INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

SYNAPTIC PLASTICITY IN THE DIRECT FEEDBACK PATHWAY OF THE ELECTROSENSORY LATERAL LINE LOBE OF *APTERONOTUS LEPTORHYNCHUS*

BY

DALIANG WANG

(M.D., B.Sc)

A thesis submitted to the School of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Anatomy and Neurobiology University of Ottawa

Ottawa, Ontario

May 1997

0-612-28381-X

ABSTRACT

The electrosensory lateral line lobe (ELL) of *Apteronotus Leptorhynchus* is a good model for study of structure-function correlations. The ELL is a rhombencephalic laminated structure and pyramidal cells, the major projection neurons, have their somata in the pyramidal cell layer; their somatic dendrites in the same layer receive inhibitory input from interneurons; their basal dendrites extending to the deep neuropil layer receive input from primary afferents; the pyramidal cell apical dendrites go up to the molecular layer where direct feedback pathway from the nucleus preeminentialis dorsalis (Pd) contact the proximal part of the dendrites in the ventral molecular layer (VML) and an indirect feedback pathway from the Pd through the eminentia granularis posterior (EGP) form synapses with the distal part of the dendrites in the dorsal molecular layer (DML). The direct feedback pathway is involved in regulation of the pyramidal cell responses. However, the neurotransmitter utilized in this pathway is not known and the synaptic plasticity remains unexplored.

Immunocytochemical techniques at both light and electron microscope levels and electrophysiology combined with pharmacological interventions have been employed to address these issues.

Immunocytochemistry showed that the primary afferent fibers to ELL, pyramidal cell and both feedback pathways to the VML and DML of ELL used glutamate as a neurotransmitter.

Pharmaco-electrophysiological results demonstrate that at the direct feedback fiber-pyramidal cell synapses in VML there was a posttetanic potentiation (PTP), which is blocked by Ca^{2+}-calmodulin dependent protein kinase II (CaMKII) antagonist KN-62 applied focally in VML. Postsynaptic application of KN-62 or CaMKII inhibitory peptide did not block PTP in VML indicating that KN-62 blockade of PTP in VML may be presynaptic. There was also a PTP at the indirect feedback fiber-pyramidal synapses in DML but it was not sensitive to KN-62; Although it has been shown that protein phosphorylation contributes to PTP in some preparations, protein phosphatase antagonists calyculin A and FK-520 failed to emhance PTP in VML.

I have also shown that long-term changes (long-term potentiation, LTP and long-term depression, LTD) were not inducible by tetanic stimulation of TSF, even at different stimulation frequencies. They could not be induced even with GABA antagonism.

When the tetanization of TSF was paired with postsynaptic hyperpolarization a potentiated excitatory response was induced in VML that lasted for about 15 min while the tetanization paired with depolarization did not produce any significant changes in response.

It is concluded that PTP regulation in different sets of synapses is different. In the direct feedback fiber-pyramidal cell synapse PTP is regulated by CaMKII while at the indirect feedback pathway-pyramidal synapse in DML PTP is not; long-term plasticity may not be induced even in glutamatergic synapses with NMDA receptors indicating that long-term changes may require other key factor(s); a novel synaptic plasticity exists in the direct feedback pathway and may regulate sensory processing.

DEDICATION

To my wife Dongmin Wu, without her encouragement and support I could not have finished my doctoral degree; to my Mom, who at the time I prepared this thesis, came over here to help me take care of my family; to my sons Jianing and Eugene, I become more energetic and tireless whenever I see them.

ACKNOWLEDGEMENTS

Many people both inside and outside the University made their contributions to this work and it's almost impossible for me to mention all their names here. Particularly, I would like to thank the professors, support staff and students in the Department of Anatomy and Neurobiology. My supervisor, Dr. Leonard Maler, worked tirelessly throughout the whole process of this project and patiently explained the details to me. His support and encouragement contributed enormously to the final completion of this work. Dr. Neil Berman enlightened me in almost all aspects of my work in this lab, from how to use the computer to how to fine tune a publication. I feel deeply indebted to him. Mr.William Ellis provided all logistical support. Whenever and whatever I needed he would get them on time. I am grateful to him. I'd also thank Mr. Vijay Kapal and Mr. Yanxun Hou for providing an initial technical support.

Outside the Department I would like to thank Dr. J. Bastian at the University of Oklahoma for some useful discussions and important experimental clues and Merck Pharmaceutical company for kindly providing FK-520.

Financial support for this project was provided by the Medical Research Council of Canada (MRC) and the University of Ottawa.

ABBREVIATIONS

ACPD:1-amino-cyclopentane-1,3-dicarboxylic acid

ACSF: artificial cerebrospinal fluid

A/D: analogue/digit

ANOVA:analysis of variance

AM: amplitude modulation

AMPA:a-amino-3-hydroxy-5-methyl-4-isoxazole proprionate

APV(apv): D.L-2-amino-5-phosphonovaleric acid

BAPTA: 1,2-bis(2-aminophenoxy)ethane N,N,N',N'-tetraacetic acid

BIC: Bicuculline

BP: basilar pyramidal cells

CaMKII: calcium calmodulin-dependent protein kinase II

CC: the crista cerebellaris

cGMP: cyclic GMP

CSL: centrolateral segment

CMS: centromedial segment

CNQX: 6-cyano-7-nitroquinoxaline-2,3-dione

CNS: central nervous system

CPP: R, S-4 (phosphonoprop-2-enyl) piperazine-2-carboxylate

CV: coefficient of variation

DAG: diacylglycerol

DFL: deep fiber layer of ELL

DML: dorsal molecular layer

DMSO:dimethyl sulfoxide

DNL(dnl): deep neuropil layer
DNQX: 6,7-dinitroquinoxaline-2,3-dione
EAA: Excitatory amino acids
EGM: eminentia granularis pars medialis
EGP: eminentia granularis posterior
ELL: electrosensory lateral line lobe
EM: electron microscope
EOD: electric organ discharge
EPSP: excitatory postsynaptic potential
FKBP: FK-506 binding protein
GABA: Υ-aminobutyric acid
GR(gr):granule cell layer
IP3: inositol 1.4.5-triphosphate
IPSP: inhibitory postsynaptic potential
KA:kainic acid
L-AP3: L-2-amino-3-phosphonopropionic acid
LL: lateral lemniscus
LM: light microscope
LS: lateral segment
LTD: long term depression
LTP: long term potentiation
MOL/ML: molecular layer
MC: medial segment
NBP: non-basilar pyramidal cells

nM: nucleus medialis
NO: Nitric oxide
NMDA: N-methyl D-aspartate
NMJ: neuromuscular junctions
nXs: sensory nucleus of the vagus
PBS: phosphate buffered solution
PCP: 1-(phenylcyclohexyl)piperidine
Pd: nucleus preeminentialis dorsalis
Pdlp: lateral part of Pd
Pdpp: principal part of Pd
Pdmp: medial part of Pd
PF: parallel fibers
PKA: protein kinase A
PKC: protein kinase C
PL(pl): plexiform layer
PP: protein phosphatase
PPF: paired pulse facilitation
PTK:protein tyrosine kinases,
PTP: post-tetanic potentiation
PTX: pertussis toxin
PYR(pyr): pyramidal cell layer
TCP: N-[1-(2-thienyl) cyclohexyl] piperidine
TMD: transmembrane domains
TS: tetanization
TSF (tsf): tractus stratum fibrosum

VAMP: vesicle associated membrane protein

VML: ventral molecular layer

SPC: spherical cells

SYB: synaptobrevin

TBS: tris-buffered saline

TPC: tractus preeminentialis cerebellaris

TS: torus semicircularis

TTX: tetrodotoxin

TABLE OF CONTENTS

III. RESULTS

LIST OF TABLES

LIST OF FIGURES AND ILLUSTRATIONS

page

I. INTRODUCTION

Sensory processing is an important function of the central nervous system. To investigate the mechanisms of this processing we need to know the circuitry of the sensory system, the physiology of the synapses in these circuits as well as the neurotransmitters used. The interpretation of the neurophysiology also requires an understanding of the behavior controlled by the sensory input. While the complexity of the higher vertebrate nervous systems makes research on these aspects difficult, the simplicity of some lower vertebrate and invertebrate systems makes them preferred systems for morphological, physiological and behavioral research.

The electrosensory system of a weakly electric fish has proven to be a good model for studies of structure-function correlations and biophysics-neural computation (Heiligenberg, 1990).

I.I. Excitatory amino acids (EAAs) as neurotransmitters

The excitatory amino acids (EAAs), mainly L-glutamate and L-aspartate, are abundant amino acids in the central nervous system. In the past 3 decades tremendous progress has been made in the identification of EAAs as putative excitatory neurotransmitters, their involvement in learning and memory, and in neurotoxicity.

In many animal species EAAs have proven to be the transmitter in the hippocampus (Heinemann, Stabel and Rausche, 1990; Lambert and Jones, 1990), neocortex (Aram, Michelson and Wong, 1991; Rosen and Morris, 1991; Sheng, Cummings and Roldan, 1994; Tsumoto, 1990), cerebellum (Larson-Prior and Slater, 1989; Lauritzen, Rice and Okada, 1988; Young, Dauth, Hollingsworth, Penney, Kaatz and Gilman, 1990) and basal ganglia (Silverstein and Naik, 1991). EAAs also mediate synaptic transmission in sensory systems, for example, neurons in the nucleus of the solitary tract of the visceral afferent system (Leone and Gordon, 1989) use EAAs as transmitters. From the ganglion cells to the visual cortex of the visual system (Cline and Constantine-Paton, 1989; Hahm, Langdon and Sur, 1991; van Deusen and Meyer, 1990), in the cochlear (Isaacson and Walmsley, 1995; Nemeth, Jackson and Parks, 1983) and vestibular nuclei (Kinney, Peterson and Slater, 1994; Niedzielski and Wenthold, 1995) of the auditory and vestibular systems, EAAs are identified to be the neurotransmitter. Glutamatergic fibers are also found in the dorsolateral funiculus in the spinal cord (King and Lopez-Garcia, 1993), thalamus (Block, Schwarz and Sontag, 1993) and the parietal lobe of the neocortex (Jaarsma, Sebens and Korf, 1991). In the electrosensory lateral line system of the weakly electric fish several lines of evidence indicate that EAAs are the transmitter.

I.I.I. EAA receptor classification

The EAA receptors are classified into ionotropic and metabotropic types according to their signal transduction mechanism.

Ionotropic receptors

The ionotropic receptors are characterized by their rapid nature of signal transduction due to direct coupling to ion channels. Based on the response to specific agonists, these receptors are further divided into N-methyl D-aspartate (NMDA), kainic acid (KA) and α-amino-3-hydroxy-5-methyl-4-isoxazole proprionate (AMPA) subtypes that are selectively activated by NMDA, KA and AMPA, respectively. The latter 2 are collectively called non-NMDA receptors.

NMDA receptors: Like other ionotropic receptors, NMDA receptors are directly coupled to ion channels. NMDA receptors have some distinct features that make them different from other ionotropic receptors. First, NMDA receptors possess a number of sites that are targeted by a unique assortment of molecules and ions. These include a site that can be blocked specifically by APV (D,L-2-amino-5-phosphonovaleric acid)

(Davies and Watkins, 1982; Ebert, Madsen, Johansen and Krogsgaard-Larsen, 1991), a site specifically blocked by CPP (R,S-4 (phosphonoprop-2-enyl) piperazine-2-carboxylate) (Hatta,Yamamato, Tori, Okuwa and Moroji, 1991), a site in the ion channel that can bind to PCP (1-(phenylcyclohexyl)piperidine) (Wood, Rao, Iyengar, Lanthorn, Monahan, Cordi, Sun, Vazquez, Gray and Conteras, 1990), a site to bind TCP (N-[1-(2-thienyl) cyclohexyl] piperidine) (MacDonald, Bartlett, Mody, Reynolds and Salter, 1990) and a site to bind (+)MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) (Monaghan, 1991). Second, the receptor has a strychnine-insensitive glycine-binding site (Alesiani, Gali, Mori, Pecorari,Carla, Cherici and Pellicciari, 1991; Clements and Westbrook, 1991). Glycine acts as a co-agonist which, when the receptor is activated, potentiates NMDA currents. Third, there is a site on the NMDA receptor complex targeted by polyamines (spermine and spermidine) (Lehmann, Colpaert and Canton, 1991; Johnson, 1996), which increases the affinity of MK-801. Lastly, the receptor complex has binding sites for magnesium (MacDonald and Nowak, 1990; Sacaan and Johnson, 1991) and zinc (Peters, Koh and Choi, 1987) in the ion channel. At resting membrane potential the ion channel is blocked by Mg^{2+} from the outside. This voltage-dependent blockade can be removed by postsynaptic depolarization up to -30 mV (Burnashev, Zhou, Neher and Sakmann, 1995). Activation of NMDA receptors leads to Ca^{2+} influx (Alford, 1993; Mayer and Westbrook, 1987), which in turn, triggers calcium-dependent biochemical cascades. The NMDA-mediated synaptic potential has been characterized by its slow rise time to peak (20 ms), bi-exponential decay with a time constant of 40-200 ms.

