**MODULATION OF LONG-TERM POTENTIATION BY
THE GLYCINE SITE OF
N-METHYL-D-ASPARTATE RECEPTOR IN RAT
HIPPOCAMPAL CA1 PYRAMIDAL CELLS**

by

© Nicholas Krasteniakov

**A thesis submitted to the Faculty of Graduate Studies and Research in
partial fulfillment of the requirements for the degree of Doctor of
Philosophy of Science**

**Department of Psychology
Carleton University
Ottawa, Canada**

Library and
Archives Canada

Bibliothèque et
Archives Canada

Published Heritage
Branch

Direction du
Patrimoine de l'édition

395 Wellington Street
Ottawa ON K1A 0N4
Canada

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file Votre référence

ISBN: 0-612-97830-3

Our file Notre référence

ISBN: 0-612-97830-3

NOTICE:

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

PAGINATION ERROR.

TEXT COMPLETE.

ERREUR DE PAGINATION.

LE TEXTE EST COMPLET.

ABSTRACT

The *N*-methyl-D-aspartate receptor (NMDAR) is an ionotropic, ligand-gated channel endowed with a number of distinct recognition sites through which endogenous and exogenous compounds can alter receptor activity. Glutamate is virtually ineffective in promoting the opening of high-conductance channel unless the site that binds glycine is also occupied. In the CA1 region of the hippocampus, NMDAR supports long-lasting increases of synaptic strength known as long-term potentiation (LTP). In the present work, I have performed patch clamp recordings from CA1 hippocampal pyramidal cells maintained *in vitro* to examine the modulation of NMDAR-dependent LTP by the glycine site.

In the first series of experiments, after morphological and physiological characterization of the recorded cells, D-serine modulation of NMDAR-mediated responses in pyramidal cells and interneurons was studied. It was found that D-serine strongly enhanced NMDAR-mediated transmission in both cell types. The augmentation of NMDAR function however, was significantly larger in pyramidal cells than in interneurons.

The second part of the study examined the induction requirements for NMDAR-dependent and NMDAR-independent LTP. It was found that NMDAR-mediated component of LTP required pairing of depolarization ($V_m \leq -50$ mV) with subthreshold synaptic stimulation, whereas NMDAR-independent LTP was obtained by pairing of stronger depolarization ($V_m \geq -40$ mV) with suprathreshold synaptic stimulation.

Collectively, these experiments revealed the induction requirements for NMDAR-dependent and NMDAR-independent LTP.

The last part of this work examined the physiological role of the glycine site in synaptic plasticity. LTP was induced by depolarizing the cell to -60, -50 and -40 mV in the presence of D-serine, glycine or 7-chlorokynurenic acid (7-Cl-KYN). It was found that D-serine and glycine were more effective in enhancing LTP during submaximal induction conditions (-60, -50 mV) than during supramaximal induction (-40 mV). The role of glycine site activation for bidirectional synaptic plasticity was investigated with the selective antagonist 7-Cl-KYN. The effect of glycine site blockade on plasticity was voltage- and concentration-dependent and generated heterogeneous responses ranging from LTP to long-term depression (LTD). Together, this work reveals the physiological role of the glycine site for NMDAR-mediated plasticity.

ACKNOWLEDGEMENTS

My acknowledgments are directed first and foremost to Dr. Richard Bergeron for providing me with the opportunity to carry out this project in his laboratory. His help, guidance and encouragements have been paramount to the successful completion of the experiments described in this thesis.

I would further like to express my gratitude to Dr. Marzia Martina for her valuable criticisms and comments. The confocal images of hippocampal neurons included in this work were made possible by Dr. Martina's expert operation of a confocal laser-scanning microscope.

I am also greatly appreciative for the technical assistance provided by Christian Metivier and Linda Richard.

TABLE OF CONTENTS

TABLE OF CONTENTS.....	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF ABBREVIATIONS.....	xiii
1. INTRODUCTION	1
1.1. General features of the rat hippocampal formation	1
1.1.1. Introductory remarks.....	1
1.1.2. Definition of the hippocampal formation	2
1.1.3. Laminar organization	3
1.1.4. Cell types	3
1.1.5. The “lamellar hypothesis” of hippocampal anatomical organization	5
1.1.6. Hippocampal information processing	7
1.1.6.1. The dentate gyrus.....	7
1.1.6.2. Field CA3.....	8
1.1.6.3. Field CA1	9
1.2. Long-term potentiation (LTP) in the hippocampus	10
1.2.1. Introductory remarks.....	10
1.2.2. LTP: a cellular model of memory and learning	11

1.2.3. Properties of hippocampal LTP	12
1.2.4. Induction of LTP: role of NMDARs and Ca^{2+}	13
1.2.5. Expression mechanisms	16
1.2.6. Signal transduction mechanisms in LTP.....	18
1.3. Excitatory amino acid (EAA) receptors.....	21
1.3.1. Overview of glutamate receptors	21
1.3.2. AMPARs.....	22
1.3.3. Kainate receptors	24
1.3.4. NMDARs	26
1.4. The glycine site of NMDAR	31
1.4.1. Introductory remarks.....	31
1.4.2. Physiological role of glycine (coagonist/modulator).....	33
1.4.3. Agonists and antagonists of the glycine site.....	36
1.4.4. Saturation of the glycine site.....	38
1.4.5. Clinical implications	41
1.4.5.1. Schizophrenia.....	42
1.5. Research proposal	43
1.5.1. Hypothesis and specific objectives	43
1.5.1.1. Study I.....	43
1.5.1.2. Study II.....	44
1.5.1.3. Study III	45
2. MATERIALS AND METHODS.....	46

2.1. Surgery.....	46
2.2. Brain slice preparation	46
2.3. Visualization and whole-cell recording	47
2.4. Identification of CA1 pyramidal cells.....	48
2.5. Drug application and solutions	49
2.6. Electrical stimulation	50
2.7. LTP induction	51
2.8. Data analysis	53
 3. RESULTS	 55
3.1. D-serine modulation of NMDAR-mediated currents in pyramidal cells and interneurons.....	55
3.1.1. Morphological and physiological properties of recorded CA1 pyramidal cells and interneurons	55
3.1.2. D-serine modulation of the evoked NMDAR-mediated currents in pyramidal cells and interneurons	56
3.1.3. D-serine enhancement of NMDAR function in response to local pressure application of NMDA	57
3.2. Pairing requirements for low-frequency NMDAR-dependent and NMDAR-independent LTP.....	58
3.2.1. Depolarization enables LTP during brief high-frequency afferent stimuli..	58
3.2.2. Minimal pairing requirements for low-frequency LTP of maximal magnitude.....	59

3.2.3. Postsynaptic voltage and the induction of LTP _{AP}	60
3.2.4. Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP of submaximal magnitude (LTP _{EPSC}).....	61
3.2.5. The effect of NMDAR antagonists APV and 7-Cl-KYN on LTP _{AP} and LTP _{EPSC}	62
3.3. Regulation of synaptic plasticity by the glycine site of NMDAR	64
3.3.1. Membrane voltage controls the induction and magnitude of LTP obtained by low-frequency pairing.....	64
3.3.2. Enhancement of LTP by D-serine and glycine is differentially affected by membrane voltage during induction	66
3.3.3. Maximal enhancement of LTP by exogenous D-serine and glycine is obtained when the agonists are present prior to synapse activation and pairing ...	68
3.3.4. Blockade of the glycine site by 7-Cl-KYN reverses the direction of synaptic plasticity.....	69
3.3.5. Competitive effects of D-serine and 7-Cl-KYN on depolarization-induced plasticity.....	71
4. DISCUSSION	73
4.1. D-serine modulation of NMDAR currents in pyramidal cells and interneurons ..	73
4.2. Pairing requirements for low-frequency NMDAR-dependent and NMDAR- independent LTP	77
4.2.1. Pairing requirements for low-frequency LTP	78
4.2.2. NMDARs and low-frequency LTP	79

4.2.3. VDCCs and low-frequency LTP	81
4.2.4. Sodium channels, action potentials and low-frequency LTP.....	84
4.3. Regulation of synaptic plasticity by the glycine site of NMDAR	87
4.3.1. Requirements for D-serine and glycine modulation of plasticity	87
4.3.2. Blockade of the glycine site by 7-Cl-KYN reverses the direction of synaptic plasticity.....	90
4.3.3. D-serine protection of NMDAR-mediated plasticity.....	92
4.4. Summary and conclusions	93
 5. TABLES	 95
 6. FIGURES	 98
 7. REFERENCES	 118

LIST OF TABLES

Table 1: Physiological properties of pyramidal cells and interneurons.

Table 2: Rise and decay time constants of the NMDAR currents in CA1 pyramidal cells and interneurons.

Table 3: Modulation of synaptic plasticity by the glycine site of NMDAR.

LIST OF FIGURES

- Figure 1:** Schematic representation of the structure and function of NMDAR complex.
- Figure 2:** Morphological and physiological properties of pyramidal cells and interneurons from the hippocampal CA1 region.
- Figure 3:** D-serine modulation of NMDAR responses to endogenous glutamate in pyramidal cells and interneurons.
- Figure 4:** Effect of D-serine on NMDAR currents at different holding potentials.
- Figure 5:** D-serine enhancement of NMDAR function during local pressure application of NMDA.
- Figure 6:** Effects of postsynaptic depolarization on brief, high-frequency synaptic stimulation.
- Figure 7:** Minimal pairing requirements for LTP of maximal magnitude.
- Figure 8:** Membrane voltage and the induction of LTP_{AP}.
- Figure 9:** Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP_{EPSC}.
- Figure 10:** Blockade of NMDARs prevents LTP_{EPSC} but has no effect on LTP_{AP}.
- Figure 11:** Depolarization controls the induction and magnitude of LTP in CA1 pyramidal cells during low-frequency afferent stimulation.
- Figure 12:** Modulation of LTP by exogenous D-serine.
- Figure 13:** LTP modulation by exogenous glycine.
- Figure 14:** Maximizing the effects of D-serine and glycine on LTP induced by pairing.
- Figure 15:** 7-Cl-KYN reverses the direction of synaptic plasticity elicited by pairing.
- Figure 16:** Competitive effects of 7-Cl-KYN and D-serine on synaptic plasticity.

LIST OF ABBREVIATIONS

5,7-diCl-KYN	5,7-dichlorokynurenic acid
7-Cl-KYN	7-chlorokynurenic acid
ACBC	1-aminocyclobutanecarboxylic acid
ACPC	1-aminocyclopropanecarboxylic acid
ACSF	artificial cerebrospinal fluid
AMPA	amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AMPA	amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AP5	2-amino-5-phosphonopentanoic acid
APV	2-amino-5-phosphonovaleric acid
CA1-3	fields of the hippocampus (Cornu Ammonis) according to Lorente de Nó
CaMKII	Ca ²⁺ /calmodulin-dependent protein kinase II
cAMP	cyclic adenosine 3,5-monophosphate
cDNA	complimentary deoxyribonucleic acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CNS	central nervous system
CPP	(±)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid
CSF	cerebrospinal fluid
DAAOX	D-amino acid oxidase
DNQX	6,7-dinitroquinoxaline-2,3-dione
EAA	excitatory amino acid
EPSC	excitatory postsynaptic current

EPSP	excitatory postsynaptic potential
GABA	gamma aminobutyric acid
GAD	glutamic acid decarboxylase
GluR1	type 1 AMPA receptor subunit
KA	kainic acid
KA1	type 1 kainate receptor subunit
LTD	long-term depression
LTP	long-term potentiation
LTP _{AP}	LTP induced with action potentials
LTP _{EPSC}	LTP induced with subthreshold excitatory postsynaptic currents
MAPK	mitogen-activated protein kinase
mEPSC	miniature excitatory postsynaptic current
mGluR	metabotropic glutamate receptor
MK-801	dizocilpine maleate
mRNA	messenger ribonucleic acid
NBQX	6-nitro-7-sulfamoylbenzo(f)-quinoxaline-2,3-dione
NMDA	<i>N</i> -methyl-D-aspartate
NMDAR	<i>N</i> -methyl-D-aspartate receptor
NR1	type 1 NMDA receptor subunit
PCP	phencyclidine
PCR	polymerase chain reaction
PHA-L	phaseolus vulgaris-leucoagglutinin
PKA	cAMP-dependent protein kinase

PKC	Ca ²⁺ /phospholipid-dependent protein kinase
PSD	postsynaptic density
STP	short-term potentiation
VDCC	voltage-dependent calcium channel
V_m	membrane potential

1. INTRODUCTION

1.1. General features of the rat hippocampal formation

1.1.1. Introductory remarks

A number of general neurobiological phenomena have been first studied in the hippocampus. The hippocampal neurons were among the first to be described by Camillo Golgi (1886) with his newly discovered silver impregnation technique. The classical Golgi studies of Ramón y Cajal (1911) and Lorente de Nó (1933, 1934) tried for the first time to relate the morphological properties of hippocampal neurons to their functional properties. With the development of Nauta's (1950) degeneration method, the intrinsic and extrinsic connections of the rat hippocampal formation were mapped (Blackstad, 1956, 1965). Comprehensive neuroanatomical studies of Blackstad et al., 1970; Zimmer, 1971; Hjorth-Simonsen, 1972, 1973; Zimmer and Hjorth-Simonsen, 1975 and others (reviewed by Amaral, 1991, 1993; Amaral and Witter, 1989, 1995) further refined concepts concerning the regional organization of the hippocampus and provided new information about the connections between different hippocampal fields and between the hippocampal formations on each side of the brain. Neurophysiologists have used the hippocampus as a model to study general phenomena such as neurotransmission, inhibitory mechanisms and neural plasticity (Gray, 1959; Eccles, 1964; Bliss and Lømo, 1973). The phenomenon of long-term potentiation of synaptic transmission believed to be a realistic model for the cellular and molecular mechanisms underlying memory and learning, has been demonstrated for the first time in the hippocampal formation (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973). In recent years, massive accumulations of data relating hippocampal structure and function have been achieved

with the help of modern techniques such as immunocytochemistry, receptor autoradiography, electron microscopy, phaseolus vulgaris-leucoagglutinin (PHA-L) anterograde tracing, intracellular labeling, and computer-based reconstruction. Combined morphological and physiological approaches provide new information about various types of hippocampal neurons, their transmitters, their axonal and dendritic organization, and synaptic connections.

1.1.2. Definition of the hippocampal formation

The term hippocampal formation comprises six cytoarchitectonically distinct areas that are linked one to the next by largely unidirectional projections (Amaral and Witter, 1995). They include: the dentate gyrus, hippocampus proper, subiculum, presubiculum, parasubiculum, and entorhinal cortex. The entorhinal cortex is topographically differentiated into medial and lateral entorhinal areas (Hjorth-Simonsen, 1972). The subiculum, presubiculum, and parasubiculum are often grouped together as the subicular complex. The hippocampus proper (Cornu Ammonis, CA) was divided by Lorente de Nó (1934) into three fields (CA3, CA2, and CA1). The CA2 field is less well defined and has been a matter of some controversy. Lorente de Nó (1934) originally defined CA2 as a narrow zone of cells interposed between CA3 and CA1 that had large cell bodies like CA3 but did not receive direct innervation from the dentate gyrus. In many ways, CA2 resembles a terminal portion of the CA3 field. In terms of its connectional and perhaps even functional characteristics, CA2 is quite distinct from CA3 and CA1 (Ishizuka et al., 1990; Leranth and Ribak, 1991). Within the hippocampus, a

similar unidirectional flow of information exists: CA3 projects to CA1 and CA1 to the subicular complex.

1.1.3. Laminar organization

The hippocampus appears to have a less complex neuronal organization when compared to the neocortex. This is due to the fact that the two main cell types of the rodent hippocampus, the pyramidal cells of Ammon's horn and the granule cells of the dentate gyrus, are arranged in single, densely packed layers. This laminar organization is similar for all fields of the hippocampus (Amaral and Witter, 1989; Amaral and Witter, 1995). Ammon's horn is usually divided into four layers. The principal cellular layer is the pyramidal cell layer in which the cell bodies of hippocampal pyramidal cells are densely packed. The narrow, cell-sparse layer located deep to the pyramidal cell layer is called stratum oriens. Deep to the oriens is the fiber-containing alveus. In the CA3 field, a narrow acellular zone located just above the pyramidal cell layer is occupied by the mossy fiber axons originating from the dentate gyrus. This layer is called the stratum lucidum. Since mossy fibers do not reach CA2 or CA1, they lack the stratum lucidum. Overlying the stratum lucidum in CA3, and immediately above the pyramidal cell layer in CA2 and CA1, is the cell-sparse stratum radiatum. The stratum radiatum can be defined as the suprapyramidal region in which CA3 to CA3 associational connections and CA3 to CA1 Schaffer collateral connections are located. The most superficial layer of the hippocampus is called the stratum lacunosum-moleculare. It is in this layer that perforant pathway fibers from the entorhinal cortex travel and terminate.

1.1.4. Cell types

The principal neuronal cell type of the hippocampus is the pyramidal cell. In the rodent hippocampus, the cell bodies of pyramidal neurons form a densely packed layer. The dendrites of CA1 pyramidal cells can be divided into basal and apical dendritic systems. The basal dendritic tree extends into the stratum oriens with most of the branching occurring close to the soma. The apical system consists of an apical trunk that leaves the soma and extends into stratum radiatum towards the hippocampal fissure (Amaral and Witter, 1995; Bannister and Larkmann, 1995a; Bannister and Larkmann, 1995b; Pyapali et al., 1998). The total dendritic lengths (~ 12 mm) are similar across pyramidal cell populations (Amaral et al., 1990; Bannister and Larkmann, 1995a). The apical dendritic trees of hippocampal pyramidal cells show a fair amount of variability. For example, some cells have one principal apical dendritic shaft while others have two. The apical dendrites give off several oblique branches in the stratum radiatum before further dividing in the stratum-lacunosum moleculare border to form an apical turf (Amaral et al., 1990; Bannister and Larkmann, 1995a). In respect to thickness and branching pattern, the dendrites in stratum oriens and radiatum are similar but they differ from those in stratum-lacunosum moleculare (Megías et al., 2001). After an initial spine-free or sparsely spiny segment, both apical and basal dendrites are densely covered with spines. Excitatory inputs terminating in the distal portion of stratum radiatum are exclusively on dendritic spines, while inhibitory inputs target predominantly dendritic shafts. The proportion of inhibitory inputs to distal stratum radiatum dendrites is small (~ 3%). In contrast, the majority (70 - 100%) of inhibitory inputs target the proximal dendrites (Megías et al., 2001). The cell body and axon initial segment receive only inhibitory inputs. The pyramidal cell axon arises from the base of the cell body and

extends into the stratum oriens. Lorente de Nó (1934) noted a variety of subtle differences in the dendritic organization of pyramidal cells in different parts of CA3 and CA1 and used these distinctions, in part, to further subdivide these fields into three subareas each (CA3a,b,c; CA1a,b,c).

A significant number of hippocampal neurons do not show the relatively uniform characteristics of pyramidal neurons. These so-called non-pyramidal cells are distributed over all hippocampal layers and are traditionally regarded as ‘interneurons’, or local circuit neurons. Most of them form a basket plexus of axons and terminals around the cell bodies of pyramidal cells. The cell types that gave rise to this plexus are called “basket cells” (Lorente de Nó, 1934). The majority of nonpyramidal neurons can be immunostained with antibodies against gamma aminobutyric acid (GABA) or its synthesizing enzyme glutamate decarboxylase (GAD) and are considered essential inhibitory elements of the hippocampus (Ribak et al., 1978, 1981; Somogyi et al., 1983; Misgeld and Frotcher, 1986; Frotcher and Zimmer, 1987; Woodson et al., 1989; Miles et al., 1996; Katona et al., 1999). Nonpyramidal cells have been classified according to cell body location in the various layers of the hippocampus and the course of their dendritic and axonal projections (Freund and Buzsaki, 1996; Parra et al., 1998). Hippocampal interneurons also have apical and basal dendritic trees, like the pyramidal cells, but their dendrites have few if any dendritic spines and have an overall beaded appearance.

1.1.5. The “lamellar hypothesis” of hippocampal anatomical organization

The “lamellar hypothesis” of hippocampal intrinsic connections was developed in the early 1970s on the basis of existing neuroanatomical and electrophysiological data

(Andersen et al., 1971). According to this proposition, the major excitatory pathways in the hippocampus were all oriented perpendicular to the long axis of the structure in such a way that a point source of entorhinal activity projects its impulses along a restricted septotemporal hippocampal segment (Andersen et al., 1971). Thus, the hippocampal formation was assumed to comprise a number of similarly organized but connectionally isolated slices, or lamellas. The “lamellar hypothesis” had tremendous influence on the conceptualization of intrahippocampal information processing (Amaral and Witter, 1989). It suggested that the hippocampus could be conceived as containing a series of independent processing units. By means of this lamellar organization, narrow hippocampal strips may operate as independent functional units. The major flow of neural activity was suggested to be within each slice rather than between different slices. Furthermore, it was presumed that the lamellar organization of the hippocampal formation entailed a physical discontinuity of lamellae. Subsequent neuroanatomical studies have demonstrated the idea that the flow of neural activity takes place within a lamella is no longer tenable (Tamamaki et al., 1984, 1988; Amaral and Witter, 1989). It was determined that the CA3 to CA3 associational and CA3 to CA1 Schaffer collateral projections are both divergently distributed along the longitudinal axis of the hippocampal formation. Tamamaki et al. (1984, 1988) have shown that single CA3 and CA2 pyramidal cells give rise to highly-arborized axonal projections that distribute to as much as 75% of the longitudinal extent of the both ipsilateral and contralateral CA1 fields. These findings argue against the idea that information is processed within the hippocampus in a lamellar fashion. The major intrahippocampal projections are much more divergent than would be consistent with a strict interpretation of the “lamellar

hypothesis". Therefore, the "lamellar hypothesis" does not represent accurately the anatomical organization of the hippocampal formation. Most of the intrinsic hippocampal connections are extensively distributed in both septotemporal and transverse axis (Amaral and Witter, 1989; Amaral and Witter, 1995). Thus, within the hippocampal formation the distribution of activity most likely takes place along both the septotemporal and transverse axis, which reflects realistically the three-dimensional organization of its circuitry.

1.1.6. Hippocampal information processing

A unique feature of the hippocampal circuitry is the largely unidirectional organization of the projections that interconnect the various hippocampal regions. Current views of intrahippocampal information processing are all based on the trisynaptic circuit, which was first outlined in the neuroanatomical studies of Ramón y Cajal (1911).

1.1.6.1. The dentate gyrus

The first stage of the intrahippocampal trisynaptic loop is the dentate gyrus. It is the target of the perforant pathway, considered the most substantial output of the entorhinal cortex. The entorhinal afferents carry sensory information of multiple modalities about the external world. The medial entorhinal cortex projections terminate in the middle one-third and those from the lateral entorhinal cortex in the outer one-third of the dentate molecular layer. Perforant path fibers use glutamate as a neurotransmitter (Storm-Mathisen 1977; Storm-Mathisen et al., 1983) and form asymmetric synapses on the spines of granule cell dendrites, where they constitute at least 85% of the total number

of synapses (Matthews et al., 1976). In addition to its massive input to the molecular layer of the dentate gyrus, both major parts of the entorhinal area send projections to the hilar area and to the molecular layer of Ammon's horn. The only major extrinsic projection of the dentate gyrus is to the adjacent CA3 field by way of granule cell mossy fibers. The dentate granule cells' axons form the second link in the classical trisynaptic circuit. The mossy fibers synapse asymmetrically on the spines of the proximal dendrites of field CA3 pyramidal cells, in the stratum lucidum, and on the dendritic shafts of basket cells (Claiborne et al., 1986; Frotscher, 1986). Similarly to the other principal intrinsic connections of the rat hippocampal formation, the mossy fibers use glutamate as a primary transmitter substance (Storm-Mathisen and Fonnum, 1972).

1.1.6.2. Field CA3

The CA3 field of the hippocampus forms the second stage of the classical trisynaptic circuit. CA3 receives the dentate mossy fiber input and in turn projects to the adjacent field CA1. The intrahippocampal projections of CA3 are considerably more complex than those of the dentate gyrus or CA1. The CA3 pyramidal cells give rise to highly collateralized axons that distribute fibers to all hippocampal fields (CA3, CA2, and CA1), to the same fields in the contralateral hippocampus (the commissural projections), and subcortically to the lateral septal nucleus. The divergent fibers to field CA3 correspond to Lorente de Nó's (1934) "longitudinal association bundle", whereas the fibers projecting to CA1 correspond to the 'Schaffer' (1892) collateral system". The longitudinal association bundle is difficult to differentiate experimentally from the Schaffer collateral system and both appear to constitute a continuous plexus of nerve

fibers and terminals. CA3 cells also contribute a small number of collaterals that project medially to the hilar region of the dentate gyrus on the same side of the brain. Individual pyramidal cells in field CA3 synapse in the stratum oriens and stratum radiatum of fields CA3 and CA1. These fibers appear to utilize glutamate as a neurotransmitter and innervate both pyramidal cells and interneurons in field CA1. An important neuroanatomical feature of CA3 projections to CA1 is that individual CA3 axon collaterals take both linear and oblique courses through CA1 and that the same CA3 axon may terminate on the apical dendrite of one neuron and the basal dendrite of another. As a result, both the stratum radiatum and the stratum oriens of CA1 are heavily innervated by CA3 pyramidal cells axons. Thus, the Schaffer collaterals are as highly associated with the apical dendrites of CA1 cells in the stratum radiatum as they are with the basal dendrites in the stratum oriens (Amaral and Witter, 1995).

1.1.6.3. Field CA1

The CA1 subfield forms the third and last stage of the classical intrahippocampal circuit. CA1 is the major target of CA3 pyramidal cell axons. The intrahippocampal projections of field CA1 are considerably simpler than those of field CA3. The CA1 field has two major intrahippocampal projections. The first is a topographically organized projection to the subicular complex. CA1 pyramidal cells project in a lamellar fashion to the pyramidal and molecular cell layers of the ipsilateral subiculum (Amaral et al., 1991; Amaral and Witter, 1995). The second projection of CA1 is to the deep layers of the entorhinal cortex (Beckstead, 1978). Unlike the CA3 and CA2 fields, the CA1 has reciprocal projections to the entorhinal cortex. The medial and lateral perforant paths

terminate along the full radial extent of the CA1 stratum-lacunosum moleculare. In return, CA1 projects to both, the medial and lateral entorhinal cortex. The fibers reaching the medial entorhinal cortex predominate. Projections of CA1 to the entorhinal areas originate from the full longitudinal and transverse extent of the field. Terminals are found in all entorhinal layers, but are most prominent in layer 4. The CA1 field gives rise to substantially greater extrinsic connections than the CA3. CA1 field projects not only to the same subcortical structures as CA3, but also to cortical areas such as the retrosplenial and perirhinal cortex (Amaral and Witter, 1995).

1.2. Long-term potentiation (LTP) in the hippocampus

1.2.1. Introductory remarks

In 1973 Tim Bliss and Terje Lømo, working on the hippocampus of an anesthetized rabbit, discovered that synaptic responses of dentate granule cells could be potentiated for hours by the delivery of high-frequency volley of pulses to the perforant pathway afferents. After the high-frequency volley, the postsynaptic excitability was drastically increased; that is the response to a mild input exceeded the level seen prior to the volley. This effect lasted for as long as they could keep the preparation intact. The potentiation was measured as increased amplitudes of excitatory postsynaptic potentials and increased amplitude of the population action potentials (Bliss and Lømo, 1973). With the development of the *in vitro* hippocampal slice, the study of LTP became much easier and popular. In the *in vitro* slice preparation it is possible to carry out intracellular or voltage clamp recording, thus LTP can be examined in individual postsynaptic neurons as well as in populations of postsynaptic neurons via an extracellular electrode.

Schwartzkroin and Wester (1975), working in the *in vitro* hippocampal slice, showed that LTP could be induced in synapses on CA1 neurons, following stimulation of the stratum radiatum, and that this effect could last for at least several hours. Since then, LTP in the CA1 region of the hippocampus has become the dominant model of activity-dependent synaptic plasticity in the mammalian brain, and much progress has been made in elucidating its mechanisms. Alternative forms of synaptic plasticity that may be responsible for the decrease of synaptic strength also exist. A phenomenon known as long-term depression (LTD), a long-lasting decrease in synaptic strength has been described at synapses in various regions of the mammalian brain including the hippocampus. LTD can be evoked in the CA1 region of the hippocampus; however its induction requirements are distinct from those necessary for LTP.

1.2.2. LTP: a cellular model of memory and learning

LTP refers to a long-lasting enhancement (minutes to days) of synaptic transmission between the presynaptic and postsynaptic neurons. LTP is a phenomenon that occurs at different kinds of central and peripheral synapses but has been discovered and most thoroughly studied in the hippocampus. Activity-dependent changes in synaptic strength have been demonstrated at all three major excitatory pathways in the hippocampus: the perforant pathway to the dentate granule cells, the mossy fibers to CA3 pyramidal cells, and the Schaffer collateral/commissural projections to CA1 pyramidal cells. LTP in the CA1 region of the hippocampus however, has generated more interest and more investigation than any other form of synaptic plasticity. LTP at the Schaffer collateral/commissural-CA1 pyramidal cell synapse requires the activation of *N*-methyl-

D-aspartate receptor (NMDAR) complex. This subtype of glutamate receptor has some special properties that allow coincidental presynaptic/postsynaptic activity to be detected and transduced into a persistent increase in synaptic strength.

Several characteristics of LTP make it a possible cellular and molecular mechanism for learning: first, pharmacological and lesions studies have unequivocally demonstrated that the hippocampal formation and related temporal lobe structures such as the entorhinal and perirhinal cortex are involved in certain forms of long-term information storage (Morris et al., 1982; Zola-Morgan and Squire, 1993; Zola-Morgan et al., 1993); second, LTP is consistent with the assumption that information is stored in the brain as changes in synaptic efficiency (Hebb, 1949); third, similarly to learning, LTP is induced rapidly and can last for many hours and days; fourth, LTP can be associative: temporally pairing 'weak' with 'strong' synaptic inputs leads to potentiation of the 'weak' inputs. The 'associativity' feature of LTP resembles some simpler forms of learning such as classical conditioning and habituation, studied by psychologists and neuropsychologists at the behavioral level.

