER, M., MICHEL, C., BETTLER, B. & MOHLER, H. (1999). γ–Aminobutyric acid type B receptor splice variant proteins GBR1a and GBR1b are both associated with GBR2 in situ and display differential regional and subcellular distributions. *Journal of Biological Chemistry* **274,** 27323-27330.

BENSON, D. M., BLITZER, R. D. & LANDAU, E. M. (1988). An analysis of the depolarization produced in guinea-pig hippocampus by cholinergic receptors stimulation. *Journal of Physiology* **404,** 479-496.

BETTLER, B., BOULTER, J. & HERMANS-BORGMEYER, I. (1990). cloning of a novel glutamate receptor subunit, GluR5-expression in the nervous system during development. *Neuron* **5,** 583-595.

BETTLER, B., EGEBJERG, J. & SHARMA, G. (1992). Cloning of a putative glutamate receptor-a low affinity kainate-binding subunit. *Neuron* **8,** 257-265.

BILLINTON, A., IGE, A. O., BOLAM, J. P., WHITE, J. H., MARSHALL, F. H. & EMSON, P. C. (2001). Advances in the molecular understanding of GABA$_B$ receptors. *Trends in Neurosciences* **24,** 277-282.

BILLINTON, A., IGE, A. O., WISE, A., WHITE, J. H., DISNEY, G. H., MARSHALL, F. H., WALDVOGEL, H. J., FAULL, R. L. & EMSON, P. C. (2000). GABA(B) receptor heterodimer-component localisation in human brain. *Brain Research. Molecular Brain Research* **77,** 111-124.

BIRD, S. J. & AGHAJANIAN, G. K. (1976). The cholinergic pharmacology of hippocampal pyramidal cells: a microionphoretic study. *Neuropharmacology* **15,** 273-282.

BISCOE, T. J. & STRAUGHAN, D. W. (1966). Micro-electrophoretic studies of neurones in the cat hippocampus. *Journal of Physiology* **183,** 341-359.

BLACKSTAD, T. W. & KJAERHEIM, A. (1961). Special axo-dendritic synapses in the hippocampal cortex. Electron and light microscope studies on the layer of mossy fibres. *Journal of Comparative Neurology* **117,** 133-159.

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

BONNER, T. I., BUCKELY, N. J., YOUNG, A. C. & BRANN, M. R. (1987). Identification of a family of muscarinic acetylcholine receptor genes. *Science* **237,** 527-532.

BONNER, T. I., YOUNG, A. C., BRANN, M. R. & BUCKELY, N. J. (1988). Cloning and expression of the human and rat m5 muscarinic acetylcholine receptor genes. *Neuron* **1,** 403-410.

BONNERT, T. P., MCKERNAN, R. M., FARRAR, S. J., BOURDELLS, L., HEAVENS, R. P., SMITH, D. W., HEWSON, L., RIGBY, M. R., SIRINATHSINGHJI, D. J., BROWN, N., WAFORD, K. A. & WHITING, P. J. (1999). Theta a novel gamma-amino butyric acid type A receptor subunit. *Proceedings of the National Academy of Sciences of the United States of America* **96,** 9891-9896.

BORMANN, J., HAMILL, O. P. & SAKMANN, B. (1987). Mechanism of anion permeation through channels gated by glycine and gamma-aminobutyric acid in mouse cultured spinal neurones. *Journal of Physiology* **385,** 243-286.

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

BOUE-GRABOT, E., ROUDBARAKI, M., BASCLES, L., CRAMU, G., BLOCH, B. & GARRET, M. (1998). Expression of GABA receptor rho subunits in rat brain. *Journal of Neurochemistry* **70,** 899-907.

BOULTER, J., CONNOLLY, J., DENERIS, E. S., GOLDMAN, D., HEINEMANN, S. & PATRICK, J. W. (1987). Functional expression of two neuronal nicotinic acetylcholine receptors from cDNA clones identifies a gene family. *Proceedings of the National Academy of Sciences of the United States of America* **84,** 7763-7767.

BOULTER, J., EVANS, K., GOLDMAN, D., MARTIN, G., TRECO, D., HEINEMANN, S. & PATRICK, J. W. (1986). Isolation of a cDNA clone coding for a possible neural nicotinic acetylcholine receptor alpha-subunit. *Nature* **319,** 368-374.

BOULTER, J., HOLLMANN, M. & O'SHEA-GREENFIELD, A. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. *Science* **49,** 1033-1037.

BOWEN, D. M., ALLEN, S. J., BENTON, J. S., GOODHARDT, M. J., HAAN, E. A., PALMER, A. M., SIMS, N. R., SMITH, C. C., SPILLANE, J. A., ESIRI, M. M., NEARY, D., SNOWDON, J. S., WILCOCK, G. K. & DAVISON, A. N. (1983). Biochemical assessment of serotonergic and cholinergic dysfunction and cerebral atrophy in Alzheimer's disease. *Journal of Neurochemistry* **41,** 266-272.

BOWERY, N. G., DOBLE, A., HILL, D. R., SHAW, J. S., TURNBULL, M. J. & WARRINGTON, R. (1981). Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. *European Journal of Pharmacology* **71,** 53-70.

BOWERY, N. G., HILL, D. R., HUDSON, A. L., DOBLE, A., MIDDLEMISS, D. N., SHAW, J. S. & TURNBULL, M. J. (1980). (-)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* **283,** 92-94.

BOWERY, N. G. & HUDSON, A. L. (1979). Gamma-aminobutyric acid reduces the evoked release of 3H-noradrenaline from sympathetic nerve terminals. *British Journal of Pharmacology* **66,** 108P.

BOWERY, N. G., HUDSON, A. L. & PRICE, G. W. (1987). GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* **20,** 365-383.

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

BRANN, M. R., JORGENSEN, H. B., BURNSTEIN, E. S., SPALDING, T. A., ELLIS, J., JONES, S. V. P. & HILL-EUBANKS, D. (1993). Studies of the pharmacology, localisation, and structure of the muscarinic acetylcholine receptors. *Annals of the New York Academy of Sciences* **707,** 225-236.

BRASHEAR, H. R., ZABORSZKY, L. & HEIMER, L. (1986). Distribution of gabaergic and cholinergic neurons in the rat diagonal band. *Neuroscience* **17,** 439-447.

BRAZHNIK, E. S. & FOX, S. E. (1999). Action potentials and relations to the theta rhythm of medial septal neurones. *Experimental Brain Research* **127,** 244-258.

BROWN, T. H. & ZADOR, A. M. (1990). Hippocampus. In *The Synaptic Organisation Of The Brain.* ed. SHEPHERD, G. M., pp. 346-388. Oxford University Press, New York.

BUCKLEY, N. J., BONNER, T. I. & BRANN, M. R. (1988). Localisation of a family of muscarinic receptor mRNAs in the rat brain. *Journal of Neuroscience* **8,** 4646-4652.

BUHL, E. H., HALASY, K. & SOMOGYI, P. (1994a). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the potentials and the number of synaptic release sites. *Nature* **368,** 823-828.

BUHL, E. H., HAN, Z. S., LORINCZI, Z., STEZHKA, V. V., KARNUP, S. V. & SOMOGYI, P. (1994b). physiological properties of anatomically identified axo-axoinc cells in the rat hippocampus. *Journal of Neurophysiology* **71,** 1289-1307.

BUHL, E. H., SZILAGYI, T., HALASY, K. & SOMOGYI, P. (1996). Physiological properties of anatomically identified basket and bistratified cells in the CA1 area of the rat hippocampus *in vitro*. *Hippocampus* **6,** 294-305.

BUHLER, A. V. & DUNWIDDIE, T. V. (2001). Regulation of the activity of hippocampal stratum oriens interneurons by α7 nicotinic acetylcholine receptors. *Neuroscience* **106,** 55-67.

BUHLER, A. V. & DUNWIDDIE, T. V. (2002). α7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons. *Journal of Neurophysiology* **87,** 548-557.

CARSI, J. M., H.H., V. & POTTER, L. T. (1999). m2-toxin: a selective ligand for M2 muscarinic receptors. *Molecular Pharmacology* **56,** 933-937.

CAULFIELD, M. P. (1993). Muscarinic receptors-characterisation, coupling and function. *Pharmacology and Therapeutics* **58,** 319-379.

CHANG, Y., WANG, R., BAROT, S. & WIESS, D. S. (1996). Stoichiometry of a recombinant $GABA_A$ Receptor. *The Journal of Neuroscience* **16,** 5415-5424.

CHARPAK, S., GAHWILER, B. H., DO, K. Q. & KNOPFEL, T. (1990). Potassium conductances in hippocampal neurones blocked by excitatory amino-acid transmitters. *Nature* **347,** 765-767.

CHEBIB, M., MEWETT, K. N. & JOHNSTON, G. A. R. (1998). $GABA_C$ receptor antagonists differentiate between human rho1 and rho2 receptors expressed in Xenopus oocytes. *European Journal of Pharmacology* **357,** 227-234.

CHERUBINI, E. & CONTI, F. (2001). Generating diversity at GABAergic synapses. *Trends in Neurosciences* **24,** 155-162.

CHIODINI, F. C., TASSONYI, E., HULO, S., BERTRAND, D. & MULLER, D. (1999). Modulation of synaptic transmission by nicotine and nicotinic antagonists in hippocampus. *Brain Research Bulletin* **48,** 623-628.

CHRISTIE, M. J. & NORTH, R. A. (1988). Agonists at 1-opiod, M2 muscarinic and $GABA_B$- receptors increase the same potassium conductance in the rat lateral parabrachial neurones. *British Journal of Pharmacology* **95,** 896-902.

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

CLARKE, B. P. & REUBEN, M. (1996). Release of [^{3}H]-noradrenaline from rat hippocampal synaptosomes by nicotine mediation by different nicotinic receptor subtypes from striatal [^{3}H]-dopamine release. *British Journal of Pharmacology* **117,** 596-606.

CLARKE, J. A., MEZEY, E., LAM, A. S. & BONNER, T. I. (2000). Distribution of $GABA_B$ receptors subunit gb2 in rat CNS. *Brain Research* **860,** 41-52.

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

CLEMENTI, F., FORNASARI, D. & GOTTI, C. (2000a). Neuronal nicotinic acetylcholine receptors:from structure to therapeutics. *Trends in Pharmacological Sciences* **21,** 35-37.

CLEMENTI, F., FORNASARI, D. & GOTTI, C. (2000b). Neuronal nicotinic receptors, important new players in brain function. *European Journal of Pharmacology* **393,** 3-10.

COBB, S. R., BUHL, E. H., HALASY, K., PAULSEN, O. & SOMOGYI, P. (1995). Synchronisation of neuronal activity by individual hippocampal GABAergic interneurons. *Nature* **378,** 75-78.

COBB, S. R., BULTERS, D. O. & DAVIES, C. H. (2000). Coincident activation of mGluRs and MAChRs imposes theta frequency patterning on synchronised network activity in the hippocampal CA3 region. *Neuropharmacology* **39,** 1933-1942.

COBB, S. R., BULTERS, D. O., SUCHAK, S., RIEDEL, G., MORRIS, R. G. M. & DAVIES, C. H. (1999). Activation of nicotinic acetylcholine receptors pattern network activity in the rodent hippocampus. *Journal of Physiology* **518,** 131-140.

COBB, S. R., HALASY, K., VIDA, I., NYIRI, G., TAMAS, G., BUHL, E. H. & SOMOGYI, P. (1997). Synaptic effects of identified interneurons innervating both interneurons and pyramidal cells in the rat hippocampus. *Neuroscience* **79,** 629-648.

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

COLE, A. E. & NICOLL, R. A. (1984a). Characterization of a slow cholinergic post-synaptic potential recorded *in vitro* from rat hippocampal pyramidal cells. *Journal of Physiology* **352,** 173-188.

COLE, A. E. & NICOLL, R. A. (1984b). The pharmacology of cholinergic excitatory responses in hippocampal pyramidal cells. *Brain Research* **305,** 283-290.