So far, at least 12 isoforms from 2 subunit families of the NMDA receptor channel have been identified. The NMDAR1 family is encoded by a single gene and has 8 members (NMDAR1A-H) that come from different splice variants (Zukin and Bennett,

1995) while NMDAR2 has 4 members (Wenzel, Sheurer, Kunzi, Fritschy, Mohler and Benke, 1995). Naturally, these isoforms are expressed in different parts of the CNS with different subunit combinations. The oligomer of NMDAR1 is able to display most of the physiological and pharmacological characteristics of native receptors, including Ca^{2+} permeability and the voltage-dependent block by Mg^{2+}. Receptors consisting of only NMDAR2 subunit do not yield functional channels but the combination of NMDAR1 subunit with any of NMDAR2 subunit produces channels with larger currents and higher sensitivity to Mg^{2+} block (Blahos and Wenthold, 1996; Nakanishi, Masu, Bessho, Nakajima, Hayashi and Shigemoto, 1994).

Unlike members in the super ligand-gated ion channel family which have 4 transmembrane domains (TMD, Barnard, Darlison and Seeberg, 1987), a glutamate receptor subunit has 4 hydrophobic domains (Wo and Oswald, 1995; Hollmann, et al., 1994); however, the putative 2nd domain does not cross the membrane. The N-terminus is located extracellularly while the C-terminal is internal; the 1st and 2nd loops stay inside while the 3rd one outside; the funnel-shaped channel is formed by the 2nd domain (Hollmann, 1997).

In NMDA receptor channels the most narrow part of the pore is ringed by a negatively charged asparagine (Moriyoshi, Masu, Ishii, Shigemoto, Mizuno and Nakanishi, 1991). In NMDAR1 the asparagine in TM2 is thought to control Mg^{2+} blocking and Ca^{2+} permeability since replacement of this residue by a glutamine decreases the Ca^{2+} permeability and reduces the Mg^{2+} block; however, the same substitution in NMDAR2 only slightly reduces the Mg^{2+} sensitivity (Burnashev, Schoepfer, Monyer, Ruppersberg, Bunther, Seeberg and Sakman, 1992; Schoepfer, Monyer, Sommer, Wisden, Sprengel, Kunner, Lomeli, Herb, Kohler, Burnashev and et al., 1994). In the intracellular C terminal domain of the NMDAR1 there are consensus sites for phosphorylation by protein kinase C (PKC) (Raymond, Tingley, Blackstone, Roche and Huganir, 1994), calcium calmodulin-dependent protein kinase II (CaMKII) (Kolaj, Cerne, Chen, Brickey and Randic, 1994). Phosphorylation of NMDAR1 by tyrosine kinase was also reported (Lau and Huganir, 1995). Protein phosphatases can also modulate NMDA receptor function (Wang, Orser, Brautigan and MacDonald, 1994).

Non-NMDA subtypes

The KA and AMPA receptors show simpler physiological and pharmacological properties. When activated, both receptor channels are permeable to Na^+ and K^+ producing a synaptic response with a rapid rate of onset and decay. Some AMPA receptor subunits are also permeable to Ca^{2+} (Wisden and Seeberg, 1993). Although they have different agonists, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and DNQX (6,7-dinitroquinoxaline-2,3-dione) can block both AMPA and KA receptor channels.

Both AMPA and KA receptor channels are heteromeric. There are 4 subunits for AMPA (GluR1-4) and 5 subunits for KA receptors (GluR5-7, KA1-2) (Keinanen, Wisden, Sommer, Werner, Herb, Verdoorm, Sakman and Seeberg, 1990; Nakanishi, 1992). Each subunit has 4 TMDs with a conformation similar to the NMDA subunit. However, the most narrow part of the channel pore is ringed by arginine (R) or glutamine (G) which determines the permeability for divalent cations, particularly Ca^{2+} (Hume, Dingledine and Heinemann, 1991). There are consensus sites in the intracellular domains for protein phosphorylation by PKC (inhibitory) and CaMKII (Roche, O'Brien, Mammen, Bernhardt and Huganir, 1996; Wright, Sefland, and Waalas, 1993). It has also been shown that the KA and AMPA currents are modulated by protein kinase A (PKA) (Raymond, Blackstone and Huganir, 1993.).

Glutamate metabotropic receptor family:

The activation of this subtype of receptors produces a prolonged response since the coupling of the receptors to effector systems are mediated by GTP-binding proteins (G proteins). The effector systems include some ion channels (K+, Ca2+, Na+, Cl- channels) (Jackson, 1991), adenylyl cyclases, phospholipases (Exton et al., 1991) and cGMP phosphodiesterase (Baskys, 1992; Pin and Duvoisin, 1995).

Eight members of metabotropic receptor family are cloned (Westbrook, 1994) which fall into 3 groups based on their effector system: Group 1 (mGluR 1 and 5) are coupled to phospholipase C and result in increases in intracellular calcium. Group 2 (mGluR 2,3) and group 3 (mGluR 4,6,7,8) are negatively coupled to adenylate cyclase, resulting in a decrease in forskolin-stimulated cAMP. Trans-ACPD (trans-1-amino-cyclopentyl-1,3-dicarboxylate) activates groups 1 and 2 mGluRs, whereas L-AP4 (2-amino-4-phosphobutyrate) activates group 3 receptors (Nakanishi et al., 1994).

Group 3 receptors are electrophysiologically defined as a presynaptic inhibitory receptors and their activation may result in closing the high threshold Ca2+ channel and reducing the transmitter release (pin and Duvoisin, 1995; Schoepp and Conn, 1993).

As other G-protein coupled receptors, the glutamate metabotropic receptors has 7 TMD with the large extracellular amino terminus and a small intracellular carboxyl terminus (Bockaert, 1991).

I.I.II. Modulation of EAA receptors and transmitter release by protein kinases and protein phosphatases

Like any other ionotropic receptor the activities of the EAA receptors undergo modulation during signal transduction. Very often, the modifications are carried out by reversible protein phosphorylation and dephosphorylation processes catalyzed by kinases and phosphatases respectively. The steady state of activity reflects the ratio of the two processes.

Protein phosphorylation is a posttranslational covalent modification used by prokaryotic and eukaryotic cells to control the activities of many kinds of proteins including ion channels, receptors and enzymes. The kinases that phosphorylate the EAA receptor channel complex include CaMKII, PKA, PKC and tyrosine kinases.

CaMKII

CaMKII is made up of at least 4 gene families (α, β, ϒ, ĉ) and two of them (α- and β-CaMKII) are expressed only in the nervous system (Tobimatsu and Fujisawa, 1989). The holoenzyme can exist in homo (α- subunit alone)- or heteromultimers in different regions of the brain. It has been indicated that CaMKII exists both pre- (Erondu and Kennedy, 1985; Lin, Sugimori, Llinás, McGuinness and Greengard, 1990) and post-synaptically (Kelly, McGuinness and Greengard, 1984) at central synapses.

Each subunit has 3 major domains (Tobimatsu and Fujisawa, 1989): 1): N-terminus catalytic protein kinase domain. The sequence of this domain is most homologous to that of other calmodulin-regulated kinases, which means it acts on substrates containing Ser/Thr consensus sequences; 2): A regulatory, calmodulin-binding domain in the middle of a subunit. This domain contains both an autoinhibitory sequence that keeps the enzyme inactive at basal state, and a calmodulin-binding sequence which binds Ca^{2+}-calmodulin. This binding is triggered by calcium. Initially, the Ca^{2+} increase stimulates binding with calmodulin, which undergoes conformational change and increases the affinity to CaMKII. In response to Ca^{2+}-calmodulin binding the autoinhibitory sequence is displaced leading to the activation of CaMKII. CaMKII subunits can autophosphorylate and, once autophosphorylation occurs, the enzyme is no longer Ca^{2+} -calmodulin dependent (Schulman, 1993), even after Ca^{2+} has returned to baseline levels and calmodulin has dissociated. The active CaMKII phosphorylates substrates that contain threonine and serine consensus sequences (Saitoh and Schwartz, 1985; Lou, Lloyd and Schulman, 1986; Lai, Nairn, Corelick and Greenspan, 1987) and the phosphorylation persists until the substrate is dephosphorylated. The autoinhibitory sequence is used pharmacologically. A synthetic peptide sequence from amino acid 281-302 in the α-subunit (Smith, Colbran, Brickey and Soderling, 1992) is used as a CaMKII inhibitor. KN-62 (1-[N,O-bis(5-isoquinolinesulphonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine) and KN-93 are found to inhibit the CaMKII selectively by blocking its binding to calmodulin (Sumi, Kiuchi, Ishikawa, Ishii, Hagiwara, Nagatsu and Hidaka, 1991; Tukumitsu,Chijiwa, Hagiwara, Mizutani and Terasawa, 1990). 3): C-terminus association domain. This domain is required for assembly of the subunits into a holoenzyme and recombinant kinase lacking this domain is fully Ca^{2+} calmodulin dependent but monomeric rather than oligomeric.

The phosphoprotein phosphatases

Phosphatases belong to several gene families. In the case of threonine/serine phosphatases three gene families are found to encode 5 phosphatases (Honkanen, Zwiller, Daily, Khatra, Dukelow and Boynton, 1991; Ingebritsen and Cohen, 1983).

The first family is phosphatase 1 (PP1). PP1 has broad substrate specificities but for phosphorylase kinases it dephosphorylates only the beta subunit. PP1 is inhibited by some small thermostable proteins (called inhibitor-1 and 2) (Ingebritsen and Cohen, 1983). A number of natural toxins are also powerful and specific inhibitors of PP1. Some of the toxins are fatty polyethers that can penetrate the cells. They include okadaic acid (OA), Calyculin A, E, F, G, H (A is the most potent one), dinophysistoxin1 and tautomycin (Cohen, Holmes and Tsukitani, 1990; Honkanen, Codispoti, Tse, Boynton and Honkanen, 1994; Ishihara, Martin, Brautigan, Karaki, Ozaki, Kato, Fusetani, Watabe, Hashimoto, Uemura and et al., 1989). The other type of toxins are cyclic peptides, which do not penetrate the cells and have to be applied intracellularly. Microcystin-LR (Honkanen et al., 1991), motoporin, nodularins, cantharidin and endothal fall into this group of inhibitors.

The second family is phosphatase 2 (PP2). PP2 is further classified into PP2A, PP2B (also called calcineurin) and PP2C. PP2 A and C have broad substrate specificity while PP2B is restricted. When phosphorylase kinases are used as substrate, the PP2 family dephosporylates only the α-subunit of the kinases. PP2 is insensitive to the thermostable proteins inhibitors 1 and 2. The PP2A catalytic subunit forms heterotrimers with 2 non-catalytic subunits which alter its specificity for substrates and activity. All of the inhibitors that act on PP1 also block the activity of PP2A (Ingebritsen and Cohen, 1983). PP2B is a Ca^{2+}-calmodulin-dependent phosphatase. Calcineurin is a heterodimer with the catalytic A-subunit and a no-catalytic B-subunit homoglous to calmodulin. Calcium activates PP2B by binding to B-subunit and Ca^{2+}-calmodulin binds to the A-subunit. The activations are synergistic and happen at very low calcium concentration. Some immunosuppressants are found to be very potent calcineurin inhibitors. The suppressants include FK-520 (Garver, Oyler, Harris, Polavarapu, Damuni, Lehman and Billingsley, 1995; Nichols, Suplick and Brown, 1994), FK-506 and cyclosporin A. FK-506 (and maybe FK-520 too) interacts with FK-506 binding protein (FKBP) and forms a complex of PP2B-FKBP-FK506. Recently the crystal structure of this complex was demonstrated (Kissinger, Parge, Knighton, Lewis, Pelletier, Tempczyk, Kalish, Tucker, Showalter, Moomaw, Gastinel, Habuka, Chen, Maldonado, Barker, Bacquet and Villafranca, 1995). Cyclosporin A interacts with cyclophilin, an endogenous protein (Braun, Kallen, J., Mikol, Walkinshaw and Wuthrich, 1995) and forms a complex similar to that of FK-

506. These complexes, resulting from the interactions, bind the activated form of calcineurin in a competitive manner with Ca^{2+}-calmodulin and block the phosphatase activity. PP2C is a Mg^{2+}-dependent phosphatase and structurally different from other protein threonine/serine phosphatase. At very high Ca^{2+}, PP2C is blocked (Wang, Santini, Qin and Huang, 1995). Some experiments showed that PP2C, which is insensitive to OA (Fukunaga, Rich and Soderling, 1989), selectively dephosphorylates the autophosphorylated CaMKII (Fukunaga, Kobayashi, Tamura and Miyamoto, 1993)

The third family is PP3. It is divalent cation-independent and is stimulated rather than inhibited by the inhibitor-2. PP3 preferentially dephosphorylates the beta-subunit of kinases when they are used as substrate (Honkanen et al., 1991). It has similar pharmacological properties to PP1 and PP2A in terms of calyculin A antagonism (Honkanen et al., 1994).