1.2.3. Properties of hippocampal LTP

LTP in the CA1 hippocampal region is characterized by three basic properties: input-specificity, cooperativity and associativity. First, LTP is input-specific. Thus, the potentiation is confined to the synapses that are activated by the high-frequency stimulation. Inactive synapses on the same cell will not be potentiated. Second, LTP induction is a cooperative process requiring coactivity of a large number of afferents. Threshold stimulus intensity is required to obtain LTP that is considerably higher than

that required to show a synaptic response at all. 'Cooperativity' suggests the existence of a postsynaptic depolarization threshold: 'weak' tetani, activating relatively few afferent fibres, do not depolarize the postsynaptic cell sufficiently and do not trigger LTP. Thus, LTP induction is a complex function of the intensity and rate of presynaptic stimulation. Finally, LTP is associative in the sense that weak input can be potentiated if it is active at the same time with strong input to the same cell. The cooccurrence of transmitter release and strong postsynaptic depolarization are sufficient to account for the 'input-specificity', 'cooperativity' and 'associativity' features of LTP. For example, postsynaptic hyperpolarization prevents LTP induction by a strong tetanic stimulus (Malinow and Miller, 1986). However, although postsynaptic depolarization is necessary, it is not in itself sufficient. Injection of depolarizing current via intracellular or patch electrode does not induce LTP in the absence of afferent stimulation. Thus, concurrent stimulation of the synaptic input, together with postsynaptic depolarization, is required for successful induction of LTP (Bliss and Collingridge, 1993).

1.2.4. Induction of LTP: role of NMDARs and Ca^{2+}

The synaptic modifications associated with LTP are confined to the excitatory glutamatergic synapses of CA1 pyramidal cells (Yuste and Denk, 1995; Nymchinsky et al., 2000; Sabatini et al., 2002). The mechanism responsible for the induction of LTP at the Schaffer collateral/commissural-CA1 pyramidal cell synapse has been determined largely by pharmacological means. There is a general agreement that LTP is triggered by Ca^{2+} influx into the postsynaptic cell (Eccles, 1983; Bliss and Collingridge, 1993). The induction of LTP could be blocked by preventing the rise in intracellular Ca^{2+} by loading

postsynaptic cells with Ca^{2+} chelators (Lynch et al., 1983; Malenka et al., 1988). Conversely, experimentally increasing the level of postsynaptic Ca^{2+} can mimic LTP (Malenka et al., 1988; Yang et al., 1999). Because NMDARs are located at dendritic spines and have high permeability to Ca^{2+} , it is generally believed that the signal necessary for LTP induction is mediated mainly by this subtype of glutamate receptor (Regehr and Tank, 1990; Perkel et al., 1993; Yuste and Denk, 1995). Furthermore, the induction of LTP is prevented by a variety of NMDAR antagonists, including those that act at the receptor, such as 2-amino-5-phosphonopentanoate (AP5), in the channel dizocilpine maleate (MK-801) and at the allosteric glycine site 7-chlorokynurenic acid (7-Cl-KYN) (Collingridge et al., 1983; Coan et al., 1987; Bashir et al., 1990). For LTP to be induced, NMDARs must be in the appropriate state. During low-frequency transmission, the excitatory neurotransmitter glutamate binds to three classes of excitatory amino acid receptors: NMDA, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate. Because NMDARs are blocked by extracellular Mg^{2+} when the cell is close to its resting potential, the majority of the inward current responsible for generating synaptic responses is mediated by the non-NMDARs (Collingridge et al., 1983; Mayer et al., 1984; Nowak et al., 1984; Bliss and Collingridge, 1993). But if the cell is already depolarized, NMDARs will open for tens of milliseconds and allow Ca^{2+} , as well as Na^+ , into the postsynaptic neuron. Thus, NMDARs detect the coincidence of synaptically released glutamate and postsynaptic depolarization. The resultant rise in second messenger ion (Ca^{2+}) through NMDARs is a necessary step for long-lasting alterations of the synapse.

The LTP properties of ‘input-specificity’, ‘cooperativity’ and ‘associativity’ can be easily explained with the special features of NMDAR. ‘Input-specificity’ is explained by the need of sufficient concentration of glutamate at the synapse. Glutamate is usually provided by action potentials arriving at the presynaptic terminals. ‘Cooperativity’ reflects the strong voltage-dependence of NMDAR and the need Mg^{2+} ions to be expelled from their binding sites by depolarization of some critical amount. ‘Associativity’ can also be explained by the need of sufficient postsynaptic depolarization. However, here the necessary depolarization has to come by electrotonic spread from a different highly activated synapse. The necessary amount of depolarization can be even provided by injecting directly current into the postsynaptic cell (Kelso et al., 1986).

Other mechanisms by which Ca^{2+} may enter the cell and contribute to LTP are also available. Voltage-dependent calcium channels (VDCCs) have been localized to the dendritic shafts and spines of CA1 pyramidal cells (Jaffe et al., 1994; Mills et al., 1994) and evoked excitatory postsynaptic potential (EPSPs) may be sufficient to activate them (Magee and Johnston, 1995; Magee et al., 1998). Intracellular Ca^{2+} elevation associated with activation of VDCC may lead to LTP, LTD, or STP (short-term potentiation) (Johnston et al., 1992). Ca^{2+} may also enter the spine through Ca^{2+} -permeable AMPA channels (Yuste et al., 1999) or be released from intracellular stores in response to NMDAR activation (Emptage et al., 1999). However, the LTP resulting from Ca^{2+} sources other than NMDARs is not well understood and most likely utilizes distinct mechanisms (Grover and Teyler, 1990; Cavus and Teyler, 1996).

In summary, the available evidence suggests that at the Schaffer collateral/commissural-CA1 synapse, Ca^{2+} permeates NMDA channels to provide a

transient signal, which is necessary for the induction of LTP. Most likely this signal is restricted to the activated spines, which function as separate compartments allowing Ca^{2+} build-up during intense synaptic stimulation.

1.2.5. Expression mechanisms

The question whether LTP expression reflects mainly presynaptic or postsynaptic modifications has been generating controversy for a long time. Evidence exists in support of both presynaptic and postsynaptic models of LTP. There is widespread agreement that the induction of LTP depends on events that occur in the postsynaptic neuron. The presynaptic involvement in LTP, most likely is during the maintenance phase of LTP, beginning at 30 min or longer after induction. The identification of the loci and nature of storage and expression mechanisms remains a major challenge.

The simplest presynaptic modification that can account for the potentiated postsynaptic response could be an increase in the amount of glutamate released per impulse. Evidence for an increase in transmitter release has been reported by studies using radiolabel or endogenous glutamate to estimate glutamate concentration before and after LTP (Bliss et al., 1986; Bekkers and Stevens, 1990; Malgaroli and Tsien, 1992). Additional data in support of presynaptic localization comes from experiments using minimal stimulation paradigm where it was found that LTP was accompanied by change in the probability of transmitter release but no change occurred in the amplitude of postsynaptic current (Stevens and Wang, 1994). Subsequent attempts to replicate the evidence for elevated glutamate in potentiated hippocampal slices have failed (Aniksztejn et al., 1989; Jay et al., 1999) and it remains uncertain whether glutamate was measured

accurately. If during LTP the probability of transmitter release is altered, then paired-pulse facilitation and other forms of short-term presynaptic plasticity should also be affected. Most investigators find that these phenomena are minimally affected by LTP (Manabe et al., 1993; Asztely et al., 1996; Selig et al., 1999). Furthermore, measurements of transmitter transporter currents in glial cells indicate that current amplitudes although sensitive to the amount of transmitter release are not altered after the induction of LTP (Manabe and Nicoll, 1994; Diamond et al., 1998; Luscher et al., 1998).

Other studies have provided evidence for purely postsynaptic modifications. Pharmacological examination of NMDAR- and non-NMDAR-mediated synaptic responses after LTP induction suggested that non-NMDAR responses were greatly increased while the NMDAR-mediated responses were not potentiated (Kauer et al., 1988; Muller and Lynch, 1988). The absence of changes in NMDAR-mediated responses focused the attention on the mechanisms responsible for the increased non-NMDAR sensitivity to glutamate during the early phases of LTP. Detailed electrophysiological and biochemical studies point to an increase in the function of AMPARs. Brief application of NMDA or intense activation of Ca^{2+} channels lead to an increase in the amplitude of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) (Manabe et al., 1992; Wyllie et al., 1994; Oliet et al., 1996). Similar results were obtained by exogenous application of agonists: AMPA responses gradually increased during LTP (Davies et al., 1989). Furthermore, it has been demonstrated that during LTP, the postsynaptic modification of AMPAR function is due in part to an increase in the single channel conductance (Benke et al., 1998; Derkach et al., 1999). This suggests that elementary channel properties can be rapidly modified during synaptic activity. The most likely

mechanism responsible for the increase of AMPAR responsiveness to glutamate is phosphorylation of the AMPAR subunit GluR1. During LTP, there is an increase of phosphorylation of GluR1 on serine 831, an effect that is prevented by protein kinase inhibitors (Barria et al., 1997). In addition, genetic knockout of GluR1 in mouse prevents LTP in CA1 pyramidal cells, which indicates that hippocampal LTP is controlled by the number or subunit composition of AMPARs (Zamanillo et al., 1999). Even stronger evidence in support for postsynaptic loci of LTP expression comes from experiments studying synapses containing only NMDARs (Rao and Graig, 1997; Gomperts et al., 1998; Liao et al., 1999; Petralia et al., 1999; Takumi et al., 1999). Synapses with only NMDARs would be functionally silent during glutamatergic stimulation at potentials close to resting. However, it was demonstrated that LTP induction causes rapid appearance of AMPARs at NMDAR-only synapses (Isaac et al., 1995; Liao et al., 1995; Durand et al., 1996). Thus, the postsynaptic expression of LTP most likely is due to both phosphorylation and rapid delivery and insertion of AMPARs within the postsynaptic membrane.

1.2.6. Signal transduction mechanisms in LTP

Several distinct biochemical mechanisms have been suggested to play a role in translating the Ca^{2+} signal into stably potentiated synaptic responses. Most interest has focused on phosphorylation cascades and in particular the role of protein kinases. Protein kinases appear to be capable of converting the probable induction signal, the entry of Ca^{2+} through the NMDA channel, into persistent increase in synaptic strength. There is general agreement that Ca^{2+} /calmodulin-dependent protein kinase II (CaMKII) is a

critical participant in the mechanisms responsible for LTP. CaMKII is found in high concentrations in the postsynaptic density (PSD). In the presence of micromolar concentrations of Ca^{2+} , a Ca^{2+} -calmodulin complex is formed. Binding of this complex to CaM kinase II subunits results in conformational changes revealing the enzyme's active catalytic domains. In addition to phosphorylating a number of cellular proteins, CaMKII has the interesting property to undergo rapid autophosphorylation on threonine 286, which renders the enzyme independent of Ca^{2+} and calmodulin. Such activation allows its activity to outlast the Ca^{2+} signal that originally activated the enzyme (Fukunaga et al., 1993; Braun and Schulman, 1995). Inhibitor studies have provided more direct evidence for the role of CaMKII in LTP. Directly loading CA1 pyramidal cells with specific CaMKII inhibitors during induction prevents LTP (Malenka et al., 1989; Malinow et al., 1989). Moreover, knockout mice, deficient in critical CaMKII subunit have been shown to have impaired LTP and spatial learning (Silva et al., 1992). In addition, injecting the postsynaptic cell with autoactivated form of CaMKII can induce LTP-like potentiation (Lledo et al., 1995). Recent studies have provided more direct evidence for the role of CaMKII in LTP. Autophosphorylation of CaMKII occurs after the induction of LTP, as does an increase in the phosphorylation level of one of CaMKII major substrates, the AMPAR GluR1 subunit (Barria et al., 1997; Giese et al., 1998).

Several other protein kinases have also been implicated to play role in LTP. These include: Ca^{2+} /phospholipid-dependent protein kinase (PKC); cyclic adenosine 3,5-monophosphate (cAMP)-dependent protein kinase (PKA); the tyrosine kinase Src; and mitogen-activated protein kinase (MAPK). PKC was the first protein kinase to be implicated in LTP. PKC is present in the brain in high concentrations with the highest

PKC activity found in the hippocampus and cerebellum. Similarly to CaMKII, PKC also has the ability to autophosphorylate and prolong its activity (Pontremoli et al., 1990). Evidence suggests that PKC has a similar to CaMKII effect on LTP. PKC inhibitors have been reported to block LTP, while loading the postsynaptic cell with PKC produces LTP-like potentiation and facilitation of synaptic transmission (Hu et al., 1987; Klann et al., 1991; Sacktor et al., 1993). The induction of NMDAR-dependent LTP is associated with increased cAMP levels, which suggests the involvement of PKA (Chetkovich et al., 1991). However, PKA may also increase the activity of CaMKII by inhibiting certain protein phosphatases (Blitzer et al., 1998; Makhinson et al., 1999). Blockade of tyrosine kinase Src has been shown to prevent LTP and Src activity was increased by stimulation producing LTP (Lu et al., 1998). The role of MAPK in LTP is less clear. One possible mechanism is a MAPK-mediated reduction in K^+ current by phosphorylation of dendritic potassium channels (Yuan et al., 2002).

In conclusion, the induction of NMDAR-dependent LTP can be nicely explained by the interesting and unusual properties of NMDAR complex. The expression of synaptic potentiation probably involves a combination of presynaptic and postsynaptic mechanisms and proceeds in stages. Ca^{2+} influx through NMDARs binds to calmodulin to activate CaMKII, which after undergoing autophosphorylation phosphorylates GluR1 subunit of the AMPARs to increase their single channel conductance. CaMKII can also contribute to LTP by participating in the delivery and insertion of additional AMPARs in the postsynaptic membrane. The late phase of LTP most likely requires changes in transmitter release and protein synthesis. Alterations in dendritic morphology and synapse number may also contribute to changes in synaptic efficiency.

1.3. Excitatory amino acid (EAA) receptors (ionotropic/metabotropic)

1.3.1. Overview of glutamate receptors

EAAAs (glutamate, aspartate, cysteate, and homocysteate) are the principal excitatory neurotransmitters in the mammalian nervous system. They produce depolarizing effects in neuronal cell types of virtually any origin. Glutamate however, is considered the most important excitatory substance in the brain that fulfills most of the criteria for neurotransmitter candidate. It occurs in high concentrations in the brain and can exert powerful stimulatory effect on neuronal activity. Glutamate is synthesized in the nerve terminals from two sources: from glucose via the Krebs cycle and transamination of α -oxoglutarate and from glutamine that is synthesized in glial cells and converted to glutamate by glutaminase after translocation to glutamate-containing nerve terminals (Nicholls, 1994). Glutamate is stored in synaptic vesicles and released by exocytosis in a Ca^{2+} -dependent manner. The action of glutamate is terminated by a high-affinity transporters located on the presynaptic terminal or on glial membranes.

The neuroactive substances in the brain alter some properties of a target neuron. This is achieved through neurotransmitter and hormone receptors that recognize the signaling molecule and convert the extracellular signal to a response. Glutamate is termed a “mixed agonist” because it activates different types of receptors. Each type of receptor has a distinct pharmacology and physiology. The receptors mediating the various glutamate effects can be broadly classified in two main categories (Nakanishi, 1992; Seeburg, 1993; Hollmann and Heinemann, 1994). The first category (ionotropic) includes receptors that mediate the fast excitatory effects of glutamate. The ionotropic receptors

are coupled directly to cationic channels whose activity they regulate. They are further subdivided into three groups and named after the agonist compounds that are specific in eliciting a given physiological response. Thus, the receptors are called AMPA, kainate, and NMDA. The second broad category of glutamate receptors comprises the metabotropic glutamate receptors (mGluRs). These receptors do not form ion channels; instead they couple to G-proteins, which, in turn, are coupled to different intracellular transduction systems (Tanabe et al., 1992). Members of the mGluR family can be divided into three subgroups according to their sequence similarities, signal transduction properties, and pharmacological profiles to agonists. The first subgroup, comprised of mGluR1 and mGluR5, is coupled to the stimulation of phosphatidylinositol hydrolysis/ Ca^{2+} signal transduction. The second group, mGluR2 and mGluR3, is negatively coupled through adenylyl cyclase to cAMP formation. The third group, mGluR4, mGluR6, mGluR7, and mGluR8, is also negatively linked to adenylyl cyclase activity but shows a different agonist preference from that of mGluR2 and mGluR3. As a group, mGluRs are widely expressed throughout the brain, but the individual subtypes show some differential distribution. The functional role of mGluRs has not been well defined because of a lack of good receptor antagonists. No agonist yet identified appears to be specific for any single metabotropic receptor subtype.

1.3.2. AMPARs

AMPARs mediate fast excitatory synaptic neurotransmission in most of the synapses in the central nervous system (CNS). This class of receptor was initially named “quisqualate receptor”, but the name was changed, since quisqualate was found to act on

mGluRs and AMPA acted more specifically on AMPARs. The AMPAR family comprises four highly homologous subunits, termed GluR1 - GluR4 (Boulter et al., 1990; Keinänen et al., 1990; Seeburg, 1993; Hollmann and Heinemann, 1994). The four subunits are of similar size (~ 900 amino acids), share 68-73% homology in their amino acid sequence and contain four distinct hydrophobic domains (M1 - M4). Each of the GluR1 - GluR4 subunits occurs in two different forms, flip and flop, created by alternative splicing of a 115-base pair region immediately preceding M4 segment (Sommer et al., 1990; Monyer et al., 1991). AMPARs are homomeric or heteromeric oligomers composed of these multiple subunits. The pharmacological and physiological properties of native AMPARs are consequences of different assemblies of these subunits. The subunit composition of native AMPARs is unknown and is likely to vary regionally.

AMPARs are found throughout the CNS, although regional differences in the distribution are conspicuous. Brain regions that are rich in high affinity [³H]AMPA binding include the hippocampus, which has higher densities in CA1 than CA3, and higher densities in the pyramidal cell layer than stratum radiatum and stratum oriens. High levels of [³H]AMPA binding are also found in the molecular layer of the dentate gyrus and in the superficial layers of the cerebral cortex (Olsen et al., 1987). Using *in situ* hybridization histochemistry, AMPAR subunit distribution was examined in more detail. In the hippocampus GluR1 - GluR3 messenger ribonucleic acids (mRNAs) are abundant in CA1 and CA3. The expression of GluR4 mRNAs is much less abundant, and is relatively higher in CA1 and the dentate gyrus (Sommer et al., 1990; Monyer et al., 1991).

The binding of glutamate or AMPA to the receptor is associated with an inward depolarizing current primarily carried by Na^+ ions. AMPARs in central neurons are also permeable to K^+ but considered almost impermeable to Ca^{2+} . However, it was found that AMPARs of cultured hippocampal neurons could be permeable to Ca^{2+} (Ozawa and Lino, 1993). The permeability of AMPARs to Ca^{2+} is determined by receptor-subunit composition. GluR1 and GluR3 can form homomeric or heteromeric receptors that are permeable to Ca^{2+} . However, inclusion of GluR2 subunits in the receptor assembly renders Ca^{2+} impermeability (Hollmann et al., 1991). The most selective and potent AMPAR antagonists available are a series of dihydroxyquinoxaline derivatives including 6-nitro-7-sulfamoylbenzo(f)-quinoxaline-2,3-dione (NBQX), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6,7-dinitroquinoxaline-2,3-dione (DNQX). However, these agents also block kainate receptors, although NBQX appears to exhibit the best selectivity for AMPARs.

1.3.3. Kainate receptors

The lack of specific antagonists for kainate receptors has been complicating the pharmacological and physiological characterization of non-NMDARs. Recombinant expression studies have demonstrated the ability of kainic acid (KA) to activate AMPARs and of AMPA to activate kainate receptors (Hollmann et al., 1989; Huettner, 1990; Keinanen et al., 1990). Although KA is a potent agonist of the AMPAR, it also binds with extremely high-affinity to a distinct subclass of KA-preferring ionotropic glutamate receptors. Subsequently, using low-stringency hybridization screening with AMPAR subunit probes, low-affinity (GluR5-GluR7) and high-affinity kainate receptor subunits

(KA1 - KA2) were cloned (Bettler et al., 1990; Egebjerg et al., 1991; Bettler et al., 1992; Koeler et al., 1993). GluR5 - GluR7 are of similar size (~ 900 amino acids) and share 75 - 80% homology in their amino acid sequence. KA1 and KA2 (~ 970 amino acids) also share high amino acid sequence identity with each other (70 - 80%), but only about 40% with either GluR1 - GluR4 or GluR5 - GluR7. The physiological and pharmacological properties of homomers and heteromers of kainate subunits vary depending upon the subunit composition.

Kainate subunits distribution throughout the brain has been determined with *in situ* hybridization histochemistry and immunohistochemistry (Herb et al., 1992; Sakimura et al., 1992; Seeburg, 1993; Wisden and Seeburg, 1993; Bahn et al., 1994; Roche and Huganir, 1995; Siegel et al., 1995). It was found that each kainate receptor subunit is differentially distributed throughout the brain. KA2 is universally distributed, whereas KA1 predominates in the dentate gyrus and CA3 hippocampal subfield. GluR5 and GluR7 are found in the subicular complex, cingulate and piriform cortex, basal ganglia, and cerebellum. GluR6 expression is high in cerebellar granule cells, basal ganglia and hippocampus. Although kainate and AMPAR subunits can coexist in the same neuron, they do not coassemble with each other (Wenthold et al., 1994).

Pharmacological discrimination can be achieved, with AMPA and quisqualate being the preferred agonists for AMPARs and domoate and KA the preferred agonists for kainate receptors. The development of AMPAR selective noncompetitive antagonists GYKI 52466 and GYKI 53655 made possible to isolate native kainate receptor-mediated responses (Lerma, 1997; Vizi et al., 1997). With the use of these compounds, it was possible to demonstrate that fast excitatory currents in the dentate gyrus and CA1

subfield lack a kainate receptor-mediated component, which suggested that kainate receptors do not mediate fast synaptic transmission at the Schaffer collateral-CA1 synapse. It was also found that AMPARs and kainate receptors have similar activation-inactivation kinetics. Furthermore, both have the same affinity to glutamate ($EC_{50} \sim 300 \mu M$).

1.3.4. NMDARs

NMDARs also belong to the family of ionotropic glutamate receptors, however they mediate excitatory neurotransmission in different ways from AMPAR and kainate receptor subtypes (Figure 1). NMDARs primary role may not be to generate excitatory (depolarizing) effects in the postsynaptic neurons, although they do so. Instead they seem to be involved in neuronal plasticity, development and survival. They mediate the above functions by a combination of coincidence detection and signal transduction by elevating intracellular Ca^{2+} . NMDARs have several unique properties. First, extracellular Mg^{2+} blocks the channel in a voltage-dependent manner (Nowak et al., 1984; Mayer and Westbrook, 1987). Second, the channel is highly permeable to Ca^{2+} ions (Mayer and Westbrook, 1987; Ascher and Nowak, 1988). Third, activation and deactivation kinetics are much slower in NMDARs than in AMPA/kainate receptors. The channel burst time is long due to its high affinity to glutamate (Lester et al., 1990). Fourth, for NMDAR activation to occur glycine-binding sites have to be occupied (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988).

The NMDAR is composed of three different subunit families: NR1, NR2A - D, and NR3A - B (Moriyoshi et al., 1991; Monyer et al., 1992; Meguro et al., 1992; Ciabarra

et al., 1995; Sucher et al., 1996; Das et al., 1998; Chatterton et al., 2002). The first NR1 subunit was isolated through expression cloning from a rat brain complementary deoxyribonucleic acid (cDNA) library by Nakanishi's group (Moriyoshi et al., 1991). Soon after a second subfamily of NMDAR subunits (NR2A - D) was cloned by PCR and cross hybridization techniques (Ikeda et al., 1992; Monyer et al., 1992; Ishii et al., 1993). NR1 can exist in eight splice variants (Zukin and Bennett, 1995). These splice variants have considerably different properties and show specific regional localization (Laurie and Seeburg, 1994; Weiss et al., 1998). The two subunit families show low homology (22 - 29% amino acid sequence identity) to other GluR subunits and only 18 - 27% amino acid sequence identity with each other. However, the NR2A - D subunits share considerable homology with each other (42 - 56%). Despite the low overall homology with AMPARs and kainate receptors, NMDARs are believed to share the same tetrameric structure and subunit transmembrane topology as non-NMDARs. Glutamate receptor subunits proved to have only three transmembrane domains (M1, M2 and M4) plus a cytoplasm-facing re-entrant membrane loop (M2). Thus, the N-terminus is located extracellularly, the C-terminus intracellularly and the large putatively extracellularly located loop is between the third and fourth membrane-associated domains (Mori and Mishina, 1995). The large loop between M3 and M4 may also participate in ligand-binding.

Functional homomeric receptor channels can be formed by expression of the NR1 subunit in *Xenopus* oocytes. Homomeric NR1 channels respond to NMDA, glutamate, ibotenate, aspartate, but not to AMPA and KA. Similarly to native NMDAR channels they are permeable to Ca^{2+} , require the presence of co-agonist glycine, and display a prominent voltage-dependent Mg^{2+} block at resting potentials. The current-voltage

relationship in the absence of Mg^{2+} was linear with reversal potential of 0 mV (Moriyoshi et al., 1991; Nakanishi et al., 1992; Sugihara et al., 1992). Thus, homomeric NR1 channels displayed most of the characteristics of the native NMDARs. However, the homomeric current responses are much smaller compared to those in native NMDARs, suggesting that co-expression of subunits from both families are necessary to give the receptor its full efficacy. In addition, transfection of NR1 subunits into mammalian cells does not result in receptors with the properties of native NMDARs. NR1 homomers in mammalian cells do not respond to glutamate or glycine, nor do they form functional glutamate binding sites. Although NR1 homomers bind glycine antagonists with affinity similar to that of native NMDARs, they produce markedly reduced levels of binding sites for the channel blocker MK-801. Evidence also suggests that NR1 homomeric receptors are functional because *Xenopus* oocytes endogenously express a unitary glutamate receptor subunit, which can functionally interact with NR1 (Barnard, 1997; Soloviev and Barnard, 1997; Soloviev et al., 1998). Thus, homomeric NR1 subunits in mammalian cells possess some of the features of native NMDARs but do not form electrophysiologically active channels and rarely reach the cell surface (Monyer et al., 1992; Laurie and Seeburg, 1994; Okabe et al., 1999).

Transfection experiments using homomeric and heteromeric expression of NR2A - D subunits failed to generate functional ion channels (Ikeda et al., 1992; Meguro et al., 1992; Monyer et al., 1992). However, when coexpressed with NR1 each of the four NR2 subunits formed functional heteromeric channels with properties closely resembling those of the native receptors (Meguro et al., 1992; Monyer et al., 1992; Monyer et al., 1994; Vicini et al., 1998). Most notably, in the presence of glycine, current amplitudes for

NR1/NR2D, NR1/NR2C, NR1/NR2A, and NR1/NR2B are 5, 20, 40 and 60 fold larger respectively than NR1 homomers. Each recombinant receptor had reversal potential between 0 and 20 mV in the presence of high N^+ and Ca^{2+} concentrations. Similarly to NR1 homomers, in the absence of external Mg^{2+} the current voltage relationship was linear for all heteromeric subunit combinations (Monyer et al., 1992). However, in the presence of physiological concentrations of extracellular Mg^{2+} , differences between the different heteromeric combinations were observed. The NR1/NR2C and NR1/NR2D combinations are much less sensitive to the voltage-dependent Mg^{2+} block compared to NR1/NR2A or NR1/NR2B (Monyer et al., 1992; Monyer et al., 1994). Distinct biophysical and pharmacological properties that depend on the specific heteromeric combination include: the strength of the Mg^{2+} block; permeability and sensitivity to Ca^{2+} ; affinities to glutamate, glycine, specific agonists and antagonists; desensitization and offset decay kinetics; sensitivity to endogenous modulation by reducing agents, polyamines and phosphorylation (Monyer et al., 1992; Vyklicky, 1993; Buller et al., 1994; Köhr et al., 1994; Monyer et al., 1994; Medina et al., 1995; Vicini et al., 1998; Dingledine et al., 1999). Thus, although NR2 subunits do not form into functional ion channels when expressed as homomeric receptors, the type of NR2 subunit coassembled with NR1 determines the NMDAR properties. In that respect NR2 subunits can be considered as modulatory (Seeburg, 1993).

More recently, using the genetic knockout strategy a novel subunit family (NR3A, NR3B) of the NMDAR has been cloned and characterized (Ciabarra et al., 1995; Das et al., 1998). Coexpression of NR3A with NR1 and NR2 results in NMDARs with decreased whole cell currents and single channel conductance. While the functional

significance of this striking inhibition is not fully understood, this finding suggests that NR3 subunits coexpression with NMDARs may serve a regulatory function. Furthermore, cerebrocortical neurons of NR3A knockout mice have increased dendritic spine density and enhanced NMDAR electrophysiological responses. Thus, NR3 coexpression may be involved in the regulation of Ca^{2+} influx through synaptic NMDARs (Das et al., 1998; Chatterton et al., 2002).

The distribution pattern of each NMDAR subunit was examined using *in situ* hybridization histochemistry (Monyer et al., 1992; Monyer et al., 1994; Mori and Mishina, 1995). The NR1 mRNA is ubiquitously distributed throughout the adult rodent brain, while the four NR2 mRNAs show distinct regional and temporal expression patterns in the developing and adult brain. NR2B transcripts are prominent in the forebrain with high expression levels in the cerebral cortex, hippocampus, basal ganglia and olfactory bulb. The NR2C is predominant in the cerebellum, olfactory bulb, and thalamus. In the hippocampus, NR2C and NR2D are found in a subset of interneurons (Monyer et al., 1994). NR2 subunit expression also shows development regulation. NR2B and NR2D are present at birth, whereas NR2A and NR2C first appear around birth. NR3 has been also shown to be both developmentally and regionally regulated. The highest level of NR3 expression occurs during the first two postnatal weeks (Ciabarra et al., 1995). Since heteromeric NMDARs have different functional differences, the known spatial and temporal expression profiles would permit a characterization of NMDAR properties in different neuronal populations (Seeburg, 1993).

The pharmacology of NMDAR is exceedingly complex (Figure 1). There are at least seven major distinct recognition sites through which compounds can alter the

receptor activity: (1) the glutamate binding site, where in addition to glutamate NMDA, ibotenic, homocysteic, and quinolinic acids can promote the channel opening (2) the strychnine-insensitive glycine-binding site where the co-agonists glycine and D-serine act; (3) the voltage-dependent Mg^{2+} -binding site; (4) the noncompetitive antagonists site (phencyclidine, MK-801, ketamine), which act most effectively when the receptor is in the open state; (5) the inhibitory divalent cation site near the mouth of the channel that binds Zn^{2+} to produce a voltage-independent block; (6) the redox modulatory site; (7) the polyamine-modulatory site whose activation by spermine and spermidine facilitates channel function.

Competitive antagonists for the glutamate-binding site include: ($\pm$)-3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and 2-amino-5-phosphonovaleric acid (APV). The binding sites for the co-agonists D-serine and glycine are blocked by 7-Cl-KYN and 5,7-dichlorokynurenic acid (5,7-diCl-KYN).