COLINO, A. & HALLIWELL, J. V. (1993). Carbachol potentiates Q current and activates a calcium dependent non-specific conductance in rat hippocampus in vitro. *European Journal of Neuroscience* **5,** 1198-1209.

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

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

COLLINGRIDGE, G. L. & SINGER, W. (1991). Excitatory amino acid receptors and synaptic plasticity. *Trends in Pharmacological Sciences: A Special Report,* 42-48.

COLQUHOUN, D., JONAS, P. & SAKMANN, B. (1992). Action of brief pulses of glutamate on AMPA/kainate receptors in patches from different neurones of rat hippocampal slices. *Journal of Physiology* **458,** 261-287.

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

COOPER, E., COUTURIER, S. & BALLIVET, M. (1991). Pentameric structure and subunit stoichiometry of a neuronal acetylcholine receptor. *Nature* **350.**

COUTURIER, S., BERTRAND, D., MATTER, J. M., HERNANDEZ, M. C. & BERTRAND, S. (1990a). A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BgTx. *Neuron* **5,** 847-856.

COUTURIER, S., ERKMAN, L., S., V., RUNGGER, D., BERTRAND, S., BOULTER, J., BALLIVET, M. & BERTRAND, D. (1990b). Alpha 5, alpha 3 and non-alpha 3. Three clustered avian genes encoding neuronal nicotinic acetylcholine receptor-related subunits. *Journal of Biological Chemistry* **265,** 17560-17567.

COVERNTON, P. O. J. & LESTER, R. A. J. (2002). Prolonged stimulation of presynaptic nicotinic acetylcholine receptors in the rat interpeduncular nucleus has differential effects on transmitter release. *International Journal of Developmental Neuroscience* **20,** 247-258.

CUEVAS, J. & ADAMS, D. J. (1997). M4 muscarinic receptor activation modulates calcium channels currents in rat intracardiac neurons. *Journal of Neurophysiology* **78,** 1903-1912.

CURTIS, D. R., DUGGAN, A. W., FELIX, D. & JOHNSTON, G. A. R. (1970). GABA, bicuculline and central inhibition. *Nature* **226,** 1222-1224.

CURTIS, D. R. & WATKINS, J. C. (1960). The excitation and depression of spinal neurones by structurally related amino acids. *Journal of Neurochemistry* **6,** 117-141.

CURTIS, D. R. & WATKINS, J. C. (1963). Acidic amino acids with strong excitatory actions on mammalian neurones. *Journal of Physiology* **166,** 1-14.

CUTTING, G. R., CURRISTIN, S., ZOGHBI, H., O'HARA, B., SELDIN, M. F. & UHL, G. R. (1992). Identification of a putative γ-aminobutyric acid (GABA) receptor subunit ρ2 cDNA and colocalization of the genes encoding ρ2 and ρ1 to human chromosome 6q 14-q21 and mouse chromosome 4. *Genomics* **12,** 801-806.

CUTTING, G. R., LU, L., OHARA, B. F., KASCH, L. M., MONTROSERAFIZSDEH, C., DONOVAN, D. M., SHIMADA, S., ANTONARAKIS, S. E., GUGGINO, W. B., UHL, G. R. & KAZAZIAN, H. H. (1991). Cloning of the gamma-aminobutyric-acid (GABA) rho-1 cDNA-A GABA receptor subunit highly expressed in the retina. *Proceedings of the National Academy of Sciences of the United States of America* **88,** 2673-2677.

DALE, H. H. (1914). The action of certain esters and ethers of choline, and their relation to muscarine. *The Journal of Pharmacology and Experimental Therapeutics* **6,** 147-190.

DALE, H. H. (1934). Chemical transmission of the effects of nerve impulses. *British Medical Journal* **I,** 835-841.

DALE, H. H. (1938). Acetylcholine as a chemical transmitter of the effects of nerve impulses. *Journal of Mount Sinai Hospital* **4**.

DALE, H. H., FELDBERG, W. & VOGT, M. (1936). Release of acetylcholine at voluntary motor nerve endings. *Journal of Physiology* **86,** 353-380.

DAMAJ, M. I., GLASSCO, W., DUKAT, M. & MARTIN, B. R. (1999). Pharmacological characterisation of nicotine-induced seizures in mice. *Journal of Pharmacology and Experimental Therapeutics* **291,** 1284-1291.

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

DAVIES, C. H., POZZA, M. F. & COLLINGRIDGE, G. L. (1993). CGP55845A: A potent antagonist of $GABA_B$ receptors in the CA1 region of the rat hippocampus. *Neuropharmacology* **32,** 1071-1073.

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

DAVIES, J., FRANCIS, A. A., JONES, A. W. & WATKINS, J. C. (1981). 2-amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. *Neuroscience Letters* **21,** 77-81.

DELLER, T., KATONA, I., COZZARI, C., FROTSCHER, M. & FREUND, T. F. (1999). Cholinergic innervation of mossy cells in the rat fasci dentata. *Hippocampus* **9,** 314-320.

DELLER, T. & LERANTH, C. (1990). synaptic connections of neuropeptide Y (NPY) immunoreactive neurons in the hilar area of the rat hippocampus. *Journal of Comparative Neurology* **300,** 433-447.

DENERIS, E. S., CONNOLLY, J., ROGERS, S. W. & DUVOISIN, R. (1991). Pharmacology and functional diversity of neuronal nicotinic acetylcholine receptors. *Trends in Pharmacological Sciences* **12,** 34-40.

DINGLEDINE, R., DODD, J. & KELLY, J. S. (1977). Intracellular recording from pyramidal neurones in the *in vitro* transverse hippocampal slice. *Journal of Physiology* **269,** 13P-15P.

DINOCOURT, C., PETANJEK, Z., FREUND, T. F., BEN-ARI, Y. & ESCLAPEZ, M. (2003). Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures. *Journal of Comparative Neurology* **459,** 407-425.

DODD, J., DINGLEDINE, R. & KELLY, J. S. (1981). The excitatory action of acetylcholine on hippocampal neurones of the guinea pig and rat maintained in vitro. *Brain Research* **207,** 109-127.

DOODS, H. N., BATINK, H. D., VAN CHARLDORP, K. J., DAVIDESKO, D., DE JONGE, A. & VAN ZWIETEN, P. A. (1987a). Similarities between peripheral and central M1, M2 and M3 binding sites. In *International symposium on muscarinic cholinergic mechanisms*. ed. COHEN, S. & SOKOLOVSKY, M., pp. 109-114. Freund, London.

DOODS, H. N., MATHY, M. J., DAVIDESKO, D., VAN CHARLDORP, K. J., DE JONGE, A. & VAN ZWIETEN, P. A. (1987b). Selectivity of muscarinic antagonists in radioligand and *in vivo*experiments for the putative M1, M2 and M3 receptors. *The Journal of Pharmacology and Experimental Therapeutics* **242,** 257-262.

DOUGHERTY, K. D. & MILNER, T. A. (1999). Cholinergic septal afferent terminals preferentially contact Neuropeptide Y-containing interneurones compared to parvalbumin-containing interneurons in the rat dentate gyrus. *Journal of Neuroscience* **19,** 10140-10152.

DREW, C. A., JOHNSTON, G. A. R. & WEATHERBY, R. P. (1984). Bicuculline-insensitive GABA receptors: studies on the binding of (-) baclofen to rat cerebellar membranes. *Neuroscience Letters* **52,** 317-321.

DUDAR, J. D. (1975). The effect of septal nuclei stimulation on the release of acetylcholine from the rabbit hippocampus. *Brain Research* **206,** 462-468.

DURAND, G. M., GREGOR, P. & ZHENG, X. (1992). cloning of an apparent splice variant of he rat N-methyl-D-aspartate receptor NMDAR1 with altered sensitivity to polyamines and activators of protein kinase C. *Proceedings of the National Academy of Sciences of the United States of America* **89,** 9359-9363.

DUTAR, P., BASSANT, M., SENUT, M. & LAMOUR, Y. (1995). The septohippocampal pathway: structure and function of a central cholinergic system. *Physiological Reviews* **75,** 393-413.

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

DUTAR, P. & NICOLL, R. A. (1988b). A physiological role for $GABA_B$ receptors in the central nervous system. *Nature* **332,** 156-158.

DUTAR, P. & NICOLL, R. A. (1988c). Pre- and postsynaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties. *Neuron* **1,** 585-591.

EGEBJERG, J., BETTLER, B., HERMANS-BORGMEYER, I. & HEINEMANN, S. (1991). Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. *Nature* **351,** 745-748.

EICHENBAUM, H. (1996). Is the rodent hippocampus just for 'place'? *Current Opinion in Neurobiology* **6,** 187-195.

EKEMA, G. M., ZHENG, W. & LU, L. (2002). Interaction of GABA receptor/channel ρ1 and γ2 subunit. *Investigative Ophthalmology and Visual Science* **43,** 2326-2333.

ELLIOT SMITH, G. (1910). Some problems relating to the evolution of the brain II. *Lancet* **i,** 147-153.

ENGELMAN, H. S. & MACDERMOT, A. B. (2004). Presynaptic ionotrophic receptors and control of transmitter release. *Nature Reviews Neuroscience* **5,** 135-145.

ENZ, R., BRANDSTATTER, J. H., HARTVEIT, E., WASSLE, H. & BORMANN, J. (1995). Expression of GABA receptor rho-1 and rho-2 subunits in the retina and brain of the rat. *European Journal of Neuroscience* **7,** 1495-1501.

ENZ, R. & CUTTING, G. R. (1999). $GABA_C$ receptor ρ subunits are heterogeneously expressed in the human CNS and form homo- and

heterooligomers with distinct physical properties. *European Journal of Neuroscience* **11,** 41-50.

ERIKSSON, M., NILSSON, A., FROELICH-FABRE, S., AKESSON, E., DUNKER, J., SEIGER, A., FOLKESSON, R., BENEDIKZ, E. & SUNDSTROM, E. (2002). Cloning and expression of the human N-methyl-D aspartate receptor subunit NR3A. *Neuroscience Letters* **321,** 177-181.

FABIAN-FINE, R., SKEHEL, P., ERRINGTON, M. L., DAVIES, H. A., SHER, E., STEWART, M. G. & FINE, A. (2001). ultrastructural distribution of the $\alpha 7$ nicotinic acetylcholine receptor subunit in the rat hippocampus. *The Journal of Neuroscience* **21,** 7993-8003.

FARRAR, S. J., WHITING, P. J., BONNERT, T. P. & MCKERNAN, R. M. (1999). Stoichiometry of a ligand gated ion channel determined by fluorescence energy transfer. *Journal of Biological Chemistry* **274,** 10100-10104.

FEIGENSPAN, A., WASSLE, H. & BORMANN, J. (1993). Pharmacology of GABA receptor Cl- channels in rat retinal bipolar cells. *Nature* **361,** 159-162.

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

FERNÁNDEZ DE SEVILLA, D. & BUÑO, W. (2003). Presynaptic inhibition of schaffer collateral synapses by stimulation of hippocampal cholinergic afferent fibres. *European Journal of Neuroscience* **17,** 555-558.

FERNÁNDEZ DE SEVILLA, D., CABEZAS, C., OSHIMA DE PRADA, A. N., SÁNCHEZ-JIMÉNEZ, A. & BUÑO, W. (2002). Selective muscarinic regulation of functional glutamatergic schaffer collateral synapses in rat CA1 pyramidal neurons. *Journal of Physiology* **545,** 51-63.

FISAHN, A., PIKE, F. G., BUHL, E. H. & PAULSEN, O. (1998). Cholinergic induction of network oscillations at 40Hz in the hippocampus in vitro. *Nature* **394,** 186-189.

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

FRASER, D. D. & MACVICAR, B. A. (1996). Cholinergic-dependent plateau potential in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience* **16,** 4113-4128.