In addition to PP1, 2A and calcineurin, some phosphatases have a dual specificity since they catalyze the substrates that have threonine/serine and tyrosine consensus sequences (Haavik, Schelling, Campbell, Andersson, Flatmark and Cohen, 1989).

Modulation of EAA receptors and transmitter release by phosphorylation events

The Ca^{2+}-calmodulin-dependent protein phosphorylation mediated by CaMKII and dephosphorylation mediated by phosphatases modulate a variety of functions, including neurotransmitter release, and receptor ion channels; these modulations are thought to underlie different forms of synaptic plasticity.

Postsynaptically, CaMKII enhances both non-NMDA (Kolaj et al., 1994; Yakel, Vissavajjhala, Derkach, Brickey and Soderling, 1995) and NMDA currents (Kitamura, Miyazaki, Yamanaka and Nomura, 1993; Kolaj et al., 1994;) in different preparations; this potentiation can be further enhanced by phosphatase inhibitors (Figurov, Boddeke and Muller, 1993; Wyllie and Nicoll, 1994).

Presynaptically, CaMK II may modulate transmitter release by phosphorylating some synaptic vesicle-associated proteins. An action potential reaches the nerve terminal and depolarizes the presynaptic plasma membrane leading to the opening of voltage-gated Ca^{2+} channels. The rise in intracellular Ca^{2+} triggers the exocytosis of synaptic vesicles resulting in the neurotransmitter release. The vesicles membrane is retrieved by endocytosis and the vesicles are eventually refilled with transmitter. For the convenience of description the release cycle is divided into several stages. In stage 1 the synaptic vesicles are filled by an active transport process and in stage 2 and 3 the vesicles translocate and dock at the active zone. When Ca^{2+} rises at the terminal the docked vesicles are extruded in stage 4. Following the exocytosis the vesicle membranes undergoes rapid endocytosis, move away from the active zone and reform the synaptic vesicles in stages 5,6,7 (Jessell and Kandel, 1993).

Some key presynaptic components can be phosphorylated: for example, synapsin I, synaptophysin, synaptotagmin and the vesicle associated membrane protein (VAMP)/synaptobrevin (SYB) (Südhof and Jahn, 1991; Greengard, Valtorta, Czernik and Benfenati, 1993) which anchor, dock and probably fuse the synaptic vesicles.

Synapsin I binds synaptic vesicles to actin filaments and also bundles the actin filaments preventing the vesicles from being released (Südhof, Czernik, Kao, Takei, Johston, Horiuchi, Kanazir, Wagner, Perin, De Camilli, and Greengard, 1989; De Camilli, Benfenati, Valtorta and Greengard, 1990). When synapsin I is phosphorylated by CaMKII, actin filaments' bundling and binding is blocked and the availability of vesicles for exocytosis increases (Fukunaga, Soderling and Miyamoto, 1992); conversely, dephosphorylation decreases transmitter release (Llinás, Gruner, Sugimori, McGuinness and Greengard, 1991). However, the role of synapsin I in transmitter release is still controversial (Rosahl, Spillane, Missler, Herz, Selig, Wolff, Hammer, Malenka and Südhof, 1995).

Synaptophysin is a major integral protein of synaptic vesicle membrane and has been proposed to form pores in the active zone by which vesicles are exocytocized. Antibodies directed against synaptophysin blocked Ca^{2+}-dependent transmitter release (Alder, Xie, Valtorta, Greengard and Poo, 1992). Synaptophysin is also a substrate for CaMKII which phosphorylates synaptophysin on its serine residues (Rubenstein, Greengard and Czernik, 1993).

Synaptotagmin is another integral membrane protein of synaptic vesicles and plays a role in the vesicle docking-fusion process (so it is also called docking protein). In synaptotagmin I-deficient mice transmitter release is decreased (Geppert, Goda, Hammer, Li, Rosahl, Stevens and Südhof, 1994). This protein possesses several sites for PKC phosphorylation (Trimble, Linial and Scheller, 1991) and its phosphorylation by PKC may facilitate transmitter release. In addition, phospho-synaptotagmin can be immunoprecipitated from endogenously phosphorylated synaptic vesicles, suggesting that synaptotagmin may also be phosphorylated by CaMKII (Popoli, 1993).

VAMP/SYB is thought to be involved in docking the vesicles at the active zone. Cleavage by some toxins blocked transmitter release (Schiavo, Benfenati, Poulain, Rossetto, Polvorino de Laurito, DasGupta and Montecucco, 1992). VAMP/SYB can also be phosphorylated by CaMKII (Nielander, Onofri, Valtorta, Schiavo, Montecucco, Greengard and Benfenati, 1995).

There is some other evidence that denies the involvement of CaMKII in transmitter release: 1) at the crayfish neuromuscular junctions, presynaptic injection of the calmodulin binding domain, or the calmodulin inhibitor calmidazolium, or KN-62, neither affects synaptic transmission nor blocks short-term plasticity; 2) bath application of calmidazolium or KN-62 in the same preparation does not have any effects on transmission (Kamiya and Zucker, 1994).

Phosphatases have been indicated to be involved in long term depression (LTD, Xiao, Karpefors, Gustafsson and Wigstrom, 1995), long term potentiation (LTP, Zhao, Bennett, Sedman and NG, 1995), ion channel activities (Murakami, Sakai, Nei, Matsuyama, Saito and Tanaka, 1994), and more importantly, glutamate transmitter release and functional regulation of glutamate receptors. Okadaic acid-incubated synaptosomes increase glutamate uptake (Pisano, Samuel, Nieoullon and Kerkerian-Le Goff, 1996) and release (Sim, Lloyd, Jarvie, Morrison, Rostas and Dunkley, 1993). Long-term glutamate desensitization induced by okadaic acid or calyculin A has been reported (Kogan and Aghajanian, 1995). In a patch clamp recording PP1 and PP2A decreased the NMDA receptor channel open probability (Wang, Orser, Brautigan, MacDonald, 1994). On hippocampal neurons synaptically stimulated-NMDA receptor desensitization was prevented by calcineurin inhibitors (Tong, Shepherd and Jahr, 1995). Blocking calcineurin increased glutamate transmitter release (Nichols et al., 1994; Sihra, Nairn, Kloppenburg, Lin and Pouzat, 1995). At the single channel recording level, FK-506 shortened the duration of NMDA receptor channel opening (Lieberman and Mody, 1994).

I.II. Synaptic plasticity

Synaptic plasticity is defined as a change in synaptic efficacy caused by some physiological process. Most commonly, synaptic plasticity is caused by repetitive stimulation of presynaptic fibers. It can be categorized in different ways. Based on the time domain, the plasticity is classified into short term and long term plasticity. Based on the direction of the change it is grouped into potentiation and depression. According to the induction condition, the plasticity can be either Hebbian, non-Hebbian or anti-

Hebbian.

I.II.I. Short term plasticity

Many synapses in the CNS and at the neuromuscular junction produce a short enhancement of synaptic efficacy after tetanization. The short term enhancement is further divided into 1) synaptic facilitation which occurs in 1 sec; 2) synaptic augmentation that lasts for less than 10 sec; 3) post-tetanic potentiation (PTP) that lasts for several minutes (Magleby, 1979). All the short term enhancements are considered to be presynaptic phenomena.

PTP has been thoroughly investigated in invertebrate synapses and vertebrate neuromuscular junctions (NMJ, Atwood and Wojtowicz 1986; Magleby 1987) and evidence indicate that it is mediated by residual calcium in the presynaptic terminals (Katz and Miledi, 1968; Charlton, Smith and Zucker, 1982; Zucker, 1996; Zucker, Delaney, Mulkey and Tank, 1991). The residual calcium theory hypothesizes that short term potentiation results from transmitter release which nonlinearly depends on intracellular Ca^{2+} concentration and the probability that after a presynaptic action potential some residual calcium still persists at sites of transmitter release (Katz and Miledi, 1968). Although this hypothesis has generally been accepted little was known about the mechanism until recently.

Some experimental data directly link the calcium buildup to phosphorylation of synaptic vesicle-related proteins that mediate the neurotransmitter release (Llinás et al., 1991; Walch-Solimena, Jahn, Südhof, 1993; Wang, Renger, Griffth, Greenspan and Wu, 1994).

As mentioned before, the phosphorylation by protein kinases mobilizes the synaptic vesicles, promotes docking and fusion to the presynaptic plasma membrane and increases the release of transmitter (but see Kamiya and Zucker, 1994).

I.II.II. Long term plasticity

The efficacy of chemical synapses undergoes consistent changes based on their recent history of activities. The synaptic changes that last longer than 40-60 min is called long term plasticity. Long term plasticity is thought to underlie learning and memory. Hebb proposed that synaptic strength follows a very simple rule: if the activity in a presynaptic neuron is repeatedly associated with the discharge of the postsynaptic neuron, then the efficiency of the synapse will increase (Hebb, 1949). Long-term plasticity can be non-Hebbian, which results from activating the presynaptic component alone, Hebbian, that is the conditions that induce the plasticity is consistent with the Hebbian rule, or anti-Hebbian, which is that the conditions are opposite to the Hebbian rule.

Non-Hebbian plasticity:

Non-Hebbian plasticity is induced in both invertebrate (Kandel and Schwartz, 1982; Carew and Sahley, 1986) and vertebrate animals. The plasticity induced from vertebrate hippocampal mossy fibers in CA3 (Alger and Teyler, 1976; Harris and Cotman, 1986) and cerebellar parallel fibers (Salin, Malenka and Nicoll, 1996) is long-term potentiation. Some agreement has been reached on the mechanism for this LTP (see review by Johnston,Williams, Jaffe and Gray, 1992): 1): High frequency presynaptic stimulation is necessary and sufficient for the induction. 2): Induction of the LTP depends in some way on Ca^{2+}. Ca^{2+} influx may be presynaptic, resulting from increased transmitter release (Katsuki, Kaneko, Tajima and Satoh, 1991; Zalutsky and Nicoll, 1990) or postsynaptic, activating voltage-dependent Ca^{2+} channels (Jeffe and Johnston, 1990), but not from NMDA channels (Harris and Cotman, 1986). More and more evidence supports the former mechanism. The calcium influx must be presynaptic rather than postsynaptic since postsynaptic application of Ca^{2+} chelator BAPTA or fluoride does not block the LTP induced at these synapses (Langdon, Johnson and Barrionuevo, 1995; Zalutsky and Nicoll,1990). Quantal analysis (Xiang, Greenwood, Kairiss and Brown, 1994) and paired pulse facilitation actually showed an increase in transmitter release (Zalutsky and Nicoll,1990). Recent results show that this LTP is mediated by presynaptic Ca^{2+} calmodulin-dependent PKA (López-García, Arancio, Kandel and Baranes, 1996; Salin, et al., 1996; Weisskopf, Castillo, Zalutsky and Nicoll, 1994). (For the transmitter release mecahnisms, see discussion for details). 3): The induction of non-Hebbian LTP does not depend on postsynaptic polarization (Langdon, et al., 1995; Zalutsky and Nicoll, 1990).

Tetanization may reduce autoinhibition via metabotropic receptors and increase the transmitter release.

Hebbian plasticity

The Hebbian theory of learning and memory was first experimentally supported by Bliss and Lømo in hippocampus (1973). Since then the hippocampus has been used as a model system to study LTP. In this model system, Long term potentiation is defined as an increase in synaptic efficacy that lasts for at least 60 min (Bliss and Collingridge, 1993) following brief high frequency stimulation.

There are 3 main excitatory pathways in the hippocampus. The perforant fibers coming from entorhinal cortical neurons synapse onto the granule cells in dentate gyrus, the mossy fibers from dentate granule cells contact pyramidal cells in CA3 area and the Schaffer collaterals that come from the CA3 pyramidal cells form synapses with pyramidal cells in the CA1 area. GABAergic interneurons exert strong inhibition of the pyramidal cells through feedback or feedforward loops (Andersen, 1975). When the Schaffer collaterals in CA1 are tetanized, the potential evoked at the synapse is potentiated and this potentiation can last for several hours or even several days (Bliss and Lømo, 1973).

The LTP induced in hippocampus is characterized by cooperativity, associativity and input-specificity. Cooperativity means that there is a threshold for the induction of LTP. The threshold is a complex function of intensity and pattern of tetanization. Weak tetani produce PTP (McNaughton, Douglas and Goddard, 1978). Typically the optimal stimulation parameter for hippocampal LTP is θ-burst (several bursts of 4 shocks at 100 Hz delivered at an interburst interval of 200 ms, Larson,Wong and Lynch, 1986) or primed-burst stimulation (a single priming stimulation followed at 200 ms by a single burst of 4 shocks at 100 Hz, Rose and Dunwiddie, 1986). These parameters are similar to those in the hippocampus during learning (Otto, Eichenbaum, Wiener and Wible, 1991). These stimulation protocol may depress GABA-mediated synaptic inhibition (Davies, Starkey, Pozza and Collingridge, 1991). Associativity is demonstrated by the fact that weak stimulation can be potentiated only if they are active at the same time as a strong tetani to a separate but convergent inputs (McNaughton et al., 1978). Input specificity means that only the synapses that are active during the tetanization are potentiated (Andersen, Sundberg, Sveen and Wigstrom, 1977; Lynch, Dunwiddie and Gribkoff, 1977).