1.4. The glycine site of NMDAR

1.4.1. Introductory remarks

Structurally glycine is the simplest amino acid. It is found in significant amounts in body fluids and tissue proteins. Glycine is also an established inhibitory neurotransmitter. Unlike GABA, which acts predominantly in the brain, glycine has more circumscribed neurotransmitter role in the CNS. It appears to mediate most inhibitory neurotransmission in the spinal cord and lower brain stem. Glycine is an exclusively vertebrate transmitter, making it unique among the transmitter substances. The inhibitory effects of glycine in the spinal cord are mediated by a strychnine-sensitive chloride

conductance. The actions of glycine are terminated by a specific sodium-dependent transport system located in presynaptic terminals (Vandenberg et al., 1992; Zafra et al., 1997).

Since the early evidence that glycine may play a physiological role in the modulation of NMDAR-mediated responses, the nature of the interaction between glycine and NMDAR has become the focus of many physiological, pharmacological and biochemical studies. Because NMDARs mediate some important physiological and pathological processes in the human CNS, the strychnine-insensitive glycine-binding site on the NMDAR has attracted a great deal of interest as a potential target for the action of drugs modulating NMDAR function.

Johnson and Ascher (1987) were the first to demonstrate the link between the strychnine-insensitive glycine recognition site and NMDAR. Submicromolar concentrations of glycine potentiated responses of cultured neurons to NMDA. This effect was not mediated by the strychnine-sensitive glycine receptor and was observed as an increase in the frequency of opening of the channels activated by NMDAR agonists. This discovery resulted from the initial observation that the magnitude of the whole cell current evoked by NMDA decreased when the perfusion rate was increased. This suggested that the culture released a substance that augmented NMDA responses, and accumulated when the perfusion rate was slow. After careful examination of the various constituents of the conditioned medium, the common amino acid glycine was found to be the active substance (Johnson and Ascher, 1987). Since then, this finding was confirmed by many studies and our understanding of the modulation of NMDAR through the glycine site has expanded enormously (Kemp et al., 1988; Kleckner and Dingledine,

1988; Mayer et al., 1989; Salt, 1989; Kemp and Leeson, 1993; Kuryatov et al., 1994; Hashimoto and Oka, 1997; Supplissson and Bergman, 1997; Bergeron et al., 1998; Kew et al., 1998; Regalado et al., 2001).

1.4.2. Physiological role of glycine (coagonist/modulator)

Glycine is required for NMDAR activation (Figure 1). Kleckner and Dingledine (1988) first demonstrated this in *Xenopus* oocytes injected with whole-brain mRNA. Neither perfusion nor rapid pressure application of NMDA caused a distinct ionic current without added glycine. The effect of glycine was concentration-dependent and was absent when glycine was carefully removed from the perfusing solution. It was also found that several analogs of glycine could substitute for glycine, among which D-serine and D-alanine were the most effective. This observation suggested that D-amino acids would be important in developing drugs targeted at the glycine site (Kleckner and Dingledine, 1988). The necessity of glycine for the activation of NMDARs was confirmed by a number of patch-clamp studies in neuronal cell cultures (Mayer et al., 1989; Benveniste et al., 1990; Henderson et al., 1990; Chen et al., 1997). Additional evidence for the obligatory role of glycine in NMDAR responses was obtained by showing that selective antagonists of the glycine site completely blocked the NMDAR-mediated response (Kleckner and Dingledine, 1988; Lerma et al., 1990; Parsons et al., 1993; Molnar and Erdo, 1996; Parsons et al., 1997). More recently, it was demonstrated that knockout mice carrying point mutations in the glycine site of the NR1 subunit have a dramatic reduction in the affinity for glycine in addition to a number of physiological and behavioral deficits associated with NMDAR hypofunction (Kew et al., 2000). Thus, without activation of the

glycine site, no activation of the channel is possible, which makes glycine an essential coagonist of NMDARs.

The enhancing effects of glycine on NMDAR responses is observed as an increase in the frequency of channel opening and to a lesser extent, increase in the duration of channel opening (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Lester et al., 1993; Parsons et al., 1993). The mechanism by which glycine regulates the NMDAR has been characterized in *Xenopus* oocytes and native mammalian neurons. Glycine greatly reduces desensitization of NMDARs (Mayer et al., 1989; Vornov and Coyle, 1991; Chen et al., 1997). D-serine was able to substitute for glycine in preventing desensitization (Mayer et al., 1989; Lerma et al., 1990; Parsons et al., 1993). In that role, glycine site agonists most likely act as modulators rather than cotransmitters. Glycine increases the rate of recovery from desensitization, without affecting its onset. Kinetic analysis of glycine modulation suggests that for maximal effect, glycine must bind to the closed state of the receptor (Benveniste et al., 1990). With increasing concentrations of glycine, the magnitude of the desensitization is decreased, which suggests that recovery from desensitization reflects the association kinetics of the agonist and the respective concentrations (Benveniste et al., 1990; Lerma et al., 1990; Parsons et al., 1993). Thus, most likely at higher glycine concentrations, the rebinding of glycine exceeds the rate of dissociation from the receptor, which in patch-clamp studies is observed as a reduced time course of desensitization (Lerma et al., 1990; Parsons et al., 1993; Danysh and Parsons, 1998).

The glycine-sensitive desensitization of NMDARs can be seen in the presence of some full glycine site antagonists (Kemp and Priestley, 1991; Parsons et al., 1993). The

ability to induce desensitization may be different for different antagonists, the most effective being 7-Cl-KYN and 5,7-diCl-KYN (Molnar and Erdo, 1996; Karcz-Kubicha et al., 1997; Parsons et al., 1997). Some partial agonists (e.g. R(+)-HA-996 and D-cyclosterine) have no effect on desensitization; instead they produce allosteric reduction in the affinity of agonists for the NMDA-binding site (Kemp and Priestley, 1991; Parsons et al., 1993). In contrast, 7-Cl-KYN does not induce any allosteric modulation, but acts more like a pure antagonist (Kemp and Priestley, 1991). Whole cell and single channel studies have confirmed that a strong mutual allosteric interaction exists between the NMDA- and glycine-binding sites (Lerma et al., 1990; Lester et al., 1993; Parsons et al., 1993). Thus, the affinities of agonists for glutamate and glycine sites during desensitization are five-fold higher than their respective affinities as agonists at the peak of the response (Chizhnikov et al., 1992). Evidence for negative reciprocal interaction are also available; in the presence of agonists for the NMDA site, glycine-sensitive desensitization is accompanied by a five- to seven-fold decrease in the affinity of the glycine site (Lerma et al., 1990; Parsons et al., 1993).

In summary, for NMDAR activation to occur both glutamate and glycine sites have to be occupied. By its action at the strychnine-insensitive binding site, glycine modulates NMDAR function by increasing the frequency of channel opening. This effect is usually observed as a reduction of NMDAR desensitization. The NMDA and glycine sites appear to be functionally coupled and their interaction is bi-directional; ligands that bind to the glycine site allosterically modulate the activity of NMDA site and *vice versa*. This process appears to be ligand- and concentration-dependent. Although much progress

has been made in elucidating the modulatory role of glycine at NMDARs, the detailed mechanisms *in vivo* largely remain to be determined.

1.4.3. Agonists and antagonists of the glycine site

Glycine was the first agonist described for the glycine site, although its usefulness *in vivo* is limited since it is also a ligand for the glycine inhibitory receptors. The structural requirements for glycine site agonists have been characterized (Huettnner, 1991; Lewin et al., 1993). Compounds with significant agonist activity include amino acids with ionized amino and carboxyl groups with small substituents on the alpha carbon (Huettnner, 1991; Lewin et al., 1993). The distance between the amino and carboxyl groups appears to be critical, since the addition of an extra carbon between the two groups reduced potency. The glycine site shows a preference for *dextro* isomers, although glycine is not a chiral molecule. The stereoselectivity is supported by the fact that L isomers of alanine, serine and cyclosterine have 30-fold lower affinity compared to the D isomers (Lewin et al., 1993). A number of studies employing a variety of techniques have established the potencies for a family of glycine analogs (Ogita et al., 1989; Chizhnikov et al., 1992; Lewin et al., 1993). The relative potencies show the following rank order: glycine > D-serine > D-alanine >> L-serine > L-alanine. In contrast to the full agonists glycine and D-serine, D-cyclosterine is a partial agonist at the glycine site. Other partial agonists include: 1-aminocyclopropanecarboxylic acid (ACPC) and 1-aminocyclobutanecarboxylic acid (ACBC).

Microdialysis studies have confirmed that the two major agonists, glycine and D-serine are present at micromolar concentrations in the mammalian CNS, including human

CNS (Hashimoto et al., 1992; Hashimoto et al., 1993a; Hashimoto et al., 1993b; Westergren et al., 1994; Hashimoto et al., 1995; Matsui et al., 1995; Hashimoto and Oka, 1997). However, substantial regional differences in the distribution of glycine and D-serine have been reported. Higher levels of glycine are detected in the cerebellum than in the frontal cortex (Matsui et al., 1995). In contrast, D-serine levels are five-ten fold higher in the frontal cortex than in the cerebellum. Based on these findings, it was suggested that glycine is the NMDAR coagonist in the cerebellum, whereas D-serine performs the same function in the forebrain (Matsui et al., 1995; Schell et al., 1997). Immunohistochemical studies confirmed the complimentary distribution of D-serine and glycine in the brain (Schell et al., 1995; Schell et al., 1997). Glycine predominates in the cerebellum, hypothalamus and hindbrain. D-serine immunoreactivity is high in the cerebral cortex, hippocampus, striatum, and amygdala (Schell et al., 1995; Schell et al., 1997). D-serine immunoreactivity shows high degree of co-localization with NMDARs in the frontal cortex (Hashimoto et al., 1995; Hashimoto and Oka, 1997; Schell et al., 1997). The highest densities of D-serine binding sites are found in the molecular layer of CA1 hippocampal field (Schell et al., 1997). In the stratum radiatum, D-serine-containing astrocytes are found in close proximity to NR2A/B-enriched dendrites of pyramidal cells (Schell et al., 1997). More recently, Mothet et al. (2000) showed that selective degradation of endogenous D-serine with D-amino acid oxidase (DAAOX) reduced NMDAR responses in brain slices and cell culture preparations. Thus, it appears that at least at some NMDARs, D-serine may be the endogenous modulator of NMDAR function.

The most selective antagonists for the glycine site are some kynurenic acid derivatives. Kynurenic acid (4-hydroxyquinoline-2-carboxylic acid) has been used as a broad spectrum EAA antagonist. It was found however, that the halogen-substituted derivative, 7-Cl-KYN is also a glycine site antagonist, with 70-fold higher affinity than kynurenic acid. 7-Cl-KYN blocked completely NMDAR responses, an effect that was reversed by increasing the concentration of glycine (Kemp et al., 1988). 7-Cl-KYN is the antagonist of choice in many studies on the glycine modulatory site. Another potent and very selective antagonist from the same group is 5,7-diCl-KYN.

1.4.4. Saturation of the glycine site

A very important question, relevant to the clinical potential of glycine site agonists and antagonists is whether the glycine site is saturated *in vivo*. If under physiological conditions the glycine sites were saturated by glycine or D-serine, then any exogenous glycine site agonists would be useless as therapeutic agents.

There are several lines of evidence suggesting that the glycine site could be saturated by extracellular background concentrations of glycine. First, extracellular and cerebrospinal fluid (CSF) levels of glycine are 4 - 10 μM , which is above the affinity for glycine (0.1 - 3 μM) (Grimwood et al., 1992; Baron et al., 1996). Second, under most experimental conditions, NMDAR responses are easily obtained without the addition of glycine. Third, glycine and D-serine reverse the effect of glycine site antagonists, suggesting that the underlying mechanisms remain intact (Fletcher and Lodge, 1988; Kemp et al., 1988). Fourth, extracellular application of glycine or D-serine potentiates

NMDAR-mediated responses only sometimes and inconsistently (Fletcher and Lodge, 1988; Kemp et al., 1988; Bashir et al., 1990).

The following arguments suggest that the glycine site may not be saturated *in vivo*. First, there are some potential problems with the *in vitro* studies reporting a lack of glycine or D-serine effects. Slice preparation and insertion of microelectrodes are conditions often associated with cell damage and release of endogenous substances (e.g. glycine) that may saturate the system. Another potential shortcoming of the studies reporting a lack of agonist effect is that NMDA application or high glutamate levels may induce glycine release. However, even under such unfavorable conditions, D-serine and glycine have been shown to enhance NMDAR responses (Minota et al., 1989; Pullan and Cler, 1989). Second, strong evidence in support of the notion that glycine sites may not be saturated comes from *in vivo* studies, which have clearly demonstrated the excitatory action of glycine and D-serine through NMDARs in central neurons (summarized by Danysz and Parsons, 1998). Third, glycine site agonists have been shown to induce behavioral phenomena such as seizures, stereotypy, and hyperactivity (Peeters and Vanderheyden, 1992; Tanii et al., 1994; Danysz and Parsons, 1998). Furthermore, D-serine and glycine positively affect learning and LTP, both of them known to depend on NMDARs (Oliver et al., 1990; Thiels et al., 1992). Fourth, NMDAR subtypes have differences in the stimulatory effects of glycine. This was first demonstrated by Monaghan et al. (1988), who showed that glycine had differential effects on NMDARs in different brain regions. Since then, the heterogeneous pharmacological characteristics of the glycine site of NMDAR subtypes have been confirmed by many groups (Kutsuwada et al., 1992; Monyer et al., 1992; Wafford et al., 1993; Wafford et al., 1995; Vicini et al.,

1998). The affinities for glycine of different NMDARs subtypes differ drastically. Most studies have found the following rank order of glycine affinities for different NMDARs: NR1/NR2D > NR1/NR2C > NR1/NR2B > NR1/NR2A (Kutsuwada et al., 1992; Priestley and Kemp, 1993; Wafford et al., 1993; Priestley et al., 1995; Buller and Monaghan, 1997; Kew et al., 1998; Vicini et al., 1998). Thus, different affinities to agonists and antagonists of NMDAR subtypes would allow subpopulations of NMDAR to remain non-saturated, depending on the endogenous glycine concentrations (Vicini et al., 1998; Cheffings and Colquhoun, 2000). Fifth, extracellular or CSF concentrations of D-serine or glycine may not reflect accurately the availability of these substances in the synaptic cleft. The concentration of any neurotransmitter substance in the synaptic cleft is strictly regulated by rapid release and active removal involving both uptake mechanisms and enzymatic degradation to inactive metabolites. Although it is not possible to measure directly the neurotransmitter levels in the synaptic cleft, it seems unlikely that active uptake mechanisms would allow glycine concentrations at the synapse to reach values equivalent to those in CSF. This assumption is supported by the existence of glycine uptake system in the cerebral cortex, striatum, hippocampus, and cerebellum (Fedele and Foster, 1992; Fedele et al., 1993). High-capacity, astroglial, glycinergic transporters have been cloned and not surprisingly their expression is high in regions with high density of NMDARs (Smith et al., 1992; Zafra et al., 1995). The role of these transporter systems is to keep the synaptic glycine to subsaturating levels (Sakata et al., 1997; Supplisson and Bergman, 1997; Berger et al., 1998; Bergeron et al., 1998; Herdon et al., 2001; Chen et al., 2003). Thus, it appears that active glycine uptake mechanisms play an important role in the

effectiveness of NMDAR-mediated synaptic transmission by lowering the synaptic concentrations of glycine.

In summary, accumulating evidence suggests that under physiological conditions the glycine sites may not be saturated. The most convincing evidence comes from *in vivo* studies where application of glycine site agonists result in significant physiological and behavioral responses. Additional factors that may control the degree of glycine site occupation include: NMDAR subtype, type and density of glycine transporters and the regional differences of local brain glycine or D-serine levels.

1.4.5. Clinical implications

Deficits in NMDAR functioning have been associated with a variety of neurological disorders. Excitotoxicity and neurodegeneration resulting from overactivation of NMDARs have been implicated in a number of acute and chronic disorders including ischemic stroke, epilepsy, anxiety, Parkinson's disease, head trauma, drug dependence and addiction, AIDS dementia, and neuropathic pain. On the other hand, hypofunction of NMDARs has been associated with learning and psychiatric disturbances, closely resembling the symptoms of schizophrenia. The glycine site has attracted a great deal of interest as a potential target for selective drugs that might be useful in improving NMDAR dysfunction. Agonists and antagonists at the glycine site could have broad therapeutical potential and more benign side effects than other NMDAR agents (Leeson and Iversen, 1994). Indeed, evidence from animal studies suggests that glycine site agonists and antagonists do not produce many of the side effects observed with competitive NMDAR antagonists and channel blockers (Rao et al., 1993;

Ginski and Witkin, 1994). For example, a glycine site partial agonist, L-687,414 has been shown to be an orally active anticonvulsant and neuroprotective in animal models of seizures at doses that unlike MK-801 did not induce blood pressure increase, abnormalities in glucose metabolism and dopamine turnover (Smith and Meldrum, 1992; Tricklebank et al., 1994). There are several potential reasons for the minimal side effects observed with glycine site drugs (Danysz and Parsons, 1998): (1) glycine site antagonists may show better selectivity for different NMDAR subtypes; (2) glycine-sensitive desensitization could be differentially modulated, depending on the degree and type of NMDAR activation; (3) glycine site agents may act preferentially on the functionally impaired NMDARs.

1.4.5.1. Schizophrenia

Hypofunction of NMDARs might be central to the neurobiology of schizophrenia. This hypothesis is based on the behavioral effects of non-competitive NMDAR antagonists and postmortem studies of glutamatergic transmission in brain samples of schizophrenics (Carlsson and Carlsson, 1990; Javitt and Zukin, 1991; Sherman et al., 1991; Olney and Farber, 1995).

Blockade of NMDARs with ketamine, phencyclidine (PCP), or MK-801 provides the best pharmacological model of a schizophrenic psychotic episode (Javitt and Zukin, 1991). PCP-like agents uniquely reproduce both positive symptoms, like hallucinations and delusions, negative symptoms like affective blunting, and cognitive deficits, including thought disorder symptoms and learning defects (Javitt and Zukin, 1991; Newcomer and Krystal, 2001). PCP and ketamine may also act on other transmitter

systems, but the available evidence indicates that the behavioral effects are mediated by NMDARs (Javitt and Zukin, 1991; Olney and Farber, 1995). It has been suggested that enhancing the neurotransmission at NMDARs by glycine site agonists would improve the symptoms of schizophrenia. Indeed, antagonizing the effects of PCP and MK-801 with glycine, D-serine, and D-alanine reduces PCP- and MK-801- induced stereotyped behavior and ataxia in rats (Contreras, 1990; Tanii et al., 1994). Furthermore, glycine has been found to improve neuroleptic-resistant negative symptoms in schizophrenic patients (Javitt et al., 1994; Heresko-Levy et al., 1999). These findings suggest that glycine site agonists have significant antipsychotic potential and offer a potentially safe and feasible approach for ameliorating persistent negative symptoms of schizophrenia. Thus, enhancement of NMDAR function through the glycine site may represent an effective treatment of neuropathological conditions associated with NMDAR hypofunction.

1.5. Research proposal

1.5.1. Hypothesis and specific objectives

1.5.1.1. Study I

Hypothesis to test:

D-serine modulation of NMDAR function differs in hippocampal pyramidal cells and interneurons.

Specific objectives:

1. To characterize the morphological and physiological properties of the recorded pyramidal cells and stratum radiatum interneurons.

2. To investigate the modulatory effect of D-serine on evoked NMDAR EPSCs in pyramidal cells and interneurons.
3. To investigate the modulatory effect of D-serine on NMDAR responses to pressure application of NMDA in pyramidal cells and interneurons.
4. To compare the kinetics of NMDAR currents in pyramidal cells and interneurons.
5. To compare the modulatory effects of D-serine on NMDAR currents in pyramidal cells and interneurons.

1.5.1.2. Study II

Hypothesis to test:

NMDAR contribution to LTP depends on the specific induction conditions.

Specific objectives:

1. To investigate the effect of the degree of postsynaptic depolarization on NMDAR contribution to LTP.
2. To investigate the effect of depolarization duration on NMDAR contribution to LTP.
3. To investigate the relationship between postsynaptic depolarization and the sign of synaptic plasticity.
4. To determine the minimal pre-/postsynaptic requirements for LTP of saturating magnitude.
5. To investigate and characterize the properties of LTP obtained with subthreshold EPSCs.
6. To investigate and characterize the properties of LTP obtained with suprathreshold EPSCs.

7. To investigate the effects of selective NMDAR antagonists on LTP.

1.5.1.3. Study III

Hypothesis to test:

NMDAR-dependent mechanisms of synaptic plasticity can be effectively modulated by the glycine site.

Specific objectives:

1. To investigate the effects of D-serine and glycine on LTP magnitude.
2. To investigate the effects of D-serine and glycine on LTP induction threshold.
3. To compare the effects of D-serine and glycine on LTP.
4. To investigate the effects of both agonists on LTP obtained with different induction protocols.
5. To identify the conditions under which both agonists are most effective.
6. To investigate and characterize the effects of 7-Cl-KYN on LTP.

2. MATERIALS AND METHODS

Whole-cell patch-clamp is a powerful technique for studying cellular physiology in *in vitro* brain slice preparations (Hamill et al., 1981). Whole cell current analysis provides valuable information about the function of ion channels, neurotransmitter receptors, and electrogenic transporters from cell types of almost any origin.

2.1. Surgery

Young adult (25 - 35 days, 50 - 150 g) Sprague-Dawley male rats (Charles River, St. Constant, Quebec, Canada) were used for the study. The animals were treated and housed in compliance with the Canadian Council for Animal Care guidelines. Coronal brain slices were prepared using standard procedures. Every effort was made to minimize animal suffering. Briefly, the rats were anaesthetized by isoflurane inhalation (1 - 3 min) and surgically decapitated at the atlanto-occipital joint following a rostro-caudal cut over the scalp to expose the skull. Following decapitation, the skull of the animal was removed with the use of surgical scissors to expose the brain. After careful removal of dura matter the brain was gently lifted with a blunt spatula and placed in ice-cold ($\sim 4^{\circ}$) artificial cerebrospinal fluid (ACSF) containing (in mM) 126 NaCl, 2.5 KCl, 1 MgCl₂, 26 NaHCO₃, 1.25 NaH₂PO₄, 2 CaCl₂ and 10 Glucose. Osmolarity was adjusted to 300 mOsm and pH equilibrated to 7.2 by continuously bubbling with 95% O₂ / 5% CO₂.

2.2. Brain slice preparation

The brain was transferred onto an iced petri dish covered with a round piece of filter paper moistened with ACSF. Using razor blades (VWR), the left and right

hemispheres were separated by a rostro-caudal cut along the longitudinal fissure. The selected hemisphere was further trimmed by two coronal cuts made at ~ 2 mm anterior and ~ 8 mm posterior to Bregma, respectively. The block of tissue was then rotated on its caudal side and further trimmed-down, removing the excess tissue ventral to the cerebrum. The block was then glued to the stainless steel vibratome stage on its caudal side using cyanoacrylate glue (Crazy GlueTM). The dorsal side of the tissue was facing the cutting blade (VALET, World Precision Instruments, Sarasota, FL). A cold rectangular block of agar (4%), previously glued to the vibratome stage was supporting the brain tissue from its ventral surface during the slicing. Fresh, ice-cold ACSF was then poured into the microtome tray, and frontal slices of 300 μm were cut using a vibrating microtome (Leica VT 1000S). The blade vibration was set on the highest frequency and the advance of the blade was set on the lowest speed. The solutions in the slicing tray and incubation chamber was bubbled with 95% O₂ / 5% CO₂. The slices were selected by naked eye and only those containing the dorsal hippocampus (from ~ 2.5 to ~ 4 mm posterior to Bregma) were transferred to the incubation chamber and allowed to recover for at least 45 min at room temperature (22 - 24°C).

2.3. Visualization and whole-cell recording

For recording, the slices were transferred to a submerged-type recording chamber and held securely in place by a nylon mesh. Oxygenated warm ACSF ($33 \pm 0.5^\circ\text{C}$) was continuously superfused at a rate of 2.5 ml/min. Whole-cell current- and voltage-clamp recordings were made from visually identified CA1 pyramidal neurons located 40 - 100 μm beneath the slice surface using a Multiclamp 700A amplifier (Axon Instruments,

Foster city, CA). CA1 subfield of the hippocampus was defined according to the coordinates provided by rat brain atlas (Paxinos and Watson, 1998). The neurons and the patch electrode were visualized with upright microscope (Leica DMLFSA, Germany) equipped with water immersion objectives, infrared video microscopy and differential interference contrast (IR - DIC). Whole cell pipettes (5 - 10 M Ω ; 1 - 2 μ m) were pulled from single-barreled borosilicate glass capillaries (World Precision Instruments, Sarasota, FL) using a P-97 Brown-Flaming puller (Sutter Instruments Co., Novato, CA) and filled with (in mM) 130 K-gluconate, 10 *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 10 KCl, 2 MgCl₂, 2 ATP-Mg and 0.2 GTP-tris(hydroxy-methyl) aminomethane (pH 7.2, 280 mOsm). The patch pipette solutions were filtered using 4-mm nylon syringe filters (0.22 μ m pore size; Corning Inc., Acton, MA). Series resistance (typically 12 - 20 M Ω) and bridge balance were monitored and compensated during the experiments. Signals were filtered at 2 kHz, digitized and stored using acquisition software pClamp 8 (Axon Instruments Inc., Foster City, CA). Additional off-line analyses were performed with Igor Pro (WaveMetrics Inc., Lake Oswego, OR).

2.4. Identification of CA1 pyramidal cells

Recorded cells were identified by their anatomical location, morphology, and electrophysiological characteristics. CA1 projection cells have well defined morphology and are localized to specific layers of CA1 subfield. The large, usually pyramidally - shaped cell body lies in stratum pyramidale, the basal dendritic trees project into stratum oriens while the proximal and distal apical dendrites extend ventrally into stratum radiatum and stratum lacunosum moleculare, respectively. For morphological

identification, the recorded cells were dialyzed with Lucifer Yellow (2 mM). The slices were fixed for 1 - 3 days in 100 mM PBS, pH 7.4, containing 4% paraformaldehyde, then washed in dimethyl-sulfoxide (DMSO) for 1 hour. The cells were visualized using a confocal laser-scanning microscope LMS 510 (Zeiss, Germany), equipped with 10 - 40x water immersion objectives. Scanning and image acquisition were controlled with custom software. Computer generated three-dimensional reconstruction of the cell.

Neuronal electrophysiological properties were examined in current clamp while the evoked postsynaptic responses were studied in voltage-clamp. Pyramidal cells generate spike trains that exhibit frequency adaptation when depolarized. Action potential amplitude, duration and afterhyperpolarization amplitude were measured. Cell membrane potential and input resistance (measured from 500 ms-long, 10 pA fixed increment pulses from rest) were monitored throughout the experiment. The input resistance (R_{in}) was estimated in the linear portion of current-voltage plots.

2.5. Drug application and solutions

For bath application, the drugs were diluted in ACSF to the desired final concentration and stored in three separate oxygenated containers connected to the main perfusion line. The pH was adjusted to 7.2, if necessary. The drugs were applied to the slice chamber by switching the perfusion solution. With this procedure, the time lag for the drugs to reach the recording chamber is 40 seconds.

For pressure application, the drugs were dissolved in ACSF and the pH adjusted to 7.2, if necessary. Pressure electrodes were pulled from the same type of pipettes used for pulling patch electrodes. Pressure electrode tips were placed over the recording area,

50 - 100 μm away from the surface of the slice. The air pressure pulses (3 - 10 ms) were applied with a picospritzer (Parker Ins., Fairfield, NJ). The pressure [3 to 100 pounds per square inch (psi)] applied to the electrode was adjusted through the pneumatic valve of the picospritzer.

For isolation of NMDAR-mediated currents, lidocaine N-ethyl bromide (QX-314, 5 mM), Cesium-BAPTA (10 mM) and D-890 (3 mM) were included in the intracellular solution. Some experiments were performed with a solution containing (in mM) 130 Cs^+ -methane sulphonate, 10 *N*-2-hydroxy-ethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 10 KCl, 2 MgCl_2 , 2 ATP-Mg and 0.2 GTP-tris(hydroxy-methyl) aminomethane, 5 QX-314, 10 Cesium-BAPTA, and 3 D-890 (pH 7.2, 280 mOsm). Cells were held at -70 mV. With this solution, the liquid junction potential was measured (10 mV) and membrane potential (V_m) was corrected accordingly. Cells were dialyzed with electrode solution for at least 15 min. The extracellular solution contained a low concentration of MgCl_2 (0.1 mM). The composition of this ACSF (low Mg^{2+} ACSF) was identical to the control ACSF. The difference in concentration of MgCl_2 was replaced with an equimolar amount of CaCl_2 .

Drugs included: NBQX, picrotoxin, bicuculline, tetrodotoxin, D-serine, glycine, NMDA, strychnine, APV, [purchased from Research Biomedicals International (RBI), Natick, MA], CGP 52432, 7-Cl-KYN (purchased from Tocris, Bristol, UK), D-890 (Knoll, Ludwingshafen, Germany).

2.6. Electrical stimulation

Schaffer collaterals/commissural afferents were stimulated by a concentric bipolar electrode (Frederick Haer Co, Brunswick, ME) placed in stratum radiatum. Baseline postsynaptic responses were evoked by 100 μ s constant current pulses (0.05 - 0.1 Hz) delivered through current output isolation unit (WPI-A365, World Precision Instruments, Sarasota, FL). In LTP experiments, stimulus location and intensity was adjusted such that active sodium conductances appeared at response amplitude between 200 and 300 pA. Next, intensity was reduced to $\sim$ 50% to elicit EPSCs of $\sim$ 100 pA. This procedure ensured maximal LTP of $\sim$ 70% magnitude in the absence of postsynaptic spiking. Before induction, an initial baseline of current data lasting at least 20 min was collected. After the induction period, responses were monitored for at least 30 min post-treatment. Drugs were applied for 4 min prior to and during depolarization for a total of 5 min. To study the effects of glycine site agonists in the absence of endogenous glutamate, in some experiments the afferent stimulation was turned off for 5 min prior to induction and restarted during depolarization.

2.7. LTP induction

High- and low-frequency LTP induction protocols were used. High-frequency induction (high-frequency LTP) consisted of single (100 or 250 ms) 100 Hz trains paired with or without postsynaptic depolarization ($V_m = -50$ mV). Low-frequency induction (low-frequency LTP) consisted of different types of somatic depolarization ($V_m = -60, -50, -40, -30, -20$ mV) paired with single afferent pulses (0.1 Hz). Stimulation intensity for all induction paradigms was the same as during the baseline period. Voltages have not been corrected for the theoretical liquid junction potential ($\sim$ 10 mV).

Single electrode whole-cell patch-clamp is prone to error when recording continuously from long multibranched neurons. To minimize space and point clamp errors and maximize uniformity throughout the experiments, the following measures were taken: First, for the analysis, only cells that met the following criteria were included: resting V_m of -65 to -69 mV; spike amplitude of 85 - 120 mV; input resistance of 90 - 140 M Ω . Second, recordings with series resistance higher than 20 M Ω were discarded. Third, recordings were discarded if during the experiment, V_m changed spontaneously by more than 2 mV and spike amplitude by more than 8 mV. Fourth, EPSC activation ($\tau < 2$ ms) mediated mainly by AMPARs were monitored throughout each experiment.