FRAZIER, C. J., BUHLER, A. V., WEINER, J. L. & DUNWIDDIE, T. V. (1998a). Synaptic potentials mediated via α-Bungarotoxin-sensitive nicotinic acetylcholine receptors in rat hippocampal interneurons. *Journal of Neuroscience* **18,** 8228-8235.

FRAZIER, C. J., ROLLINS, Y. D., BREESE, C. R., LEONARD, S., FREEDMAN, R. & DUNWIDDIE, T. V. (1998b). Acetylcholine activates an α-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *Journal of Neuroscience* **18,** 1187-1195.

FREEDMAN, R., ADAMS, C. E. & LEONARD, S. (2000). The alpha 7-nicotinic acetylcholine receptor and the pathology of hippocampal interneurones in schizophrenia. *Journal of Chemical Neuroanatomy* **20,** 299-306.

FREEDMAN, R., COON, H., MYLES-WORSLEY, M., ORR-URTREGER, A., OLINCY, A., DAVIS, A., POLYMEROPOULOS, M., HOLIK, J., HOPKINS, J., HOFF, M., ROSENTHAL, J., WALDO, M. C., REIMHERR, F., WENDER, P., YAW, J., YOUNG, D. A., BREESE, C. R., ADAMS, C., PATTERSON, D., ALDER, L. E., KRUGLYAK, L., LEONARD, R. J. & BYERLEY, W. (1997). Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus. *Proceedings of the National Academy of Sciences of the United States of America* **94,** 587-592.

FREEDMAN, R., WETMORE, C., STROMBERG, I., LEONARD, S. & OLSEN, L. (1993). α-bungarotoxin binding to hippocampal interneurons-immunocytochemical characterisation and effects on growth factor expression. *Journal of Neuroscience* **13,** 1965-1975.

FREUND, T. F. (2003). Interneuron diversity series: Rhythm and mood in perisomatic inhibition. *Trends in Neurosciences* **26,** 489-495.

FREUND, T. F. & ANTAL, M. (1988). GABA-containing neurons in the septum control inhibitory interneurons in the hippocampus. *Nature* **336,** 170-173.

FREUND, T. F. & BUZSAKI, G. (1996). Interneurons of the hippocampus. *Hippocampus* **6,** 347-470.

FRITSCHY, J.-M. & BRUNIG, I. (2003). Formation and plasticity of GABAergic synapses: physiological mechanisms and pathophysiological implications. *Pharmacology and Therapeutics* **98,** 299-323.

FROTSCHER, M. & LERANTH, C. (1985). Cholinergic innervation of the rat hippocampus as revealed by choline acetyltransferase immunocytochemistry: a combined light and electron microscopic study. *Journal of Comparative Neurology* **239,** 237-246.

FROTSCHER, M., SCHLANDER, M. & LERANTH, C. (1986). Cholinergic neurons in the hippocampus: a combined light and electron microscopic immunocytochemical study in the rat. *Cell Tissue Research* **246,** 237-246.

FROTSCHER, M., VIDA, I. & BENDER, R. (2000). Evidence for the existence of non-gabaergic, cholinergic interneurons in the rodent hippocampus. *Neuroscience* **96,** 27-31.

FUJII, S., JI, Z., MORITA, N. & SUMIKAWA, K. (1999). Acute and chronic nicotine exposure differentially facilitate the induction of LTP. *Brain Research* **846,** 137-143.

FUJII, S., JIA, Y., YANG, A. & SUMIKAWA, K. (2000). Nicotine reverses GABAergic inhibition of long-term potentiation induction in the hippocampal CA1 region. *Brain Research* **863,** 259-265.

GAHWILER, B. H. & BROWN, D. A. (1985). Functional innervation of cultured hippocampal neurones by cholinergic afferents from co-cultured septal explants. *Nature* **313,** 577-579.

GARTNER, U., HARTIG, W., BRAUER, K., BRUCKNER, G. & ARENDT, T. (2001). Electron microscope evidence for different myelination of rat septohippocampal fibres. *Neuroreport* **12,** 17-20.

GEIGER, J. R. P., MELCHER, T., KOH, D.-S., SAKMANN, B., SEEBURG, P. H., JONAS, P. & MONYER, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability on AMPA receptors in principal neurons and interneurones in rat CNS. *Neuron* **15,** 193-204.

GEREAU, R. W. & CONN, P. J. (1995). Multiple presynaptic metabotrophic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. *Journal of Neuroscience* **15,** 6879-6889.

GERZANICH, V., ANAND, R. & LINDSTROM, J. (1994). Homomers of $\alpha 8$ and $\alpha 7$ subunits of nicotinic receptors exhibit similar channel but contrasting binding site properties. *Molecular Pharmacology* **45,** 212-220.

GIACHETTI, A., MICHELETTI, R. & MONTAGNA, E. (1986). Cardioselective profile of AF-DX 116, a muscarinic M2 receptor antagonist. *Life Sciences* **38,** 1663-1672.

GIROD, R. & ROLE, L. W. (2001). Long-lasting enhancement of glutamatergic synaptic transmission by acetylcholine contrasts with response adaptation after exposure to low-level nicotine. *Journal of Neuroscience* **21,** 5182-5190.

GOEBEL, D. J. & POOSCH, M. S. (1999). NMDA receptor subunit gene expression in the rat brain: a quantitative analysis of endogenous mRNA levels of $NR1_{com}$, NR2A, NR2B, NR2C, NR2D and NR3A. *Brain Research. Molecular Brain Research* **69,** 164-170.

GOLDBACH, R., ALLGAIER, C., HEIMRICH, B. & JACKISCH, R. (1998). Postnatal development of muscarinic autoreceptors modulating acetylcholine release in the septohippocampal cholinergic system I. Axon terminal region:hippocampus. *Developmental Brain Research* **108,** 23-30.

GOTTI, C., FORNASARI, D. & CLEMENTI, F. (1997). Human neuronal nicotinic receptors. *Progress in Neurobiology* **53,** 199-237.

GRAY, R., RAJAN, A. S., RADCLIFFE, K. A., YAKEHIRO, M. & DANI, J. A. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* **383,** 713-716.

GUERINEAU, N. C., BOSSU, J.-L., GAHWILER, B. H. & GERBER, U. (1995). Activation of a non-selective cation conductance by metabotrophic glutamatergic and muscarinic agonists in CA3 pyramidal neurones of the rat hippocampus. *Journal of Neuroscience* **15,** 4395-4407.

GUERINEAU, N. C., GAHWILER, B. H. & GERBER, U. (1994). reduction of resting K^+ current by metabotrophic glutamate and muscarinic receptors in rat CA3 cells: mediation by G-proteins. *Journal of Physiology* **474,** 27-33.

GULYAS, A. I., ACSADY, L. & FREUND, T. F. (1999a). Structural basis of the cholinergic and serotonergic modulation of GABAergic neurons in the hippocampus. *Neurochemistry International* **34,** 359-372.

GULYAS, A. I. & FREUND, T. F. (1996). Pyramidal cell dendrites are the primary targets of calbindin D_{28K}- immunoreactive interneurons in the hippocampus. *Hippocampus* **6,** 525-534.

GULYAS, A. I., HAJOS, N. & FREUND, T. F. (1996). Interneurons containing calretinin are specialized to control other interneurones in the rat hippocamus. *Journal of Neuroscience* **16,** 3397-3411.

GULYAS, A. I., HAJOS, N., KATONA, I. & FREUND, T. F. (2003). Interneurons are the local targets of hippocampal inhibitory cells which project to the medial septum. *European Journal of Neuroscience* **17,** 1861-1872.

GULYAS, A. I., MEGIAS, M., EMRI, Z. & FREUND, T. F. (1999b). Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. *Journal of Neuroscience* **19,** 10082-10097.

GULYAS, A. I., MILES, R., HAJOS, N. & FREUND, T. F. (1993a). Precision and variability in postsynaptic target selection of inhibitory cells in the hippocampal CA3 region. *European Journal of Neuroscience* **5**.

GULYAS, A. I., MILES, R., SIK, A., TOTH, K., TAMAMAKI, N. & FREUND, T. F. (1993b). Hippocampal pyramidal cells excite inhibitory neurones through a single release site. *Nature* **366,** 683-687.

GULYAS, A. I., TOTH, K., DANOS, P. & FREUND, T. F. (1991). Subpopulations of GABAergic neurons containing parvalbumin, calbindin D28k, and cholecystokinin in the rat hippocampus. *Journal of Comparative Neurology* **312,** 371-378.

HAAS, H. L. (1982). Cholinergic disinhibition in hippocampal slices of the rat. *Brain Research* **233,** 200-204.

HAJ-DAHMANE, S. & ANDRADE, R. (1999). muscarinic receptors regulate two different calcium-dependent cation currents in the prefrontal cortex. *European Journal of Neuroscience* **11,** 1973-1980.

HÁJOS, N. & MODY, I. (1997). Synaptic communication among hippocampal interneurons: Properties of spontaneous IPSCs in morphologically identified cells. *The Journal of Neuroscience* **17,** 8427-8442.

HÁJOS, N., PAPP, E. C., ACSADY, L., LEVEY, A. I. & FREUND, T. F. (1998). Distinct interneuron types express M2 muscarinic receptor immunoreactivity on their dendrites or axon terminals in the hippocampus. *Neuroscience* **82,** 355-376.

HALLIWELL, J. V. & ADAMS, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research* **250,** 71-92.

HAMMER, R., BERRIE, C. P., BIRDSALL, N. J. M., BURGEN, A. S. V. & HULME, E. C. (1980). Pirenzepine distinguishes between different subclasses of muscarinic receptors. *Nature* **283,** 90-92.

HAMMER, R., GIRALDO, E., SCHIAVI, G. B., MONFERINI, E. & LADINSKY, H. (1986). Binding profile of a novel cardioselective muscarinic receptor antagonist, AF-DX 116, to membranes of peripheral tissues and brain in the rat. *Life Sciences* **38,** 1653-1662.

HAN, Z. S., BUHL, E. H., LORINCZI, Z. & SOMOGYI, P. (1993). A high degree of spatial selectivity in the axonal and dendritic domains of physiologically identified local-circuit neurones in the dentate gyrus of the rat hippocampus. *European Journal of Neuroscience* **5,** 395-410.

HARRIS, K. M., MARSHALL, P. E. & LANDIS, D. M. (1985). Ultrastructural study of the cholecystokinin-immunoreactive cells and processes in area CA1 of the rat hippocampus. *Journal of Comparative Neurology* **233,** 147-158.

HARVEY, R. J., KIM, H.-C. & DARLISON, M. G. (1993). Molecular cloning reveals the existence of a fourth gamma subunit of the vertebrate brain $GABA_A$ receptor. *FEBS Letters* **331,** 211-216.

HASSELMO, M. E. (1999). Neuromodulation: acetylcholine and memory consolidation. *Trends in Cognitive Sciences* **3,** 351-359.

HASSELMO, M. E. & FEHLAU, B. P. (2001). Differences in time course of ACh and GABA modulation of excitatory synaptic potentials in slices of rat hippocampus. *Journal of Neurophysiology* **86,** 1792-1802.

HAYASHI, T. (1952). A physiological study of epileptic seizures following cortical stimulation in animals and its application to human clinics. *Japanese Journal of Physiology* **3,** 46-64.

HAYASHI, T. (1954). Effects of sodium glutamate on the nervous system. *Keio Journal of Medicine* **3,** 183-192.

HEFFT, S., HULO, S., BERTRAND, D. & MULLER, D. (1999). Synaptic transmission at nicotinic acetylcholine receptors in rat hippocampal organotypic cultures and slices. *Journal of Physiology* **515,** 769-776.

HEIMRICH, B. & FROTSCHER, M. (1993). Formation of the septohippocampal projection *in vitro*: An electron microscopic immunocytochemical study of cholinergic synapses. *Neuroscience* **52,** 815-827.

HERB, A., BURNASHEV, N. & WERNER, P. (1992). The KA-2 subunit of excitatory amino acid receptors shows widespread expression in the brain and forms ion channels with distantly related subunits. *Neuron* **8,** 775-785.