The identification of glutamate in synapses of CA1 provides insight into the LTP mechanism. Both NMDA and non-NMDA receptor types are found in the pyramidal cells and tetanization of Schaffer collaterals triggers the release of glutamate and or aspartate. Strong tetanization first activates non-NMDA (particularly the AMPA) receptors, leading to partial depolarization of the postsynaptic neurons. This partial depolarization removes the Mg^{2+} blockade from the outside of the NMDA receptor-channel pore, activates NMDA receptors, and induces LTP (Collingridge, Kehl and McLennan, 1983; Collingridge, Herron and Lester, 1988). Selective NMDA receptor antagonists or channel blockers completely prevent induction of LTP without blocking the ongoing synaptic response (Collingridge et al.,1983; Harris, Ganong and Cotman, 1984). Therefore, induction of LTP requires simultaneous presynaptic activation and postsynaptic depolarization, which is consistent with the Hebbian principle.

Activation of NMDA receptor channels permits Ca^{2+} influx. As a second messenger, Ca^{2+} triggers biochemical cascades that ultimately lead to LTP. Thus LTP is not induced in the presence of Ca^{2+} chelators (Lynch, Larson, Kelso, Barrionuevo and Schottler, 1983; Malenka, Kauer, Zucker and Nicoll, 1988). Several effects of these biochemical cascades may underlie the mechanism for the induction and/or maintenance of LTP: 1): protein phosphorylation and /or persistent protein kinase activity; 2): intracellular Ca^{2+} release; 3): the production of a retrograde transmitter; 4): increase in transmitter release.

The phosphorylation of NMDA and non-NMDA receptors by CaMKII (Malenka et al, 1989; Malinow, Schulman and Tsien, 1989) and PKC (Akers, Lovinger, Colley, Linden and Routtenberg, 1986; Klann, Chen and Sweatt, 1991) is implicated and is consistent with the presence of consensus phosphorylation sites in the intracellular loop of receptors for CaMKII and PKC. Recent reports demonstrate that the α-subunit of CaMKII is essential for the production of LTP since in the mutant animal, which lacks α-CaMKII, LTP is not induced (Silva, Stevens, Tonegawa and Wang, 1992b; Silva, Paylor, Wehner and Tonegawa, 1992a). Once CaMKII is activated it becomes autophosphorylated for a long time and no longer needs Ca^{2+}. This long lasting autophosphorylation has been suggested to underlie the molecular basis for memory (Lisman and Goldring, 1988). It has also been demonstrated that LTP is blocked by a PKC inhibitor (Malinow et al., 1989) and is induced by PKC (Hu, Hvalby, Walaas, Albert, Skjeflo, Andersen and Greengard, 1987) or its agonist (Malenka. Madison and Nicoll , 1986). Activation of PKC selectively enhances the NMDA current in hippocampal (Ben-Ari. Aniksztejn and Bregestovski, 1992) and spinal neurons (Gerber, Kangrga, Ryu, Larew and Randic, 1989). LTP is partially maintained by persistent CaMKII and PKC activity since H-7, an inhibitor of the activated kinases, interrupts LTP (Malinow, Madison and Tsien, 1988).

The involvement of glutamatergic metabotropic receptors in LTP is also reported (Bortolotto, Collingridge and Edgbaston, 1992; Bashir, Bortolotto, Davies, Berretta, Irving, Seal. Henley, Jane, Watkins and Collingridge, 1993). The activation of mGluR may be coupled to phospholipase C via a G-protein which, in turn, catalyzes phosphatidylinositol 4,5-biphosphate into IP3 (inositol 1.4.5-triphosphate) and diacylglycerol (DAG), both of which act as second messengers. While DAG activates PKC, IP3 couples to IP3 receptors in the endoplasmic reticulum leading to internal calcium release. It has been shown that postsynaptic calcium magnitude is a critical variable controlling the duration of LTP. The metabotropic receptor-mediated increase in PKC activity has also been reported (Otani, Ben-Ari and Roisin-Lallemand, 1993).

Since the induction of LTP requires activation of postsynaptic NMDA receptors and maintenance might require increased presynaptic transmitter release (Dolphin, Errington and Bliss 1982; Skrede and Malthe-Sørenssen, 1981), some message must be sent from the postsynaptic to the presynaptic. Nitric oxide (NO) has been proposed to act as such a retrograde messenger to increase transmitter release (Bliss, Erringnton and Lynch, 1990). The gaseous NO is generated by a calcium calmodulin-dependent NO synthase using L-arginine. NMDA receptor activation causes the release of NO postsynaptically, which diffuses to the presynaptic neuron and promotes transmitter release (Hawkins, Zhuo and Arancio, 1994).

The site of LTP expression has been a controversial topic (Bliss and Collingridge, 1993; Kullmann and Siegelbaum, 1995).

There is some evidence supporting a presynaptic expression of LTP. The overflow of radiolabelled or endogenous glutamate from the hippocampus after LTP shows an increase over before (Bliss et al., 1990; Dolphin et al., 1982; Skrede and Malthe-Sørenssen, 1981) and this increase can last for several hours (Bliss, Errington and Lynch, 1990). Quantal analysis shows that LTP is associated with a decrease in the trial-to-trial variability in the size of the postsynaptic signal or a decrease in the coefficient of variation (CV), which is compatible with an increase in average number of transmitter quanta (Kullmann and Siegelbaum, 1995).

There are also several lines of evidence that support a postsynaptic expression of LTP. The paired pulse facilitation (PPF), which is an indicator of increased transmitter release, does not accompany LTP (Manabe, Wyllie, Perkel and Nicoll, 1993; McNaughton, 1982). During LTP the two components of postsynaptic glutaminergic signal, mediated by AMPA and NMDA, appear to be increased differentially: a number of studies showed an increase in AMPA current with little or no change in NMDA current (Kauer, Malenka and Nicoll, 1988; Muller and Lynch, 1988). Presynaptic expression would be expected to increase both currents. During PTP, which is thought as a purely presynaptic phenomenon, an increase in both currents was seen. Despite this, the isolated NMDA current failed to exhibit LTP (Bashir et al.,1991; Xie, Berger and Barrionuevo, 1992).

In reconciliation of the pre- and postsynaptic LTP expression, a "silent synapse" hypothesis was proposed which interprets the increase in transmitter release as uncovering of previously silent AMPA receptors in the postsynaptic membrane (Edwards, 1991). The first line of evidence supporting the silent hypothesis came from a comparison of the probabilistic behavior of AMPA and NMDA before and after low frequency presynaptic stimulation (Kullmann, 1994). Before stimulation, the CV of

AMPA was larger than that of NMDA under voltage-clamp at various membrane potentials. After induction of LTP the CV of AMPA fell while the amplitude or CV of NMDA did not change. This was interpreted as activation of AMPA receptors that were silent and coexisted with NMDA receptors under baseline conditions. In other words, LTP induction increases the number of AMPA receptors. This interpretation was further supported by others (Liao, Hessler and Malinow, 1995; Isaac, Nicoll and Malenka, 1995) which showed that very low intensity presynaptic stimulation, which activated NMDA receptors at a positive holding potential, failed to elicit AMPA currents at a negative potential. When paired with postsynaptic depolarization to induce Ca^{2+} influx from NMDA receptors, the low intensity stimulation induced AMPA-mediated currents when the potential became negative, suggesting the unmasking of AMPA receptors. Finally, the proportion of synaptic transmission failure was shown to decrease after the induction of LTP when measured at negative potentials where excitatory transmission is mediated only by AMPA (Liao, et al., 1995).

Anti-Hebbian Plasticity:

This type of plasticity can be depression, which is induced by co-exciting the pre- and postsynaptic elements, or potentiation, which is triggered by exciting the presynaptic while inhibiting the postsynaptic components.

Anti-Hebbian depression has been thoroughly investigated in mammalian cerebellar Purkinje cells. Purkinje cells, which serve as output of cerebellar cortex, receive 2 different excitatory inputs. The climbing fibers coming from the inferior olive nuclei make powerful one to one connections with the dendritic tree of Purkinje cells while the parallel fibers from vast numbers of granule cells form synapses on the spines of the Purkinje dendrites (Eccles, Ito and Széntagothai, 1967). The conjunctive activation of the parallel and the climbing fibers induces a persistent depression at parallel fiber-purkinje synapses (Ito, Sakurai and Tongroach, 1982). This depression consists of an initial phase lasting for 10 min and a late phase lasting for 1 to several hours depending on the preparation. The depression is expressed as an amplitude reduction by 30-90% (Ekerot and Kano, 1985; Sakurai 1987) of AMPA-mediated EPSPs (Linden, Dickinson, Smeyne and Connor, 1991). Some good evidence indicates that the cerebellar anti-Hebbian LTD is induced and expressed postsynaptically.

The stimulation of climbing fibers can be replaced by intra-Purkinje cell depolarization (Hirano, 1990), and this depolarization is strong enough to activate the voltage-gated Ca^{2+} channels leading to the Ca^{2+} accumulation (Konnerth, Dressen and Augustine, 1992). Postsynaptic application of a Ca^{2+} chelator (Sakurai, 1990) or hyperpolarization of Purkinje cells (Hirano, 1990) blocks the induction of LTD during climbing/parallel fibers conjunctive stimulation. The production of cGMP catalysed by NO is also observed with the induction of cerebellar LTD. NO synthase inhibitor or scavenger (hemoglobin) blocked LTD by climbing/parallel fiber coactivation (Shibuki and Okada, 1991) or by depolarization/parallel fiber conjunction (Crepel and Jaillard, 1990; Deniel, Hemart Jaillard and Crepel, 1993). Bath application of NO donor (sodium nitroprusside) or membrane permeable cGMP analogs, to replace climbing fiber activation, also induced LTD (Deniel et al., 1993; Shibuki and Okada, 1991). However, some conflicting results were also reported (Glaum, Slater, Rossi and Miller, 1992; Linden and Connor, 1992). The mechanism of NO in the LTD induction is still unclear.

The activation of parallel fibers can be replaced by iontophoretic application of AMPA and trans-ACPD (Crepel and Krupa, 1988; Linden et al., 1991). The activation of ACPD receptors couples the signal to the effector system via a G-protein since L-AP3 (metabotropic receptor antagonist), PTX (a toxin that inhibits G-protein coupled receptor-effector interaction), or mGluR1 antibodies (Linden et al., 1991; Shigemoto, Nakanishi and Hirano, 1993) all block the induction LTD. Purkinje cells have a rich distribution of mGluR1 (Masu, Tanabe, Tsuchida, Shigemoto and Nakanishi, 1991), which is linked to phosphoinositide turnover and subsequent PKC activation. A role for PKC in the induction of LTD is suggested by the observations that a PKC inhibitor blocks the induction if applied during depolarization/glutamate conjunction (Linden and Connor, 1991) while phorbol esters, PKC activators, induced depression when exogenous glutamate or AMPA was applied (Crepel and Krupa, 1988).

AMPA receptors appear to act through a Na^+-mediated process since the replacement of Na^+ with other permeant cations blocks LTD during quisqualate/depolarization (Linden, Smeyne and Connor, 1993). The role of Na^+ in the induction of LTD is not clear. It's speculated that a Na^+-Ca^{2+} exchange mechanism may reduce the Ca^{2+} efflux and help increase internal Ca^{2+}.

LTD is expressed postsynaptically as a depression of AMPA-mediated currents resulting from alterations in the number or sensitivity of AMPA receptors. Although exogenous AMPA is not sufficient to replace parallel fiber stimulation it is sufficient to detect LTD once it is induced. It has been observed that LTD can be induced in cultured Purkinje cells by quisqualate/depolarization conjunction and detected with test pulses of AMPA in the presence of TTX and adenosine to eliminate action potentials and spontaneous transmitter release (Linden et al., 1991; Linden and Connor, 1991).

In recent years anti-Hebbian LTD in hippocampus has became a focus of intense study (Linden and Conner, 1995; Malenka, 1994). This LTD is readily inducible by pairing low frequency stimulation (1-5 Hz) of the Schaffer collateral pathway with pyramidal cell depolarization (Bolshakov and Siegelbaum, 1994) *in vitro* or by tetanizing the presynaptic cell in conjunction with postsynaptic depolarization in cultured hippocampal cells (Goda and Stevens, 1996). Hippocampal LTD is different from that in the cerebellum in that the hippocampal LTD is postsynaptically induced and presynaptically expressed (Bolshakov and Siegelbaum, 1994; Stevens and Wang, 1994; Xiao, Wigström and Gustaffson, 1994). Its induction also requires the activation of the postsynaptic NMDA receptor subtype and of L-Ca^{2+} channels (Bolshakov and Siegelbaum, 1994 ; Dudek and Bear, 1992; Mulkey ana Malenka, 1992) and its induction can be blocked by postsynaptic hyperpolarization (Goda and Stevens, 1996). Quantal analysis shows that the LTD is expressed by a decrease in the presynaptic transmitter release while the postsynaptic responsiveness is unaltered (Bolshakov and Siegelbaum, 1994; Goda and Stevens, 1996; Stevens and Wang, 1994). The molecular basis for the hippocampal LTD is still obscure.