To examine the pairing requirements for LTP, it was necessary first to identify the electrophysiological parameters of CA1 pyramidal cells that may influence the impact of synaptic potentials. This was done with the help of current clamp $I - V$ plots and depolarizing steps in the voltage-clamp mode applied from $V_m = -65$ mV. The action potential threshold (~ -49 mV) was determined by the voltage preceding each spike. This value was relatively stable despite the irregular firing behavior of pyramidal cells. The threshold was further tested and confirmed by prolonged voltage clamp pulses, conditions that mimicked closely the low-frequency induction. In our suboptimal voltage clamp conditions the peak of sodium action potentials appeared at ~ -30 mV. Despite the usual variability EPSCs were clamped well and peak amplitudes were inversely related to the holding current (V_m from -65 to 0 mV). The holding current for the subthreshold induction ranged from 0.03 to 0.2 nA whereas suprathreshold induction required ≥ 0.2 nA.

The scope of this work is limited to the Hebbian LTP which requires co-occurrence of synapse activation by glutamate and postsynaptic depolarization (Kelso et al., 1986; Brown et al., 1990). In control experiments, the Hebbian requirements and the locus of LTP expression were examined directly. Subthreshold or suprathreshold somatic depolarization of variable duration applied in the absence of afferent stimulation did not result in any lasting changes in EPSC amplitude. Both, control and potentiated EPSCs were reduced to the same amplitude by 20 μ M NBQX, proving that the synaptic enhancement was due to changes in AMPAR activity at the stimulated pathway. In addition, several EPSC parameters indicative for input specificity were monitored throughout the experiments. LTP was accepted as monosynaptic (input specific) if EPSCs before and after induction exhibited short constant latency, identical initial slope and were of similar duration.

2.8. Data analysis

All acquisition and measurements were made using pClamp 8 software. Analyses were performed off-line using pClamp 8 (Clampfit) and IGOR Pro (Wave Metrics Inc., Lake Oswego, OR).

The most important dependent variable in the study was the current amplitude (EPSC), measured in voltage clamp experiments. Data for EPSCs (Ampere) was averaged with six individual sweeps per waveform in LTP experiments and 20 - 25 sweeps in any other experiment. The peak amplitude of the averaged synaptic response was measured with respect to its baseline a few ms before the stimulus artifact.

Independent variables were: drug and LTP induction paradigm. Variables held constant by elimination/inclusion include: resting membrane potential, action potential amplitude, action potential duration, input resistance, EPSC baseline amplitude, EPSC duration and series resistance.

In experiments designed to study the kinetics of isolated NMDAR currents, rise and decay time constants were measured. The rise times were measured at 10 - 90% peak. Their decays were fitted with the exponential functions: $y = A_f e^{-t/\tau_f} + A_s e^{-t/\tau_s}$ for double and $y = A_1 e^{-t/\tau}$ for single exponential decay, where A is the amplitude, τ is the decay time constant, and the subscripts f and s denote fast and slow components.

For LTP experiments, in figures describing individual experiments, a data point represents an individual response. In figures that summarize the average experiments, individual values represent time-matched mean value normalized first to each cell baseline and then across all subjects. The normalized values from all subjects were used to calculate $\pm$ SEM. Sample traces are 5-min averages taken just before induction and between 10 and 15 min after induction. LTP magnitude was calculated from the averaged data 10 min before induction *versus* 20 to 30 min post-induction. In the experiments with disrupted stimulation, the 10 min period before interruption was used. The same time windows were used for statistical comparisons.

In drug experiments, comparisons were made between potentiation values for drug *versus* corresponding control condition. Averaged data is presented as mean $\pm$ SEM. In competitive experiments (7-Cl-KYN/D-serine) comparisons were made *versus* D-serine data. Statistical significance was assessed by one- and two-tailed unpaired Student's *t*-tests ($p < 0.05$).

3. RESULTS

3.1. D-serine modulation of NMDAR-mediated currents in pyramidal cells and interneurons

3.1.1. Morphological and physiological properties of recorded CA1 pyramidal cells and interneurons

Cells were visually identified in slices with IR - DIC and selected for recordings based on their morphology and localization in specific layers of the CA1 region. Pyramidal cells were recorded in the stratum pyramidale while all interneurons were selected from the stratum radiatum. A total of 20 pyramidal cells and 30 interneurons were morphologically identified after injection with Lucifer Yellow and scanning with a confocal microscope. All pyramidal cells had spiny dendrites (Figure 2A3). The soma measured: longest axis $27.3 \pm 2.04 \mu\text{m}$ and shortest axis $17.6 \pm 2.18 \mu\text{m}$ ($n = 10$). The pyramidal cells had long apical dendrites ($592 \pm 32.59 \mu\text{m}$; $n = 10$) extending the entire stratum radiatum layer and ending in the stratum lacunosum moleculare. In contrast, the interneurons had aspiny, sparsely spiny or varicose dendrites (Figure 2B3). Interneuronal soma measured: longest axis $24.07 \pm 1.18 \mu\text{m}$ and shortest axis $15.47 \pm 0.71 \mu\text{m}$ ($n = 30$). The longest dendrite of interneurons measured $244.68 \pm 21.44 \mu\text{m}$ ($n = 30$). The dendritic branches radiated in stellate or bitufted configuration. Overall, the dendritic trees were restricted to the stratum radiatum. In one cell the dendrites extended into the stratum pyramidale.

The physiological properties of pyramidal cells and interneurons are summarized in Table 1. In response to depolarizing current injection pyramidal cells generate spike train that exhibited frequency adaptation (Figure 2A4). In contrast, interneurons could

sustain high firing rates with little or no accommodation (Figure 2B4). In addition, interneurons had more depolarized resting potential, higher input resistance and more pronounced fast afterhyperpolarization than pyramidal cells (4.84 ± 0.22 mV in pyramidal cells, $n = 39$ vs 13.65 ± 0.91 mV in interneurons, $n = 19$, $p < 0.005$).

3.1.2. D-serine modulation of the evoked NMDAR-mediated currents in pyramidal cells and interneurons

To examine the modulatory effect of D-serine on the evoked NMDAR-mediated responses, the glutamatergic Schaffer collaterals were stimulated with a bipolar electrode. The evoked responses were recorded at $V_m = -70$ mV for both pyramidal cells and interneurons. The NMDAR-mediated component was pharmacologically isolated in a low Mg^{2+} ACSF with: NBQX (20 μ M), picrotoxin (50 μ M), CGP 52432 (10 μ M) and strychnine (0.5 μ M) to block AMPA-, GABA_A-, GABA_B- and glycine-mediated responses, respectively. In control condition, peak NMDAR current measured 20.31 ± 2.62 pA in pyramidal cells ($n = 17$) and 14.83 ± 2.68 pA in interneurons ($n = 12$). D-serine (10 μ M) induced strong enhancement of NMDAR current amplitudes of $51.66 \pm 7.89\%$, $n = 17$, $p < 0.005$ in pyramidal cells and $25.07 \pm 5.65\%$, $n = 12$, $p < 0.005$ in interneurons (Figures 3A1, A3 and 3B1, B3) without affecting the τ_{act} (Table 2). The decay of the NMDAR currents in pyramidal cell was best fitted with a single exponential function (τ_s , fast decay) whereas in interneurons the decay was fitted with two exponentials (τ_f and τ_s , fast and slow decay components) (Table 2). NMDAR currents were completely blocked by APV (50 μ M) and 7-Cl-KYN (50 μ M) applied separately but simultaneously with D-serine, data not shown.

The enhancing effects of D-serine on the amplitude of NMDAR currents were also observed at +30 mV. The responses were isolated in ACSF containing picrotoxin (50 μ M), CGP 52432 (10 μ M), strychnine (0.5 μ M) and NBQX (20 μ M). NMDAR current amplitudes were significantly increased by D-serine in pyramidal cells $33.31 \pm 2.62\%$, $n = 9$, $p < 0.005$ and in interneurons $18.97 \pm 3.06\%$, $n = 19$, $p < 0.005$ (Figure 4A1, B1). D-serine did not alter significantly the voltage-dependent properties of NMDARs in pyramidal cells and interneurons as indicated by the current-voltage curves obtained in the presence and absence of exogenous D-serine (Figure 4A2, B2).

3.1.3. D-serine enhancement of NMDAR function in response to local pressure application of NMDA

D-serine modulation of NMDAR function in pyramidal cells and interneurons was examined during pressure application of the selective glutamate site agonist NMDA. NMDA (100 μ M) was applied in low Mg^{2+} (0.1 mM), TTX-(0.5 μ M) containing ACSF. NMDAR-mediated current amplitudes in response to NMDA were 41.23 ± 6.85 pA in pyramidal cells ($n = 10$) and 30.14 ± 4.78 pA in interneurons ($n = 10$). Current amplitudes were strongly enhanced by adding D-serine (10 μ M) to the perfusate (Table 1, Figure 5). Application of APV (50 μ M) and 7-Cl-KYN (50 μ M) completely abolished the responses to NMDA in pyramidal cells and interneurons, data not shown. Similarly to the responses to endogenous glutamate, D-serine enhancement of NMDAR responses was more pronounced in pyramidal cells than in interneurons.

The present results demonstrate that D-serine strongly potentiates NMDAR-mediated responses in neurons from the rat hippocampal CA1 region. However, D-

serine-mediated enhancement was significantly larger in pyramidal cells than in interneurons.

3.2. Pairing requirements for low-frequency NMDAR-dependent and NMDAR-independent LTP

3.2.1. Depolarization enables LTP during brief high-frequency afferent stimuli

Tetanic stimulation applied to the Schaffer collaterals is a standard procedure for LTP induction in CA1 pyramidal cells (Bliss and Collingridge, 1993). The effect of depolarization on high-frequency LTP was examined with the help of single, 100 Hz, brief (25 and 10 pulses) trains that did not induce LTP when applied alone. The synaptic responses during the tetanic stimulation were continuously monitored (Figure 6A-D, lower traces). When the cell was clamped at -65 mV during the trains, no potentiation was observed (Figure 6A,C). However, when the cell was clamped at -50 mV for the duration of the trains, potentiation invariably was observed (Figure 6B,D). Pooled data illustrate that LTP was obtained only when the trains were paired with somatic depolarization to -50 mV (Figure 6E). In both cases, potentiation was significant ($57.43 \pm 8.4\%$, $n = 5$, $p < 0.01$, for a 25 pulse train and $52.31 \pm 7.6\%$, $n = 4$, $p < 0.01$, for a 10 pulse train) and of maximal magnitude. Stronger depolarization than that provided by the afferent stimulation must be therefore required for enabling LTP during brief high-frequency synaptic stimulation.

During the experiments, it was observed that for LTP induction, the cell must fire prolonged trains of action potentials. It was also noticed that in different cells, the bursts were of variable duration. The prolonged action potential discharge during the train led to

the build up of additional depolarization (Figure 6B,D, lower trace). Thus, depolarization enabled the cell to fire persistently and at levels well above the applied holding potential. The after-depolarization observed here was similar to that reported previously for hippocampal (Jensen et al., 1996) and cortical (Sjöström et al., 2001) pyramidal neurons. The mechanism responsible for the build-up of membrane after-depolarization involves interaction between the membrane time constant and voltage-gated persistent Na^+ currents (Azouz et al., 1996; Jensen et al., 1996). The spike after-depolarization build-up during high-frequency stimulation may have contributed to LTP as previously shown for cortical pyramidal neurons (Sjöström et al., 2001).

From the high-frequency LTP experiments summarized in Figure 6, it was concluded that: 1) somatic depolarization enables LTP under conditions where the afferent stimulation alone is insufficient; 2) lowering the threshold for postsynaptic spiking is one possible mechanism depolarization may have contributed to LTP; 3) LTP induction is associated with prolonged trains of action potentials, which lead to the build-up of prolonged after-depolarization; 4) postsynaptic voltage requirements for LTP cannot be determined precisely with the help of high-frequency stimulation due to the variability in postsynaptic spiking and more importantly due to the variability in amplitude and duration of spike after-depolarization.

3.2.2. Minimal pairing requirements for low-frequency LTP of maximal magnitude

Low-frequency pairing protocols were used to examine the postsynaptic voltage requirements for LTP without the confounding effect of massive, high-frequency induced spike after-depolarization. A number of pairing protocols were tested that differed in

duration (1, 5 and 10 min), degree of depolarization ($V_m = -60, -50, -40, -30, -20$ and 0 mV), and frequency (0.05, 0.1 and 0.2 Hz) of afferent stimulation (data not shown). During these experiments, it was observed that LTP was most easily obtained when the postsynaptic cells fired action potentials during induction (LTP_{AP}). It was also determined that 60 sec of depolarization during which the postsynaptic cell fired consistently in response to the afferent pulses (0.1 Hz) resulted in LTP_{AP} of maximal magnitude. Pairing depolarization to -40 mV with 6, 5 and 4 single synaptically initiated action potentials for 60, 50, and 40 sec respectively, resulted in LTP_{AP} of maximal magnitude (Figure 7A,B,C). Potentiation was: $52.86 \pm 7.2\%$, $n = 7$, $p < 0.01$, for 6 action potentials; $52.23 \pm 7.8\%$, $n = 6$, $p < 0.01$, for 5 action potentials; $51.1 \pm 7.1\%$, $n = 5$, $p < 0.01$, for 4 action potentials (Figure 7F,G). Pairing -40 mV with 3 and 2 action potentials (Figure 7D,E) for 30 and 20 sec, resulted from rapid reduction of LTP_{AP} size for 3 action potentials ($21.35 \pm 9.1\%$, $n = 5$, $p < 0.01$), to complete loss of potentiation for 2 action potentials ($1.27 \pm 5.6\%$, $n = 4$) (Figure 7F,G). It was concluded that the minimal requirements for obtaining LTP_{AP} of maximal magnitude is $V_m = -40$ mV paired with 4 - 6 synaptically initiated single action potentials (0.1 Hz).

3.2.3. Postsynaptic voltage and the induction of LTP_{AP}

The postsynaptic voltage requirements for LTP_{AP} were examined by pairing depolarization of constant duration (60 sec) but of different amplitude ($V_m = -40, -30$ and -20 mV) with 6 suprathreshold synaptic responses (0.1 Hz; Figure 8A-D). In all three conditions, potentiation was of similar and maximal magnitude: $52.86 \pm 7.2\%$, $n = 7$, $p < 0.01$, for -40 mV; $57.17 \pm 8.1\%$, $n = 5$, $p < 0.01$, for -30 mV and $58.22 \pm 8.9\%$, $n = 5$, $p < 0.01$, for -20 mV.

0.01 for -20 mV, respectively (Figure 8D). These results indicate that once the threshold for action potentials is reached, very little or no additional potentiation is added even when the cell is further depolarized. In the presence of membrane depolarization ($V_m \geq -40$), the action potential threshold is also the threshold for obtaining maximal LTP_{AP}.

3.2.4. Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP of submaximal magnitude (LTP_{EPSC})

It has been demonstrated that blockade of the postsynaptic somatic action potential does not prevent the induction of LTP (Kelso et al., 1986; Golding et al., 2002). Although this finding indicates that the generation of somatic sodium spikes is not required for LTP, little is known about LTP obtained with depolarization that is subthreshold for postsynaptic spikes. Here small depolarization was paired with subthreshold EPSCs to examine the properties of LTP obtained in the absence of sodium spikes (LTP_{EPSC}) (Figure 9). Pairing somatic depolarization to -60 mV with 6 EPSCs (0.1 Hz) did not result in significant potentiation ($2.79 \pm 4.7\%$, $n = 7$) (Figure 9A,D). Depolarization to -50 mV was associated with two different types of postsynaptic response during the afferent shocks: either EPSCs or action potentials (Figure 9B,C). However, the action potentials in most cases did not persist during the entire induction time and a significant number of failures were observed. To control for this difference, the cells that fired during induction were analyzed separately from the cells that did not fire. Pooled data indicate that for both groups, potentiation was present: $16.4 \pm 5.9\%$, $n = 5$, $p < 0.01$, for the purely LTP_{EPSC} group and $29.64 \pm 7.9\%$, $n = 6$, $p < 0.01$, for the spiking group (Figure 9B,C,D).

It was found that the size of LTP_{EPSC} obtained with -50 mV is $\sim 16\%$. Since the upper limit of LTP in our conditions is $\sim 70\%$, LTP_{EPSC} ($V_m = -50$ mV) magnitude translates into 20 - 25% of total potentiation. LTP_{EPSC} was found to be voltage-dependent since depolarization to -60 mV did not induce any potentiation while at $V_m = -50$ mV, potentiation was maximal. With further increase of the depolarization, pure LTP_{EPSC} was more difficult to obtain due to the appearance of postsynaptic spiking. These results suggest that postsynaptic firing is not a prerequisite for LTP and synapse modification can take place under conditions where local depolarization and synaptic activation coexists with enhanced perisomatic inhibition (Yuste and Tank, 1996; Häusser et al., 2000; Golding et al., 2002).

3.2.5. The effect of NMDAR antagonists APV and 7-Cl-KYN on LTP_{AP} and LTP_{EPSC}

Next the effects of APV (50 μ M) on LTP_{AP} and LTP_{EPSC} were examined (Figure 10). During the experiments, it was observed that although APV did not change baseline EPSC amplitude, it increased the threshold for action potential during depolarization. Pyramidal cells, voltage clamped at -40 mV, usually spike in control ACSF in response to afferent stimulation (Figures 7 and 8). In the presence of APV, approximately 50% of the cells did not spike ($n = 6$, data not shown). Depolarizing the cell to -50 mV was often associated with irregular spiking in response to the stimulus. The occasional spiking was absent in the presence of APV ($n = 8$, data not shown). The effects of APV on the action potential threshold are consistent with a reduction of inward cationic currents mediated by NMDAR (Mayer et al., 1984; Nowak et al., 1984). In the pooled data for the effects of

APV on LTP_{EPSC}, cells that did not fire during depolarization to -40 mV were analyzed together with the cells depolarized to -50 mV (Figure 10A,B).

In the presence of APV, no potentiation was observed when the postsynaptic cell did not spike during depolarization. Pairing depolarization ($V_m = -60, -50$ and -40 mV) with 6 subthreshold EPSCs in the presence of APV was found to have no lasting effect on EPSC amplitude ($0.62 \pm 5.7\%$, $n = 4$ for -60 mV and $1.27 \pm 5.8\%$, $n = 6$ for $-50/-40$ mV, respectively) (Figure 10A,C). However, APV did not prevent LTP induced by pairing depolarization to -40 mV with 6 synaptically initiated spikes (LTP_{AP}) ($51.17 \pm 7.6\%$, $n = 4$, $p < 0.01$) (Figure 10A,B). The magnitude of LTP_{AP} obtained in the presence of APV was not significantly different from that obtained in control ACSF (Figure 10C). With stronger background depolarization ($V_m \geq -40$ mV), APV cannot prevent postsynaptic firing and the induction of maximal LTP_{AP}. Thus, at subthreshold induction conditions, APV through its action on NMDARs increases the threshold for LTP_{AP} and reliably blocks LTP_{EPSC}.

This is the first study to report the resistance of LTP induced by single afferent pulses to NMDAR antagonism. Therefore, the role of NMDARs in the induction of both types of LTP was further examined with the help of 7-Cl-KYN. Glycine is required for NMDAR activation and antagonist blockade of the glycine site prevents NMDAR-mediated responses (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988; Lerma et al., 1990). 7-Cl-KYN ($3 \mu\text{M}$) applied during the pairing mimicked the effect of APV on both types of LTP. Blockade of the glycine site during induction eliminated the potentiation obtained by pairing depolarization with subthreshold EPSCs ($1.1 \pm 5.9\%$, $n = 4$ for -60 mV and $0.89 \pm 6.2\%$, $n = 5$ for -50 mV) (Figure 10B,C). Increasing

depolarization to -40 mV in most cases did not prevent postsynaptic firing and the induction of strong LTP ($51.67 \pm 8.9\%$, $n = 5$, $p < 0.01$) (Figure 10B,C). In the cases where postsynaptic spiking was absent, LTP did not develop ($n = 3$, data not shown). Blockade of LTP_{EPSC} with 3 μ M 7-Cl-KYN, which is in the concentration range (0.3 - 10 μ M) previously shown to block the glycine site of NMDARs (Kemp et al., 1988; Izumi et al., 1990; Kemp and Priestley, 1991; Parsons et al., 1993) indicates that the glycine site activation is required for LTP_{EPSC}.

From the experiments with APV and 7-Cl-KYN it was concluded that: 1) LTP obtained by pairing depolarization with EPSCs (LTP_{EPSC}) is purely NMDAR-dependent; 2) LTP_{EPSC} requires small depolarization (≤ 15 mV); 3) Bath perfused NMDAR antagonists can not prevent the development of input specific LTP when the depolarized cell fires action potentials in response to single afferent pulses; 4) NMDAR antagonism has no effect on the magnitude of LTP induced by pairing depolarization ($V_m \geq -40$ mV) with synaptically initiated single action potentials.

Thus, by pairing different degrees of somatic depolarization with single sub- or suprathreshold synaptic responses, two different types of LTP can be differentiated: the purely NMDAR-dependent LTP_{EPSC}, which is of submaximal magnitude and LTP_{AP}, being of maximal magnitude and resistant to NMDAR antagonism.

3.3. Regulation of synaptic plasticity by the glycine site of NMDAR

3.3.1. Membrane voltage controls the induction and magnitude of LTP obtained by low-frequency pairing

It has been suggested that the occurrence, magnitude and mechanism of LTP depend on various induction parameters including type and intensity of afferent stimulation and degree of postsynaptic depolarization (Grover and Teyler, 1990; Morgan and Teyler, 2001). LTP obtained by direct postsynaptic depolarization is a controlled way of LTP induction. This type of induction avoids the confounding effects of various mechanisms including altered transmitter release, build-up of synaptic glutamate, overwhelmed transporters, spatial and temporal integration of excitatory and inhibitory postsynaptic responses and changes in back-propagating action potentials. To study the glycine site modulation of plasticity LTP was induced by direct postsynaptic depolarization (-60, -50 and -40 mV; 1 min) in the presence of low-frequency afferent stimulation (0.1 Hz). Depolarizing the cell to -60 mV was subthreshold for the generation of somatic action potentials and did not result in potentiation (Table 3, Figure 11A). Depolarization to -50 mV was also subthreshold for somatic spikes but resulted in small potentiation (Table 3, Figure 11B). Depolarization to -40 mV was suprathreshold for somatic spiking and resulted in LTP on average four times stronger than that induced by pairing to -50 mV (Table 3, Figure 11C). In our conditions depolarizing the pyramidal cell to -40 mV (supramaximal induction) resulted in LTP of maximal magnitude (maximal LTP). These control experiments suggest that postsynaptic membrane voltage controls the induction and magnitude of LTP during low-frequency synaptic stimulation. The same pairing protocols were used to investigate the requirements for glycine site modulation of plasticity.

3.3.2. Enhancement of LTP by D-serine and glycine is differentially affected by membrane voltage during induction

The effects of D-serine (1 μ M) and glycine (3 μ M) on LTP were studied by depolarizing the cell to -60, -50 and -40 mV. The agonists were applied prior to and during depolarization for a total of 5 min (Figures 12 and 13). In preliminary experiments, different agonist concentrations were tested and it was determined that concentrations lower than 0.5 μ M for D-serine and 1 μ M for glycine had no detectable effects on baseline synaptic responses or depolarization-induced plasticity (data not shown). On the other hand, concentrations higher than 1 μ M and 3 μ M of D-serine and glycine, respectively, often resulted in drug related increases or decreases of the baseline EPSCs. Interestingly, washout of high agonist concentrations was usually followed by potentiation lasting 5 - 10 min. Earlier studies have described similar potentiation upon washout of high glycine concentrations and this effect was found to be NMDAR-mediated (Shahi et al., 1993; Musleh et al., 1997). It was observed however that this type of potentiation was followed by a long lasting reduction of the evoked EPSC amplitudes (data not shown). The effects of high agonist concentrations on baseline responses were drug specific with D-serine being on average more potent than glycine.

Depolarizing the cell to -60 mV in D-serine, despite being subthreshold for somatic spike generation, resulted in LTP (Table 3, Figure 12A). Thus, D-serine induced LTP by pairing protocol, which applied in control ACSF did not result in potentiation. Depolarizing the postsynaptic cell to -50 mV in the presence of D-serine was associated with the appearance of somatic action potentials superimposed on the EPSCs. Potentiation induced under these conditions was robust and similar to that obtained by the

most intense induction protocol used in the present study (depolarization to -40 mV) applied in control ACSF (Figure 12B). Thus, D-serine during depolarization to -50 mV lowered the action potential threshold and facilitated the induction of maximal LTP. LTP induced by depolarization to -40 mV in the presence of D-serine was slightly stronger but not different from control, suggesting that under supramaximal induction conditions D-serine adds very little or no additional potentiation (Figure 12C).

To examine the modulatory effects of glycine and compare them to those of D-serine, the same groups of experiments were performed in the presence of 3 μ M glycine during induction (Table 3, Figure 13). Glycine mimicked the effect of D-serine on LTP by all three induction protocols although the amount of potentiation was somewhat smaller (Table 3, Figure 13B). Thus, depolarization to -60 mV in glycine resulted in the induction of LTP. During depolarization to -50 mV, perfusion with glycine facilitated the appearance of sodium spikes and the induction of large magnitude LTP. Potentiation induced by depolarization to -40 mV was not statistically different when carried out in the presence of glycine, suggesting that glycine site agonists do not contribute additional potentiation when LTP is obtained by supramaximal induction (Table 3, Figure 13B).

The experiments with D-serine and glycine demonstrate the voltage dependence of glycine site agonist enhancement of LTP. Both agonists were most effective when small depolarization (-60 and -50 mV) was paired with subthreshold EPSCs. These findings suggest that the glycine site can exert strong modulatory control over LTP threshold. Glycine and D-serine effects were very similar, indicating that both agonists can perform the same function in CA1 pyramidal cells. However, D-serine was a more

potent agent than glycine since three-times lower concentration of D-serine produced slightly stronger enhancement of LTP (Table 3, Figures 12 and 13).

3.3.3. Maximal enhancement of LTP by exogenous D-serine and glycine is obtained when the agonists are present prior to synapse activation and pairing

It has been suggested that glycine site agonists positively modulate the glutamate binding site by increasing the affinity for glutamate (Fadda et al., 1988; Grimwood et al., 1993; Lester et al., 1993; Priestley and Kemp, 1994; Grimwood et al., 1995; Danysz and Parsons, 1998). To explore further the dependence of glycine site modulation on induction conditions, pairing protocols were used that allowed glycine site agonists to be present before afferent stimulation and LTP induction (Table 3, bottom portion; Figure 14). The induction procedure required the afferent stimulation to be stopped during perfusion with D-serine (1 μ M) or glycine (3 μ M) and resumed after the cell was depolarized to the desired level (-60 or -50 mV). An important requirement for this induction procedure was that depolarization onset had to precede the pairing (Figure 14A). This allowed the agonists to act at the receptors during depolarized V_m for $\sim$ 5 sec in the absence of afferent stimulation. Interestingly, when the order was reversed (resume stimulation, then depolarize), potentiation was not different from control (data not shown).

Depolarizing the cell to -60 mV and restarting the afferent stimulation in the presence of D-serine and glycine resulted in stronger potentiation than that obtained by the standard pairing protocols (Table 3, Figure 14B). Pairing to -50 mV also resulted in greater LTP, although the difference was not significant (Table 3, Figure 14B). To rule

out the possibility that the differences in LTP magnitude were not mediated through the glycine site, the protocols were compared in control ACSF. It was found that in the absence of agonists the amount of potentiation was of similar magnitude suggesting that the effect was mediated by the glycine site (Table 3, Figure 14B). D-serine and glycine enhancement of LTP was stronger during depolarization to -60 mV confirming the voltage-dependence of glycine site modulation.

These experiments suggest that D-serine and glycine have strong priming effects on LTP when present prior to afferent stimulation and LTP induction. Thus, maximal enhancement of LTP can be achieved by applying exogenous D-serine and glycine before glutamate release.

3.3.4. Blockade of the glycine site by 7-Cl-KYN reverses the direction of synaptic plasticity

7-Cl-KYN is a halogen-substituted derivative of kynurenic acid with high selectivity for the glycine site (Kemp et al., 1988; Kemp and Priestley, 1991). Antagonizing NMDAR responses by 7-Cl-KYN results in significant current decay (Kemp et al., 1988; Kemp and Priestley, 1991; Parsons et al., 1993). Extracellular field potential studies have demonstrated that 7-Cl-KYN inhibits LTP *in vivo* and *in vitro* (Bashir et al., 1990; Oliver et al., 1990; Thiels et al., 1992; Watanabe et al., 1992; Ito and Hicks, 2001). To examine the effects of glycine site blockade on depolarization induced plasticity, 3, 10 and 30 μ M 7-Cl-KYN were used (Table 3, Figure 15). Perfusion of 3 μ M 7-Cl-KYN had no detectable effect on baseline EPSC amplitudes. Perfusion of 10 μ M 7-Cl-KYN resulted in a small reduction ($\sim$ 5%) of the evoked EPSC amplitude in $\sim$ 50% of

the slices. Perfusion of 30 μM 7-Cl-KYN caused $\sim 8\%$ reduction of baseline responses. 7-Cl-KYN effect on baseline currents was reversible upon drug withdrawal.

Similar to control, depolarizing the cell to -60 mV in the presence of 3 μM 7-Cl-KYN did not result in any long-lasting changes in EPSC amplitude (Figures 11 and 15). Perfusion with 3 μM 7-Cl-KYN however, prevented LTP by depolarization to -50 mV (Table 3, Figure 15B). In contrast, perfusion of 3 μM 7-Cl-KYN had no effect on LTP magnitude obtained by depolarization to -40 mV (Figure 15). Perfusion of 10 μM 7-Cl-KYN during induction blocked LTP by depolarization to -50 mV. Depolarizing the postsynaptic cell to -40 mV in the presence of 10 μM 7-Cl-KYN resulted in long lasting reduction of EPSC amplitudes (Table 3, Figure 15B). Perfusion of 30 μM 7-Cl-KYN has confirmed that glycine site blockade during induction results in LTD. Depolarizing the cell (-60, -50 and -40 mV) in the presence of 30 μM 7-Cl-KYN consistently resulted in LTD. These findings suggest that LTP protocols applied during glycine site blockade result in the induction of LTD. LTD obtained in this way was also voltage-dependent since the largest amount of depression was obtained during depolarization to -40 mV. Thus, LTD induction by glycine site blockade required $\geq 10 \mu\text{M}$ 7-Cl-KYN and ≥ -50 mV depolarization.