HERRICK, C. J. (1910). The morphology of the forebrain in amphibia and reptilia. *Journal of comp. Neurol. Psychol.* **20,** 413-547.

HILL, D. R. & BOWERY, N. G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in the rat brain. *Nature* **290,** 149-152.

HOLLMANN, M., BOULTER, J. & MARON, C. (1993). Zinc potentiates agonist-induced currents at certain spliced variants of the NMDA receptor. *Neuron* **10,** 943-954.

HOLLMANN, M., O'SHEA-GREENFIELD, A., ROGERS, S. W. & HEINEMANN, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. *Nature* **342,** 643-648.

HOUAMED, K. M., KUIJPER, J. L., GILBERT, T. L., HALDEMAN, B. A., O'HARA, P. J., MULVIHILL, E. R., ALMERS, W. & HAGEN, F. S. (1991). Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. *Science* **252,** 1318-1321.

HOUNSGAARD, J. (1978). Presynaptic inhibitory action of acetylcholine in area CA1 of the hippocampus. *Experimental Neurology* **62,** 787-797.

HUETTNER, J. E. (2003). Kainate receptors and synaptic transmission. *Progress in Neurobiology* **70,** 387-407.

HULME, E. C., BIRDSALL, N. J. M. & BUCKLEY, N. J. (1990). Muscarinic receptor subtypes. *Annual Review of Pharmacology and Toxicology* **30,** 633-673.

HULO, S. & MULLER, D. (2001). Tetrodotoxin-sensitive enhancement of inhibition in CA1 pyramidal neurones by nicotine. *Neuroreport* **12,** 1351-1354.

HUNT, R. & TAVEAU, R. D. (1906). On the physiological action of certain cholin derivatives and new methods for detecting cholin. *British Medical Journal* **II,** 1788-1791.

HYMAN, B. T., VAN HOESEN, G. W. & DAMASIO, A. R. (1990). Memory-related neural systems in Alzheimer's disease: an anatomical study. *Neurology* **40,** 1721-1729.

ISHIZUKA, N., COWAN, W. M. & AMARAL, D. G. (1995). A quantitative analysis of the dendritic organization of pyramidal cells in the rat hippocampus. *Journal of Comparative Neurology* **362,** 17-45.

ITO, I., KOHDA, A., TANABE, S., HIROSE, E., HAYASHI, M., MITSUNAGA, S. & SUGIYAMA, H. (1992). 3,5-Dihydroxyphenylglycine: a potent agonist of metabotrophic glutamate receptors. *Neuroreport* **3,** 1013-1016.

JACKSON, P. A., KESNER, R. P. & AMANN, K. (1998). Memory for duration: role of hippocampus and medial prefrontal cortex. *Neurobiology of Learning and Memory* **70,** 328-348.

JAFFARD, R. & MEUNIER, M. (1993). Role of the hippocampal formation in learning and memory. *Hippocampus* **3,** 203-218.

JAKAB, R. L. & LERANTH, C. (1995). Septum. In *The Rat Nervous System.*

JAMES, J. R. & NORDBERG, A. (1995). Genetic and environmental aspects of the role of nicotinic receptors in neurodegenerative disorder: emphasis on Alzheimer's disease and Parkinson's disease. *Behavior Genetics* **25,** 149-159.

JARRARD, L. E. (1993). On the role of the hippocampus in learning and memory in the rat. *Behavioral and Neural Biology* **60,** 9-26.

JEFFERYS, J. G. R. (1993). The pathophysiology of epilepsies. In *A text book of epilepsy*. ed. LAIDLAW, J., RICHENS, A. & CHADWICK, D. W. Churchill Livingston, Edinburgh.

JI, D. & DANI, J. A. (2000). Inhibition and disinhibition of pyramidal neurons by activation of nicotine receptors on hippocampal interneurons., 2682-2690.

JOHNSON, J. W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature* **325,** 529-531.

JOHNSTON, G. A. R. (1996a). GABAA receptor pharmacology. *Pharmacology and Therapeutics* **69,** 173-198.

JOHNSTON, G. A. R. (1996b). $GABA_C$ receptors: relatively simple transmitter-gated ion-channels? *Trends in Pharmacological Sciences* **17,** 319-323.

JOHNSTON, G. A. R., CURTIS, D. R., BEART, P. M., GAME, C. J. A., MCCULLOCH, R. M. & TWITCHEN, B. (1975). Cis and trans-4-aminocrotonic acid as GABA analogues of restricted conformation. *Journal of Neurochemistry* **24,** 157-160.

JOHNSTON, G. A. R., CURTIS, D. R., DAVIES, J. & MCCULLOCH, R. M. (1974). Spinal interneurone excitation by conformationally restricted analogues of L-glutamic acid. *Nature* **248,** 804-805.

JONAS, P. & SAKMANN, B. (1992). Glutamate receptor channels in isolated patches from CA1 and CA3 pyramidal cells of rat hippocampal slices. *Journal of Physiology* **455,** 143-147.

JONES, K. A., BOROWSKY, B., TAMM, J. A., CRAIG, D. A., DURKIN, M. M., DAI, M., YAO, W.-J., JOHNSON, M., GUNWALDSEN, C., HUANG, L.-Y., TANG, C., SHEN, Q., SALON, J. A., MORSE, K., LAZ, T., SMITH, K. E., NAGARATHNAM, D., NOBLE, S. A., BRANCHEK, T. A. & GERALD, C. (1998). GABAB receptors function as a heteromeric assembly of the subunits GABABR1 and GABABR2. *Nature* **396,** 674-679.

JONES, S., SUDWEEKS, S. & YAKEL, J. L. (1999). Nicotinic receptors in the brain: correlating physiology with function. *Trends in Neurosciences* **22,** 555-561.

JONES, S. & YAKEL, J. L. (1997). Functional nicotinic ACh receptors on interneurones in the rat hippocampus. *Journal of Physiology* **504,** 603-610.

KÁSA, P., RAKONCZAY, Z. & GULYA, K. (1997). The cholinergic system in Alzheimer's disease. *Progress in Neurobiology* **52,** 511-535.

KAUPMANN, K., HUGGEL, K., HEID, J., FLOR, P. J., BISCHOFF, S., MICKEL, S. J., MCMASTER, G., ANGST, C., BITTIGER, H., FROESTL, W. & BETTLER, B. (1997). Expression cloning of GABAB receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386,** 239-246.

KAUPMANN, K., MALITSCHEK, B., SCHULER, V., HEID, J., FROESTL, W., BECK, P., MOSBACHER, J., BISCHOFF, S., KULIK, A., SHIGEMOTO, R., KARSCHIN, A. & BETTLER, B. (1998). GABAB-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396,** 683-687.

KAWAI, H., ZAGO, W. & BERG, D. K. (2002). Nicotinic receptor clusters on hippocampal GABAergic neurons: Regulation by synaptic activity and neurotrophins. *The Journal of Neuroscience* **22,** 7903-7912.

KEINANEN, K., WISDEN, W. & SOMMER, B. (1990). A family of AMPA-selective glutamate receptors. *Science* **249,** 556-560.

KITT, C. A., PRICE, D. L., DELONG, M. R., STRUBLE, R. G., MITCHELL, S. J. & HEDREEN, J. C. (1982). The nucleus basalis of Meynert: projections to the cortex, amygdala, and hippocampus. *Proceedings of the Society for Neuroscience* **8,** 212.

KLAUSBERGER, T., MAGILL, P. J., MÁRTON, L. F., ROBERTS, J. D. B., COBDEN, P. M., BUZSÁKI, G., SOMOGYI, P. & . (2003). Brain-state- and Cell-type-specific firing of hippocampal interneruons *in vivo*. *Nature* **421,** 844-848.

KOBAYASHI, M. & BUCKMASTER, P. S. (2003). Reduced inhibition of dentate granule cells in a model of temporal lobe epilepsy. *Journal of Neuroscience* **23,** 2440-2452.

KOHLER, C. & CHAN-PALAY, V. (1982). Somatostatin-like immunoreactive neurons in the hippocampus: an immunocytochemical study in the rat. *Neuroscience Letters* **34,** 259-264.

KOHLER, C., CHAN-PALAY, V. & WU, J.-Y. (1984). Septal neurones containing glutamic acid decarboxylase immunoreactivity project to the hippocampal region in the rat brain. *Anatomy and Embryology* **169,** 41-44.

KOSAKA, T., KATSUMARU, H., HAMA, K., WU, J.-Y. & HEIZMANN, C. W. (1987). GABAergic neurons containing the Ca^{2+}-binding protein parvalbumin in the rat hippocampus and dentate gyrus. *Brain Research* **419,** 119-130.

KOSAKA, T., KOSAKA, K., TATEISHI, K., HAMAOKA, Y., YANAIHARA, N., WU, J.-Y. & HAMA, K. (1985). GABAergic neurons containing CCK-8-like

and/or VIP-like immunoreactivities in the rat hippocampus and dentate gyrus. *Journal of Comparative Neurology* **239,** 420-430.

KRNJEVIC, K. & PHILLIS, J. W. (1963). Acetylcholine-sensitive cells in the cerebral cortex. *Journal of Physiology* **166,** 296-327.

KRNJEVIC, K. & SCHWARTZ, S. (1967). The action of γ-aminobutyric acid on cortical neurones. *Experimental Brain Research* **3,** 320-366.

KUBO, T., FUKUDA, K., MIKAMI, A., MAEDA, A., TAKAHASHI, H., MISHINA, M., HAGA, T., HAGA, K., ICHIYAMA, A., KANGAWA, K., KOJIMA, M., MATSUO, H., HIROSE, T. & NUMA, S. (1986). Cloning, sequencing and expression of complimentary DNA encoding the muscarinic acetylcholine receptor. *Nature* **323,** 411-416.

KULLMAN, D. M. (2001). presynaptic kainate receptors in the hippocampus: slowly emerging from obscurity. *Neuron* **32,** 561-564.

KUNKEL, D. D., LACAILLE, J.-C. & SCHWARTZKROIN, P. A. (1988). Ultrastructure of stratum lacunosum-moleculare interneurons of hippocampal CA1 region. *Synapse* **2,** 382-394.

KURIYAMA, K. & OHMORI, Y. (1990). Solubilization and partial purification of cerebral $GABA_B$ receptors. In *$GABA_B$ receptors in mammalian function.* ed. BOWERY, N. G., BITTEGER, H. & OLPE, H.-R., pp. 183-196. Wiley, Chichester.

LACAILLE, J.-C. (1991). Postsynaptic potentials mediated by excitatory and inhibitory amino acids in interneurones of *stratum pyramidale* of the CA1 region of rat hippocampal slices. *Journal of Neurophysiology* **66,** 1441-1454.

LACAILLE, J.-C. & SCHWARTZKROIN, P. A. (1988). Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. intracellular response characteristics, synaptic responses and morphology. *Journal of Neuroscience* **8,** 1400-1410.

LANFUMEY, L., ADRIEN, J. & GRAY, J. A. (1982). Septal driving of hippocampal theta rhythm as a function of frequency in the infant male rat. *Experimental Brain Research* **45,** 230-232.

LANGLEY, J. N. (1907). On the contraction of muscle, chiefly in relation to the presence of "receptive" substances. *Journal of Physiology* **36,** 347-384.

LEBLANC, M. O. & BLAND, B. H. (1979). Developmental aspects of hippocampal electrical activity and motor behaviour in the rat. *Experimental Neurology* **66,** 220-237.

LERANTH, C. & FROTSCHER, M. (1987). Cholinergic innervation of hippocampal GAD- and somatostatin-immunoreactive commissural neurons. *Journal of Comparative Neurology* **261,** 33-47.

LERANTH, C., SZEIDEMANN, Z., HSU, M. & BUZSAKI, G. (1996). AMPA receptors in the rat and primate hippocampus: a possible absence of GluR2/3 subunits in most interneurones. *Neuroscience* **70**.