I.III. The electrosensory system of a weakly electric fish

The weakly electric fish has been recognized as a good experimental animal for studies of neural structure-function correlations (Bullock, 1986; Heiligenberg, 1990). These fish are nocturnal and their visual system is regressed (Sas and Maler, 1986). The weakly electric fish have evolved electrosensory/motor systems for the purpose of electrolocation and electrocommunication by means of an electric organ discharge (EOD). Some species of electric fish generate very regular, continuous high frequency EODs (wave-type) while others produce brief pulses at irregular intervals (pulse type). Gymnotiform *Apteronotus leptorhynchus* is a wave type electric fish and has been intensely studied in the past several decades. The electrosensory system of this electric fish, like other sensory systems, consists of peripheral and central components.

I.III.I. Electrosensory periphery

In the periphery the electric organs of the apteronotus are distributed in the hypaxial muscle along the spinal column. Unlike the organs of other species, in which they are myogenic, the electric organs of apteronotus are derived from modified spinal motor axons, which give out regular and continuous EODs, a sine-wave of 500-1000 Hz (Bass, 1986). The electroreceptors are distributed over most of the body surface. There are 2 functional types of receptors: the ampullary receptors which respond to low frequency electric fields (no more than several tens of Hz, Zakon, 1986) and tuberous receptors that respond optimally to the frequency of the fish's own EOD (tens to several hundreds Hz). Tuberous electroreceptors are further divided into time coding (T-type) receptors that encode the zero-crossing point of the EOD, and amplitude coding (P-type) receptors which encode the peak amplitude of the EOD by their probability of discharge (Zakon, 1986). Each receptor is innervated by a single primary afferent fiber (Carr, Maler and Sas, 1982) which has its soma in the ganglion of the anterior lateral line nerve. These ganglion cells project centrally to the electrosensory lateral line lobe (ELL) of the medulla (Carr et al., 1982; Carr and Maler, 1986).

I.III.II. Electrosensory lateral line lobe (ELL)

The ELL is the first processing center for the electroreceptive input. It is a hypertrophied extension of the dorsal medulla. Internally the ELL is divided into four somatotopically organized segments (medial segment, MC; centromedial, CMS; centrolateral, CLS; and lateral, LS). Each segment has 8 similar laminae (Maler, 1979). From ventral to dorsal these are 1): The deep fiber layer contains the primary afferents. 2): In the deep neuropil layer these primary afferents contact ELL target cells and dendrites. 3): The granule cell layer has 2 types of inhibitory interneurons. The GABAergic Type-2 has long apical dendrites extending to the molecular layer, while the non-GABAergic type-1 cell does not; the axons of both cell types contact pyramidal cells (Maler and Mugnaini, 1994). Deep pyramidal cells are also located in this layer (Bastian and Courtright, 1991). 4): The plexiform layer is composed of efferent fibers of pyramidal cells. 5): The pyramidal

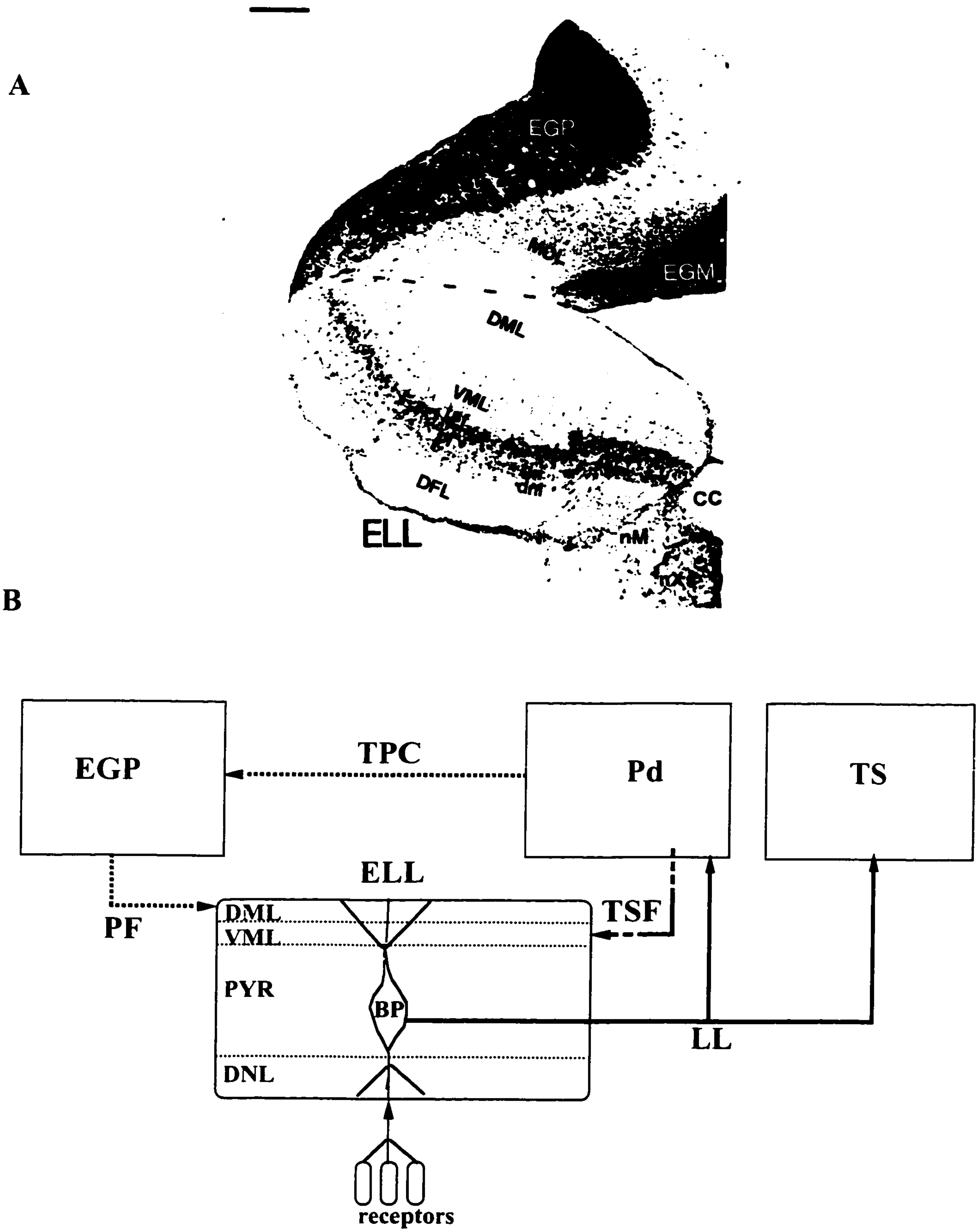


Figure 1: Electrosensory lateral line lobe

Figure 1. Electrosensory lateral line lobe.

A: Cresyl violet-stained section (25 μm thick) through medulla showing the topographic relations and the laminar structure of the electrosensory lateral line lobe (ELL. Scale bar= 250 μm).

B: Schematic of the connections between the ELL and Pd.

BP: basilar pyramidal cells; CC: the crista cerebellaris; DFL: deep fiber layer of ELL; DML: dorsal molecular layer of ELL; DNL (dnl): deep neuropil layer of ELL; EGM: eminentia granularis pars medialis; EGP: eminentia granularis posterior; gr: granule cell layer of ELL; MOL: molecular layer of EGP; LL: lateral lemniscus; nM: nucleus medialis; nXs: sensory nucleus of the vagus; Pd: nucleus preeminentialis dorsalis; PF: parallel fibers; pl: plexiform layer of ELL; PYR (pyr): pyramidal cell layer of ELL; TPC: tractus preeminentialis cerebellaris; TS: torus semicircularis; TSF (tsf): tractus stratum fibrosum of ELL; VML: ventral molecular layer of ELL.

cell layer is characterized by large efferent neurons, the pyramidal cells. There are 2 types of pyramidal cells: basilar and non-basilar. Both types of pyramidal cells extend apical dendrites up to the molecular layer.

The basilar pyramidal cells extend their basal dendrites down to the deep neuropil layer contacting the P-type primary afferents and the non-basilar cell is activated indirectly by these afferents via granule cells. 6): The stratum fibrosus (TSF) consists of myelinated fiber bundle of a direct feedback pathway from nucleus preeminentialis (Maler, 1979; Maler, Sas and Rogers, 1981). 7): The ventral molecular layer (VML) is the termination zone of the TSF feedback pathway (Bastian and Bratton, 1990; Maler, Sas, Carr and Mitsubara, 1982). These fibers form synapses with the spines of proximal apical dendrites as well as with GABAergic VML interneurons which inhibit the pyramidal cells. Recent immunocytochemistry has shown a rich distribution of α–CaMKII in the ventral molecular layer (L.Maler, personal communication) and it' s likely that CaMKII plays an important role in regulating the function of the direct feedback system. 8): The dorsal molecular layer (DML) is the termination zone of parallel fibers from the overlying caudal cerebellar granule cells, which form an indirect feedback system (Bastian and Bratton, 1990; Maler, Finger and Karten, 1974; Sas and Maler,1987). They excite the pyramidal cells by contacting the spines of pyramidal apical dendrites and strongly inhibit the pyramidal cells by activating type-1 granule cells, VML cells and stellate cells.

The four segments have different functions. While the medial segment receives input from the low frequency electrosensory ampullary receptors (the low frequency information processing part will not be discussed further), the other three segments receive information encoded by the high frequency tuberous receptors (Carr and Maler, 1986; Heiligenberg and Dye, 1982).

Even among the 3 tuberous segments there are differences in structure and function (Turner, Plant and Maler, 1996). The axons of T- and P-type afferents trifurcate in ELL and terminate somatotopically in each of the 3 segments (Lannoo, Maler and Tinner, 1989), there is a smaller convergence of the primary afferents in the CMS than in the LS (Shumway, 1989b). This difference indicates that CMS may have a smaller receptive field and higher spatial resolution than LS (Shumway, 1989b). The CMS has 10 times fewer inhibitory VML neurons than the other 2 segments. The VML neurons are activated by the inhibitory component of the direct feedback pathway and their activation inhibits the pyramidal cells. Fewer VML neurons in CMS means that inhibitory components of the direct feedback pathway may not play an important role in regulating the pyramidal cell activity. Physiological data also show that in a center-surround receptor field of the pyramidal cells the strength of surround inhibition is the strongest in CMS. While the pyramidal cells in LS prefer a high frequency EOD amplitude modulation (AM), those in CMS strongly respond to a low frequency EOD amplitude modulation (Shumway, 1989a). The ELL projects bilaterally to 2 higher centers: torus semicircularis and nucleus preeminentialis dorsalis (Pd).

I.III.III. Torus semicircularis (torus)

Torus is a laminated midbrain structure that receives electrosensory as well as mechanoreceptive and auditory inputs. The electrosensory input from the ELL terminates in the dorsal torus. The efferent fibers of the spherical cells project contralaterally to lamina 6 of the torus where the differential phase is computed (Carr, Maler, Heiligenberg and Sas, 1981). The axons of the pyramidal cells gather in a large bundle at the rostromedial pole of the ELL and turn ventrally where most fibers decussate forming the lateral lemniscus (LL). The fiber bundle runs towards the torus and fan out to distribute in several layers of torus. The vertical connections between different layers of the torus produces a convergence of amplitude and phase information

(Heiligenberg, 1986).

The toral efferents come from neurons in many layers and have several targets, including the Pd. The neurons projecting to Pd are found in all layers except 1,6,8B and these projections are ipsilateral. The axons enter the Pd laterodorsally and distribute approximately to the same parts of the nucleus as those for the axons from the ELL (see below). These descending projections to Pd are belived to regulate the Pd feedback pathways (Carr et al., 1981; Carr and Maler, 1986).

I.III.IV. Nucleus preeminentialis (Pd) and the feedback pathways

While the lateral lemniscus fibers are on the way to the torus, they go rostrolaterally to the medial aspect of the nucleus preeminentialis (Pd) and give off collaterals which project bilaterally and somatotopically (Maler, et al., 1982; Sas and Maler, 1983). Pd is further divided into 3 parts. The medial part of Pd (Pdmp) receives ELL projections. The principal part of Pd (Pdpp) is divided into dorsal, central and ventral zones which are reciprocally and topographically connected with the ELL segments: MS with the ventral zone, CMS and CLS with the central zone, and LS with the dorsal zone. The projection neurons send out feedback fibers to the ELL directly or via posterior eminentia granularis (EGP), a cerebellar structure overlying the ELL (Bastian and Bratton, 1990; Bratton and Bastian, 1990; Sas and Maler, 1983). The lateral part (Pdlp) receives inputs from the torus.