Collectively, the experiments with 7-Cl-KYN demonstrate the complex regulation of plasticity by glycine site antagonism. Blockade of the glycine site during depolarization-induced plasticity generates heterogeneous responses ranging from LTP to LTD. The outcome is determined largely by antagonist concentration and degree of depolarization.

3.3.5. Competitive effects of D-serine and 7-Cl-KYN on depolarization-induced plasticity

Kinetic studies on cultured neurons have demonstrated that 7-Cl-KYN can induce NMDAR desensitization in the presence of exogenous glycine (Kemp et al., 1988; Parsons et al., 1993). This effect is sensitive to the levels of background glycine and it is due to the faster association/dissociation kinetics of 7-Cl-KYN (Kemp et al., 1988; Vyklícký et al., 1990; Parsons et al., 1993). To examine the competitive effects of D-serine and 7-Cl-KYN on depolarization-induced plasticity, D-serine (1 μ M) and 7-Cl-KYN (3, 10 and 30 μ M,) were co-applied during LTP induction by depolarization (-60, -50 and -40 mV) (Table 3, Figure 16). Perfusion of 1 μ M D-serine and 3 μ M 7-Cl-KYN had no effect on baseline responses evoked by afferent stimuli. Perfusion of 1 μ M D-serine and 10 μ M 7-Cl-KYN in most cells resulted in small increases or decreases ($\sim$ 5 %) of evoked EPSC amplitudes. The drug-related reduction of EPSC amplitudes ($\sim$ 8%) observed previously with 30 μ M 7-Cl-KYN was also evident in the presence of 1 μ M D-serine and 30 μ M 7-Cl-KYN.

Potentiation obtained in the presence of 1 μ M D-serine and 3 μ M 7-Cl-KYN (-60, -50 and -40 mV) was not different from that obtained in the presence of 1 μ M D-serine alone (Table 3, Figure 16B). In contrast, perfusion of 1 μ M D-serine and 10 μ M 7-Cl-KYN blocked potentiation by depolarization to -60 mV. Application of 1 μ M D-serine and 10 μ M 7-Cl-KYN did not block LTP by depolarization to -50 and -40 mV. The amount of potentiation however was significantly reduced (Table 3, Figure 16). These findings indicate that exogenous D-serine saves LTP induced by depolarization to -50 and -40 mV. In contrast, in the absence of exogenous D-serine, perfusion of 10 μ M 7-Cl-

KYN blocked LTP by all three pairing paradigms (Table 3, Figure 15B). The induction of LTD by glycine site blockade during depolarization was confirmed in the presence of exogenous D-serine. Thus, perfusion of 1 μ M D-serine and 30 μ M 7-Cl-KYN resulted in LTD by all three induction protocols (-60, -50, -40 mV). Interestingly, the amount of depression obtained during co-application of 1 μ M D-serine and 30 μ M 7-Cl-KYN was larger than that obtained in the presence of 30 μ M 7-Cl-KYN alone (Table 3, Figures 15 and 16). These results indicate that D-serine is ineffective in rescuing LTP to any degree in the presence of high antagonist concentration (30 μ M). Furthermore, in high antagonist concentrations, D-serine seems to facilitate LTD since the amount of depression was larger than that obtained in the presence of 30 μ M 7-Cl-KYN alone.

Overall, the competitive effects of D-serine and 7-Cl-KYN on plasticity confirmed the differential effect of membrane voltage on glycine site modulation. The reversal of direction of plasticity by 7-Cl-KYN was also evident in the presence of D-serine. The present results indicate significant neuroprotective effect of D-serine on NMDAR-mediated plasticity. D-serine appears to counteract 7-Cl-KYN-mediated reversal of plasticity towards LTD. D-serine protection however, is ineffective in the presence of high concentrations of 7-Cl-KYN (> 10 μ M). Furthermore, in these conditions D-serine seems to facilitate the induction of LTD.

4. DISCUSSION

As reviewed in the introduction of this document, the NMDAR is an ionotropic, ligand-gated channel that appears to have a pivotal role in neuronal development, survival and plasticity. NMDAR supports these functions by controlling the influx of potent second messenger Ca^{2+} into the postsynaptic cell. This receptor subtype is widely distributed in the mammalian brain and spinal cord, with particularly high receptor densities found in the hippocampus and cerebral cortex.

In the results section, I provided evidence that activation of the glycine binding site by exogenous D-serine leads to a significant enhancement of NMDAR responses in CA1 pyramidal cells and interneurons. After the modulatory role of D-serine on the isolated NMDAR EPSCs has been established, the work was extended to the modulation of hippocampal synaptic plasticity through the glycine site. So far, the modulation of NMDAR plasticity by the glycine site has not been studied at the single cell level. Therefore, the induction requirements for NMDAR-dependent LTP had to be examined. The results of these experiments led to the development of improved LTP induction protocols. The new protocols in combination with selective glycine site compounds were used to study the glycine site role in plasticity. The presented neurophysiological data demonstrate the central role of the glycine site in regulating the magnitude and direction of NMDAR-dependent plasticity.

4.1. D-serine modulation of NMDAR currents in pyramidal cells and interneurons

In the first series of experiments the modulatory effects of D-serine on NMDAR-mediated EPSCs in pyramidal cells and interneurons have been characterized. Pyramidal cells and interneurons were visually identified with IR - DIC and selected for recordings based on their morphology and localization. Physiological identification of each cell was also performed. The effect of D-serine on NMDAR function was studied in response to evoked glutamate release and to pressure application of NMDA. Exogenous D-serine strongly enhanced NMDAR responses in pyramidal cells and interneurons. D-serine enhancement was stronger in pyramidal cells than in interneurons suggesting that excitation-inhibition balance in the hippocampal network may be altered by the glycine site.

The saturation of the glycine site under physiological conditions continues to be a subject of considerable debate. Many investigators assume that extracellular and cerebrospinal fluids provide sufficient glycine or D-serine in the vicinity of NMDARs to saturate the glycine sites (Crawford and Roberts, 1989; Ramson and Deschenes, 1989; Mothet et al., 2000; Ballard et al., 2002). In contrast, other groups using *in vivo* (Salt, 1989; Wood et al., 1989; Thiels et al., 1992; Danysz and Parsons, 1998) and *in vitro* (Wilcox et al., 1996; Bergeron et al., 1998) paradigms have demonstrated that application of glycine and D-serine enhances NMDAR-mediated responses. The present experiments strongly argue against glycine site saturation in hippocampal CA1 pyramidal cells and interneurons. D-serine increases the NMDAR-mediated responses to synaptically released glutamate and to local application of NMDA in both cell types, suggesting that NMDARs in the CA1 hippocampal region are not saturated by endogenous glycine and D-serine. These findings are in agreement with previous *in vitro* studies describing

enhancement of NMDAR function by exogenously applied glycine or D-serine (Minota et al., 1989; Wilcox et al., 1996; Bergeron et al., 1998). Recent evidence suggests that the degree of saturation of the glycine site appears to be determined by a number of factors, among them the regional differences in the expression of NMDAR subtypes, the type and density of glycine transporters and fluctuations in synaptic glycine or D-serine concentrations (Supplisson and Bergman, 1997; Danysz and Parsons, 1998).

The effects of D-serine observed here appeared to be mediated by NMDAR subtypes not saturated by background glycine or D-serine concentrations. NMDARs are heteromultimeric channels composed of three different subunit families (NR1, NR2A - D, NR3A - B). Functional NMDARs are formed by combination of NR1 and NR2 subunits, which contain the glycine and glutamate recognition sites, respectively (Dingledine et al., 1999). Receptor biophysical and pharmacological properties depend on the specific heteromeric combination. Thus, coexpression of NR2A-D with NR1 determines the pharmacological profile, the gating properties and the strength of Mg^{2+} block of the NMDAR complex (Nakanishi et al., 1990; Nakanishi, 1992; Monyer et al., 1992; Hollmann and Heinemann, 1994; Sucher et al., 1996). NR2A subunit first appears around birth and when coexpressed with NR1 forms a channel with low affinity for glycine and D-serine (Monyer et al., 1994; Kew et al., 1998). The complexity of NMDAR structure and function is further increased by post-translational modifications such as alternative splicing and RNA editing (Seeburg, 1996). In the present work, the effects of D-serine appear to be mediated by NMDARs with low affinity for glycine. Thus, the strong enhancement of NMDAR function by D-serine observed here is most

likely mediated by a subpopulation of NMDARs expressing a high degree of NR2A subunit (Buller et al., 1994; Laurie and Seeburg, 1994; Kew et al., 1998).

The hippocampal formation is a complex network tightly regulated by excitatory and inhibitory neuronal interactions. Inhibitory interneurons play a crucial role in regulating the activity of pyramidal cells including population oscillations, synaptogenesis and plasticity, epileptic synchronization, and hormonal responses. Depending on the subtypes and location, CA1 hippocampal interneurons receive two types of excitatory inputs: feedback and feed-forward (Schwartzkroin and Mathers, 1978; Knowles and Schwartzkroin, 1981; Lacaille et al., 1987; Riback and Peterson, 1991; Kneisler and Dingledine, 1995a; Kneisler and Dingledine, 1995b). In the stratum radiatum, glutamatergic inputs to interneurons are predominantly from local pyramidal cell collaterals or Schaffer collateral afferents (Freund and Buzsáki, 1996; Parra et al., 1998). Despite the important role of interneurons, little is known about the properties of their NMDAR-mediated responses.

The present results indicate that NMDAR currents in pyramidal cells and interneurons are different. Furthermore, it was found that D-serine enhancement of NMDAR function was significantly larger in pyramidal cells than in interneurons. Although, the rise time of NMDAR currents was the same in both cell types, the decay time course of NMDAR currents was much slower in interneurons. Previous studies have shown that in the adult rat hippocampus, NR2A and NR2B mRNA are prominent in CA1 pyramidal cells, but NR2C and NR2D occur in different subsets of interneurons (Perouansky et al., 1993; Monyer et al., 1994). Studies with both recombinant and native systems have suggested that the expression of NR1/NR2A subunits produce channels

with faster deactivation than NR1/NR2B or NR1/NR2C (Vicini et al., 1998). In contrast, NMDARs containing the NR1/NR2D subunits display very slow kinetics (Vicini et al., 1998; Misra et al., 2000). Thus, the much longer currents in interneurons observed here most likely arise from a different ratio of NR2A, /NR2B, /NR2C, /NR2D subunits. The slower time course of NMDAR responses in interneurons may be required for shaping the integrative properties of these cells. Facilitation of the integration of excitatory inputs could be a functional consequence of the prolonged NMDAR currents in interneurons.

Abnormalities in NMDAR transmission in interneurons may be of significant clinical importance. Antagonists of the NMDAR provide a realistic model of psychosis in humans and experimental animals (Javitt and Zukin, 1991). Data from circuit analysis in the CA1 hippocampal region have suggested that NMDAR antagonist psychosis may in fact result from loss of inhibitory drive onto excitatory amino acid neurons (Traub et al., 1993; Olney and Farber, 1995; Grunze et al., 1996; Newcomer et al., 1999). Blockade of NMDARs in interneurons that maintain tonically active inhibitory feedback synapses on pyramidal cells may lead to unmodulated excessive excitation (Olney and Farber, 1995; Olney et al., 1999). Indeed, a reduction of GABA transmission in this area has been known to generate epileptiform activity (Traub et al., 1993). Thus, pharmacological blockade or other impairments affecting the prolonged NMDAR-mediated responses in interneurons may have serious deleterious effect on interneuron tonic activity and particularly on their feedback regulatory function.

4.2. Pairing requirements for low-frequency NMDAR-dependent and NMDAR-independent LTP

4.2.1. Pairing requirements for low-frequency LTP

The goal of these experiments was to examine the induction requirements for LTP obtained by pairing depolarization with single afferent pulses. It was found that: 1) LTP of maximal magnitude ($> 40\%$) was induced when depolarization ($V_m \geq -40$ mV, 40 - 60 sec) was paired with four to six single, synaptically initiated action potentials (LTP_{AP}); 2) LTP_{AP} was NMDAR-independent; 3) Pairing mild depolarization ($V_m = -50$ mV, 60 sec) with six single subthreshold EPSCs resulted in LTP of submaximal magnitude ($< 30\%$) (LTP_{EPSC}); 4) LTP_{EPSC} was purely NMDAR-mediated.

In agreement with previous reports, (Kelso et al., 1986; Malinow and Miller, 1986; Gustafsson et al., 1987; Debanne et al., 1996; Magee and Johnston, 1997; Sjöström et al., 2001) it was found that background depolarization enables high- and low-frequency LTP to be induced under conditions where those same afferent stimuli, when applied alone, did not induce any significant postsynaptic modification. In both types of induction, robust LTP was obtained only when the synaptically initiated depolarization, combined with the background somatic depolarization, was above the threshold for action potential. These findings further demonstrate that in the presence of prolonged depolarization, the number of afferent pulses required for LTP can be reduced to as low as four given at 0.1 Hz. Depolarizing the postsynaptic cell above the threshold for action potential is sufficient for the induction of maximal LTP. NMDAR-mediated component of LTP was obtained by pairing small depolarization with six single subthreshold EPSCs. Collectively, these results demonstrate that postsynaptic depolarization regulates the induction and magnitude of LTP during low-frequency afferent stimulation.

4.2.2. NMDARs and low-frequency LTP

NMDAR, often described as a Hebbian molecule, serves as a coincident detector allowing Ca^{2+} influx only when glutamate is present and the postsynaptic membrane is sufficiently depolarized (Bliss and Collingridge, 1993). Functional and calcium imaging studies have demonstrated that NMDARs are active and generate Ca^{2+} signals at potentials close to resting (Mayer et al., 1984; Regehr and Tank, 1994; Swadlow and Hicks, 1997; Mainen et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). However, at potentials close to resting, LTP has been difficult to obtain and it was suggested that at that level of postsynaptic activity, LTD is more readily induced (Dudek and Bear, 1992; Mulkey and Malenka, 1992; Ngezahayo et al., 2000; Shouval et al., 2002).

The present experiments demonstrate that pairing mild depolarization with subthreshold EPSCs results in the induction of purely NMDAR-dependent LTP (LTP_{EPSC}). This component is of relatively small magnitude ($< 30\%$) and can be prevented independently by APV and 7-Cl-KYN. The induction parameters of LTP_{EPSC} suggest that while membrane depolarization is required for NMDAR plasticity, clear separation of the NMDAR-dependent component of LTP can be obtained by keeping the synaptic stimulation subthreshold for the generation of somatic action potentials. These findings are in agreement with previous reports and demonstrate that somatic spiking is not necessary for NMDAR-mediated strengthening of the active synapses (Kelso et al., 1986; Golding et al., 2002).

Depolarizing the somatic V_m has been used routinely in plasticity studies for recruitment of synaptic NMDARs blocked at more hyperpolarized potentials (Kelso et

al., 1986; Gustafsson et al., 1987; Stricker et al., 1996; Sjöström et al., 2001). The present observation that increasing membrane depolarization from -60 to -50 mV led to substantial increase of the NMDAR-mediated component of LTP is consistent with the recruitment of NMDARs relatively inactive at close to resting potentials. The mechanism of electrotonic recruitment of distal NMDARs along the somatodendritic axis was beyond the scope of the study. Accumulating evidence indicates however that enhancement of sodium and calcium channel activity and inactivation of potassium currents contribute to the electrogenesis necessary for activation of synaptic NMDARs (Stuart and Sakmann, 1995; Sjöström et al., 2001; Stuart and Hausser, 2001).

A potentially important finding of the present study is that no LTD was observed during the subthreshold and suprathreshold induction conditions. It has been demonstrated that LTD is produced in response to “weaker” prolonged low-frequency trains that insufficiently depolarize the post-synaptic membrane and fail to activate NMDARs (Dudek and Bear, 1992; Mulkey and Malenka, 1992). Furthermore, it was proposed that clamping the postsynaptic cell between -60 and -52.5 mV would be sufficient to induce LTD while with more positive potentials LTP would be obtained (Shouval et al., 2002). LTD induction by pairing at even more depolarized potentials has also been reported (Feldman et al., 1998; Ngezahayo et al., 2000). The discrepancies between some of the above studies and the present results are most likely due to the different induction parameters used. Specifically, the duration of depolarization used for LTP induction here is smaller to that used by most studies. Another potentially important difference is that LTP was induced by limited number of pairings. Pairing prolonged depolarization with four to six single afferent pulses (0.1 Hz) is sufficient for the

development of input specific LTP. The degree of depolarization in the presence of afferent stimulation however, seems to be the most important factor for the induction and magnitude of LTP.

4.2.3. VDCCs and low-frequency LTP

The synaptic modifications associated with LTP are confined to the excitatory glutamatergic synapses located in dendrites and dendritic spines of CA1 pyramidal cells (Yuste and Denk, 1995; Malenka and Nicoll, 1999; Sabatini et al., 2002). The necessary signal for LTP is Ca^{2+} , which may enter the spine through NMDARs, VDCCs, Ca^{2+} permeable AMPA channels (Yuste et al., 1999) or be released from intracellular stores (Emptage et al., 1999).

In contrast to the input specific NMDAR-dependent LTP, NMDAR-independent LTP can be input specific (homosynaptic), not input specific (heterosynaptic) or a combination of both (Brown et al., 1990; Kullmann et al., 1992; Bliss and Collingridge, 1993; Hanse and Gustafsson, 1995; Chen et al., 1998). During induction, the input specificity of LTP may be affected by the intensity and type of afferent stimulation and the properties of postsynaptic Ca^{2+} signals. Since for the input specificity of LTP afferent stimulation is required, potentiation obtained without afferent stimulation is not input specific (Kullmann et al., 1992; Chen et al., 1998).

When LTP is obtained by tetanic stimulation, the rate and intensity of stimulation determines the form of LTP through activation of different postsynaptic mechanisms of plasticity. NMDAR-dependent LTP usually requires lower frequency (≤ 30 Hz) afferent stimulation (Grover and Teyler, 1990; Grover and Teyler, 1992; Morgan and Teyler,

2001). Increasing the intensity of afferent stimulation increases the contribution of Ca^{2+} influx via VDCCs. Thus, very high-frequency (≥ 200 Hz) tetanization or high-intensity theta-burst stimulation induces potentiation by Ca^{2+} entry through L-type VDCCs (Grover and Teyler, 1990; Grover and Teyler, 1992; Hanse and Gustafsson, 1995; Morgan and Teyler, 2001). Pairing studies have confirmed that VDCC-dependent LTP requires strong postsynaptic depolarization (Kullmann et al., 1992; Chen et al., 1998; Stricker et al., 1999). The present results are in agreement with these studies and further clarify the induction requirements for both types of LTP. This study presents the first report of NMDAR-independent LTP induced by single afferent pulses. Thus, NMDAR-dependent LTP requires pairing of depolarization ($V_m \leq -50$ mV) with subthreshold synaptic stimulation whereas NMDAR-independent LTP can be obtained by pairing depolarization ($V_m \geq -40$ mV) with suprathreshold stimulation. The voltage requirements for LTP were estimated through separation of voltage clamp depolarization from that induced by afferent stimulation. This was achieved by reducing the frequency and duration of afferent stimulation. Furthermore, the low-frequency afferent rate allowed the monitoring of the postsynaptic response during induction and the conclusion that NMDAR antagonism is ineffective when the afferent pulses discharge the depolarized pyramidal cell. Thus, the appearance of somatic action potentials during induction is a potential indicator for the occurrence, magnitude and mechanism of LTP. Taken together these findings suggest that NMDAR-independent LTP requires stronger depolarization and the generation of somatic action potentials, conditions most likely occurring during high-frequency tetanization (Grover and Teyler, 1990; Hanse and Gustafsson, 1995; Grover, 1998).

In the present study, low-frequency NMDAR-dependent and NMDAR-independent LTP were induced by single postsynaptic manipulation (depolarization). LTP obtained by postsynaptic current injection is a controlled way of LTP induction. Reducing the rate and number of afferent pulses circumvents a number of confounding mechanisms including altered transmitter release, temporal and spatial integration of EPSCs, build-up of synaptic and extrasynaptic glutamate, and saturation of uptake systems. Most of these mechanisms may affect the input specificity of LTP by recruitment of new release sites. In the present study, the possibility of heterosynaptic spread of potentiation was minimized by two additional measures. First, the number and intensity of afferent pulses were kept to minimal levels and second, potassium- and chloride-mediated inhibitory systems were kept intact. Thus, regardless of the mechanism, the present results suggest that the locus of expression of synaptic modification for NMDAR-dependent LTP and NMDAR-independent LTP is postsynaptic and confined to the same set of activated inputs.

Previous studies have suggested that VDCC-dependent LTP and NMDAR-dependent LTP require distinct transduction cascades (Grover and Teyler, 1990; Cavus and Teyler, 1996). Pharmacological treatments with APV during high-frequency tetanization decrease the magnitude of potentiation suggesting that this LTP is a compound potentiation consisting of VDCC-mediated and NMDAR-mediated components (Grover and Teyler, 1990; Cavus and Teyler, 1996; Grover, 1998). In the present study, NMDAR antagonists had no effect on the magnitude of low-frequency NMDAR-independent LTP. This finding indicates that during supramaximal induction conditions postsynaptic Ca^{2+} levels are above the threshold for activation of NMDAR-

coupled biochemical pathways. If this is the case, this result would suggest a cross-talk between Ca^{2+} influx through VDCCs and NMDAR-dependent mechanisms of plasticity. This interpretation is consistent with a common expression mechanism of NMDAR-independent and NMDAR-dependent LTP. Indeed, recent evidence suggests that the expression and maintenance of NMDAR-independent LTP appears to require enhancement of postsynaptic AMPAR function (Grover, 1998; Stricker et al., 1999).

4.2.4. Sodium channels, action potentials and low-frequency LTP

Distal and proximal dendrites of pyramidal neurons contain voltage-gated sodium channels, which main function seems to be the integration of excitatory synaptic input (Magee and Johnston, 1995; Golding and Spruston, 1998; Magee, 2000). Somatic depolarization, similar to that used in the present study, has been the tool for investigating the role of sodium channels in plasticity and in shaping the integrative properties of pyramidal neurons (Deisz et al., 1991; Magee and Johnston, 1995; Stuart and Sakmann, 1995; Magee and Johnston, 1997; Markram et al., 1997; Andreassen and Lambert, 1999; Golding et al., 2002). Thus, activation of axosomatic sodium channels by depolarizing current injection amplifies dendritic EPSPs while TTX prevents the depolarization-induced amplification and reduces the size of large EPSPs (Stuart and Sakmann, 1995; Andreassen and Lambert, 1999).

The contribution of sodium channels to synaptic plasticity has been established by demonstrating that activation of dendritic sodium channels provides the electrogenesis required for Ca^{2+} influx through NMDARs and VDCCs during LTP (Magee and Johnston, 1997; Markram et al., 1997; Magee, 2000; Golding et al., 2002). Although in

the present study the role of sodium channels in LTP was examined indirectly and mostly in respect to the generation of somatic action potential, several observations were consistent with contribution of these channels to plasticity. First, the amount of potentiation was generally proportional to the degree of membrane depolarization. Second, potentiation reached its maximum only when the depolarization was paired with suprathreshold synaptic responses, conditions presumably reflecting full activation of axosomatic and dendritic sodium channels. Third, LTP induced by strong depolarization and somatic action potentials was least affected by NMDAR antagonists. These findings are consistent with other plasticity studies demonstrating the contribution of sodium channels to the generation of dendritic spikes and the spread of back-propagating action potentials (Jaffe et al., 1992; Magee and Johnston, 1995; Magee and Johnston, 1997; Markram et al., 1997; Golding et al., 2002). The present results also confirm however, that depolarization and postsynaptic action potentials without afferent stimulation, does not lead to input specific, Hebbian plasticity (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Conti and Lisman, 2002).

The main source of postsynaptic depolarization under physiological conditions arises from the temporal and spatial integration of dendritic EPSPs (Cash and Yuste, 1998; Cash and Yuste, 1999; Hausser et al., 2000; Magee and Cook, 2000). Activation of unitary synaptic contacts however, seems to be too weak for plasticity induction and more than one presynaptic cell must fire simultaneously for the postsynaptic membrane to be depolarized sufficiently (Debanne et al., 1996). This is the essence of the 'cooperativity' requirement and is best supported by the fact that stronger LTP is

obtained when the number of discharging presynaptic fibers is increased (McNaughton et al., 1978; Lee, 1983; Debanne et al., 1996).

The back-propagating somatic action potential is another mechanism capable of providing the dendritic depolarization necessary for LTP (Stuart and Sakmann, 1994; Stuart et al., 1997). Action potentials initiated at the soma could function as an enabling signal for LTP by invading the distal dendrites and activating NMDARs and VDCCs (Magee and Johnston, 1997; Markham et al., 1997). Thus, pairing subthreshold EPSPs with postsynaptic action potentials induced by somatic current injection resulted in persistent changes in EPSP amplitude (Magee and Johnston, 1997; Markram et al., 1997). The compound depolarization from properly timed EPSPs and postsynaptic burst firing appears to be equivalent to the background depolarization used here and in other pairing studies. Indeed, it has been shown that postsynaptic bursting and complex spike bursting, which are common in the intact hippocampus, enable LTP under conditions where single postsynaptic spikes were ineffective (Thomas et al., 1998; Pike et al., 1999). Thus, the prolonged somatic depolarization may be a laboratory equivalent of the electrogenesis associated with pyramidal cell bursting in intact animals during exploratory and learning behaviors (Buzsáki, 1989; Buzsáki et al., 1996). This is consistent with the observation that in the presence of background depolarization, neither presynaptic nor postsynaptic bursting is necessary and LTP can be elicited by single synaptically evoked action potentials.

In summary, these results demonstrate the effects of membrane voltage on the induction, magnitude and type of LTP in CA1 pyramidal cells. They suggest that purely NMDAR-dependent LTP is obtained by pairing small membrane depolarization with

subthreshold EPSCs. In the depolarized cell, synaptically evoked low-frequency somatic action potentials lead to robust LTP, of a form that is insensitive to NMDAR antagonists. Finally, in the presence of dendritic depolarization, NMDAR-mediated plasticity mechanisms can function independently from the neuron output regions and the somatic action potential.

4.3. Regulation of synaptic plasticity by the glycine site of NMDAR

4.3.1. Requirements for D-serine and glycine modulation of plasticity

Plasticity studies have suggested that by manipulating various stimulation parameters, LTP can be induced in such a way to depend either on Ca^{2+} influx through NMDARs or VDCCs. Thus, induction protocols that use lower-frequency afferent stimulation or smaller postsynaptic depolarization require Ca^{2+} influx through NMDARs (Grover and Teyler, 1990; Cavus and Teyler, 1996; Morgan and Teyler, 2001). The induction requirements for NMDAR-dependent LTP are in agreement with functional and calcium imaging studies demonstrating strong NMDAR activity at potentials close to resting (Regehr and Tank; Swadlow and Hicks, 1997; Mainen et al., 1999; Yuste et al., 1999; Kovalchuk et al., 2000; Sabatini et al., 2002). LTP obtained with very high-frequency afferent stimulation, high-intensity theta-burst stimulation or stronger depolarization require VDCCs (Kullmann et al., 1992; Hanse and Gustafsson, 1995; Cavus and Teyler, 1996; Chen et al., 1998; Stricker et al., 1999; Morgan and Teyler, 2001).

The present results are consistent with the induction requirements for NMDAR-dependent and NMDAR-independent LTP. The study suggests that membrane voltage

during induction differentially affects glycine site modulation of plasticity. D-serine and glycine enhancement of LTP is most effective during submaximal induction conditions. Thus, both agonists induced potentiation with pairing protocol (depolarization to -60 mV) that applied in control ASCF had no effect on plasticity. During depolarization to -50 mV, D-serine and glycine drastically increase the amount of potentiation. At this level of postsynaptic activity, glycine site agonists promote membrane excitability, depolarization and the generation of somatic action potentials. During depolarization to -50 mV, D-serine and glycine effects on LTP appear to be mediated by further enhancement of NMDAR-dependent component of LTP and by lowering the threshold for Ca^{2+} influx through voltage-gated channels contributing to plasticity (Magee and Johnston, 1997; Morgan and Teyler, 2001; Golding et al., 2002). During supramaximal induction (-40 mV/action potentials), LTP magnitude was not affected by D-serine and glycine, indicating that at that level of postsynaptic activity different mechanisms of plasticity dominate and further enhancement of NMDAR function through the glycine site has no measurable effect on LTP magnitude (Magee and Johnston, 1997; Morgan and Teyler, 2001; Bauer et al., 2002; Golding et al., 2002; Raymond and Redman, 2002).

Kinetic analysis of NMDAR activation suggests that glycine promotes transitions to open states and for this glycine must first bind to the closed state of the receptor (Benveniste et al., 1990; Vyklicky et al., 1990; Lerma et al., 1990). Thus, the fastest rising responses to NMDA have been observed when exogenous glycine is applied 5 - 10 sec prior to NMDA application (Lerma et al., 1990). Furthermore, binding and functional studies have demonstrated that full and partial glycine site agonists positively modulate the glutamate binding site by increasing the affinity for glutamate (Fadda et al., 1988;

Grimwood et al., 1993; Lester et al., 1993; Priestley and Kemp, 1994; Grimwood et al., 1995; Danysz and Parsons, 1998). Using modified induction paradigms, the priming effect of exogenous glycine site agonists on LTP magnitude was examined directly. Experimentally, this was achieved by restarting the afferent stimulation for LTP induction after D-serine and glycine application. Under these conditions, D-serine and glycine augmentation of LTP was maximal. Although the molecular mechanism responsible for the priming effect of glycine site agonists can not be corroborated from the present experiments, these findings suggest that glycine site agonist presence before glutamate release strongly facilitate NMDAR function and that this effect can be detected during physiological conditions involving NMDARs. As with the standard induction, however, the involvement of glycine site modulation appears depolarization- and induction-dependent.

Microdialysis and immunohistochemical studies have confirmed that D-serine and glycine are present in the mammalian CNS, including human CNS (Westergren et al., 1994; Matsui et al., 1995; Schell et al., 1995; Hashimoto and Oka, 1997; Schell et al., 1997). However, substantial regional differences in the distribution of glycine and D-serine exist. Glycine immunoreactivity is very low in the cortex and hippocampus, whereas D-serine immunoreactivity is highest in the hippocampus (Schell et al., 1995; Schell et al., 1997). In the present study, the effects of D-serine and glycine were compared in parallel experiments and the key findings have been replicated with both agents. The results suggest that in CA1 pyramidal cells NMDAR-mediated plasticity can be modulated to a similar degree by exogenous D-serine and glycine.

Ultrastructural analysis has demonstrated that D-serine-containing astrocytes ensheath CA1 pyramidal cell dendrites, heavily expressing NMDAR subunits (Schell et al., 1997). Biosynthesis of D-serine in protoplasmic astrocytes and a mechanism for activity-dependent release have also been described (Schell et al., 1995; Mothet et al., 2000). Thus, it appears that at least at some NMDARs, D-serine may be the endogenous modulator of NMDAR function. In support for D-serine being the predominant endogenous ligand for the glycine site, it was found that D-serine was more potent agent than glycine. Thus, the effect of glycine on LTP was matched by three-fold lower concentration of D-serine. The present D-serine results are consistent with those of other neurophysiological studies demonstrating strong enhancement of NMDAR function by D-serine in hippocampal pyramidal cells and interneurons (Oliver et al., 1990; Thiels et al., 1992; Matsui et al., 1995; Mothet et al., 2000).