LESTER, R. A. J., CLEMENTS, J. D., WESTBROOK, G. L. & JAHR, C. E. (1990). Channel kinetics determine the time course of NMDA receptor mediated synaptic currents. *Nature* **346,** 565-567.

LEVEY, A. I., EDMUNDS, S. M., KOLIATSAS, V., WILEY, R. G. & HEILMAN, C. J. (1995). Expression of m1-m4 muscarinic acetylcholine receptor protein in the rat hippocampus and regulation by cholinergic innervation. *Journal of Neuroscience* **15,** 4077-4092.

LEWIS, P. R. & SHUTE, C. C. D. (1967b). The cholinergic limbic system: projections to hippocampal formation, medial cortex, nuclei of the ascending cholinergic reticular system and subfornical organ and supraoptic crest. *Brain* **90**.

LEWIS, P. R., SHUTE, C. C. D. & SILVER, A. (1967a). Confirmation from choline acetylase analyses of a massive innervation to the rat hippocampus. *Journal of Physiology* **191,** 215-224.

LIANG, J. S., CARSI-GABRENAS, J., KRAJEWSKI, J. L., MCCAFFERTY, J., PURKERSON, S. L., SANTIAGO, M., STRAUSS, W., VALENTINE, H. H. & POTTER, L. T. (1996). Antimuscarinic toxins from *Dendroaspis angusticeps*. *Toxicon* **34,** 1257-1267.

LINO, M., OZAWA, S. & TSUZUKI, K. (1990). Permeation of calcium through excitatory amino acid receptor channels in cultured hippocampal neurons. *Journal of Physiology* **424,** 151-165.

LIU, Z. W., YANG, S., ZHANG, Y. X. & LIU, C. H. (2003). Presynaptic alpha-7 nicotinic acetylcholine receptors modulate excitatory synaptic transmission in hippocampal neurons. *Sheng Li Xue Bao* **55,** 731-735.

LOMELI, H., WISDEN, W., KOHLER, M., KEINANEN, K., SOMMER, B. & SEEBURG, P. H. (1992). High-affinity kainate and domoate receptors in rat brain. *FEBS Letters* **307,** 139-143.

LORENTE DE NO, R. (1934). Studies on the structure of the cerebral cortex II. Continuation of the study of the ammonic system. *Journal of Psychol. and Neurol.* **46,** 113-177.

LOSCHER, W., POTSCHKA, H., WLAZ, P., DANYSZ, W. & PARSONS, C. G. (2003). Are neuronal nicotinic receptors a target for antiepileptic drug development? Studies in different seizure models in mice and rats. *European Journal of Pharmacology* **466,** 99-111.

LUJAN, R., NUSSER, Z., ROBERTS, J. D. B., SHIGEMOTO, R. & SOMOGYI, P. (1996). Perisynaptic location of metabotrophic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *European Journal of Neuroscience* **8,** 1488-1500.

MACCAFERRI, G. & LACAILLE, J.-C. (2003). *Interneruone Diversity series*:Hippocampal interneuron classifications- making things as simple as possible, not simpler. *Trends in Neurosciences* **26,** 564-571.

MACDONALD, R. L. & OLSEN, R. W. (1994). $GABA_A$ receptor channels. *Annual Review of Neuroscience* **17,** 569-602.

MADISON, D. V., LANCASTER, B. & NICOLL, R. A. (1987). Voltage clamp analysis of cholinergic action in the hippocampus. *Journal of Neuroscience* **7,** 733-741.

MANN, D. M. & YATES, P. O. (1986). neurotransmitter deficits in Alzheimer's disease and in other dementing disorders. *Human Neurobiology* **5.**

MARCHI, M. & RAITERI, M. (1989). Interaction acetylcholine-glutamate in rat hippocampus: involvement of two subtypes of M-2 muscarinic receptors. *Journal of Pharmacology and Experimental Therapeutics* **248,** 1255-1260.

MARGETA-MITROVIC, M., MITROVIC, I., RILEY, R., JAN, L. Y. & BASBAUM, A. I. (1999). Immunohistochemical localization of $GABA_B$ receptors in the rat central nervous system. *Journal of Comparative Neurology* **405,** 299-321.

MARINO, M. J., ROUSE, S. T., LEVEY, A. I., POTTER, L. T. & CONN, P. J. (1998). Activation of the genetically defined m1 muscarinic receptor potentiates NMDA receptor currents in hippocampal pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America* **95,** 11465-11470.

MARTIN, I. L. & DUNN, S. M. J. (2002). GABA receptors. *Tocris Reviews* **20.**

MASU, M., TANABE, Y., TSUCHIDA, K., SHIGEMOTO, R. & NAKANISHI, S. (1991). Sequence and expression of a metabotrophic glutamate receptor. *Nature* **349,** 760-765.

MATSUDA, K., KAMIYA, Y., MATSUDA, S. & YUZAKI, M. (2002). Cloning and characterisation of a novel NMDA receptor subunit NR3: a dominant

subunit that reduces calcium permeability. *Brain Research. Molecular Brain Research* **100,** 43-52.

MATTHEWS, D. A., NADLER, J. V., LYNCH, G. S. & COTMAN, C. W. (1974). Development of cholinergic innervation in the hippocampal formation in the rat
1. Histochemical demonstration of acetylcholinesterase activity. *Developmental Biology* **36,** 130-141.

MATTHEWS, D. A., SALVATERRA, P. M., CRAWFORD, G. D., HOUSER, C. R. & VAUGHN, J. E. (1987). An immunocytochemical study of choline acetyltransferase-containing neurons and axon terminals in normal and partially deafferented hippocampal formation. *Brain Research* **402,** 30-43.

MAX, S. I., LIANG, J. S. & POTTER, L. T. (1991). m1-toxin: isolation, characterisation and selectivity for m1 muscarinic receptors. *Neuroscience Abstracts* **17,** 389.

MAX, S. I., LIANG, J. S. & POTTER, L. T. (1993a). Purification and properties of m1-toxin, a specific antagonist of m1 muscarinic receptors. *Journal of Neuroscience* **13,** 4293-4300.

MAX, S. I., LIANG, J. S., PURKERSON, S. L. & POTTER, L. T. (1993b). m4 toxin, a selective, reversible allosteric antagonist of m4 muscarinic receptors. *Neuroscience Abstracts* **19,** 462.

MAYCOX, P. R., HELL, J. W. & JAHN, R. (1990). Amino acid neurotransmission: spotlight on synaptic vesicles. *Trends in Neurosciences* **13,** 83-87.

MAYER, M. L., WESTBROOK, G. L. & GUTHRIE, P. B. (1984). Voltage-dependent block by Mg^{+} of NMDA response in spinal cord neurones. *Nature* **309,** 261-263.

MCBAIN, C. J., DICHIARA, T. J. & KAUER, J. A. (1994). Activation of metabotrophic glutamate receptors differentially affects two classes of hippocampal interneurons and potentiates excitatory synaptic transmission. *Journal of Neuroscience* **14,** 4433-4445.

MCBAIN, C. J. & DINGLEDINE, R. (1993). Heterogeneity of synaptic glutamate receptors on CA3 stratum radiatum interneurons of rat hippocampus. *Journal of Physiology* **462,** 373-392.

MCBAIN, C. J. & FISAHN, A. (2001). Interneurones unbound. *Nature Reviews Neuroscience* **2,** 11-23.

MCCULLOCH, R. M., JOHNSTON, G. A. R., GAME, C. J. A. & CURTIS, D. R. (1974). The differential sensitivity of spinal interneurones and renshaw

cells to kainate and N-methyl-D-aspartate. *Experimental Brain Research* **21,** 515-518.

MCGEHEE, D. S., HEATH, M. J. S., GELBER, S., DEVAY, P. & ROLE, L. W. (1995). Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science* **269,** 1692-1696.

MCGEHEE, D. S. & ROLE, L. (1995). Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annual Review of Physiology* **57,** 521-546.

MCINTYRE, D. C. & RACINE, R. J. (1986). kindling mechanisms: current progress on an experimental epilepsy model. *Progress in Neurobiology* **27,** 1-12.

MCKERNAN, R. M. & WHITING, P. J. (1996). Which $GABA_A$-receptor subtypes really occur in the brain? *Trends in Neurosciences* **19,** 139-143.

MCNAUGHTON, B. L. (1980). Evidence for two physiological distinct perforant pathways to the fascia dentata. *Brain Research* **199,** 1-19.

MCQUISTON, A. R. & MADISON, D. V. (1999a). Muscarinic receptor activity has multiple effects on the resting membrane potentials of CA1 hippocampal interneurons. *The Journal of Neuroscience* **19,** 5693-5702.

MCQUISTON, A. R. & MADISON, D. V. (1999b). Muscarinic receptor activity induces an afterdepolarisation in a subpopulation of hippocampal CA1 interneurons. *The Journal of Neuroscience* **19,** 5703-5710.

MCQUISTON, A. R. & MADISON, D. V. (1999c). Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. *The Journal of Neuroscience* **19,** 2887-2896.

MIETTINEN, R., GULYAS, A. I., BAIMBRIDGE, K. G., JACOBOWITZ, D. M. & FREUND, T. F. (1992). Calretinin is present in non-pyramidal cells of the rat hippocampus. II co-existence with other calcium binding proteinsand GABA. *Neuroscience* **48,** 29-43.

MILES, R., TOTH, K., GULYAS, A. I., HAJOS, N. & FREUND, T. F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16,** 815-823.

MILLIGAN, C. J., BUCKLEY, N. J., GARRET, M., DEUCHARS, J. & DEUCHARS, S. A. (2004). Evidence for inhibition mediated by Coassembly of $GABA_A$ and $GABA_C$ receptor subunits in native central neurones. *Journal of Neuroscience* **24,** 7241-7250.

MILNER, T. A., LOY, R. & AMARAL, D. G. (1983). An anatomical study of the development of the septo-hippocampal projection in the rat. *Developmental Brain Research* **8,** 343-371.

MINAKAMI, R., KATSUKI, F., YAMAMOTO, T., NAKAMURA, K. & SUGIYAMA, H. (1994). Molecular cloning and the functional expression of two isoforms of human metabotrophic glutamate receptor subtype 5. *Biochemical and Biophysical Research Communications* **199,** 1136-1143.

MISHINA, M., TAKAI, T., IMOTO, K., NODA, M., TAKAHASHI, T., NUMA, S., METHFESSEL, C. & SAKMANN, B. (1986). Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. *Nature* **321,** 406-411.

MITCHELL, S. J., RAWLINS, J. N. P., STEWARD, O. & OLTON, D. S. (1982). Medial septal area lesions disrupt θ rhythm and cholinergic staining in medial entorhinal cortex and produce impaired radial arm maze behaviour in rats. *Journal of Neuroscience* **2,** 292-302.

MONAGHAN, D. T., YAO, D. & COTMAN, C. W. (1985). l-[^{3}H]Glutamate binds to kainate-, NMDA- and AMPA-sensitive binding sites: an autoradiographic analysis. *Brain Research* **340,** 378-383.

MONYER, H. & SEEBURG, P. H. (1992). Developmental expression of NMDA receptor subtypes. *Society for Neuroscience Abstracts* **18,** #172.171.

MONYER, H., SEEBURG, P. H. & WISDEN, W. (1991). Glutamate-operated channels -developmentally early and mature forms arise by alternative splicing. *Neuron* **6,** 799-810.

MONYER, H., SPRENGEL, R., SCHOEPFER, R., HERB, A., HIGUCHI, M., LOMELI, H., BURNASHEV, N., SAKMANN, B. & SEEBURG, P. H. (1992). Heteromeric NMDA receptors- molecular and functional distinction of subtypes. *Science* **256,** 1217-1221.

MORIYOSHI, K., MASU, M., ISHII, T., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1991). Molecular cloning and characterisation of the rat NMDA receptor. *Nature* **354,** 31-37.

MORTON, R. A. (1998). Modulation Of Cholinergic Synaptic Transmission in The Rat Hippocampus. In *The Department of Neuroscience*, pp. 171. The University of Edinburgh, Edinburgh.