1. The direct feedback pathway:

This direct feedback pathway originates in Pdpp stellate cells, which give out excitatory part of the pathway, and bipolar cells, which give out inhibitory part.

An *in vivo* electrophysiological experiment (Bratton and Bastian, 1990) showed that the stellate cells are stimulated by amplitude modulation of the EOD and they respond phasically to 4-16 Hz of amplitude modulation frequency. Given optimal inputs the stellate cells discharge at 100-300 Hz in the first 100 ms. However, they do not respond to amplitude modulation (AM) frequency of more than 16 Hz. Below 10 Hz of AM frequency the stellate cells fire at an average of 100 Hz. These cells also respond well to moving electrolocation targets.

The output fibers from stellate and bipolar cells in Pd form a myelinated fiber tract (the tractus stratum fibrosus, TSF) and this tract goes down to the ELL. In the ELL, the excitatory fibers from stellate cells are located in the dorsal part of the TSF, where they lose their myelination and synapse on the proximal apical dendrites of pyramidal cells in VML and therefore the pyramidal cells and stellate cells form an excitatory reciprocal and topographical circuit. Paralleling the excitatory fibers, the inhibitory fibers in ventral TSF diffusely terminate on the pyramidal cell somata (Maler, 1979; Maler and Mugnaini 1993). Recent electrophysiological results showed that the TSF-VML synapse has an NMDA receptor component with non-linear conductance (see discussion for explanation of function).

The anatomical and physiological characteristics suggest that the direct feedback pathway may serve as a searchlight mechanism, as proposed by Crick (1984) (see discussion).

A function of "central negative image" has also been proposed. It has been found that the neurons in the ELL of mormyrid electric fish developed an insensitivity to some repetitive pattens of sensory input and to reafferent stimuli (Bell, 1981). This insensitivity results from the cancellation of these unwanted inputs by centrally produced opposing synaptic inputs termed "negative image inputs". (see discussion for details).

In addition, the feedback pathway has also been proposed to enhance electrolocation. The EOD amplitude modulation comes from moving electrolocation targets (0.6-2.5 Hz) and neighbouring electric fish. When the beat frequencies (the frequency difference between 2 fish's EODs) are very low (1-10 Hz) the EOD modulations caused by electrolocation targets and the beat frequencies are similar and the ability of the system to locate the target is reduced since the stellate cells are very sensitive to both EOD AM modulations.

2. The indirect feedback pathway: The indirect feedback pathway originates from multipolar cells in Pdpp, which connects Pd, EGP and pyramidal cells. It acts as a gain control circuit to the pyramidal cells. When the average amplitude of the EOD changes the indirect pathway ensures a stable output from pyramidal cells (Bastian, 1986a; 1986b).

I.III.V. Functions of the electrosensory system

The electrosensory system has 2 major functions: electrolocation and electrocommunication (Bullock, 1986; Heiligenberg, 1986). Electric fish use electrolocation to locate objects in its immediate surrounding. The high frequency EOD generated by its electric organs forms an electric field surrounding its body. An object that has a different conductance from the ambient water may distort the EOD field. This distortion alters the firing rate of primary afferents. Since the inputs to the higher centers are topographically arranged the exact site of distortion can be detected (Bastian, 1986c). The fish use electrocommunication during social activity. For example, the neighbouring fish detect each other through the interference with their own EODs. The system can detect the frequency difference between 2 fish (beat frequency) (Heiligenberg, 1990).

I.III.VI. Comparison with other sensory systems

The electrosensory system shares some commonalities with other sensory systems (auditory or visual) in extracting four elementary attributes of a stimulus: the modality, intensity, duration and location.

Receptive field:

The adequate stimulus to the tuberous electrosensory receptors is the EOD (Bastian, 1976; Scheich, Bullock and Hamstra, 1973) rather than sound, light or touch. Each receptor has its own receptive field where it detects the EOD and, upon activation, it transduces the electric stimulus into action potentials of primary afferent fibers. The firing frequency of these fibers depends on the intensity of the EOD (coded by P-type receptors). A second order neuron has a larger receptive field than a single receptor since a number of primary afferents converge onto a pyramidal cell or a spherical cell (Shumway, 1989a,b). Firing of a spherical cell requires synchronized activation of all the T-unit afferents that converge to it. It faithfully relays the timing signals to the higher center (TS) at 1:1 ratio. P-unit afferents from a small area of skin excite a basilar pyramidal cell of the ELL directly and inhibit the surrounding basilar pyramidal cells via inhibitory interneurons (center on/ surround off); thus a basilar pyramidal cell is defined as an E-unit electrophysiologically. The input from these receptors may inhibit the nonbasilar pyramidal cells via the inhibitory type-2 granule cells and the surrounding nonbasilar pyramidal cells may be excited due to the disinhibition (center off/surround on); thus a nonbasilar pyramidal cell is defined as an I-unit electrophysiologically. This is much like the ON and OFF signal pathways in ganglion cells of the retina.

Topography:

The electric receptors over the fish's body are represented in an orderly fashion in the ELL, torus and Pd in such a manner that the neighbouring relations in the periphery are faithfully preserved. There are 4 copies of the body image in the ELL (Carr et al., 1982; Heiligenberg and Dye, 1982). They are remapped in the dorsal portion of Pd (Maler et al., 1982; Sas and Maler, 1983) and converge to one map in the dorsal torus (Carr, et al., 1981). Similar topographical organizations define the somatotopy, tonotopy and retinotopy of the somatosensory, auditory and visual systems, respectively.

Feedback regulation:

In higher animals the auditory system has an extensive set of feedback pathways in its hierarchial structures. The auditory cortex sends its axons back to the medial geniculate nucleus and to the inferior colliculus, which in turn, send fibers down to the cochlear nucleus or to the hair cells to affect sensitivity of receptors to the sound and to affect feature extraction of sound as well as sound location by the subcortical nuclei (Huffman and Henson, 1990). Similar feedback is seen in the visual and somatosensory systems.

The electrosensory system of the electric fish also has feedback pathways from the torus through ELL. Most studies have focused on the Pd-ELL feedback pathway.

I.IV. Objectives

I.IV.I. To determine if EAAs are used as transmitters in the direct feedback pathway

Morphological (Maler et al., 1981) and physiological (Saunders and Bastian, 1984) studies demonstrated that both the electrosensory afferents and the feedback inputs to the ELL are excitatory, but their transmitter is not clear. Biochemical (Nadi and Maler, 1987), binding (Maler and Monaghan, 1991) and electrophysiological (Bastian, 1993) studies indicated that excitatory amino acids are likely the transmitter candidates in the ELL. I propose that EAAs are the main excitatory neurotransmitter in the ELL, particularly in the direct feedback pathway.

I.IV.II. To characterize plasticity of the direct feedback pathway

Some forms of plasticity may exist in the direct feedback pathway in order for the Pd to regulate the response of the pyramidal cells in the ELL to EOD modulation.

II. MATERIALS AND METHODS

II.I. Materials and methods for immunocytochemistry

Five adult brown ghost fish (*Apteronotus leptorhynchus*) were used. The fish were immersed in a diluted solution (1:15000) of MS-222 (Sigma). After being completely anesthetized they were perfused with 4% paraformaldehyde and 0.3% glutaraldehyde (in 0.1 M PBS, PH 7.4), the brains were removed and postfixed overnight. Three brainstems were cut into 1 mm horizontal blocks, which were embedded in L-R White. Semi-thin sections (~1 μm thick) were cut through the brainstem and transferred to slides. The primary antibodies (rabbit), directed against glutamate or aspartate (Hepler, Toomim, McCarthy, Conti, Battaglia, Rustioni and Petrusz, 1988) conjugated to keyhole limpet hemocyanine with glutaraldehyde (Arnel Products Co.Inc., 119 Washington Place, NY, NY 10014), were used at dilutions of 1:10,000 (glutamate) and 1:8,000 (aspartate) in 0.1 M phosphate buffered saline (PBS). After overnight incubation in the primary antibody at room temperature, the sections were rinsed and incubated with biotinylated secondary antibodies (donkey anti-rabbit, Amersham) at a concentration of 1:200 in PBS for 2 hours at room temperature. Sections were rinsed and then incubated in streptavidin conjugated to the fluorophore CY3 (1:100, Sigma) in PBS for 1 hour at room temperature. Omission of the primary antibody or preabsorption with glutamate conjugated to keyhole limpet hemocyanin eliminated all immunoreactivity (Figure 2). Immunofluorescent staining was analyzed and photographed with an Olympus BH-2 microscope.

One millimeter thick vibratome sections of the electrosensory lateral line lobe (from 2 brains) were osmicated (1% in PBS for 2 hours) and embedded in araldite. Grey or silver sections were cut, collected on nickel grids and incubated at 37°C for 2 hours in the primary antibody as for light microscope (LM) but diluted in 0.05 M Tris-buffered

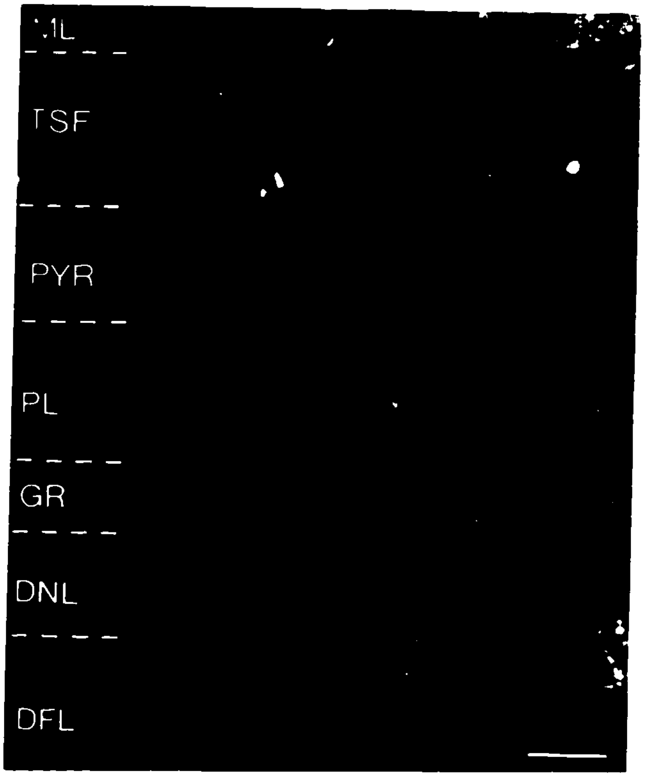


Figure 2: Control to immunofluorescent staining of ELL

Figure 2. Control to immunofluorescentstaining of ELL.

This control was done with preabsorbed antiglutamate antibody in semithin sections. No specific glutamate immunoreactivity is seen. **DFL**: deep fiber layer of ELL: **DNL**: deep neuropil layer of ELL; **GR**: granule cell layer of ELL; **ML**: molecular layer: **PL**: plexiform layer of ELL; **PYR**: pyramidal cell layer of ELL: **TSF**: tractus stratum fibrosum of ELL. Scale bar=15 μm.

saline (TBS). After washing in TBS the grids were incubated at 37°C for 1 hr in the goat anti-rabbit IgG secondary antibody conjugated to 10 nm gold particles. Finally, the grids were stained with uranyl acetate and lead citrate.

The gold particle density analysis for the ELL synapses was done with Image (N.I.H.).

II.II. Materials and methods for electrophysiology

II.II.I. Slice preparation

Brain slices were prepared from adult brown ghost fish of either sex (*Apteronotus leptorhynchus*). The fish were anesthetized with MS-222 (Sigma, 1:15000) and respirated (aerated water) on a holder via a pipette inserted into the mouth. The water flow was controlled by a flow meter (Fisher Scientific). The surgical operation was done using a Zeiss dissecting microscope. During the operation the body skin was moistened and the skin above the skull was cut and peeled off. The cranium was deflected laterally and cut off to expose the brain and cranial nerves; they were kept moist continuously by superfusion of artificial cerebrospinal fluid (ACSF). At this point the gill movement and the integrity of blood circulation on the ELL, which are indications of viability, must be observed. The cranial nerves were severed with iris scissors, the brain was cut with a micro scalpel at an angle of 30-45 degrees to the surface through the level of the diencephalon in the true transverse plane of the ELL (Mathieson and Maler, 1988) and transected from the spinal cord. The angle is critical for the continuity of TSF fibers in the ELL, improper cutting angles can result in weak evoked potentials or failure of recordings. The brainstem was removed, glued to a vibratome chuck with cyanoacrylate and embedded in 4% Agar (4 g/100 ml ACSF, boiled and cooled to 40-50 ^{0}C) for further support. Three or 4 slices of 300 μm thick were cut in cold (4 ^{0}C) oxygenated (95% O_2 / 5% CO_2) ACSF (for its components see

Table 1). The slices were flipped over and transferred to an interface chamber so that the rostral face of the ELL was uppermost. Since TSF fibers run rostrocaudally in the ELL, placing the slice rostral face up allows for optimal activation of the TSF. The slices were perfused with oxygenated ACSF at a flow rate of 2.5-3 ml/min, illuminated from above by a fiberoptic lighting system and visualized with a dissecting microscope. These procedures provided a clear view of ELL segment boundaries and lamination. Recording started after a 60-90 minute recovery period.