4.3.2. Blockade of the glycine site by 7-Cl-KYN reverses the direction of synaptic plasticity

Previous studies have suggested that glycine site activation is required for LTP because pharmacological treatments with 7-Cl-KYN abolished the development of LTP induced by high-frequency stimulation *in vitro* and *in vivo* (Bashir et al., 1990; Oliver et al., 1990; Thiels et al., 1992; Watanabe et al., 1992; Ito and Hicks, 2001). This study also strongly supports the central role of glycine site activation for LTP induction. In addition, the study reveals the complexity of glycine site antagonist modulation. Present electrophysiological findings demonstrate that the sign and magnitude of synaptic plasticity are determined by activity at the glycine site of NMDAR. Thus, blockade of the

glycine site during prolonged depolarization reverses the direction of plasticity from LTP to LTD. This process appears to be affected by antagonist concentration and degree of membrane depolarization during induction.

In the present study, it was found that LTP protocols applied in the presence of 7-Cl-KYN result in the induction of LTD. Previous studies have suggested that it is possible for LTP protocols to induce LTD on the same synapses when certain conditions are met (Stanton and Sejnowski, 1989; Artola et al., 1990; Cummings et al., 1996). This can be achieved by altering the level of postsynaptic depolarization during constant afferent stimulation (Stanton and Sejnowski, 1989; Artola et al., 1990), by changing the precise timing of pre- and postsynaptic action potentials (Markram et al., 1997; Bi and Poo, 1998; Debanne et al., 1998; Feldmann, 2000) or by partial blockade of NMDARs during LTP induction (Cummings et al., 1996; Nishiyama et al., 2000).

Although the present study examines the effect of glycine site blockade on synaptic plasticity, the reversal of plasticity here appears similar to that reported by Cummings et al., 1996. These authors have demonstrated that brief high-frequency tetanus that normally induces potentiation, in the presence of modest concentrations (10-20 μ M) of APV results in LTD. In the study of Cummings et al., 1996 however, increasing the concentration of APV blocked LTD, whereas increasing 7-Cl-KYN concentration in the present study increased the amount of depression. The reason for this discrepancy appears to be the different contribution of VDCCs to LTD in both studies. The present induction requires stepwise depolarization that leads to simultaneous activation of NMDARs and voltage-gated channels. Thus, the increased amount of depression during stronger depolarization (≥ -50 mV) most likely reflects the contribution

of those channels to LTD. In contrast, Cummings et al., 1996 examined the role of VDCCs to LTD independently from NMDAR-mediated LTD. Based on the observation that prior induction of monosynaptic LTD largely occluded the generation of VDCC-mediated LTD, Cummings et al., 1996 suggested that VDCC-mediated LTD and NMDAR-mediated LTD shared important mechanisms. Furthermore, Cummings et al., 1996 proposed that a reduction of Ca^{2+} influx through NMDAR is responsible for the reversal of plasticity from LTP to LTD. The present results are consistent with this interpretation and indicate that the sign and magnitude of NMDAR-dependent plasticity can also be regulated by the glycine site. Further support for this hypothesis was found by the competitive experiments with D-serine and 7-Cl-KYN. In the presence of 7-Cl-KYN ($\leq 10 \mu\text{M}$), D-serine systematically reverses the direction of plasticity back to potentiation, suggesting that the underlying mechanism responsible for the sign of synaptic plasticity is intrinsically related to NMDAR function. Future studies will be needed to determine the degree of voltage-gated channels contribution to NMDAR plasticity.

4.3.3. D-serine protection of NMDAR-mediated plasticity

Co-application of D-serine and 7-Cl-KYN produced a profound attenuation of 7-Cl-KYN inhibition of LTP. D-serine saved LTP by counteracting the 7-Cl-KYN-mediated reversal of synaptic plasticity. D-serine attenuation was most effective at $\leq 10 \mu\text{M}$ 7-Cl-KYN. However, the protective effect of D-serine was lost at higher concentrations ($> 10 \mu\text{M}$) of 7-Cl-KYN. These results suggest that D-serine acting at the glycine site of NMDAR may have powerful protective effect on glutamatergic

neurotransmission in the CA1 hippocampal region. Pretreatment with glycine site agonists may enhance channel activity and diminish the overall effectiveness of NMDAR antagonists.

The present experiments represent an attempt for systematic examination of the requirements for glycine site modulation of NMDAR plasticity. As such, they may have relevance for clinical trials in psychiatric disorders associated with NMDAR dysfunction and schizophrenia. Enhancement of glutamatergic transmission through the glycine site of NMDAR has been a useful approach for reducing schizophrenia-like symptoms in animal models and in human patients (Javitt et al., 1994; Tani et al., 1994; Nilsson et al., 1997; Heresco-Levy et al., 1999; Javitt et al., 2001; Coyle et al., 2002). Since NMDAR-mediated plasticity is considered the best available neurobiological model for memory and learning, understanding its underlying mechanisms and routes of modulation may stimulate the development of new therapeutic strategies targeting the cognitive abnormalities in schizophrenia.

4.4. Summary and conclusions

The aim of the current study was to further our knowledge of the mechanisms regulating NMDAR function and the excitatory synaptic transmission in general. Experiments aimed at the role of NMDARs in plasticity determined the induction requirements for low-frequency LTP. Thus, NMDAR-dependent LTP requires pairing of depolarization ($V_m = -50$ mV, 60 sec) with six single subthreshold EPSCs. NMDAR-independent LTP is obtained by pairing depolarization ($V_m \geq -40$ mV, 40 - 60 sec) with four to six single suprathreshold synaptic responses.

In this work, the physiological role of the glycine site of NMDAR for synaptic plasticity has been examined and characterized. Together, these findings provide clear evidence that the glycine site plays a central role in the modulation of excitatory synaptic transmission in the hippocampal region. By determining the postsynaptic requirements for glycine site modulation of plasticity, this study expands our knowledge of NMDAR function.

The present findings may have clinical relevance in neurological disorders associated with NMDAR dysfunction. Enhancement of NMDAR-mediated transmission through the glycine site has been a useful approach in clinical trials with schizophrenic patients. Understanding the mechanisms and parameters of glycine site modulation of synaptic plasticity may stimulate the development of new strategies for the treatment of cognitive deficits associated with NMDAR dysfunction.

*

These findings have been presented, in part, at the annual international meetings of the *Society for Neuroscience* (Krasteniakov et al., 2002, 2003, Abstracts). Part of these findings has been published in the *Journal of Physiology* (Martina et al., 2003). Furthermore, a second manuscript has been accepted for publication in *Neuroscience* (Krasteniakov et al., 2004). A third manuscript has been submitted to *Neuropsychopharmacology*. Within the next few months, I anticipate to submit a review describing the physiological action of glycine site compounds on synaptic plasticity.

* * *

5. TABLES

Table 1. Physiological properties of pyramidal cells and interneurons

Cell Type	Physiological properties				Effect of D-serine on NMDARs		
	Resting V_m mV	R_{in} M Ω	τ ms	Spike Amplitude mV	Spike duration at half-amplitude ms	Electrical Stimuli [†] % increase	Pressure application of NMDA [‡] % increase
Pyramidal cells	-76.25 ± 0.62 $n = 39$	99.26 ± 1.88 $n = 39$	25.49 ± 1.78 $n = 39$	99.26 ± 1.88 $n = 39$	1.17 ± 0.05 $n = 39$	51.66 ± 7.89 $n = 17$	49.20 ± 8.29 $n = 10$
Interneurons	-62.11 ± 1.54 $n = 19$	346.85 ± 21.63 $n = 19$	63.03 ± 5.0 $n = 19$	80.24 ± 1.89 $n = 19$	1.38 ± 0.10 $n = 19$	25.07 ± 5.65 $n = 12$	28.15 ± 3.84 $n = 10$

[†]NMDAR responses to endogenous glutamate recorded in a low Mg^{2+} ACSF (0.1 mM) containing NBQX (20 μ M), picrotoxin (50 μ M), CGP 52432 (10 μ M) and strychnine (0.5 μ M). [‡]Responses to local pressure application of NMDA (100 μ M) in presence of TTX (0.5 μ M).

Table 2. Rise and decay time constants of the NMDAR currents in CA1 pyramidal cells and interneurons

Cell Type	Low Mg^{2+} ACSF [†]			D-serine [‡]		
	Inactivation			Inactivation		
	τ_{act} ms	τ_f ms	τ_s ms	τ_{act} ms	τ_f ms	τ_s ms
Pyramidal cells	13.18 ± 2.64 $n = 17$	56.62 ± 7.44 $n = 17$	=	13.28 ± 2.68 $n = 17$	71.52 ± 6.46 $n = 17$	=
Interneurons	11.46 ± 2.49 $n = 12$	36.83 ± 2.76 $n = 12$	242.4 ± 33.79 $n = 12$	12.94 ± 1.88 $n = 12$	55.78 ± 5.38 $n = 12$	244.0 ± 31.35 $n = 12$

[†]NMDAR responses to endogenous glutamate recorded in a low Mg^{2+} ACSF (0.1 mM) containing NBQX (20 μ M), picrotoxin (50 μ M), CGP 52432 (10 μ M) and strychnine (0.5 μ M). The fast and slow decay components are designated τ_f and τ_s . [‡]The same perfusion solution as in [†] plus D-serine (10 μ M). In both cell types, D-serine increased τ_f ($p < 0.05$).

Table 3. Modulation of synaptic plasticity by the glycine site of NMDAR

	Pairing to -60 mV % increase $\pm$ SEM	Pairing to -50 mV % increase $\pm$ SEM	Pairing to -40 mV % increase $\pm$ SEM
Control (ACSF)	2.86 $\pm$ 4.6 <i>n</i> = 7	16.4 $\pm$ 5.2 <i>n</i> = 5	52.57 $\pm$ 6.9 <i>n</i> = 7
D-serine 1 μ M	17.33 $\pm$ 4.4 * <i>n</i> = 6	52.6 $\pm$ 7.1 ** <i>n</i> = 5	64.17 $\pm$ 9.1 <i>n</i> = 6
Glycine 3 μ M	16.4 $\pm$ 5.1 * <i>n</i> = 5	49.12 $\pm$ 7.9 ** <i>n</i> = 6	57.5 $\pm$ 7.8 <i>n</i> = 4
7-Cl-KYN 3 μ M	0.75 $\pm$ 4.1 <i>n</i> = 4	0.4 $\pm$ 4.3 * <i>n</i> = 5	50.6 $\pm$ 7.5 <i>n</i> = 5
7-Cl-KYN 10 μ M	0.5 $\pm$ 7.2 <i>n</i> = 4	1.2 $\pm$ 3.9 * <i>n</i> = 5	-17.5 $\pm$ 6.3 ** <i>n</i> = 4
7-Cl-KYN 30 μ M	-5.2 $\pm$ 6.7 <i>n</i> = 5	-17.5 $\pm$ 8.5 ** <i>n</i> = 4	-28.4 $\pm$ 8.5 ** <i>n</i> = 5
D-serine 1 μ M + 7-Cl-KYN 3 μ M	14.6 $\pm$ 5.2 <i>n</i> = 5	54.4 $\pm$ 6.9 <i>n</i> = 5	57.5 $\pm$ 6.3 <i>n</i> = 4
D-serine 1 μ M + 7-Cl-KYN 10 μ M	0.67 $\pm$ 9.7 † <i>n</i> = 3	20.8 $\pm$ 7.4 † <i>n</i> = 4	33.75 $\pm$ 9.4 † <i>n</i> = 4
D-serine 1 μ M + 7-Cl-KYN 30 μ M	-14.8 $\pm$ 9.2 †† <i>n</i> = 5	-23.75 $\pm$ 6.8 †† <i>n</i> = 4	-48.33 $\pm$ 7.3 †† <i>n</i> = 3
Control (ACSF) (no stimulus)	2.6 $\pm$ 5.3 <i>n</i> = 5	15.81 $\pm$ 8.8 <i>n</i> = 4	-
D-serine 1 μ M (no stimulus)	31.5 $\pm$ 5.2 † <i>n</i> = 4	61.2 $\pm$ 7.1 <i>n</i> = 5	-
Glycine 3 μ M (no stimulus)	26.7 $\pm$ 3.1 ‡ <i>n</i> = 6	55.6 $\pm$ 6.2 <i>n</i> = 5	-

* *p* < 0.05 compared to pairing in control ACSF.
 ** *p* < 0.01 compared to pairing in control ACSF.
 † *p* < 0.05 compared to pairing in 1 μ M D-serine.
 †† *p* < 0.01 compared to pairing in 1 μ M D-serine.
 ‡ *p* < 0.05 compared to pairing in 3 μ M glycine.

6. FIGURES

Figure 1. Schematic representation of the structure and function of NMDAR complex. (From Feldman et al., 1997).

Figure 2. Morphological and physiological properties of pyramidal cells and interneurons from the hippocampal CA1 region. A1 and B1, confocal images of a pyramidal cell (PC) and an interneuron (Int) injected with Lucifer Yellow. The cells were recorded at the positions indicated in A2 and B2 (asterisks; confocal and DIC image superimposed). A3 and B3, confocal images of the boxed dendritic regions shown in A1 and B1, respectively. The arrowheads indicate dendritic spines. A4 and B4 show the voltage responses of the same neurons to a series of intracellular current pulses. Abbreviations: alv, alveus; so, stratum oriens; sp, stratum pyramidale; sr, stratum radiatum; slm, stratum lacunosum moleculare.

Figure 3. D-serine modulation of NMDAR responses to endogenous glutamate in pyramidal cells and interneurons. Each trace is an average of 20 traces. A and B, responses evoked by electrical stimuli at $V_m = -70$ mV in a pyramidal cell (PC) and in an interneuron (Int). A1 and B1, the isolated NMDAR currents recorded in low Mg^{2+} ACSF (thin line), during application of D-serine (thick line) and after recovery from the drug (dotted line). A2 and B2, decay time course of NMDAR currents in low Mg^{2+} ACSF (thin line) and during application of D-serine (thick line). NMDAR currents in low Mg^{2+} ACSF (thin line) are normalized to those in D-serine (thick line). A3 and B3, graph showing the effect of D-serine (10 μ M) on NMDAR currents in both cell types.

Figure 4. Effect of D-serine on NMDAR currents at different holding potentials. NMDAR currents were isolated in normal ACSF containing picrotoxin ($50 \mu\text{M}$), CGP 52432 ($10 \mu\text{M}$), strychnine ($0.5 \mu\text{M}$) and NBQX ($20 \mu\text{M}$). Each trace is an average of 5 traces. A1 and B1, NMDAR responses from sample neurons recorded at different membrane potentials before (thin line) and during (thick line) D-serine application. A2 and B2, peak current-voltage relations are shown for both cell types before (■) and during (●) D-serine application. The peak current-voltage relations are averages of 3 pyramidal cells and 3 interneurons.

Figure 5. D-serine enhancement of NMDAR function during local pressure application of NMDA. A and B, NMDA-evoked responses in pyramidal cells and interneurons recorded in low Mg²⁺ ACSF (thick line) and during application of D-serine (thin line), and after recovery (dotted line). NMDA (100 μ M) was applied through a patch pipette positioned directly above the proximal dendrites. TTX (0.5 μ M) was present throughout the experiments. The cells were voltage-clamped at $V_m = -70$ mV. Each trace is an average of 4 traces. Time base is 1 s (A) and 500 ms (B). The inset represents the traces in B at a slower time base.

Figure 6. Effects of postsynaptic depolarization on brief, high-frequency synaptic stimulation. A, failure of LTP during high-frequency afferent stimulation. Individual experiment in which single train (25 pulses, 100 Hz) was given to the Schaffer collaterals. Top graph shows response amplitudes before and after the train (arrow).

Induction protocol and synaptic responses during induction (lower traces) are shown. Inset, superimposed 5-minute response averages taken immediately prior to (dotted line) and between 10 and 15 min after induction. B, depolarization to -50 mV co-applied with the train results in LTP. Prolonged firing and spike after-depolarization build-up (lower trace) during induction seems necessary for high-frequency LTP. C, similar results were obtained with 10-pulse train (100 Hz). Synaptic stimulation alone did not induce any potentiation. D, postsynaptic spikes and spike after-depolarization rescue LTP induced by pairing 10-pulse train with depolarization to -50 mV. E, pooled data illustrate the effects of depolarization to -50 mV on LTP during brief high-frequency afferent stimulation.

Figure 7. Minimal pairing requirements for LTP of maximal magnitude. A, pairing depolarization to -40 mV with 6 single (0.1 Hz) suprathreshold synaptic responses leads to LTP of maximal magnitude. Low-frequency induction protocol and a whole cell single response during induction are shown. B,C,D and E, four different pairing protocols and representative synaptic responses. Depolarization to -40 mV paired with suprathreshold synaptic responses, sequentially reduced from 5 to 2. F, summary graph showing potentiation time course under the different induction conditions. G, pooled data indicate potentiation for the different pairing protocols. Pairing depolarization to -40 mV with 4, 5 or 6 synaptically initiated action potentials resulted in LTP of maximal magnitude. Reducing further the number of suprathreshold responses paired with depolarization to 3 and 2 led to gradual loss of potentiation.

Figure 8. Membrane voltage and the induction LTP_{AP} . Pairing three different types of somatic depolarization with suprathreshold synaptic responses are used to examine the effects of V_m on LTP_{AP} . A, pairing -20, -30 and -40 mV with 6 single suprathreshold responses. On the right, single synaptic response during induction from sample experiment. B, summary graph shows potentiation magnitude for the different induction protocols. There is no significant difference in LTP_{AP} under the three induction conditions. Inset, superimposed averaged traces showing the response before (dotted line) and after pairing from sample experiment.

Figure 9. Pairing postsynaptic depolarization with subthreshold EPSCs results in LTP_{EPSC}. A, pairing depolarization to -60 mV with 6 subthreshold EPSCs. Induction protocol and single synaptic response during induction from sample experiment are shown. B and C, pairing postsynaptic depolarization to -50 mV with single afferent pulses may result in subthreshold or suprathreshold responses. B, pairing -50 mV with 6 subthreshold EPSCs. C, pairing -50 mV with 6 suprathreshold EPSCs. D, summary graph shows potentiation time course under the three induction conditions. Insets, averaged traces from individual experiments represent the evoked EPSCs before (dotted lines) and after pairing.

Figure 10. Blockade of NMDARs prevents LTP_{EPSC} but has no effect on LTP_{AP} . A, graph showing the effects of APV on LTP_{EPSC} and LTP_{AP} . APV blocks LTP induced by pairing depolarization with subthreshold EPSCs but is ineffective in preventing LTP induced by pairing depolarization with suprathreshold synaptic responses. Insets, averaged sample traces show EPSCs before (dotted lines) and after LTP induction for the three pairing protocols. B, 7-Cl-KYN (3 μ M) has similar to APV effects on LTP_{EPSC} and

LTP_{AP}. Pairing depolarization with subthreshold EPSCs in the presence of 7-Cl-KYN did not result in potentiation. 7-Cl-KYN did not prevent LTP induced by pairing depolarization with postsynaptic action potentials. C, summary data show the magnitude of potentiation in control slices and in the presence of APV and 7-Cl-KYN. Although pairing to -60 mV was not associated with potentiation, NMDAR antagonists were also tested with this protocol.

Figure 11. Depolarization controls the induction and magnitude of LTP in CA1 pyramidal cells during low-frequency afferent stimulation. A, pairing membrane depolarization to -60 mV with six subthreshold EPSCs. Pairing protocol and single synaptic response during induction from representative experiment. B, pairing membrane depolarization to -50 mV with six subthreshold EPSCs. C, pairing depolarization to -40 mV with six suprathreshold synaptic responses. D, summary graph showing the effects of

varying degrees of depolarization on plasticity. Insets, superimposed response averages taken prior to (dotted line) and after pairing from sample experiments.

Figure 12. Modulation of LTP by exogenous D-serine. A, LTP induction by pairing depolarization to -60 mV with six subthreshold EPSCs in the presence of D-serine.

Induction protocol and single synaptic response during induction from sample experiment are shown. D-serine is applied for total of 5 min, beginning 4 min before pairing. B, pairing depolarization to -50 mV with six synaptic responses in the presence of D-serine. Depolarizing the cell to -50 mV in the presence of D-serine resulted in the generation of somatic action potentials during the afferent pulse. C, pairing depolarization to -40 mV with six synaptic responses. D, summary graph showing LTP in D-serine treated and control slices. Insets, response averages from representative D-serine experiments. Comparisons by one-tailed Student's *t*-test, **p* < 0.05, ***p* < 0.01.

Figure 13. LTP modulation by exogenous glycine. A, pairing protocols and glycine application. B, summary data showing normalized EPSC amplitudes before and after pairing in glycine. Insets, superimposed EPSC averages taken prior to (dotted line) and after induction from representative experiments in which pairing was done in the presence of 3 μ M glycine. Comparisons by one-tailed Student's *t*-test, **p* < 0.05, ***p* < 0.01.

Figure 14. Maximizing the effects of D-serine and glycine on LTP induced by pairing. A, induction protocols and drug application. D-serine and glycine were applied in the absence of afferent stimulation prior to induction. Stimulation resumes ~ 5 sec after cell depolarization for the pairing. B, summary graph showing LTP magnitude under the different induction conditions. Comparisons by one-tailed Student's *t*-test vs data obtained by standard induction (constant afferent stimulation) in the presence of agonists, **p* < 0.05.

Figure 15. 7-Cl-KYN reverses the direction of synaptic plasticity elicited by pairing. A, induction protocols and 7-Cl-KYN application. B, summary graph showing normalized EPSC amplitudes after pairing (-60, -50 or -40 mV) in the presence of 7-Cl-KYN (3, 10 or 30 μ M). LTD induction by 7-Cl-KYN is concentration- and depolarization-dependent. Comparisons by two-tailed Student's *t*-test, **p* < 0.05, ***p* < 0.01.

Figure 16. Competitive effects of 7-Cl-KYN and D-serine on synaptic plasticity. A, induction protocols and co-application of 7-Cl-KYN (3, 10 or 30 μ M) and D-serine (1 μ M). B, summary data showing normalized EPSC amplitudes after pairing in 7-Cl-KYN and D-serine. D-serine counteracts the inhibitory effect of 7-Cl-KYN (3 and 10 μ M) on LTP. In the presence of 30 μ M 7-Cl-KYN, D-serine facilitates LTD. Comparisons by two-tailed Student's *t*-test, **p* < 0.05, ***p* < 0.01.

7. REFERENCES

- Amaral, D.G. 1991. Is there channeling of information through the intrinsic circuit of the rat hippocampus? *Brain Res Rev.* 16:193-220.
- Amaral, D.G. 1993. Emerging principles of intrinsic hippocampal organization. *Curr Opin Neurobiol.* 3:225-229.
- Amaral, D.G., Dolorfo, C., and Alvarez-Royo, P. 1991. Organization of CA1 projections to the subiculum: a PHA-L analysis in the rat. *Hippocampus.* 1:415-435.
- Amaral, D.G., Ishizuka N., and Claiborne, B. 1990. Neurons, numbers and the hippocampal network. *Prog Brain Res.* 83:1-11.
- Amaral, D.G., and Witter, M.P. 1989. The three-dimensional organization of the hippocampal formation: a review of anatomical data. *Neuroscience.* 31:571-591.
- Amaral, D.G., and Witter, M.P. 1995. Hippocampal formation. In G. Paxinos (Ed.), *The rat nervous system*, 2nd ed. (pp. 443-494). New York: Academic Press.
- Andersen, P., Bliss, V.P., and Sorede, K. 1971. Lamellar organization of hippocampal pathways. *Exp Brain Res.* 13:222-238.
- Andreassen, M., and Lambert, J.D. 1999. Somatic amplification of distally generated subthreshold EPSPs in rat hippocampal pyramidal neurones. *J Physiol.* 519:85-100.
- Aniksztejn, L., Roisin, M.P., Amsellem, R., and Ben-Ari, Y. 1989. Long-term potentiation in the hippocampus of the anaesthetized rat is not associated with a sustained enhanced release of endogenous excitatory amino acids. *Neuroscience.* 28:387-392.
- Artola, A., Brocher, S., and Singer, W. 1990. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature.* 347:69-72.
- Ascher, P., and Nowak, L. 1988. The role of divalent cations in the N-methyl-D-aspartate responses of mouse central neurones in culture. *J Physiol.* 399:247-266.
- Asztely, F., Xiao, M.Y., and Gustafsson, B. 1996. Long-term potentiation and paired-pulse facilitation in the hippocampal CA1 region. *Neuroreport.* 7:1609-1612.
- Azouz, R., Jensen, M.S., and Yaari, Y. 1996. Ionic basis of spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol.* 492:211-223.

- Bahn, S., Volk, B., and Wisden, W. 1994. Kainate receptor gene expression in the developing rat brain. *J Neurosci.* 14:5525-5547.
- Ballard, T.M., Pauly-Evers, M., Higgins, G.A., Ouagazzal, A-M., Mutel, V., Borroni, E., Kemp, J.A., Bluethmann, H., and Kew, J.N. 2002. Severe impairment of NMDA receptor function in mice carrying targeted point mutations in the glycine binding site results in drug-resistant nonhabituating hyperactivity. *J Neurosci.* 22:6713-6723.
- Bannister, N.J., and Larkmann, A.U. 1995a. Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: I. Branching patterns. *J Comp Neurol.* 360:150-160.
- Bannister, N.J., and Larkmann, A.U. 1995b. Dendritic morphology of CA1 pyramidal neurones from the rat hippocampus: II. Spine distributions. *J Comp Neurol.* 360:161-171.
- Barnard, E.A. 1997. Ionotropic glutamate receptors: new types and new concepts. *Trends Pharmacol Sci.* 18:141-148.
- Baron, B.M., Seigel, B.W., Harrison, B.L., Gross, R.S., Hawes, C., and Towers, P. 1996. [3H]MDL 105,519, a high-affinity radioligand for the N-methyl-D-aspartate receptor-associated glycine recognition site. *J Pharmacol Exp Ther.* 279:62-68.
- Barria, A., Muller, D., Derkach, V., Griffith, L.C., and Soderling, T.R. 1997. Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science.* 276:2042-2045.
- Bashir, Z.I., Tam, B., and Collingridge, G.L. 1990. Activation of the glycine site in the NMDA receptor is necessary for the induction of LTP. *Neurosci Lett.* 108: 261-266.
- Bauer, E.P., Schafe, G.E., and LeDoux, J.E. 2002. NMDA receptors and L-type voltage-gated calcium channels contribute to long-term potentiation and different components of fear memory formation in the lateral amygdala. *J Neurosci.* 22:5239-5249.
- Beckstead, R.M. 1978. Afferent connections of the entorhinal area in the rat as demonstrated by retrograde cell-labeling with horseradish peroxidase. *Brain Res.* 152:249-264.
- Bekkers, J.M., and Stevens, C.F. 1990. Presynaptic mechanism for long-term potentiation in the hippocampus. *Nature.* 346:724-729.
- Benke, T.A., Lüthi, A., Isaac, J.T., and Collingridge, G.L. 1998. Modulation of AMPA receptor unitary conductance by synaptic activity. *Nature.* 393:793-797.

- Benveniste, M., Clements, J., Vyklicky, L. Jr., and Mayer, M.L. 1990. A kinetic analysis of the modulation of N-methyl-D-aspartic acid receptors by glycine in mouse cultured hippocampal neurones. *J Physiol.* 428:333-357.
- Bettler, B., Boulter, J., Hermans-Borgmeyer, I., O'Shea-Greenfield, A., Deneris, E.S., Moll, C., Borgmeyer, U., Hollmann, M., and Heinemann, S. 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., Pecht, G., Hermans-Borgmeyer, I., Moll, C., Stevens, C.F., and Heinemann, S. 1992. Cloning of a putative glutamate receptor: a low affinity kainate-binding subunit. *Neuron.* 8:257-265.
- Berger, A.J., Dieudonne, S., and Ascher, P. 1998. Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synapses. *J Neurophysiol.* 80:3336-3340.
- Bergeron, R., Meyer, T.M., Coyle, J.T., and Greene, R.W. 1998. Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proc Natl Acad Sci USA.* 95:15730-15734.
- Bi, G.Q., and Poo, M.M. 1998. Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type. *J Neurosci.* 18:10464-10472.
- Blackstad, T.W. 1956. Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. *J Comp Neurol.* 105:417-537.
- Blackstad, T.W. 1965. Mapping of experimental axon degeneration by electron microscopy of Golgi preparations. *Z Zellforsch Mikrosk Anat.* 67:819-834.
- Blackstad, T.W., Brink, K., Hem, J., and Jeune, B. 1970. Distribution of hippocampal mossy fibers in the rat. An experimental study with silver impregnation methods. *J Comp Neurol.* 138:433-449.
- Bliss, T.V., and Collingridge, G.L. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature.* 361:31-39.
- Bliss, T.V., Douglas, R.M., Errington, M.L., and Lynch, M.A. 1986. Correlation between long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats. *J Physiol.* 377:391-408.
- Bliss, T. V., and Gardner-Medwin, A.R. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the unanesthetized rabbit following stimulation of the perforant path. *J Physiol.* 232:357-374.

- Bliss, T. V., and Lømo, T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol.* 232:331-356.
- Blitzer, R.D., Connor, J.H., Brown, G.P., Wong, T., Shenolikar, S., Iyengar, R., and Landau, E.M. 1998. Gating of CaMKII by cAMP-regulated protein phosphatase activity during LTP. *Science.* 280:1940-1942.
- Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron, C., and Heinemann, S. 1990. Molecular cloning and functional expression of glutamate receptor subunit genes. *Science.* 249:1033-1037.
- Braun, A.P., and Schulman, H. 1995. The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. *Annu Rev Physiol.* 57:417-445.
- Brown, T.H., Kairiss, E.W., and Keenan, C.L. 1990. Hebbian synapses: biophysical mechanisms and algorithms. *Annu Rev Neurosci.* 13:475-511.
- Buller, A.L., Larson, H.C., Schneider, B.E., Beaton, J.A., Morrisett, R.A., and Monaghan, D.T. 1994. The molecular basis of NMDA receptor subtypes: Native receptor diversity is predicted by subunit composition. *J Neurosci.* 14:5471-5484.
- Buller, A.L., and Monaghan, D.T. 1997. Pharmacological heterogeneity of NMDA receptors: Characterization of NR1a/NR2D heteromers expressed in *Xenopus* oocytes. *Eur J Pharmacol.* 320:87-94.
- Buzsaki, G. 1989. Two stage model of memory trace formation: a role for "noisy" brain states. *Neuroscience.* 31:551-570.
- Buzsaki, G., Penttonen, M., Nadasdy, Z., and Bragin, A. 1996. Pattern and inhibition-dependent invasion of pyramidal cell dendrites by fast spikes in the hippocampus *in vivo*. *Proc Natl Acad Sci USA*, 93:9921-9925.
- Carlsson, M., and Carlsson, A. 1990. Interaction between glutamatergic and monoaminergic systems within the basal ganglia: Implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* 13:272-276.
- Cash, S., and Yuste, R. 1998. Input summation by cultured pyramidal neurons is linear and position-independent. *J Neurosci.* 18:10-15.
- Cash, S., and Yuste, R. 1999. Linear summation of excitatory inputs by CA1 pyramidal neurons. *Neuron.* 22:383-394.
- Cavus, I., and Teyler, T.J. 1996. Two forms of long-term potentiation in area CA1 activate different signal transduction cascades. *J Neurophysiol.* 76:3038-3047.