MORTON, R. A., BULTERS, D. O. & DAVIES, C. H. (1997). $GABA_B$ receptor-mediated modulation of muscarinic acetylcholine receptor-mediated synaptic responses in the rat hippocampus. *British Journal of Pharmacology* **122,** 274P.

MORTON, R. A. & DAVIES, C. H. (1997). Regulation of muscarinic acetylcholine receptor-mediated synaptic responses by adenosine receptors in the rat hippocampus. *Journal of Physiology* **502,** 75-90.

MORTON, R. A., MANUEL, N. A., BULTERS, D. O., COBB, S. R. & DAVIES, C. H. (2001). Regulation of muscarinic acetylcholine receptor-mediated synaptic responses by $GABA_B$ receptors in the rat hippocampus. *Journal of Physiology* **535,** 757-766.

MÜLLER, W. & MISGELD, U. (1986). Slow cholinergic excitation of guinea pig hippocampal neurons is mediated by two types of muscarinic receptor. *Neuroscience Letters* **67,** 107-112.

MUMBY, D. G., PINEL, J. P. J. & KORNECOOK, T. J. (1992). Dissociating the effects of hippocampal and amygdalar lesions in rats with a battery of nonspatial memory tasks. *Society for Neuroscience Abstracts* **18.**

MUMBY, D. G., PINEL, J. P. J., KORNECOOK, T. J., SHEN, M. J. & REDILA, V. A. (1995). Memory deficits following lesions of the hippocampus or amygdala: assessment by an object-memory test battery. *Psychobiology* **23,** 26-36.

MURATA, Y., WOODWARD, R. M., MILEDI, R. & OVERMAN, L. E. (1996). The first selective antagonist or a $GABA_C$ receptor. *Trends in Pharmacological Sciences* **17,** 319-323.

NAKAJIMA, Y., IWAKABE, H., AKAZAWA, C., NAWA, H., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, N. (1993). Molecular characterisation of a novel retinal metabotrophic glutamate receptor mGluR6 with a high agonist selectivity for 2-amino-4-phosphonobutyrate. *Journal of Biological Chemistry* **268,** 11868-11873.

NAKAJIMA, Y., NAKAJIMA, S., LEONARD, R. J. & YAMAGUCHI, K. (1986). Acetylcholine raises excitability by inhibiting the fast transient potassium current in cultured hippocampal neurons. *Proceedings of the National Academy of Sciences of the United States of America* **83,** 3022-3026.

NAKANISHI, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. *Science* **258,** 597-603.

NAKANISHI, S., AXEL, R. & SHNEIDER, N. A. (1992). Alternative splicing generates functionally distinct N-methyl-D-asparte receptors. *Proceedings of the National Academy of Sciences of the United States of America* **89,** 8552-8556.

NAYEEM, N., GREEN, T. P., MARTIN, I. L. & BARNARD, E. A. (1994). Quaternary structure of the native GABAA receptor determined by electron microscopic image analysis. *Journal of Neurochemistry* **62,** 815-818.

NEF, P., ONEYSER, C., ALLIOD, C., COUTURIER, S. & BALLIVET, M. (1988). Genes expressed in the brain define three distinct neuronal nicotinic acetylcholine receptor. *EMBO Journal* **7,** 595-601.

NICHOLLS, D. G. (1989). Release of glutamate, aspartate and γ-aminobutyric acid from isolateral nerve terminals. *Journal of Neurochemistry* **52,** 331-341.

NICHOLLS, D. G. & ATTWELL, D. (1990). The release and uptake of excitatory amino acids. *Trends in Pharmacological Sciences* **11,** 477-478.

NITSCH, R., SORIANO, E. & FROTSCHER, M. (1990). The parvalbumin-containing nonpyramidal neurons in the rat hippocampus. *Anatomy and Embryology* **181,** 413-425.

NORDBERG, A. (2001). Nicotinic receptor abnormalities of Alzheimer's disease: Therapeutic implications. *Biological Psychiatry* **49,** 200-210.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBERT, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307,** 462-465.

NUNZI, M. G., GORIO, A., MILAN, F., FREUND, T. F., SOMOGYI, P. & SMITH, A. D. (1985). Cholecystokinin-immunoreactive cells form symmetrical synaptic contacts with pyramidal and nonpyramidal neurons in the hippocampus. *Journal of Comparative Neurology* **237,** 485-505.

OGURUSU, T. & SHINGAI, R. (1996). Cloning of a putative γ-aminobutyric acid (GABA) receptor subunit rho3 cDNA. *Biochimica et Biophysica Acta* **1305,** 15-18.

OGURUSU, T., TAIRA, H. & SHINGAI, R. (1995). Identification of $GABA_A$ receptor subunits in rat retina; cloning of the rat $GABA_A$ receptor rho2-subunit cDNA. *Journal of Neurochemistry* **65,** 964-968.

OKAMOTO, N., HORI, S., AKAZAWA, C., HAYASHI, Y., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1994). Molecular characteristion of a new metabotrophic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. *Journal of Biological Chemistry* **269,** 1231-1236.

O'KEEFE, J. (1993). Hippocampus, theta, and spatial memory. *Current Opinion in Neurobiology* **3,** 917-924.

O'KEEFE, J. & NADEL, L. (1978). *the hippocampus as a cognitive map*. Oxford University Press, Oxford.

OLIVA, A. A., JIANG, M., LAM, T., SMITH, K. L. & SWANN, J. W. (2000). Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. *Journal of Neuroscience* **20,** 3354-3368.

OLSEN, R. W. & TOBIN, A. J. (1990). Molecular biology of $GABA_A$ receptors. *FASEB Journal* **4,** 1469-1480.

PAN, Z.-H., ZHANG, D., ZHANG, X. & LIPTON, S. A. (2000). Evidence for coassembly of mutant $GABA_C$ ρ1 with $GABA_A$ γ2s, glycine α1 and glycine α2 receptor subunits *in vitro*. *European Journal of Neuroscience* **12,** 3137-3145.

PAPKE, R. L. (1993). The kinetic properties of neuronal nicotinic receptors:genetic basis of functional diversity. *Progress in Neurobiology* **41,** 509-531.

PAPKE, R. L., BENCHERIF, M. & LIPPIELLO, P. (1996). An evaluation of neuronal nicotinic acetylcholine receptor activation by quaternary nitrogen compounds indicates that choline is selective for the α7 subtype. *Neuroscience Letters* **213,** 201-204.

PARRA, P., GULYAS, A. I. & MILES, R. (1998). How many subtypes of inhibitory cells in the hippocampus? *Neuron* **20,** 983-993.

PATERNAIN, A., MORALES, M. & LERMA, J. (1995). Selective antagonism of AMPA receptors unmasks kainate receptor-mediated responses in hippocampal neurons. *Neuron* **14,** 185-189.

PAULSEN, O. & MOSER, E. I. (1998). A model of hippocampal memory encoding and retrieval: GABAergic control of synaptic plasticity. *Trends in Neurosciences* **21,** 273-278.

PERALTA, E. G., ASHKENAZI, A., WINSLOW, J. W., RAMACHANDRAN, J. & CAPON, D. J. (1988). Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. *Nature* **334,** 434-437.

PHILLIPS, H. A., SCHEFFER, I. E., CROSSLAND, K. M., BHATIA, K. P., FISH, D. R., MARSDEN, C. D., HOWELL, S. J., STEPHENSON, J. B., TOLMIE, J., PLAZZI, G., EEG-OLOFSSON, O., SINGH, R., LOPES-CENDES, I., ANDERMANN, E., ANDERMANN, F., BERKOVIC, S. F. & MULLEY, J. C. (1998). Autosomal dominant nocturnal frontal-lobe epilepsy: genetic heterogenity and evidence for a second locus at 15q24. *American Journal of Human Genetics* **63**.

PIN, J. P., GALVEZ, T. & PREZEAU, L. (2003). Evolution, structure and activation mechanism of family 3/C G-protein coupled receptors. *Pharmacology and Therapeutics* **98,** 325-354.

PIN, J. P., WAEBER, C. & PREZEAU, L. (1992). Alternative splicing generates metabotrophic glutamate receptors inducing different patterns of calcium release in xenopus oocytes. *Proceedings of the National Academy of Sciences of the United States of America* **89,** 10331-10335.

PITLER, T. A. & ALGER, B. E. (1990). Activation of the pharmacologically defined M_3 muscarinic receptor depolarizes hippocampal pyramidal cells. *Brain Research* **534,** 257-262.

PITLER, T. A. & ALGER, B. E. (1992). Cholinergic excitation of GABAergic interneurons in the rat hippocampus. *Journal of Physiology* **450,** 127-142.

POLENZANI, L., WOODWARD, R. M. & MILEDI, R. (1991). Expression of mammalian γ–aminobutyric acid receptors with distinct pharmacology in Xenopus oocytes. *Proceedings of the National Academy of Sciences of the United States of America* **88,** 4318-4322.

POWER, J. M. & SAH, P. (2002). Nuclear Calcium Signaling Evoked by Cholinergic Stimulation in Hippocampal CA1 Pyramidal Neurons. *Journal of Neuroscience* **22,** 3454-3462.

PRITCHETT, D. B. & SEEBURG, P. H. (1990). Gamma-aminobutyric $acid_A$ receptor α5-subunit creates novel type II benxodiazepine receptor pharmacology. *Journal of Neurochemistry* **54,** 1802-1804.

PRITCHETT, D. B., SONTHEIMER, H., SHIVERS, B. D., YMER, S., KETTENMANN, H., SCHOFIELD, P. R. & SEEBURG, P. H. (1989). Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. *Nature* **338,** 582-585.

QIAN, H. & DOWLING, J. E. (1993). Novel GABA response from rod-driven retinal horizontal cells. *Nature* **361,** 162-164.

QIAN, H. & DOWLING, J. E. (1994). Pharmacology of novel GABA receptors found on rod horizontal cells of the white perch retina. *Journal of Neuroscience* **14,** 4299-4307.

QIAN, H., DOWLING, J. E. & RIPPS, H. (1999). A single amino acid in the second transmembrane domain of GABA rho subunits is a determinant of the response kinetics of GABAC receptors. *Journal of Neurobiology* **40,** 67-76.

QIAN, H. & RIPPS, H. (1999). Response kinetics and pharmacological properties of heteromeric receptors formed by coassembly of GABA rho- and gamma 2-subunits. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **266,** 2419-2425.

QIAN, J. & SAGGAU, P. (1997). Presynaptic inhibition of synaptic transmission in the rat hippocampus by activation of muscarinic receptors; involvement of presynaptic calcium influx. *British Journal of Pharmacology* **122,** 511-519.

QUIRION, R., ARAUJO, D., RENEGOLD, W. & BOSKA, P. (1989a). Characterisation and quantitative autoradiographic distribution of ^{3}H-acetylcholine muscarinic receptors in mammalian brain. Apparent labelling of an M2-like receptor subtype. *Neuroscience* **29,** 271-289.

QUIRION, R., AUBERT, I., LAPCHAK, P. A., SCHAUM, R. P., TEOLIS, S., GAUTHIER, S. & ARAUJO, D. (1989b). Muscarinic receptor subtypes in human neurodegenerative disorders: focus on Alzheimer's disease. *Trends in Pharmacological Sciences* **Supplement 5,** 80-84.

RADCLIFFE, K. A. & DANI, J. A. (1998). Nicotinic stimulation produces multiple forms of increased Glutamatergic synaptic transmission. *The Journal of Neuroscience* **18,** 7075-7083.

RADCLIFFE, K. A., FISHER, J. L., GRAY, R. & DANI, J. A. (1999). Nicotinic modulation of glutamate and GABA synaptic transmission in hippocampal neurons. *Annals of the New York Academy of Sciences* **868,** 591-610.

RAGOZZINO, D., WOODWARD, R. M., MURATA, Y., EUSEBI, F., OVERMAN, L. E. & MILEDI, R. (1996). Design and in vitro pharmacology of a selective gamma-aminobutyric acid C receptor antagonist. *Molecular Pharmacology* **50,** 1024-1030.