II.II.II. Stimulation

Stimulation was delivered via a unipolar tungsten electrode placed on the dorsal part of the TSF in the medial segment of the ELL; since TSF fibers run caudally and laterally within the ELL (Maler et al., 1982), this provides optimal activation of the TSF terminals within VML while preventing direct stimulation of CMS interneurons. Stimulus pulses were 10 μs in duration with an intensity that produced 2/3 of the maximal EPSP amplitude (30-50 volts for field and 3-10 volts for intracellular recording).

Baseline field EPSPs were collected for at least 15 minutes before tetanic stimulation; each pre-tetanus trial was the average of 20 recordings. I only initiated further exeriments in cases where the baseline recordings produced stable EPSPs (mean change <5%).

A burst containing 10 pulses at frequencies (1-350 Hz, in control trials no tetanization pulses were given) covering the physiological range was delivered 3-5 times at intervals of 1-3 sec.

In experiments designed to examine transient effects of tetanus (PTP), post-tetanic recordings were initiated 5 sec after the tetanus. Preliminary intracellular experiments had shown that tetanic stimulation could evoke long-lasting potentials (either plateau depolarizations or IPSPs often >1 sec) or entrain subthreshold oscillations (Turner et al., 1996); EPSPs evoked 1-3 sec post-tetanus were therefore very variable in amplitude which precluded analysis of very short term synaptic plasticity. StF-evoked EPSPs were recorded at 5 sec intervals for 2 minutes; because short term enhancement decayed rapidly, no averaging was done for the post-tetanic recordings. In experiments designed to study possible long term plasticity (LTP, LTD), I wished to avoid possible contamination by short term synaptic enhancement; I therefore initiated recording trials 5 minutes post-tetanus and 10 field EPSPs were averaged (1/30 sec; 5 minutes) to obtain the "10 minute" data point; in similar fashion, 5 minutes of data was averaged every 10 minutes for 60 minutes.

Timing of the stimulation was controlled by A/dvance software used to run the experiment (University of British Columbia and McKellar Design, Fine Science Tools Inc.). The digital signals were converted into analogue by a D/A converter (Acquisition board, ITC-16 MAC computer interface, Instrutech Corporation, Great Neck, NY), which drove an Axoclamp-2A (Figure 3: Hardware connections).

II.II.III. Field recordings

Field potentials were recorded from the VML in the ELL centromedial segment (CMS) using glass microelectrodes (1-20 mΩ) filled with 3 M NaCl. Recording pipettes were placed in slices at a depth of about 50 μm (Figure 4) using a Narishige hydraulic manipulator. A stable baseline (mean change ≤5%) was collected for at least 15 min (3 trials). Each pretetanus trial came from the average of 20 recordings collected individually at an interval of 15 second. In experiments designed to examine transient effects of tetanus (posttetanic potentiation; PTP) posttetanic recordings were initiated 5 sec after the tetanus with an attempt to avoid the contamination by a plateau or inhibitory potentials produced by the tetanization (see discussion for explanation). The data were collected at 5 s intervals for 2 min ; because the expression of PTP was transient, no

TABLE 1: Normal ACSF

Supplies	Molecular weight	Concentration (mM)	Weight for 4L
NaCl	58.44	124	28.99
$NaHCO_3$	84.01	24	8.06
D-Glucose	180.16	10	7.21
$KH2PO_4$	136.09	1.25	0.68
KCl	74.56	2	0.60
$MgSO_4$	120.37	2	0.96
$CaCl_2$	110.99	2	0.89

TABLE 2: Mn^{2+} Containing ACSF

Supplies	Molecular weight	Concentration (mM)	Weight for 1L
NaCl	58.44	124	7.25
HEPES	238.30	20	4.766
D-Glucose	180.16	10	1.81
Tris*	121.10	11.4	1.38
KC l	74.56	3.25	0.24
$CaCl_2$	110.99	0.2	0.022
$MnCl_2$	197.91	4	0.79

* The pH before putting $MnCl_2$ should be 7.2.

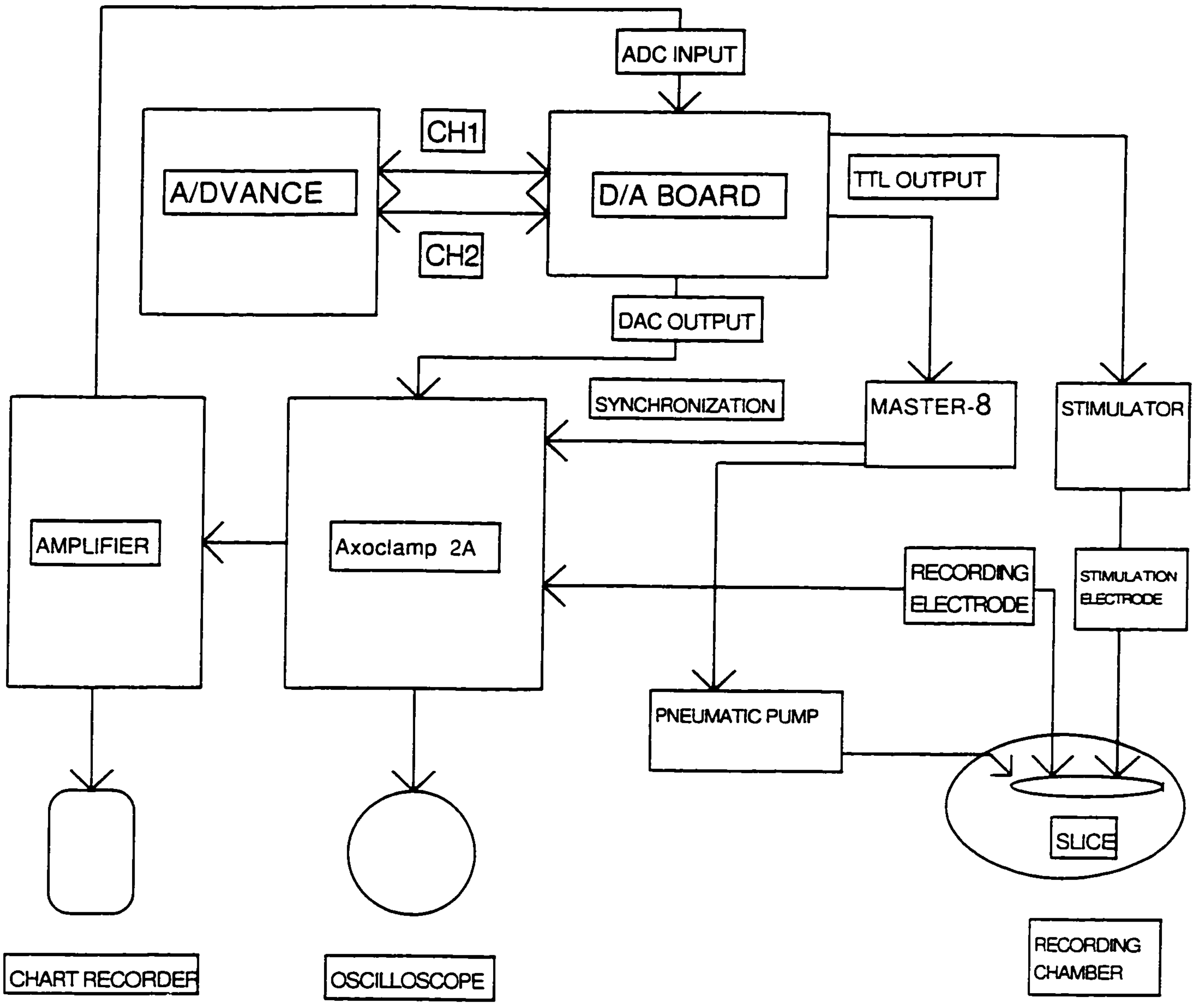


Figure 3: Hardware connections

Figure 3: Hardware connections. The computer-controlled data collection is carried out by connecting equipment to the data acquisition unit. The TTL output port of the unit controls the stimulators and Master-8 which synchronizes stimulation and recording. The DAC port controls the Axoclamp-2A for current or voltage ejection. Data collected by the Axoclamp-2A is fed to the ADC port of the unit.

averaging was done for the post-tetanic recordings. In experiments designed to study long term plasticity (long term potentiation or depression: LTP, LTD), recordings started 5 min after tetanus and 10 recordings were averaged (1/30 sec; for 5 minutes) to obtain from each cell the "10 minute" data point; in similar fashion, 5 minutes of data was averaged every 10 minutes for 60 minutes.

II.II.IV. Intracellular recording

Intracellular recordings were made from the pyramidal cell layer of the ELL with glass micropipettes (Figure 4) pulled on a Flaming/Brown micropipette puller (Model P-87, Sutter Instrument Company, San Rafael, California), filled with 3M potassium acetate and bevelled to a resistance of 70-130 mΩ (K.T.Brown type micropipette beveller, Model BV-10, Sutter Instrument Company, San Rafael, California). I recorded from the pyramidal cell layer which contains mainly the pyramidal cells (>90%, Maler, 1979; Turner, Maler, Deerinck, Levinson and Ellisman, 1994). A Burleigh microdrive (Model 6000, Burleigh Instruments Inc. Fishers, NY) was used to impale pyramidal cells.

Output from the preamplifier (AxoClamp 2A) were amplified (by a Tektronik 502) and digitized by the A/D converter. The A/dvance software (see above) was used to collect and average the data. Further data analysis was done with IgorPro (Wavemetrics) and Statistica (Release 4.1) on a Macintosh computer (see Figure 3).

Input resistance was monitored by injection of hyperpolarizing current pulses.

The data for PTP, LTP and LTD was collected as described above.

In evaluating the effects of some drugs on the presynaptic vs postsynaptic elements paired pulse facilitation was examined. The time interval between the 2 pulses was 50 ms to ensure that the 2nd pulse started after the 1st returned to the baseline. The paired pulse change was routinely expressed as the percentage increase of the 2nd EPSP from the 1st EPSP and such percentage changes were compared before and after drug application.

The drug effects on plasticity were also evaluated by comparing the plasticity changes before and after the drug application.

Recent *in vivo* work by Bastian (1996a.b) has demonstrated an anti-Hebbian plasticity in this direct feedback system. I therefore attempted to mimic Bastian's *in vivo* experiment in the slice preparation. The pre-test trials were collected in the same way as before and the tetanization frequency was 100 Hz. which falls within the physiological range (Bratton and Bastian.1990). A 160 ms hyper- or depolarizing current pulse of 0.6 nA was applied 50 ms before the tetanus started to get a stable membrane potential. I was concerned that possible long term alterations in TSF transmission might decrement upon testing (Bastian. 1996b). I therefore collected only a single sample of data following tetanus (at 5. 10. 15 or 20 min posttetanus). The input resistance was also monitored (see above). In some experiments I simultaneously recorded field potentials in the TSF in order to monitor the stability of the fiber volley.

II.II.V. Application of pharmacological agents

Drugs were administered by bath. pneumatic pressure ejection. and direct intracellular application (Figure 4).

Drugs administered by bath application were delivered to the slices in ACSF to affect the slices globally. The change between normal ACSF and drug-containing ACSF was controlled by an electronic valve (General Valve. Fairefield. New Jersey). Four mM $MnCl_2$ ($MnCl_2$. Fisher Scientific Company; with 0.02 mM $CaCl_2$) in ACSF (see Table 2 for components) was used to block Ca^{2+}-mediated synaptic transmission (Bagust and Kerkut. 1980; Berman. Plant. Turner and Maler. In press: Mathieson and Maler. 1988). The TSF-evoked VML field potential was recorded 1 hr after the Mn^{2+}

application and compared with those evoked in normal ACSF.

Seventy micromole bicuculline methochloride (BIC., from Sigma). dissolved in distilled water at 70 mM and further diluted in ACSF to 70 µM prior to use. was applied to the pyramidal cell layer to block the GABA-A inhibition. The VML field potential evoked by TSF stimulation was recorded from both the pyramidal cell layer and VML at least 15 min after the BIC application and compared to those evoked in normal ACSF.

One hundred nM D.L-2-amino-5-phosphonovaleric acid (APV. from Sigma). dissolved in NaOH at 100 µM and further diluted in ACSF to 100 nM. was applied for 15 min before starting the intracellular recording to block NMDA-receptor mediated synaptic transmission. The data were compared with those in normal ACSF.

Those drugs applied by pneumatic pressure ejection were administered over the synaptic termination of TSF fibers in VML or DML.

The pipettes were pulled with the same puller mentioned above. broken back to a tip diameter of 1 micrometer and backfilled with drugs. The ejection pressure was kept at 20 psi and delivered at 1 drop per 50 s. The timing was controlled by the Master-8.