- Chatterton, J.E., Awobuluyi, M., Premkumar, L.S., Takahashi, H., Talantova, M., Shin, Y., Cui, J., Tu, S., Sevarino, K.A., Nakanishi, N., Tong, G., Lipton, S.A., and Zhang, D. 2002. Excitatory glycine receptors containing the NR3 family of NMDA receptor subunits. *Nature*. 415:793-798.
- Cheffings, C.M., and Colquhoun, D. 2000. Single channel analysis of a novel NMDA channel from *Xenopus* oocytes expressing recombinant NR1a, NR2A and NR2D subunits. *J Physiol*. 526:481-491.
- Chen, H.X., Hanse, E., Pananceau, M., and Gustafsson, B. 1998. Distinct expressions for synaptic potentiation induced by calcium through voltage-gated calcium and N-methyl-D-aspartate receptor channels in the hippocampal CA1 region. *Neuroscience*. 86:415-422.
- Chen, L., Muhlhauser, M., and Yang, C.R. 2003. Glycine transporter-1 blockade potentiates NMDA-mediated responses in rat prefrontal cortical neurons in vitro and in vivo. *J Neurophysiol*. 89:691-703.
- Chen, N., Moshaver, A., and Raymond, L.A. 1997. Differential sensitivity of recombinant N-methyl-D-aspartate receptor subtypes to zinc inhibition. *Mol Pharmacol*. 51:1015-1023.
- Chetkovich, D.M., Gray, R., Johnston, D., and Sweatt, J.D. 1991. N-methyl-D-aspartate receptor activation increases cAMP levels and voltage-gated Ca²⁺ channel activity in area CA1 of hippocampus. *Proc Natl Acad Sci USA*. 88:6467-6471.
- Chizhnikov, I.V., Kiskin, N.I., and Krishtal, O.A. 1992. Two types of steady-state desensitization of N-methyl-D-aspartate receptor in isolated hippocampal neurones of rat. *J Physiol*. 448:453-472.
- Ciabarra, A.M., Sullivan, J.M., Gahn, L.G., Pecht, G., Heinemann, S. and Savarino, K.A. 1995. Cloning and characterization of chi-1: a developmentally regulated member of a novel class of the ionotropic glutamate receptor family. *J Neurosci*. 15:6498-6508.
- Claiborne, B.J., Amaral, D.G., and Cowan, W.M. 1986. A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus. *J Comp Neurol*. 246:435-458.
- Coan, E.J., Saywood, W., and Collingridge, G.L. 1987. MK-801 blocks NMDA receptor-mediated synaptic transmission and long term potentiation in rat hippocampal slices. *Neurosci Lett*. 80:111-114.

- Collingridge, G.L., Kehl, S.J., and McLennan, H. 1983. The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. *J Physiol.* 334:19-31.
- Conti, R., and Lisman, J. 2002. A large sustained Ca^{2+} elevation occurs in unstimulated spines during the LTP pairing protocol but does not change synaptic strength. *Hippocampus.* 12:667-679.
- Contreras, P.C. 1990. D-Serine antagonized phencyclidine-induced and MK-801-induced stereotyped behavior and ataxia. *Neuropharmacology.* 29:291-293.
- Coyle, J.T., Tsai, G., and Goff, D.C. 2002. Ionotropic glutamate receptors as therapeutic targets in schizophrenia. *Curr Drug Target CNS Neurol Disord.* 1:183-189.
- Crawford, M., and Roberts, P.J. 1989. 1-hydroxy-3-aminopyrrolid-2-one (HA-966) and kynurenate antagonize N-methyl-D-aspartate induced enhancement of [^3H]dopamine release from rat striatal slices. *Biochem Pharmacol.* 38:4165-4168.
- Cummings, J.A., Mulkey, R.M., Nicoll, R.A., and Malenka, R.C. 1996. Ca^{2+} signaling requirements for long-term depression in the hippocampus. *Neuron.* 16:825-833.
- Danysz, W., and Parsons, C.G. 1998. Glycine and N-Methyl-D-Aspartate receptors: physiological significance and possible therapeutic applications. *Pharmacol Rev.* 50:597-664.
- 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.V., Lipton, S.A., and Nakanishi, N. 1998. Increased NMDA current and spine density in mice lacking the NMDA receptor subunit NR3A. *Nature.* 393:377-381.
- Davies, S.N., Lester, R.A., Reymann, K.G., and Collingridge, G.L. 1989. Temporally distinct pre- and post-synaptic mechanisms maintain long-term potentiation. *Nature.* 338:500-503.
- Debanne, D., Gähwiler, B.H., and Thompson, S.M. 1996. Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3-CA1 cell pairs in vitro. *Proc Natl Acad Sci USA.* 93:11225-11230.
- Debanne, D., Gähwiler, B.H., and Thompson, S.M. 1998. Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. *J Physiol.* 507:237-247.
- Deisz, R.A., Fortin, G., and Zieglgansberger, W. 1991. Voltage dependence of excitatory postsynaptic potentials of rat neocortical neurons. *J Neurophysiol.* 65:371-382.

- Derkach, V., Barria, A., and Soderling, T.R. 1999. Ca^{2+} /calmodulin-kinase II enhances channel conductance of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Natl Acad Sci USA*. 96:3269-3274.
- Diamond, J.S., Bergles, D.E., and Jahr, C.E. 1998. Glutamate release monitored with astrocyte transporter currents during LTP. *Neuron*. 21:425-433.
- Dingledine, R., Borges, K., Bowie, D., and Traynelis, S.F. 1999. The glutamate receptor ion channels. *Pharmacol Rev*. 51:7-61.
- Dudek, S.M., and Bear, M.F. 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proc Natl Acad Sci USA*. 89:4363-4367.
- Durand, G.M., Kovalchuk, Y., and Konnerth, A. 1996. Long-term potentiation and functional synapse induction in developing hippocampus. *Nature*. 381:71-75.
- Eccles, J.C. 1964. *The physiology of synapses*. Berlin: Springer-Verlag.
- Eccles, J.C. 1983. Calcium in long-term potentiation as a model for memory. *Neuroscience*. 10:1071-1081.
- Egebjerg, J., Bettler, B., Hermans-Borgmeyer, I., and Heinemann, S. 1991. Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. *Nature*. 351:745-748.
- Emptage, N., Bliss, T.V., and Fine, A. 1999. Single synaptic events evoke NMDA receptor-mediated release of calcium from internal stores in hippocampal dendritic spines. *Neuron*. 22:115-124.
- Fadda, E., Danysz, W., Wroblewski, J.T., and Costa, E. 1988. Glycine and D-serine increase the affinity of N-methyl-D-aspartate sensitive glutamate binding sites in rat brain synaptic membranes. *Neuropharmacology*. 27:1183-1185.
- Fedele, E., and Foster A.C., 1992. ^3H -Glycine uptake in rat hippocampus: Kinetic analysis and autoradiographic localization. *Brain Res*. 572:154-163.
- Fedele, E., Smith, D., and Foster, A.C. 1993. Autoradiographical evaluation of [^3H]glycine uptake in rat forebrain: Cellular localization in the hippocampus. *Neurosci Lett*. 161:4-8.
- Feldman, D.E. 2000. Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron*. 27:45-56.

- Feldman, D.E., Nicoll, R.A., Malenka, R.C., and Isaac, J.T. 1998. Long-term depression at thalamocortical synapses in developing rat somatosensory cortex. *Neuron*. 21:347-357.
- Feldman, R.S., Meyer, J.S., and Quenzer, L.F. 1997. *Principles of Neuropsychopharmacology*. Sunderland MA: Sinauer Associates, Inc.
- Fletcher, E.J., and Lodge, D. 1988. Glycine reverses antagonism of N-methyl-D-aspartate (NMDA) by 1-hydroxy-3-aminopyrrolidone-2 (HA-966) but not by D-2-amino-5-phosphonovalerate (D-AP5) on rat cortical slices. *Eur J Pharmacol*. 151:161-162.
- Freund, T. F., and Buzsáki, G. 1996. Interneurons of the hippocampus. *Hippocampus*. 6:347- 470.
- Frotscher, M. 1986. Mossy fibres form synapses with identified pyramidal basket cells in the CA3 region of the guinea-pig hippocampus: a combined Golgi-electron microscope study. *J Neurocytol*. 14:245-259.
- Frotscher, M., and Zimmer, J. 1987. GABAergic nonpyramidal neurons in intracerebral transplants of the rat hippocampus and fascia dentata: a combined light and electron microscopic immunocytochemical study. *J Comp Neurol*. 259:266-276.
- Fukunaga, K., Stoppini, L., Miyamoto, E., and Muller, D. 1993. Long-term potentiation is associated with an increased activity of Ca²⁺/calmodulin-dependent protein kinase II. *J Biol Chem*. 268:7863-7867.
- Giese, K.P., Fedorov, N.B., Filipkowski, R.K., and Silva, A.J. 1998. Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science*. 279:870-873.
- Ginski, M.J., and Witkin, J.M. 1994. Sensitive and rapid behavioral differentiation of N-methyl-D-aspartate receptor antagonists. *Psychopharmacology*. 114:573-582.
- Golding, N.L., and Spruston, N. 1998. Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. *Neuron*. 21:1189-1200.
- Golding, N.L., Staff, N.P., and Spruston, N. 2002. Dendritic spikes as a mechanism for cooperative long-term potentiation. *Nature*. 418:326-331.
- Golgi, C. 1886. *Sulla fina anatomia degli organi centrali del sistema nervoso*. Milan: U Hoepli.
- Gomperts, S.N., Rao, A., Craig, A.M., Malenka, R.C., and Nicoll, R.A. 1998. Post-synaptically silent synapses in single neuron cultures. *Neuron*. 21:1443-1451.

- Gray, E.G. 1959. Axo-somatic and axo-dendritic synapses of the cerebral cortex: an electron microscopic study. *J Anat.* 93:420-433.
- Greene, R. 2001. Circuit analysis of NMDAR hypofunction in the hippocampus, in vitro, and psychosis of schizophrenia. *Hippocampus.* 11:569-577.
- Grimwood, S., Kulagowski, J.J., Mawer, I.M., Rowley, M., Leeson, P.D., and Foster, A.C. 1995. Allosteric modulation of the glutamate site on the NMDA receptor by four novel glycine site antagonists. *Eur J Pharmacol.* 290:221-226.
- Grimwood, S., Moseley, A.M., Carling, R.W., Leeson, P.D., and Foster, A.C. 1992. Characterization of the binding of [³H]L-689,560, an antagonist for the glycine site on the N-methyl-D-aspartate receptor, to rat brain membranes. *Mol Pharmacol.* 41:923-930.
- Grimwood, S., Wilde, G.J., and Foster, A.C. 1993. Interactions between the glutamate and glycine recognition sites of the N-methyl-D-aspartate receptor from rat brain, as revealed from radioligand binding studies. *J Neurochem.* 60:1729-1738.
- Grover, L.M. 1998. Evidence for postsynaptic induction and expression of NMDA receptor independent LTP. *J Neurophysiol.* 79:1167-1182.
- Grover, L.M., and Teyler, T.J. 1990. Two components of long-term potentiation induced by different patterns of afferent activation. *Nature.* 347:477-479.
- Grover, L.M., and Teyler, T.J. 1992. N-methyl-D-aspartate receptor-independent long-term potentiation in area CA1 of rat hippocampus: input-specific induction and preclusion in a non-tetanized pathway. *Neuroscience.* 49:7-11.
- Grunze, H.C., Rainnie, D.G., Hasselmo, M.E., Barkai, E., Hearn, E.F., McCarley, R.W., and Greene, R.W. 1996. NMDA-dependent modulation of CA1 local circuit inhibition. *J Neurosci.* 16:2034-2043.
- Gustafsson, B., Wigström, H., Abraham, W.C., and Huang, Y.Y. 1987. Long-term potentiation in the hippocampus using depolarizing current pulses as the conditioning stimulus to single volley synaptic potentials. *J Neurosci.* 7:774-780.
- Hamill, O.P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflüg Arch.* 391:85-100.
- Hanse, E., and Gustafsson, B. 1995. Long-term potentiation in the hippocampal CA1 region in the presence of N-methyl-D-aspartate receptor antagonists. *Neuroscience.* 67:531-539.

- Hashimoto, A., Kumashiro, S., Nishikawa, T., Oka, T., Takahashi, K., Mito, T., Takashima, S., Doi, N., Mizutani, Y., Yamazaki, T., Kanek, T., and Ootomo, E. 1993a. Embryonic development and postnatal changes in free D-aspartate and D-serine in the human prefrontal cortex. *J Neurochem.* 61:348-351.
- Hashimoto, A., Nishikawa, T., Hayashi, T., Fujii, N., Harada, K., Oka, T., and Takahashi, K. 1992. The presence of D-serine in rat brain. *FEBS Lett.* 296:33-36.
- Hashimoto, A., Nishikawa, T., Oka, T. and Takahashi, K. 1993b. Endogenous D-serine in rat brain: N-methyl-D-aspartate receptor-related distribution and aging. *J Neurochem.* 60:783-786.
- Hashimoto, A., and Oka, T. 1997. Free D-aspartate and D-serine in the mammalian brain and periphery. *Prog Neurobiol.* 52:325-353.
- Hashimoto, A., Oka, T., and Nishikawa, T. 1995. Extracellular concentration of endogenous free D-serine in the rat brain as revealed by in vivo microdialysis. *Neuroscience.* 66:635-643.
- Häusser, M., Spruston, N., and Stuart, G.J. 2000. Diversity and dynamics of dendritic signaling. *Science.* 290:739-744.
- Hebb, D.O. 1949. *The Organization of Behaviour*. New York: Wiley.
- Henderson, G., Johnson, J.W., and Ascher, P. 1990. Competitive antagonists and partial agonists at the glycine modulatory site of the mouse N-methyl-D-aspartate receptor. *J Physiol.* 430:189-212.
- Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W., and Seeburg, P.H. 1992. The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. *Neuron.* 8:775-785.
- Herdon, H.J., Godfrey, F.M., Brown, A.M., Coulton, S., Evans, J.R., and Cairns, W.J. 2001. Pharmacological assessment of the role of the glycine transporter GlyT-1 in mediating high-affinity glycine uptake by rat cerebral cortex and cerebellum synaptosomes. *Neuropharmacology.* 41:88-96.
- Heresko-Levy, U., Javitt, D.C., Ermilov, M., Mordel, C., Silipo, G., and Lichtenstein, M. 1999. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. *Arch Gen Psychiatry.* 56:29-36.
- Hjorth-Simonsen, A. 1972. Projection of the lateral part of the entorhinal area to the hippocampus and fascia dentata. *J Comp Neurol.* 146:219-232.

- Hjorth-Simonsen, A. 1973. Some intrinsic connections of the hippocampus in the rat: an experimental analysis. *J Comp Neurol.* 147:145-161.
- Hollmann, M., Hartley, M., and Heinemann, S. 1991. Ca^{2+} permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science.* 252:851-853.
- Hollmann, M., and Heinemann, S. 1994. Cloned glutamate receptors. *Annu Rev Neurosci.* 17:31-108.
- Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W., and Heinemann, S. 1989. Cloning by functional expression of a member of the glutamate receptor family. *Nature.* 342:643-648.
- Hu, G.-Y., Hvalby, O., Walaas, S.I., Albert, K.A., Skjeflo, P., Andersen, P., and Greengard, P. 1987. Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. *Nature.* 328:426-429.
- Huettnner, J.E. 1990. Glutamate receptor channels in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron.* 5:255-266.
- Huettnner, J.E. 1991. Competitive antagonism of glycine at the N-methyl-D-aspartate (NMDA) receptor. *Biochem Pharmacol.* 41:9-16.
- Ikeda, K., Magasawa, M., Mori, H., Araki, K., Sakimura, K., Watanabe, M., Inoue, Y., and Mishina, M. 1992. Cloning and expression of the $\epsilon 4$ subunit of the NMDA receptor channel. *FEBS Lett.* 313:34-38.
- Isaac, J.T., Nicoll, R.A., and Malenka, R.C. 1995. Evidence for silent synapses: implications for the expression of LTP. *Neuron.* 15:427-434.
- Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., Kadotani, H., Yokoi, M., Akazawa, C., Shigemoto, R., Mizuno, N., Masu, M., and Nakanishi, S. 1993. Molecular characterization of the family of the N-methyl-D-aspartate receptor subunit. *J Biol Chem.* 268:2836-2843.
- Ishizuka, N., Weber, J., and Amaral, D.G. 1990. Organization of intrahippocampal projections originating from CA3 pyramidal cells in the rat. *J Comp Neurol.* 295:580-623.
- Ito, K.-I., and Hicks, T.P. 2001. Effect of the glycine modulatory site of the N-methyl-D-aspartate receptor on synaptic responses in kitten visual cortex. *Neurosci Lett.* 303:95-98.

- Izumi, Y., Clifford, D.B., and Zorumski, C.F. 1990. Glycine antagonists block the induction of long-term potentiation in CA1 of rat hippocampal slices. *Neurosci Lett.* 112:251-256.
- Jaffe, D., Fisher, S., and Brown, T. 1994. Confocal laser scanning microscopy reveals voltage-gated calcium signals within hippocampal dendritic spines. *J Neurobiol.* 25:220-233.
- Jaffe, D.B., Johnston, D., Lasser-Ross, N., Lisman, J.E., Miyakawa, H., and Ross, W.N. 1992. The spread of Na⁺ spikes determines the pattern of dendritic Ca²⁺ entry into hippocampal neurons. *Nature.* 357:244-246.
- Javitt, D.C., Silipo, G., Cienfuegos, A., Shelley, A.M., Bark, N., Park, M., Lindenmayer, J.P., Suckow, R., and Zukin, S.R. 2001. Adjunctive high-dose glycine in the treatment of schizophrenia. *Int J Neuropsychopharmacol.* 4:385-391.
- Javitt, D.C., and Zukin, S.R. 1991. Recent advances in the phencyclidine model of schizophrenia. *Am J Psychiatry.* 148:1301-1308.
- Javitt, D.C., Zylberman, I., Zukin, S.R., Heresco-Levy, U., and Lindenmayer, J.P. 1994. Amelioration of negative symptoms in schizophrenia by glycine. *Am J Psychiatry.* 151:1234-1236.
- Jay, T.M., Zilkha, E., and Obrenovitch, T.P. 1999. Long-term potentiation in the dentate gyrus is not linked to increased extracellular glutamate concentration. *J Neurophysiol.* 81: 1741-1748.
- Jensen, M.S., Azouz, R., and Yaari, Y. 1996. Spike after-depolarization and burst generation in adult rat hippocampal CA1 pyramidal cells. *J Physiol.* 492:199-210.
- Johnson, J.W., and Ascher, P. 1987. Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature.* 325: 529-531.
- Johnston, D., Williams, S., Jaffe, D., and Gray, R. 1992. NMDA-receptor-independent long-term potentiation. *Annu Rev Physiol.* 54:489-505.
- Karcz-Kubicha, M., Jessa, M., Nazar, M., Plaznik, A., Hartmann, S., Parsons, C.G., and Danysz, W. 1997. Anxiolytic activity of glycine-B antagonists and partial agonists: No relation to intrinsic activity in the patch clamp. *Neuropharmacology.* 36:1355-1367.
- Katona, I., Acsady, L., and Freund, T.F. 1999. Postsynaptic targets of somatostatin-immunoreactive interneurons in the rat hippocampus. *Neuroscience.* 88:37-55.

- Kauer, J.A., Malenka, R.C., and Nicoll, R.A. 1988. A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. *Neuron*. 1:911-917.
- Keinanen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T.A., Sakmann, B., and Seeburg, P.H. 1990. A family of AMPA-selective glutamate receptors. *Science*. 249:556-560.
- Kelso, S.R., Ganong, A.H., and Brown, T.H. 1986. Hebbian synapses in hippocampus. *Proc Natl Acad Sci USA*. 83:5326-5330.
- Kemp, J.A., Foster, A.C., Leeson, P.D., Priestley, T., Tridgett, R., Iversen, L.L., and Woodruff, G.N. 1988. 7-Chlorokynurenic acid is a selective antagonist at the glycine modulatory site of the N-methyl-D-aspartate receptor complex. *Proc Natl Acad Sci USA*. 85:6547-6550.
- Kemp, J.A., and Leeson, P.D. 1993. The glycine site of the NMDA receptor-five years on. *Trends Pharmacol Sci*. 14:20-25.
- Kemp, J.A., and Priestley, T. 1991. Effects of (+)-HA-966 and 7-chlorokynurenic acid on the kinetics of N-methyl-D-aspartate receptor agonist responses in rat cultured cortical neurons. *Mol Pharmacol*. 39:666-670.
- Kew, J.N., Koester, A., Moreau, J.L., Jenck, F., Ouagazzal, A.M., Mutel, V., Richards, J.G., Trube, G., Fischer, G., Montkowski, A., Hundt, W., Reinscheid, R.K., Pauly-Evers, M., Kemp, J.A., and Bluethmann, H. 2000. Functional consequences of reduction in NMDA receptor glycine affinity in mice carrying targeted point mutations in the glycine binding site. *J Neurosci*. 20:4037-4049.
- Kew, N., Richards, J.G., Mutel, V., and Kemp, J.A. 1998. Developmental changes in NMDA receptor glycine affinity and ifenprodil sensitivity reveal three distinct populations of NMDA receptors in individual rat cortical neurons. *J Neurosci*. 18:1935-1943.
- Klann, E., Chen, S.J., and Sweatt, J.D. 1991. Persistent protein kinase activation in the maintenance phase of long-term potentiation. *J Biol Chem*. 266:24253-24256.
- Kleckner, N.W., and Dingledine, R. 1988. Requirement for glycine in activation of NMDA receptors expressed in *Xenopus* oocytes. *Science*. 241:835-837.
- Kneisler, T.B., and Dingledine, R. 1995a. Spontaneous and synaptic input from granule cells and perforant path to dentate basket cells in rat hippocampus. *Hippocampus*. 5:151-164.
- Kneisler, T.B., and Dingledine, R. 1995b. Synaptic input from CA3 pyramidal cells to dentate basket cells in rat hippocampus. *J Physiol*. 487:125-146.

- Knowles, W.D., and Schwartzkroin, P.A. 1981. Local circuit synaptic interactions in hippocampal brain slices. *J Neurosci.* 1:318-322.
- Koeler, M., Burnashev, N., Sakmann, B., and Seeburg, P.H. 1993. Determinants of Ca^{2+} permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity by RNA editing. *Neuron.* 10:491-500.
- Köhr, G., Eckardt, S., Luddens, H., Monyer, H., and Seeburg, P.H. 1994. NMDA receptor channels: subunit-specific potentiation by reducing agents. *Neuron.* 12:1031-1040.
- Kovalchuk, Y., Eilers, J., Lisman, J., and Konnerth, A. 2000. NMDA receptor-mediated subthreshold Ca^{2+} signals in spines of hippocampal neurons. *J Neurosci.* 20:1791-1799.
- Kullmann, D.M., Perkel, D.J., Manabe, T., and Nicoll, R.A. 1992. Ca^{2+} entry via postsynaptic voltage-sensitive Ca^{2+} channels can transiently potentiate excitatory synaptic transmission in the hippocampus. *Neuron.* 9:1175-1183.
- Kuryatov, A., Laube, B., Betz, H., and Kuhse, J. 1994. Mutational analysis of the glycine-binding site of the NMDA receptor: Structural similarity with bacterial amino acid-binding proteins. *Neuron.* 12:1291-1300.
- Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., Meguro, H., Masaki, H., Kumanishi, T., Arakawa, M., and Mishina, M. 1992. Molecular diversity of the NMDA receptor channel. *Nature.* 358:36-41.
- Lacaille, J.C., Muller, A.L., Kunkel, D.D., and Schwartzkroin, P.A. 1987. Local circuit interactions between oriens/alveus interneurons and CA1 pyramidal cells in hippocampal slices: electrophysiology and morphology. *J Neurosci.* 8:1411-1424.
- Laurie, D.J., and Seeburg, P.H. 1994. Regional and developmental heterogeneity in splicing of the rat brain NMDAR1 mRNA. *J Neurosci.* 13:3180-3194.
- Lee, K.S. 1983. Cooperativity among afferents for the induction of long-term potentiation in the CA1 region of the hippocampus. *J Neurosci.* 3:1369-1372.
- Leeson, P.D., and Iversen, L.L. 1994. The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. *J Med Chem.* 37:4053-4067.
- Leranth, C., and Ribak, C.E. 1991. Calcium-binding proteins are concentrated in the CA2 field of the monkey hippocampus: a possible key to this region's resistance to epileptic damage. *Exp Brain Res.* 85:129-136.
- Lerma, J. 1997. Kainate reveals its targets. *Neuron.* 19:1155-1158.

- Lerma, J., Zukin, R.S., and Bennett, M.V. 1990. Glycine decreases desensitization of N-methyl-D-aspartate (NMDA) receptors expressed in *Xenopus*-oocytes and is required for NMDA responses. *Proc Natl Acad Sci USA*. 87:2354-2358.
- Lester, R.A.J., Clements, J.D., Westbrook, G.L., and Jahr, C.E. 1990. Channel kinetics determine the time course of NMDA receptor-mediated synaptic currents. *Nature*. 346:565-567.
- Lester, R.A.J., Tong, G., and Jahr, C.E. 1993. Interactions between the glycine and glutamate binding sites of the NMDA receptor. *J Neurosci*. 13:1088-1096.
- Lewin, A.H., Skolnick, P., Marvizon, J.C., Paul, L.A., and Bowen, J.P. 1993. Requirements for high affinity binding of glycine analogs to the glycine site of the NMDA receptor complex. *Eur J Pharmacol*. 247:1-10.
- Liao, D.Z., Hessler, N.A., and Malinow, R. 1995. Activation of postsynaptically silent synapses during pairing induced LTP in CA1 region of hippocampal slice. *Nature*. 375:400-404.
- Liao, D., Zhang, X., O'Brien, R., Ehlers, M.D., and Huganir, R.L. 1999. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nature Neurosci*. 2:37-43.
- Lledo, P.M., Hjelmstad, G.O., Mukherji, S., Soderling, T.R., Malenka, R.C., and Nicoll, R.C. 1995. Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proc Natl Acad Sci USA*. 92:11175-11179.
- Lorente de Nó, R. 1933. Studies on the structure of the cerebral cortex. *J Psychol Neurol*. 45:381-438.
- Lorente de Nó, R. 1934. Studies on the structure of the cerebral cortex. II. Continuation of the study of the ammonic system. *J Psychol Neurol*. 46:113-177.
- Lu, Y.M., Roder, J.C., Davidow, J., and Salter, M.W. 1998. Src activation in the induction of long-term potentiation in CA1 hippocampal neurons. *Science*. 279:1363-1367.
- Lüscher, C., Malenka, R.C., and Nicoll, R.A. 1998. Monitoring glutamate release during LTP with glial transporter currents. *Neuron*. 21:435-441.
- Lynch, G., Larson, J., Kelso, S., Barrionuevo, G., and Schottler, F. 1983. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature*. 305:719-721.

- Magee, J.C. 2000. Dendritic integration of excitatory synaptic input. *Nat Rev Neurosci.* 1:181-190.
- Magee, J.C., and Cook, E.P. 2000. Somatic EPSP amplitude is independent of synapse location in hippocampal pyramidal neurons. *Nat Neurosci.* 3:895-903.
- Magee, J., Hoffman, D., Colbert, C., and Johnston, D. 1998. Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. *Annu Rev Physiol.* 60:327-346.
- Magee, J.C., and Johnston, D. 1995. Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. *Science.* 268:301-304.
- Magee, J.C., and Johnston, D. 1997. A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science.* 275:209-213.
- Mainen, Z.F., Malinow, R., and Svoboda, K. 1999. Synaptic calcium transients in single spines indicate that NMDA receptors are not saturated. *Nature.* 399:151-155.
- Makhinson, M., Chotiner, J.K., Watson, J.B., and O'Dell, T.J. 1999. Adenylyl cyclase activation modulates activity-dependent changes in synaptic strength and Ca²⁺/calmodulin-dependent kinase II autophosphorylation. *J Neurosci.* 19:2500-2510.
- Malenka, R.C., Kauer, J.A., Perkel, D.J., Mauk, M.D., Kelly, P.T., Nicoll, R.A., and Waxham, M.N. 1989. An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. *Nature.* 340:554-557.
- Malenka, R.C., Kauer, J.A., Zucker R.S., and Nicoll, R.A. 1988. Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science.* 242:81-84.
- Malenka, R.C., and Nicoll, R.A. 1999. Long-term potentiation - a decade of progress? *Science.* 285:1870-1874.
- Malgaroli, A., and Tsien, R.W. 1992. Glutamate-induced long-term potentiation of the frequency of miniature synaptic currents in cultured hippocampal neurons. *Nature.* 357:134-139.
- Malinow, R., and Miller, J.P. 1986. Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. *Nature.* 320:529-530.
- Malinow, R., Schulman, H., and Tsien, R.W. 1989. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science.* 245:862-866.