RAMIREZ-LATORRE, J., YU, C. R., QU, X., PERIN, F., KARLIN, A. & ROLE, L. (1996). Functional contributions of α5 subunit to neuronal acetylcholine receptor channels. *Nature* **380,** 347-351.

RANSOM, R. W. & STEC, N. L. (1988). Cooperative modulation of [^{3}H]MK-801 binding to the N-methyl-D-aspatate receptor-ion channel complex by L-glutamate, glycine and polyamines. *Journal of Neurochemistry* **51,** 830-836.

RAPIER, C., LUNT, G. G. & WONNACOTT, S. (1990). Nicotine modulation of [^{3}H] dopamine release from striatal synaptosomes: pharmacological characterisation. *Journal of Neurochemistry* **54,** 937-945.

RAYMON Y CAJAL, S. (1891). Sur la structure de l'ecorce cerebrale de quelque mammiferes. *La Cellule* **7,** 125-176.

RAYMON Y CAJAL, S. (1893). Estructura del asta de Ammon y fascia dentata. *Ann Soc Esp Hist Nat* **22.**

REECE, L. J. & SCHWARTZKROIN, P. A. (1991). Effects of cholinergic agonists on two non-pyramidal cell types in rat hippocampal slices. *Brain Research* **566,** 115-126.

REMPEL-CLOWER, N. L., ZOLA, S. M., SQUIRE, L. R. & AMARAL, D. G. (1996). Three cases of enduring memory impairment after bilateral damage limited to the hippocampal formation. *Journal of Neuroscience* **16,** 5233-5255.

RIBAK, C. E., VAUGHN, J. E. & SAITO, K. (1978). Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibtion of axonal transport. *Brain Research* **140,** 315-332.

ROMMELSPACHER, H. & KUHAR, M. J. (1974). Effects of electrical stimulation on acetylcholine levels in central cholinergic nerve terminals. *Brain Research* **81,** 243-251.

ROSHAN-MILANI, S., FERRIGAN, L., KHOSHNOOD, M. J. & COBB, S. R. (2003). Regulation of epileptiform activity in the hippocampus by nicotinic acteylcholine receptor activation. *Epilepsy Research* **56,** 51-65.

ROTHMAN, S. M. & OLNEY, J. W. (1987). Excitotoxicity and the NMDA receptor. *Trends in Neurosciences* **7,** 299-302.

ROUSE, S. T., MARINO, M. J., POTTER, L. T., CONN, P. J. & LEVEY, A. I. (1999). Muscarinic receptor subtypes involved in hippocampal circuits. *Life Sciences* **64,** 501-509.

ROVIRA, C., BEN-ARI, Y. & CHERUBINI, E. (1983). Dual cholinergic modulation of hippocampal somatic and dendritic field potentials by the septo-hippocampal pathway. *Experimental Brain Research* **49,** 151-155.

ROVIRA, C., CHERUBINI, E. & BEN-ARI, Y. (1982). Opposite actions of muscarinic and nicotinic agents on hippocampal dendritic negative fields recorded in rats. *Neuropharmacology* **21,** 933-936.

RUDOLPH, U., CRESTANI, F. & MOHLER, H. (2001). $GABA_A$ receptor subtypes: dissecting their pharmacological functions. *Trends in Pharmacological Sciences* **22,** 188-194.

SACAAN, A. I., MENZAGHI, F., DUNLOP, J. L., CORREA, L. D., WHELAN, K. T. & LLOYD, G. K. (1996). Epibatidine: a nicotinic acetylcholine receptor

agonist releases monoaminergic neurotransmitters: in vitro and in vivo evidence in rats. *Journal of Pharmacology and Experimental Therapeutics* **276,** 509-515.

SAUGSTAD, J. A., KINZIE, J. M., MULVIHILL, E. R., SEGERSON, T. P. & WESTBROOK, G. L. (1994). Cloning and expression of a new member of the L-2-amino-4-phosphonobutyric acid sensitive class of metabotrophic glutamate receptors. *Molecular Pharmacology* **45,** 367-372.

SAXENA, N. C. & MACDONALD, R. L. (1994). Assembly of $GABA_A$ receptor subunits: Role of the δ subunit. *The Journal of Neuroscience* **14,** 7077-7086.

SCHAFFER, K. (1892). Beitrag zur histologie der Ammonshornformation. *Arch Mikrosk Anat* **39,** 611-632.

SCHIFFER, H. H., SWANSON, G. T. & HEINEMANN, S. (1997). Rat GluR7 and a carboxy-terminal spliced variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. *Neuron* **19,** 1141-1146.

SCHOEPP, D. D., JOHNSON, B. G., SALHOFF, C. R., VALLI, M. J., DESAI, M. A., BURNETT, J. P., MAYNE, N. G. & MONN, J. A. (1995). Selective inhibition of forskolin-stimulated cyclic AMP formation in rat hippocampus by a novel mGluR agonist, 2R, 4R-4-aminopyrrolidine-2-4-dicarboxylate. *Neuropharmacology* **34,** 843-850.

SCHOFIELD, P. R., DARLISON, M. G., FUJITA, N., BURT, D. R., STEPHENSON, F. A., RODRIGUEZ, H., RHEE, L. M., RAMACHANDRAN, J., REALE, V., GLENCOURSE, T. A., SEEBURG, P. H. & BARNARD, E. A. (1987). Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature* **328.**

SCHWARTZKROIN, P. A. (1994). Role of the hippocampus in epilepsy. *Hippocampus* **4,** 239-242.

SCOVILLE, W. B. (1954). The Limbic Lobe in Man. *Journal of Neurosurgery* **11,** 64-66.

SCOVILLE, W. B. & MILNER, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurol. Neurosurg.psychia.* **20,** 11-21.

SEGAL, M. (1982). Multiple actions of acetylcholine at a muscarinic receptor studied in the rat hippocampal slice. *Brain Research* **246,** 77-87.

SEGAL, M. (1988). Synaptic activation of a cholinergic receptor in rat hippocampus. *Brain Research* **452,** 79-86.

SEGAL, M. (1989). Presynaptic cholinergic inhibition in hippocampal cultures. *Synapse* **4,** 305-312.

SEGUELA, P., WADICHE, J., DINELEY-MILLER, K., DANI, J. A. & PATRICK, J. W. (1993). Molecular cloning, functional properties and distribution of rat brain α7: a nicotinic cation channel highly permeable to calcium. *Journal of Neuroscience* **13,** 596-604.

SERESS, L. & POKOMY, J. (1981). Structure of the granule layer of the rat dentate gyrus. A light microscopic and Golgi study. *Journal of Anatomy* **133,** 181-195.

SHARPLES, C. G. V. & WONNACOTT, S. (2001). Neuronal Nicotinic Receptors. *Tocris Reviews* **19.**

SHIGEMOTO, R., KINOSHITA, A., WADA, E., NOMURA, S., OHISHI, H., TAKADA, M., FLOR, P. J., NEKI, A., ABE, T., NAKANISHI, S. & MIZUNO, N. (1997). Differential presynaptic localization of metabotrophic glutamate receptor subtypes in the rat hippocampus. *Journal of Neuroscience* **17,** 7503-7522.

SHIGEMOTO, R., KULIK, A., ROBERTS, J. D. B., OHISHI, H., NUSSER, Z., KANEKO, T. & SOMOGYI, P. (1996). Target-cell specific concentration of a metabotrophic glutamate receptor in the presynaptic active zone. *Nature* **381,** 523-525.

SHIVERS, B. D., KILLISCH, I., SPRENGEL, R., SONTHEIMER, H., KOHLER, C., SCHOFIELD, P. R. & SEEBURG, P. H. (1989). Two novel $GABA_A$ receptor subunits exist in distinct neuronal subpopulations. *Neuron* **3,** 327-337.

SIK, A., PENTTONEN, M., YLINEN, A. & BUZSAKI, G. (1995). Hippocampal CA1 interneurons: an in vivo intracellular labelling study. *Journal of Neuroscience* **15,** 6651-6665.

SLADECZEK, F., PIN, J. P., RECASENS, M., BOCKAERT, J. & WEISS, S. (1985). Glutamate stimulates inositol phosphate formation in striatal neurones. *Nature* **317,** 717-718.

SLOVITER, R. S. & NILAVER, G. (1987). Immunocytochemical localization of GABA-cholecystokinin, vasoactive intestinal polypeptide- and somatostatin-like immunoreactivity in the area dentata and hippocampus of the rat. *Journal of Comparative Neurology* **256,** 42-60.

SMITH, A. J., ALDER, L., SILK, J., ADKINS, C. E., FLETCHER, A. E., SCALES, T., KERBY, J., MARSHALL, G., WAFFORD, K. A., MCKERNAN, R. M. & J.R., A. (2001). Effect of alpha subunit on allosteric modulation of ion channel function in stably expressed human recombinant gamma-aminobutyric

acid(A) receptors determined using Cl-36 ion flux. *Molecular Pharmacology* **59,** 1108-1118.

SMITH, C. M. (1972). The release of acetylcholine from rabbit hippocampus. *British Journal of Pharmacology* **45,** 172P.

SMITH, C. M. (1974). Acetylcholine release from cholinergic septo-hippocampal pathway. *Life Sciences* **14,** 2159-2166.

SOMMER, B., BURNASHEV, N. & VERDOORN, T. A. (1992). A glutamate receptor channels with high affinity for domoate and kainate. *EMBO Journal* **11,** 1651-1656.

SOMMER, B., KEINANEN, K. & VERDOORN, T. A. (1990). Flip and flop: a cell specific functional switch in glutamate -operated channels of the CNS. *Science* **249,** 1580-1585.

SOMMER, B., KOHLER, C., SPRENGEL, R. & SEEBURG, P. H. (1991). RNA editing in brain controls a determinant of ion flow in glutamate -gated channels. *Cell* **67,** 11-19.

SOMOGYI, P., FREUND, T. F., HODGSON, A. J., SOMOGYI, J., BEROUKAS, D. & CHUBB, I. W. (1985). Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. *Brain Research* **332,** 143-149.

SOMOGYI, P., HODGSON, A. J., SMITH, A. D., NUNZI, M. G., GORIO, A. & WU, J.-Y. (1984). Different populations of GABAergic neurons in the visual cortex and hippocampus of the cat contain somatostatin- or cholecystokinin-immunoreactive material. *Journal of Neuroscience* **4,** 2590-2603.

SOMOGYI, P., NUNZI, M. G., GORIO, A. & SMITH, A. D. (1983). A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segment of pyramidal cells. *Brain Research* **259,** 137-142.

SPEHLMANN, R. (1963). Acetylcholine and prostigmine electrophoresis at visual cortex neurons. *Journal of Neurophysiology* **26,** 127-139.

SPENCER, D. G., HORVÁTH, E. & TRABER, J. (1986). Direct autoradiographic determination of M1 and M2 muscarinic acetylcholine receptor distribution in the rat brain: Relation to cholinergic nuclei and projections. *Brain Research* **380,** 59-68.

SPENCER, S. S., SO, N. K., ENGEL, J. J., WILLIAMSON, P. D., LEVESQUE, M. F. & SPENCER, D. D. (1993). Surgical treatment of the epilepsies. ed. ENGEL, J. J., pp. 359-377. Raven Press, New York.

STALEY, K. J., LONGACHER, M., BAINS, J. S. & YEE, A. (1998). Presynaptic modulation of CA3 network activity. *Nature Neuroscience* **1,** 201-209.

STEINLEIN, O. K., MULLEY, J. C., PROPPING, P., WALLACE, R. H., PHILLIPS, H. A., SCHEFFER, I. E., SUTHERLAND, G. R. & BERKOVIC, S. F. (1995). A missense mutation in the neuronal nicotinic acetylcholine receptor α4 subunit is associated with autosomal dominant nocturnal frontal lobe epilepsy. *Nature Genetics* **11,** 201-203.

STEWART, M. & FOX, S. E. (1990). Do septal neurons pace the hippocampal theta rhythm. *Trends in Neurosciences* **13,** 163-168.

SUCHER, N. J., AKBARIAN, S., CHI, C. L., LECLERC, C. L., AWOBULUYI, M., DEITCHER, D. L., WU, M. K., YUAN, J. P., JONES, E. G. & LIPTON, S. A. (1995). Developemental and regional expression of a novel NMDA receptor-like subunit (NMDAR-L) in the rodent brain. *Journal of Neuroscience* **15,** 6509-6520.

SUDWEEKS, S. & YAKEL, J. L. (2000). Functional and molecular characterisation of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons. *Journal of Physiology* **527,** 515-528.

SUGIHARA, H., MORIYOSHI, K. & ISHII, T. (1992). Structures and properties of 7 isoforms of the NMDA receptor generated by alternative splicing. *Biochemical and Biophysical Research Communications* **185,** 862-832.

SUGIYAMA, H., ITO, I. & HIRONO, C. (1987). A new type of glutamate receptor linked to inositiol phospholipid metabolism. *Nature* **325,** 531-533.

SWANSON, L. W., WYSS, J. M. & COWAN, W. M. (1978). An autoradiographic study of the organisation of intrahippocampal association pathways in the rat. *Journal of Comparative Neurology* **181,** 517-531.

SWARTZ, K. J. & BEAN, B. P. (1992). Inhibition of calcium channels in rat CA3 pyramidal neurons by a metabotrophic glutamate receptor. *Journal of Neuroscience* **12,** 4358-4371.

TANABE, Y., MASU, M., ISHII, T., SHIGEMOTO, R. & NAKANISHI, S. (1992). A family of metabotrophic glutamate receptors. *Neuron* **8,** 169-179.

TAYLOR, G. W., MERLIN, L. R. & WONG, R. K. S. (1995). Synchronized oscillations in hippocampal CA3 neurons induced by metabotrophic glutamate receptor activation. *Journal of Neuroscience* **15,** 8039-8052.

TERRY, R. D. & KATZMAN, R. (1983). Senile dementia of the Alzhiemer type. *Annals of Neurology* **14,** 497-506.

TOTH, K. & FREUND, T. F. (1992). Calbindin D28k-containing nonpyramidal cells in the rat hippocampus: their immunoreactivity for GABA and projection to the medial septum. *Neuroscience* **49,** 793-805.

TOTH, K., FREUND, T. F. & MILES, R. (1997). Disinhibition of rat hippocampal pyramidal cells by gabaergic afferents from the septum. *Journal of Physiology* **500,** 463-474.

TRETTER, V., EHYA, N., FUCHS, K. & SIEGHART, W. (1997). Stoichiometry and assembly of a recombinant GABAA receptor subtype. *The Journal of Neuroscience* **17,** 2728-2737.

TRUSSELL, L. O. & FISCHBACH, G. D. (1989). Glutamate receptor desensitization and its role in synaptic transmission. *Neuron* **3,** 209-218.

UNWIN, N. (1995). Acetycholine receptor channel imaged in the open state. *Nature* **373,** 37-43.

VALENTINO, R. J. & DINGLEDINE, R. (1981). Presynaptic inhibitory effect of acetylcholine in the hippocampus. *Journal of Neuroscience* **1,** 784-792.

VANDERWOLF, C. H. (1975). Neocortical and hippocampal activation in relation to behaviour: effects of atropine, eserine, phenothiazines and amphetamine. *Journal of Comparative and Physiological Psychology* **88,** 300-323.

VERDOORN, T. A., DRAGUHN, A., YMER, S., SEEBURG, P. H. & SAKMANN, B. (1990). Functional properties of recombinant rat GABAA receptors depend upon subunit composition. *Neuron* **4,** 919-928.

VIDA, I., HALASY, K., SZINYEI, C., SOMOGYI, P. & BUHL, E. H. (1998). Unitary IPSPs evoked by interneurons at the stratum radiatum-stratum lacunosum-moleculare border in the CA1 area of the rat hippocampus in vitro. *Journal of Physiology* **506,** 755-773.

VIGNES, M. & COLLINGRIDGE, G. L. (1997). The synaptic activation of kainate receptors. *Nature* **388,** 179-182.

VIZI, E. S. & LENDVAI, B. (1999). Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. *Brain Research Reviews* **30,** 219-235.

VOGT, K. E. & REGEHR, W. G. (2001). cholinergic modulation of excitatory synaptic transmission in the CA3 area of the hippocampus. *The Journal of Neuroscience* **21,** 75-83.

WANG, T. L., GUGGINO, W. B. & CUTTING, G. R. (1994). A novel γ-aminobutyric acid receptor subunit (ρ2) cloned from human retina form bicuculline insensitive homooligomeric receptors in *Xenopus* oocytes. *Journal of Neuroscience* **14,** 6524-6531.

WATKINS, J. C. & EVANS, K. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology and Toxicology* **21,** 165-204.

WATKINS, J. C., KROGSGAARD-LARSEN, P. & HONORE, T. (1991). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. *Trends in Pharmacological Sciences: A Special Report*, 4-12.

WEGELIUS, K., PASTERNACK, M., HILTUNEN, A. O., RIVERA, C., KAILA, K., SAARMA, M. & REEBEN, M. (1998). Distribution of GABA receptor rho subunit transcript in the rat brain. *European Journal of Neuroscience* **10,** 350-357.

WERNER, P., VIOGT, M. & KEINANEN, K. (1991). Cloning of a putative high-affinity kainate receptor expressed predominantly in hippocampal CA3 cells. *Nature* **351,** 742-744.

WEST, M. J., KAWAS, C. H., MARTIN, L. J. & TRONCOSO, J. C. (2000). The CA1 region of the human hippocampus is a hot spot in Alzheimer's disease. *Annals of the New York Academy of Sciences* **908,** 255-259.

WESTBROOK, G. L. & MAYER, M. L. (1987). Micromolar concentrations of Zn^{2+} antagonize NMDA and GABA responses of hippocampal neurones. *Nature* **328,** 640-643.

WHITE, J. H., WISE, A., MAIN, M. J., GREEN, A., FRASER, N. J., DISNEY, G. H., BARNES, A. A., EMSON, P., FOORD, S. M. & MARSHALL, F. H. (1998). Heterodimerization is required for the formation of a functional GABAB receptor. *Nature* **396,** 679-682.

WHITING, P. J., MCKERNAN, R. M. & IVERSEN, L. L. (1990). Another mechanism for creating diversity in γ-aminobutyrate type A receptors: RNA splicing directs expression of two forms of γ2 subunit, one of which contains a protein kinase C phospohorylation site. *Proceedings of the National Academy of Sciences of the United States of America* **87,** 9966-9970.

WHITTINGTON, M. A., TRAUB, R. G. & JEFFERYS, J. G. R. (1995). Synchronised oscillations in interneuron networks driven by metabotrophic glutamate receptor activation. *Nature* **373,** 612-615.

WILLIAMS, J. H. & KAUER, J. A. (1997). Properties of carbachol-induced oscillatory activity in rat hippocampus. *Journal of Neurophysiology* **78,** 2631-2640.

WILLIAMS, K., ROMANO, C., DICHTER, M. A. & MOLINOFF, P. B. (1991). Modulation of the NMDA receptor by polyamines. *Life Sciences* **48,** 469-498.

WILLIAMS, S., SAMULACK, D. D., BEAULIEU, C. & LACAILLE, J.-C. (1994). Membrane properties and synaptic responses of interneurons located near the stratum lacunosum-moleculare/radiatum border of area CA1 in whole-cell recordings from rat hippocampal slices. *Journal of Neurophysiology* **71,** 2217-2235.

WINSON, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science* **201,** 160-163.

WISDEN, W., HERB, A., WIELAND, H., KEINANEN, K., LUDDENS, H. & SEEBURG, P. H. (1991). Cloning, pharmacological characteristics and expression pattern of the rat $GABA_A$ receptor α4 subunit. *FEBS Letters* **289,** 227-230.

WISDEN, W., LAURIE, D. J., MONYER, H. & SEEBURG, P. H. (1992). The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *Journal of Neuroscience* **12,** 1040-1062.

WONG, H.-K., LIU, X.-B., MATOS, M. F., CHAN, S. F., PEREZ-OTANO, I., BOYSEN, M., CUI, J., NAKANISHI, N., TRIMMER, J. S., JONES, E. G., LIPTON, S. A. & SUCHER, N. J. (2002). Temporal and regional expression of the NMDA receptor subunit NR3A in the mammalian brain. *Journal of Comparative Neurology* **450,** 303-317.

WONNACOTT, S. (1997). Presynaptic nicotinic ACh receptors. *Trends in Neurosciences* **20,** 92-98.

WONNACOTT, S., ALBUQUERQUE, E. X. & BERTRAND, D. (1993). Methylcaconitine: A new probe that discriminates between nicotinic acetylcholine receptor subclasses. *Methods in Neuroscience* **12,** 263-275.

WOODSON, W., NITECKA, L. & BEN-ARI, Y. (1989). Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *Journal of Comparative Neurology* **280,** 254-271.

WU, L. G. & SAGGAU, P. (1995). $GABA_B$ receptor mediated presynaptic inhibition in guinea pig hippocampus is caused by reduction of presynaptic Ca^{2+} influx. *Journal of Physiology* **485,** 649-657.

YAMAZAKI, M., MORI, H. & ARAKI, K. (1992). Cloning, expression and modulation of a mouse NMDA receptor subunit. *FEBS Letters* **300,** 39-45.

YLINEN, A., SOLTESZ, I., BRAGIN, A., PENTTONEN, M., SIK, A. & BUZSAKI, G. (1995). Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* **5,** 78-90.

YMER, S., DRAGUHN, A., WISDEN, W., WERNER, P., KEINANEN, K., SCHOFIELD, P. R., SPRENGEL, R., PRITCHETT, D. B. & SEEBURG, P. H. (1990). Structural and functional characterisation of the γ1 subunit of the $GABA_A$ benzodiazepine receptors. *EMBO Journal* **9,** 3261-3267.

YMER, S., SCHOFIELD, P. R., DRAGUHN, A., WERNER, P., KOHLER, M. & SEEBURG, P. H. (1989). $GABA_A$ receptor beta subunit heterogeneity: functional expression of cloned cDNAs. *EMBO Journal* **8,** 1665-1670.

YOUNG, A. B. & FAGG, G. E. (1991). Excitatory amino acid receptors in the brain: membrane binding and receptor autoradiographic approaches. *Trends in Pharmacological Sciences: A Special Report,* 18-24.

ZHANG, D., PAN, Z.-H., AWOBULUYI, M. & LIPTON, S. A. (2001). Structure and function of $GABA_C$ receptors: a combination of native versus recombinant receptors. *Trends in Pharmacological Sciences* **22,** 121-132.

ZHANG, D., PAN, Z.-H., ZHANG, X., BRIDEAU, A. D. & LIPTON, S. A. (1995). cloning of a γ-aminobutyric acid type C receptor subunit in the rat retina with a methionine residue critical for picrotoxinin channel block. *Proceedings of the National Academy of Sciences of the United States of America* **92,** 11756-11760.

ZHOU, F.-M., LIANG, Y. & DANI, J. A. (2001). Endogenous nicotinic cholinergic activity regulates dopamine release in the striatum. *Nature Neuroscience* **4,** 1224-1229.

ZHU, P. J. & CHIAPPINELLI, V. A. (1999). Nicotine modulates evoked gabaergic transmission in the brain. *Journal of Physiology* **82,** 3041-3045.

ZOLA-MORGAN, S., SQUIRE, L. R. & AMARAL, D. G. (1986). Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. *Journal of Neuroscience* **6,** 2950-2967.

ZOLA-MORGAN, S., SQUIRE, L. R., REMPEL, N. L., CLOWER, R. P. & AMARAL, D. G. (1992). Enduring memory impairment in monkeys after ischemic damage to the hippocampus. *Journal of Neuroscience* **11,** 4355-4370.