- Manabe, T., and Nicoll, R.A. 1994. Long-term potentiation: evidence against an increase in transmitter release probability in the CA1 region of the hippocampus. *Nature*. 265:1888-1892.
- Manabe, T., Renner, P., and Nicoll, R.A. 1992. Postsynaptic contribution to long-term potentiation revealed by the analysis of miniature synaptic currents. *Nature*. 355:50-55.
- Manabe, T., Wyllie, D. J., Perkel, D. J., and Nicoll, R. A. 1993. Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J Neurophysiol*. 70:1451-1459.
- Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. 1997. Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science*. 275:213-215.
- Matsui, T., Sekiguchi, M., Hashimoto, A., Tomita, U., Nishikawa, T., and Wada, K. 1995. Functional comparison of D-serine and glycine in rodents: The effect on cloned NMDA receptors and the extracellular concentration. *J Neurochem*. 65:454-458.
- Matthews, D.A., Cotman, C., and Lynch, G. 1976. An electron microscopic study of lesion-induced synaptogenesis in the dentate gyrus of the adult rat. II. Reappearance of morphologically normal synaptic contacts. *Brain Res*. 115:23-41.
- Mayer, M.L., Vyklicky, L., and Clements, J. 1989. Regulation of NMDA receptor desensitization in mouse hippocampal neurons by glycine. *Nature*. 338:425-427.
- Mayer, M.L. and Westbrook, G. L. 1987. Permeation and block of N-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. *J Physiol*. 394:501-27.
- Mayer, M. L., Westbrook, G. L., and Guthrie, P. B. 1984. Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurons. *Nature*. 309:261-263.
- McNaughton, B.L., Douglas, R.M., and Goddard, G.V. 1978. Synaptic enhancement in fascia dentata: cooperativity among coactive afferents. *Brain Res*. 157:277-293.
- Medina, I., Filippova, N., Charton, G., Rougeole, S., Ben-Ari, Y., Khrestchatisky, M., and Bregestovski, P. 1995. Calcium-dependent inactivation of heteromeric NMDA receptor channels expressed in human embryonic kidney cells. *J Physiol*. 482:567-573.
- Megías, M., Emri, Z., Freund, T.F., and Gulyas, A.L. 2001. Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. *Neuroscience*. 102:527-540.

- Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K., and Mishina, M. 1992. Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. *Nature*. 357:70-74.
- Miles, R., Toth, K., Gulyas, A.I., Hajos, N., and Freund, T.F. 1996. Differences between somatic and dendritic inhibition in the hippocampus. *Neuron*. 16:815-823.
- Mills, L. R., Niesen, C. E., So, A. P., Carlen, P. L., Spigelman, I., and Jones, O. T. 1994. N-type Ca^{2+} channels are located on somata, dendrites, and a subpopulation of dendritic spines on live hippocampal pyramidal neurons. *J Neurosci*. 14:6815-6824.
- Minota, S., Miyazaki, T., Wang, M.Y., Read, H.L., and Dun, N.J. 1989. Glycine potentiates NMDA responses in rat hippocampal CA1 neurons. *Neurosci Lett*. 100:237-242.
- Misgeld, U., and Frotcher, M. 1986. Postsynaptic-GABAergic inhibition of non-pyramidal neurons in the guinea-pig hippocampus. *Neuroscience*. 19:193-206.
- Misra, C., Brickley, S.G., Wyllie, D.J.A., and Cull-Candy, S.G. 2000. Slow deactivation kinetics of NMDA receptors containing NR1 and NR2D subunits in rat cerebellar Purkinje cells. *J Physiol*. 525:299-305.
- Molnar, P., and Erdo, S.L. 1996. Differential effects of five glycine site antagonists on NMDA receptor desensitization. *Eur J Pharmacol*. 311:311-314.
- Monaghan, D.T., Olverman, H.J., Nguyen, L., and Watkins, J.C. 1988. Two classes of N-methyl-D-aspartate recognition sites: Differential distribution and differential regulation by glycine. *Proc Natl Acad Sci USA*. 85:9836-9840.
- Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B., and Seeburg, P.H. 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron*. 12:529-540.
- Monyer, H., Seeburg, P.H. and Wisden, W. 1991. Glutamate-operated channels: Developmentally early and mature forms arise by alternative splicing. *Neuron*. 6:779-810.
- Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B., and Seeburg, P.H. 1992. Heteromeric NMDA receptors: Molecular and functional distinctions of subtypes. *Science*. 256:1217-1221.
- Morgan, S.L., and Teyler, T.J. 2001. Electrical stimuli patterned after the theta-rhythm induce multiple forms of LTP. *J Neurophysiol*. 86:1289-1296.

- Mori, H., and Mishina, M. 1995. Structure and function of the NMDA receptor channel. *Neuropharmacology*. 34:1219-1237.
- Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N., and Nakanishi, S. 1991. Molecular cloning and characterization of the rat NMDA receptor. *Nature*. 354:31-37.
- Morris, R.G., Garrud, P., Rawlins, J.N., and O'Keefe, J. 1982. Place navigation impaired in rats with hippocampal lesions. *Nature*. 297:681-683.
- Mothet, J.P., Parent, A.T., Wolosker, H., Brady, R.O. Jr., Linden, D.J., Ferris, C.D., Rogawski, M.A., and Snyder, S.H. 2000. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc Natl Acad Sci USA*. 97:4926-4931.
- Mulkey, R.M., and Malenka, R.C. 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron*. 9:967-975.
- Muller, D., and Lynch, G. 1988. Long-term potentiation differentially affects two components of synaptic responses in hippocampus. *Proc Natl Acad Sci USA*. 85:9346-9350.
- Musleh, W., Bi, X., Tocco, G., Yaghoubi, S., and Baudry, M. 1997. Glycine-induced long-term potentiation is associated with structural and functional modifications of alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid receptors. *Proc Natl Acad Sci USA*. 94:9451-9456.
- Nakanishi, N., Axel, R., and Shneider, N.A. 1992. Alternative splicing generates functionally distinct N-methyl-D-aspartate receptors. *Proc Natl Acad Sci USA*. 89:8552-8556.
- Nakanishi, N., Shneider, N.A., and Axel, R. 1990. A family of glutamate receptor genes: evidence for the formation of heteromultimeric receptors with distinct channel properties. *Neuron*. 5:569-582.
- Nakanishi, S. 1992. Molecular diversity of glutamate receptors and implications for brain function. *Science*. 258:597-603.
- Nauta, W.J.H. 1950. Über die sogenannte terminale Degeneration im Zentralnervensystem und ihre Darstellung durch Silberimpragnation. *Schweiz Arch Neurol Psychiatr*. 66:353-376.

- Newcomer, J.W., Farber, N.B., Jevtovic-Todorovic, V., Selke, G., Melson, A.K., Hershey, T., Craft, S., and Olney, J.W. 1999. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. *Neuropsychopharmacology*. 20:106-118.
- Newcomer, J.W., and Krystal, J.H. 2001. NMDA receptor regulation of memory and behavior in humans. *Hippocampus*. 11:529-542.
- Ngezahayo, A., Schachner, M., and Artola, A. 2000. Synaptic activity modulates the induction of bidirectional synaptic changes in adult mouse hippocampus. *J Neurosci*. 20:2451-2458.
- Nicholls, D.G. 1994. *Proteins, transmitters and synapses*. Cambridge, MA: Blakwell Science.
- Nilsson, M., Carlsson, A., and Carlsson, M.L. 1997. Glycine and D-serine decrease MK-801-induced hyperactivity in mice. *J Neural Transm*. 104:1195-1205.
- Nimchinsky, E.A., Sabatini, B.L., and Svoboda, K. 2000. Structure and function of dendritic spines. *Annu Rev Physiol*. 64:313-353.
- Nishiyama, M., Hong, K., Mikoshiba, K., Poo, M.M., Kato, K. (2000). Calcium stores regulate the polarity and input specificity of synaptic modification. *Nature*. 408:584-588.
- Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., and Prochiantz, A. 1984. Magnesium gates glutamate-activated channels in mouse central neurones. *Nature*. 307:462-465.
- Ogita, K., Suzuki, T., and Yoneda, Y. 1989. Strychnine-insensitive binding of [3H]glycine to synaptic membranes in rat brain, treated with Triton X-100. *Neuropharmacology*. 28:1263-1270.
- Okabe, S., Miwa, A., and Okado, H. 1999. Alternative splicing of the C-terminal domain regulates cell surface expression of the NMDA receptor NR1 subunit. *J Neurosci*. 19:7781-7792.
- Oliet, S.H., Malenka, R.C., and Nicoll, R.A. 1996. Bidirectional control of quantal size by synaptic activity in the hippocampus. *Science*. 271:1294-1297.
- Oliver, M.W., Kessler, M., Larson, J., Schottler, F., and Lynch, G. 1990. Glycine site associated with the NMDA receptor modulates long-term potentiation. *Synapse*. 5:265-270.
- Olney, J.W., and Farber, N.B. 1995. Glutamate receptor dysfunction and schizophrenia. *Arch Gen Psychiatry*. 52:998-1007.

- Olney, J.W., Newcomer, J.W., and Farber, N.B. 1999. NMDA receptor hypofunction model of schizophrenia. *J Psychiatr Res.* 33:523-533.
- Olsen, R.W., Szamrai, O., and Houser, C.R. 1987. [3H]AMPA binding to glutamate receptor subpopulations in rat brain. *Brain Res.* 402:243-254.
- Ozawa, S., and Iino, M. 1993. Two distinct types of AMPA responses in cultured rat hippocampal neurons. *Neurosci Lett.* 155:187-190.
- Parra, P., Gulyás, A.I. and Miles, R. 1998. How many subtypes of inhibitory cells in the hippocampus? *Neuron.* 20:983-993.
- Parsons, C.G., Danysz, W., Quack, G., Hartmann, S., Lorenz, B., Wollenburg, C., Baran, L., Przegalinski, E., Kostowski, W., Krzascik, P., Chizh, B., and Headley, P.M. 1997. Novel systemically active antagonists of the glycine site of the *N*-methyl-D-aspartate receptor: Electrophysiological, biochemical and behavioral characterization. *J Pharmacol Exp Ther.* 283:1264-1275.
- Parsons, C.G., Zong, X.G., and Lux, H.D. 1993. Whole cell and single channel analysis of the kinetics of glycine-sensitive N-methyl-D-aspartate receptor desensitization. *Br J Pharmacol.* 109:213-221.
- Paxinos, G., and Watson, C. 1998. *The rat brain in stereotaxic coordinates.* (4 ed) Academic press.
- Peeters, B.W., and Vanderheyden, P.M. 1992. In vitro and in vivo characterization of the NMDA receptor-linked strychnine-insensitive glycine site. *Epilepsy Res.* 12:157-162.
- Perkel, D. J., Petrozzino, J. J., Nicoll, R. A., and Connor, J. A. 1993. The role of Ca^{2+} entry via synaptically activated NMDA receptors in the induction of long-term potentiation. *Neuron.* 11:817-823.
- Perouansky, M., and Yaari, Y. 1993. Kinetic properties of NMDA receptor-mediated synaptic currents in rat hippocampal pyramidal cells *versus* interneurons. *J Physiol.* 465:223-244.
- Petralia, R.S., Esteban, J.A., Wang, Y.X., Partridge, J.G., Zhao, H.M., Wenthold, R.J., and Malinow, R. 1999. Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nature Neurosci.* 2:31-36.
- Pike, F.G., Meredith, R.M., Olding, A.W., and Paulsen, O. 1999. Rapid report: postsynaptic bursting is essential for "Hebbian" induction of associative long-term potentiation at excitatory synapses in rat hippocampus. *J Physiol.* 518:571-576.

- Pontremoli, S., Michetti, M., Melloni, E., Sparatore, B., Salamino, F., and Horecker, B.L. 1990. Identification of the proteolytically activated form of protein kinase C in stimulated human neutrophils. *Proc Natl Acad Sci USA*. 87:3705-3707.
- Priestley, T., and Kemp, J.A. 1993. Agonist response kinetics of N-methyl-D-aspartate receptors in neurons cultured from rat cerebral cortex and cerebellum: evidence for receptor heterogeneity. *Mol Pharmacol*. 44:1252-1257.
- Priestley, T., and Kemp, J.A. 1994. Kinetic study of the interactions between the glutamate and glycine recognition sites on the N-methyl-D-aspartic acid receptor complex. *Mol Pharmacol*. 46:1191-1196.
- Priestley, T., Laughton, P., Myers, J., Lebourdelles, B., Kerby, J., and Whiting, P.J. 1995. Pharmacological properties of recombinant human N-methyl-D-aspartate receptors comprising NR1a/NR2a and NR1a/NR2b subunit assemblies expressed in permanently transfected mouse fibroblast cells. *Mol Pharmacol*. 48:841-848.
- Pullan, L.M., and Cler, J.A. 1989. Schild plot analysis of glycine and kynurenic acid at the N-methyl-D-aspartate excitatory amino acid receptor. *Brain Res*. 497:59-63.
- Pyapali, G.K., Sik, A., Penttonen, M., Buzsaki, G., and Turner, D.A. 1998. Dendritic properties of hippocampal CA1 pyramidal neurons in the rat: intracellular staining in vivo and in vitro. *J Comp Neurol*. 391:335-352.
- Ramón y Cajal, S. 1911. "*Histologie du Systeme Nerveux de l'Homme et des Vertebres*." Maloine, Paris.
- Ramson, R.W., and Deschenes, N.L. 1989. Glycine modulation of NMDA-evoked release of [³H]acetylcholine and [³H]dopamine from rat striatal slices. *Neurosci Lett*. 96:323-328.
- Rao, A., and Craig, A.M. 1997. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron*. 19:801-812.
- Rao, T.S., Gray, N.M., Dappen, M.S., Cler, J.A., Mick, S.J., Emmett, M.R., Iyengar, S., Monahan, J.B., Cordi, A.A., and Wood, P.L. 1993. Indole-2-carboxylates, novel antagonists of the N-methyl-D-aspartate (NMDA)-associated glycine recognition sites: In vivo characterization. *Neuropharmacology*. 32:139-147.
- Raymond, C.R., and Redman, S.J. 2002. Different calcium sources are narrowly tuned to the induction of different forms of LTP. *J Neurophysiol*. 88:249-255.
- Regalado, M.P., Villarroel, A., and Lerma, J. 2001. Intersubunit cooperativity in the NMDA receptor. *Neuron*. 32:1085-1096.
- Regehr, W., and Tank, D. 1994. Dendritic calcium dynamics. *Curr Opin Neurobiol*. 4:373-382.

- Ribak, C.E., Vaughn, J.E., and Barber, R.P. 1981. Immunocytochemical localization of GABAergic neurones at the electron microscopical level. *Histochem J.* 13:555-582.
- Ribak, C.E., Vaughn, J.E., and Saito, K. 1978. Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. *Brain Res.* 140:315-332.
- Roche, K.W., and Huganir, R.L. 1995. Synaptic expression of the high-affinity kainate receptor subunit KA2 in hippocampal cultures. *Neuroscience.* 69:383-393.
- Sabatini, B.L., Oertner, T.G., and Svoboda, K. 2002. The life cycle of Ca(2+) ions in dendritic spines. *Neuron.* 33:439-452.
- Sacktor, T.C., Osten, P., Valsamis, H., Jiang, X., Naik, M.U., and Sublette, E. 1993. Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation. *Proc Natl Acad Sci USA.* 90:8342-8346.
- Sakata, K., Sato, K., Schloss, P., Betz, H., Shimada, S., and Tohyama, M. 1997. Characterization of glycine release mediated by glycine transporter 1 stably expressed in HEK-293 cells. *Mol Brain Res.* 49:89-94.
- Sakimura, K., Morita, T., Kushiya, E., and Mishina, M. 1992. Primary structure and expression of the gamma 2 subunit of the glutamate receptor channel selective for kainate. *Neuron.* 8:267-274.
- Salt, T.E. 1989. Modulation of NMDA receptor-mediated responses by glycine and D-serine in the rat thalamus in vivo. *Brain Res.* 481:403-406.
- Schaffer, K. 1892. Beitrag zur Histologie der Ammonshornformation. *Arch Mikrosk Anat.* 39:611-632.
- Scharfman, H.E., and Sarvey, J.M. 1985. Postsynaptic firing during repetitive stimulation is required for long-term potentiation in hippocampus. *Brain Res.* 331:267-274.
- Schell, M.J., Molliver, M.E., and Snyder, S.H. 1995. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA.* 92:3948-3952.
- Schwartzkroin, P.A., and Mathers, L.H. 1978. Physiological and morphological identification of nonpyramidal hippocampal cell type. *Brain Res.* 157:1-10.
- Schwartzkroin, P.A., and Wester, K. 1975. Long-lasting facilitation of a synaptic potential following tetanization in the in vitro hippocampal slice. *Brain Res.* 89:107-119.

- Seeburg, P.H. 1993. The molecular biology of mammalian glutamate receptor channels. *Trends Neurosci.* 16:359-365.
- Seeburg, P.H. 1996. The role of RNA editing in controlling glutamate receptor channel properties. *J Neurochem.* 66:1-5.
- Selig, D.K., Nicoll, R.A., and Malenka, R.C. 1999. Hippocampal long-term potentiation preserves the fidelity of postsynaptic responses to presynaptic bursts. *J Neurosci.* 19:1236-1246.
- Shahi, K., Marvizon, J.C., and Baudry, M. 1993. High concentrations of glycine induce long-lasting changes in synaptic efficacy in rat hippocampal slices. *Neurosci Lett.* 149:185-188.
- Sherman, A.D., Hegwood, T.S., Baruah, S., and Waziri, R. 1991. Deficient NMDA-mediated glutamate release from synaptosomes of schizophrenics. *Biol Psychiatry.* 30:1191-1198.
- Shouval, H.Z., Bear, M.F., and Cooper, L.N. 2002. A unified model of NMDA receptor-dependent bidirectional synaptic plasticity. *Proc Natl Acad Sci USA.* 99:10831-10836.
- Siegel, S.J., Janssen, W.G., Tullai, J.W., Rogers, S.W., Moran, T., Heinemann, S.F., and Morrison, J.H. 1995. Distribution of the excitatory amino acid receptor subunits GluR2(4) in monkey hippocampus and colocalization with subunits GluR5-7 and NMDAR1. *J Neurosci.* 15:2707-2719.
- Silva, A.J., Paylor, R., Wehner, J.M., and Tonegawa, S. 1992. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science.* 257:206-211.
- Sjöström, P.J., Turrigiano, G.G., and Nelson, S.B. 2001. Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. *Neuron.* 32:1149-1164.
- Smith, K.E., Borden, L.A., Hartig, P.R., Branchek, T., and Weinshank, R.L. 1992. Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. *Neuron.* 8:927-935.
- Smith, S.E., and Meldrum, B.S. 1992. The glycine-site NMDA receptor antagonist R-(+)-cis- β -methyl-3-amino-1-hydroxypyrrolid-2-one, L-687,414, is anticonvulsant in baboons. *Eur J Pharmacol.* 211:109-111.
- Soloviev, M.M., Abutidze, K., Mellor, I., Streit, P., Grishin, E.V., Usherwood, P.N., and Barnard, E.A. 1998. Plasticity of agonist binding sites in hetero-oligomers of the unitary glutamate receptor subunit XenU1. *J Neurochem.* 71:991-1001.

- Soloviev, M.M., and Barnard, E.A. 1997. *Xenopus* oocytes express a unitary glutamate receptor endogenously. *J Mol Biol.* 273:14-18.
- Sommer, B., Keinänen, K., Verdoorn, T.A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. and Seeburg, P.H. 1990. Flip and flop: A cell-specific functional switch in glutamate-operated channels of the CNS. *Science.* 249:1580-1585.
- Somogyi, P., Smith, A.D., Nunzi, M.G., Gorio, A., Takagi, H., and Wu, J.Y. 1983. Glutamate decarboxylase immunoreactivity in the hippocampus of the cat: distribution of immunoreactive synaptic terminals with special reference to the axon initial segment of pyramidal neurons. *J Neurosci.* 3:1450-1468.
- Stanton, P.K., and Sejnowski, T.J. 1989. Associative long-term depression in the hippocampus induced by hebbian covariance. *Nature.* 339:215-218.
- Stevens, C.F., and Wang, Y. 1994. Changes in reliability of synaptic function as a mechanism for plasticity. *Nature.* 371:704-707.
- Storm-Mathisen, J. 1977. Glutamic acid and excitatory nerve endings: reduction of glutamic acid uptake after axotomy. *Brain Res.* 120:379-386.
- Storm-Mathisen, J., and Fonnum, F. 1972. Localization of transmitter candidates in the hippocampal region. *Prog Brain Res.* 36:41-58.
- Storm-Mathisen, J., Leknes, A.K., Bore, A.T., Vaaland, J.L., Edminson, P., Haug, F.M., and Ottersen, O.P. 1983. First visualization of glutamate and GABA in neurones by immunocytochemistry. *Nature.* 301:517-520.
- Stricker, C., Cowan, A.I., Field, A.C., and Redman, S.J. 1999. Analysis of NMDA-independent long-term potentiation induced at CA3-CA1 synapses in rat hippocampus *in vitro*. *J Physiol.* 520:513-525.
- Stricker, C., Field, A.C., and Redman, S.J. 1996. Changes in quantal parameters of EPSCs in rat CA1 neurones *in vitro* after the induction of long-term potentiation. *J Physiol.* 490:443-454.
- Stuart, G. J., and Sakmann, B. 1994. Active propagation of somatic action potentials into neocortical pyramidal cell dendrites. *Nature.* 367:69-72.
- Stuart, G., and Sakmann, B. 1995. Amplification of EPSPs by axosomatic sodium channels in neocortical pyramidal neurons. *Neuron.* 15:1065-1076.
- Stuart, G., Spruston, N., Sakmann, B., and Hausser, M. 1997. Action potential initiation and backpropagation in neurons of the mammalian CNS. *Trends Neurosci.* 20:125-131.

- Stuart, G.J., and Hausser, M. 2001. Dendritic coincidence detection of EPSPs and action potentials. *Nat Neurosci.* 4:63-71.
- Sucher, N.J., Awobuluyi, M., Choi, Y.B., and Lipton, S.A. 1996. Nmda receptors: from genes to channels. *Trends Pharmacol Sci.* 17:348-355.
- Sugihara, H., Moriyoshi, K., Ishii, T., Masu, M., and Nakanishi, S. 1992. Structure and properties of seven isoforms of the NMDA receptor generated by alternative splicing. *Biochem Biophys Res Commun.* 185:826-832.
- Supplisson, S., and Bergman, C. 1997. Control of NMDA receptor activation by a glycine transporter co-expressed in *Xenopus* oocytes. *J Neurosci.* 17:4580-4590.
- Swadlow, H.A., and Hicks, T.P. 1997. Subthreshold receptive fields and baseline excitability of "silent" S1 callosal neurons in awake rabbits: contributions of AMPA/kainate and NMDA receptors. *Exp Brain Res.* 115:403-409.
- Takumi, Y., Ramirez-Leon, V., Laake, P., Rinvik, E., and Ottersen, O.P. 1999. Different modes of expression of AMPA and NMDA receptors in hippocampal synapses. *Nature Neurosci.* 2:618-624.
- Tamamaki, N., Abe, K., and Nojyo, Y. 1987. Columnar organization in the subiculum formed by axon branches originating from single CA1 pyramidal neurons in the rat hippocampus. *Brain Res.* 412:156-160.
- Tamamaki, N., Watanabe, K., and Noiyo, Y. 1984. A whole image of the hippocampal pyramidal neuron revealed by intracellular pressure-injection of horseradish peroxidase. *Brain Res.* 307:336-340.
- Tanabe, S., Masu, M., Ischii, T., Shigemoto, R., and Nakanishi, S. 1992. A family of metabotropic glutamate receptors. *Neuron.* 8:169-179.
- Tanii, Y., Nishikawa, T., Hashimoto, A. and Takahashi, K. 1994. Stereoselective antagonism by enantiomers of alanine and serine of phencyclidine-induced hyperactivity, stereotypy and ataxia in the rat. *J Pharmacol Exp Ther.* 269:1040-1048.
- Thiels, E., Weisz, D.J., and Berger, T.W. 1992. In vivo modulation of N-methyl-D-aspartate receptor-dependent long-term potentiation by the glycine modulatory site. *Neuroscience.* 46:501-509.
- Thomas, M.J., Watabe, A.M., Moody, T.D., Makhinson, M., and O'Dell, T.J. 1998. Postsynaptic complex spike bursting enables the induction of LTP by theta frequency synaptic stimulation. *J Neurosci.* 18:7118-7126.

- Traub, R.D., Miles, R., and Jefferys, J.G. 1993. Synaptic and intrinsic conductances shape picrotoxin-induced synchronized after-discharges in the guinea-pig hippocampal slice. *J Physiol.* 461:525-547.
- Tricklebank, M.D., Bristow, L.J., Hutson, P.H., Leeson, P.D., Rowley, M., Saywell, K., Singh, L., Tattersall, F.D., Thorn, L., and Williams, B.J. 1994. The anticonvulsant and behavioural profile of L-687,414, a partial agonist acting at the glycine modulatory site on the N-methyl-D-aspartate (NMDA) receptor complex. *Br J Pharmacol.* 113:729-736.
- Vandenberg, R.J., Handford, C.A., and Schofield, P.R. 1992. Distinct agonist- and antagonist-binding sites on the glycine receptor. *Neuron.* 9:491-496.
- Vicini, S., Wang, J.F., Li, J.H., Zhu, W.J., Wang, Y.H., Luo, J.H., Wolfe, B.B., and Grayson, D.R. 1998. Functional and pharmacological differences between recombinant N-methyl-D-aspartate receptors. *J Neurophysiol.* 79:555-566.
- Vizi, E.S., Mike, A., and Tarnawa, I. 1997. The functional study of kainate receptors-hopes and doubts. *Trends Neurosci.* 20:396-397.
- Vornov, J.J., and Coyle, J.T. 1991. Glutamate neurotoxicity and the inhibition of protein synthesis in the hippocampal slice. *J Neurochem.* 56:996-1006.
- Vyklicky, L. Jr. 1993. Calcium-mediated modulation of N-methyl-D-aspartate (NMDA) responses in cultured rat hippocampal neurones. *J Physiol.* 470:575-600.
- Vyklicky, L. Jr., Benveniste, M., and Mayer, M.L. 1990. Modulation of N-methyl-D-aspartic acid receptor desensitization by glycine in mouse cultured hippocampal neurones. *J Physiol.* 428:313-331.
- Wafford, K.A., Bain, C.J., Lebourdelles, B., Whiting, P.J., and Kemp, J.A. 1993. Preferential co-assembly of recombinant NMDA receptors composed of three different subunits. *Neuroreport.* 4:1347-1349.
- Wafford, K.A., Kathoria, M., Bain, C.J., Marshall, G., Lebourdelles, B., Kemp, J.A., and Whiting, P.J. 1995. Identification of amino acids in the N-methyl-D-aspartate receptor NR1 subunit that contribute to the glycine binding site. *Mol Pharmacol.* 47:374-380.
- Watanabe, Y., Himi, T., Saito, H., and Abe, K. 1992. Involvement of glycine site associated with the NMDA receptor in hippocampal long-term potentiation and acquisition of spatial memory in rats. *Brain Res.* 582:58-64.
- Weiss, S.W., Albers, D.S., Iadarola, M.J., Dawson, T.M., Dawson, V.L., and Standaert, D.G. 1998. NMDAR1 glutamate receptor subunit isoforms in neostriatal,

- neocortical, and hippocampal nitric oxide synthase neurons. *J Neurosci.* 18:1725-1734.
- Wenthold, R.J., Trumpp, V.A., Zhu, W.S., and Petralia, R.S. 1994. Biochemical and assembly properties of GluR6 and KA2, two members of the kainate receptor family, determined with subunit-specific antibodies. *J Biol Chem.* 269:1332-1339.
- Westergren, I., Nystrom, B., Hamberger, A., Nordborg, C., and Johansson, B.B. 1994. Concentrations of amino acids in extracellular fluid after opening of the blood-brain barrier by intracarotid infusion of protamine sulfate. *J Neurochem.* 62:159-165.
- Wilcox, C.S., Fitsimonds, R.M., Johnson, B., and Dichter, M.A. 1996. Glycine regulation of synaptic NMDA receptors in hippocampal neurons. *J Neurophysiol.* 76:3415-3424.
- Wisden, W., and Seeburg, P.H. 1993. A complex mosaic of high-affinity kainate receptors in rat brain. *J Neurosci.* 13:3582-3598.
- Wood, P.L., Emmett, M.R., Rao, T.S., Mick, S., Cler, J., and Iyengar, S. 1989. *In vivo* modulation of the N-methyl-D-aspartate receptor complex by D-serine: potentiation of ongoing neuronal activity as evidenced by increased cerebellar cyclic GMP. *J Neurochem.* 53:979-981.
- Woodson, W., Nitecka, L., and Ben-Ari, Y. 1989. Organization of the GABAergic system in the rat hippocampal formation: a quantitative immunocytochemical study. *J Comp Neurol.* 280:354-271.
- Wyllie, D.J., Manabe, T., and Nicoll, R.A. 1994. A rise in postsynaptic Ca^{2+} potentiates miniature excitatory postsynaptic currents and AMPA responses in hippocampal neurons. *Neuron.* 12:127-138.
- Yang, S.N., Tang, Y.G., and Sucker, R.S. 1999. Selective induction of LTP and LTD by postsynaptic $[\text{Ca}^{2+}]_i$ elevation. *J Neurophysiol.* 81:781-787.
- Yuan, L.L., Adams, J.P., Swank, Sweatt, J.D., and Johnston, D. 2002. Protein kinase modulation of dendritic K^+ channels in hippocampus involves a mitogen-activated protein kinase pathway. *J Neurosci.* 22:4860-4868.
- Yuste, R., and Denk, W. 1995. Dendritic spines as basic units of synaptic integration. *Nature.* 375:682-684.
- Yuste, R., Majewska, A., Cash, S.S., and Denk, W. 1999. Mechanisms of calcium influx into spines: Heterogeneity among spines, coincidence detection by NMDA receptors and optical quantal analysis. *J Neurosci.* 19:1976-1987.

- Yuste, R., and Tank, D.W. 1996. Dendritic integration in mammalian neurons, a century after Cajal. *Neuron*. 16:701-716.
- Zafra, F., Aragon, C., and Gimenez, C. 1997. Molecular biology of glycinergic neurotransmission. *Mol Neurobiol*. 14:117-142.
- Zafra, F., Aragon, C., Olivares, L., Danbolt, N.C., Gimenez, C., and Storm-Mathisen, J. 1995. Glycine transporters are differentially expressed among CNS cells. *J Neurosci*. 15:3952-3969.
- Zamanillo, D., Sprengel, R., Hvalby, O., Jensen, V., Burnashev, N., Rozov, A., Kaiser, K. M., Koster, H.J., Borchardt, T., Worley, P., Lubke, J., Frotscher, M., Kelly, P.H., Sommer, B., Andersen, P., Seeburg, P.H., and Sakmann, B. 1999. Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science*. 284:1805-1811.
- Zimmer, J. 1971. Ipsilateral afferents to the commissural zone of the fascia dentata, demonstrated in decommissurated rats by silver impregnation. *J Comp Neurol*. 142:393-416.
- Zimmer, J., and Hjorth-Simmons, A. 1975. Crossed pathways from the entorhinal area to the fascia dentata. *J Comp Neurol*. 161:71-101.
- Zola-Morgan, S., and Squire, L.R. 1993. Neuroanatomy of memory. *Annu Rev Neurosci*. 16:547-563.
- Zola-Morgan, S., Squire, L.R., Clower, R.P., and Rempel, N.L. 1993. Damage to the perirhinal cortex exacerbates memory impairment following lesions to the hippocampal formation. *J Neurosci*. 13:251-265.
- Zukin, R.S., and Bennett, M.V. 1995. Alternatively spliced isoforms of the NMDAR1 receptor subunit. *Trends Neurosci*. 18:306-313.