Three point five µM 1-(N.O-bis-[5-isoquinolinesulfonyl]-N-methyl-L-tyrosyl)-4-phenyl-piperazine (KN-62 from Sigma. dissolved in DMSO at 3.5 mM and further diluted in ACSF to 3.5 µM with DMSO<0.1%). is a selective protein CaMKII antagonist (Tukumitsu et al.. 1990). was applied to VML or DML at least 10-15 min before data collection. The data were then compared with the control containing DMSO and N-[1-[N-methyl-p-(5-isoquinolinesulphonyl)benzyl]-2-(4-phenylpiperazine)ethyl]-5-isoquinolinesulfonamide (KN-04. from Seikagaku America. Inc.. 30 West Gude Drive. Suite 260. Rockville. Maryland 20850-1161. USA.. dissolved in DMSO at 10 mM and further diluted in ACSF to 3.3 µM. DMSO<0.1%). KN-04 is a structural analog to KN-62 but does not antagonize the CaMKII (Ishikawa. Hashiba and Hidaka. 1990). therefore it was used as a control; 3.3 µM KN-04 was used in the same way as

KN-62.

Point five µM Calyculine-A (from RBI, Dissolved in DMSO at 1 mM and then further diluted prior to use in ACSF to 0.5 µM, DMSO<0.1%), which is a specific antagonist to both protein phosphatases 1 and 2A, was applied 10-15 min prior to the recordings.

One to 10 µM FK-520 (a gift from Merck & Co. Inc., Dissolved in DMSO at 10 mM and then further diluted prior to use to 1 µM and 10 µM, DMSO<=0.1%) was utilized to antagonize the protein phosphatase 2B. Initially, 1 µM FK-520 was puffed to the slice surface for 10-15 min before the data collection. Since no inhibitory effect was seen, the concentration was increased to 10 µM and the incubation time to 30 min.

A mixed solution of 1 µM FK-520 and 0.5 µM calyculine-A (FK-520 was diluted from 10 mM to 2 µM with DMSO=0.02%; calyculine-A was diluted from 1 mM to 1 µM with DMSO=0.1%, such that the final concentrations were: FK-520=1 µM, calyculine-A=0.5 µM, DMSO=0.06%). Since the antagonist to either PP1, 2A or PP2B alone did not show any effect, these antagonists were applied together with at least 15 min incubation time.

One point one mM CaMKII inhibitory peptide (from RBI, sequence: Met(+-1)-His(+2-1)-Arg(+2-1)-Gln(+-1)-Ala(+-1)-Val(+-1)-Asp(+-1)-Cys(+-1)-Leu(+-1)-Lys(+2-1)-Lys(+2-1)-Phe(+-1)-Asn(+-1)-Ala(+-1)-Arg(+2-1)-Arg(+2-1)-Lys(+2-1)-Leu(+-1)-Lys(+2-1)-Gly(+-1)-Ala(+-1) (Σ+=29, Σ-=21, net charge=+8)). The peptide was dissolved prior to use by warming it to room temperature in a desiccator and then dissolving it completely in double distilled water at 1.1 mM; 3 M potassium methyl sulphate ($KmeSo_4$) was then added to the 1.1 mM peptide solution (Malinow et al., 1989). The recording electrodes had tip resistance of 80-100 mΩ and were back filled from the tip with 1.1 mM peptide solution; the shaft was filled with 3M KCl. An

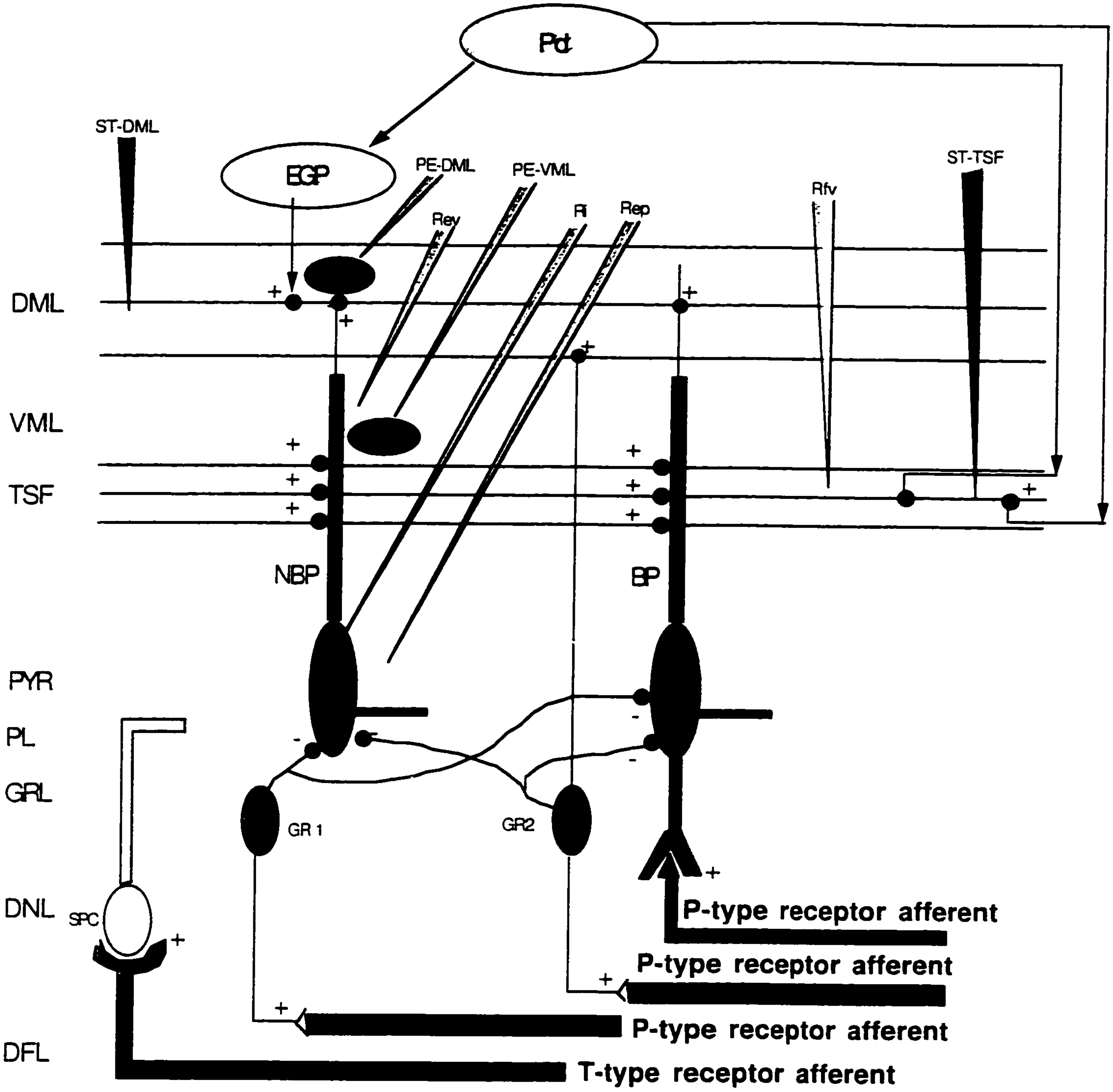


Figure 4: Schematic of locations for stimulation, recording and drug application

Figure 4: Schematic of locations for stimulation, recording and drug application.

In all intra- and extracellular recordings from VML, stimulation electrode (ST-TSF) was placed in TSF; The field recording electrode was positioned in VML (Rev), or PYR (Rep); Ri represents the position of the electrode for intracellular recording. The sites for pharmacological application via pneumatic pressure ejection are indicated by PE-VML. For some intracellular recordings the fiber volley change was also monitored at the location indicated by Rfv. For DML intracellular recording, the sites of stimulation and drug application are shown by ST-DML and PE-DML respectively. DFL: deep fiber layer; DNL: deep neuropil layer; GRL: granule cell layer; PL: plexiform layer; PYR: pyramidal cell layer; TSF: the tractus stratum fibrosum; VML: ventral molecular layer; DML: dorsal molecular layer; Pd: nucleus preeminentialis dorsalis; EGP: eminentia granularis posterior; NBP: non-basilar pyramidal cells; BP: basilar pyramidal cells; GR1: granule cell type 1; GR2: granule cell type 2; SPC: spherical cells.

incubation time of 30 min was allowed for the peptide to get into the cell.

KN-62 is membrane permeable and its effects can also be expressed by intracellular application (Stanton and Gage, 1996). Prior to use, KN-62 was dissolved to 10.5 μM in DMSO and the recording electrodes were filled in a similar way as with the CaMKII inhibitory peptide. A minimum of 20 min incubation time was allowed for the drug to diffuse into the cell.

II.II.VI. Data analysis

Standard measurements included the peak amplitude of the evoked EPSP. time to peak and slope for both extra- and intracellular recordings; in all intracellular recordings the measurements reflecting the electrophysiological properties of the pyramidal cells (resting membrane potential, input resistance from an I-V curve, spike amplitude. and time to the half amplitude) were routinely taken. In some cases. amplitude at 40 ms was also measured to monitor the change in IPSP (Figure 5). Statistics were done with the raw data unless stated otherwise and significance was assessed by either a T-test or Anova. For clarity in the text and graphs most values were expressed as a percentage and the mean ± standard error were calculated. The N, the number of observations was given in recordings. slices or cells wherever applicable: if the number of cells equals to that of the slices. the N was given in cells/slices.

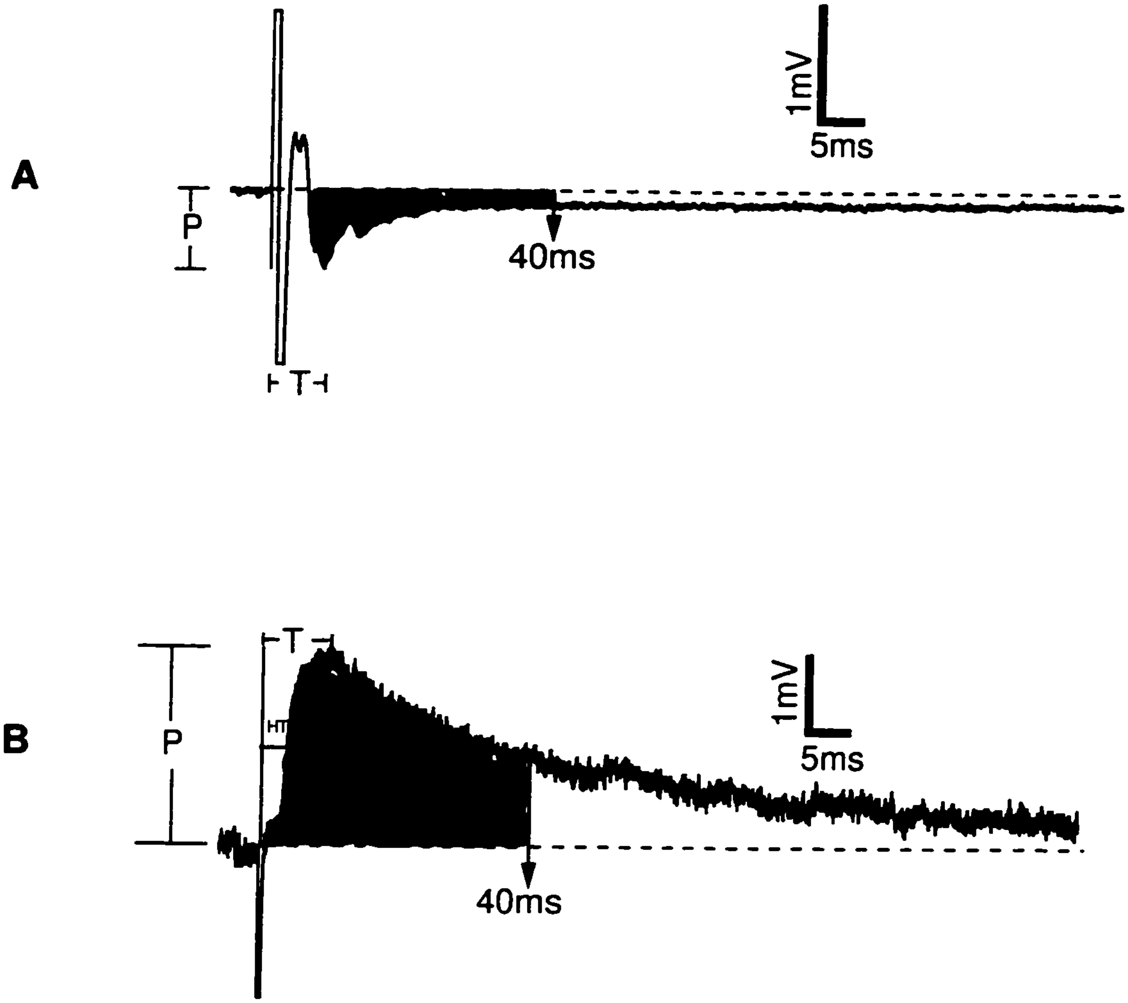


Figure 5: Standard measurements in field and intracellular recordings

Figure 5: Standard measurements in field and intracellualr recordings.

A: In field recordings the peak amplitude (P) was measured as the difference between baseline (dashed line) and and the peak of field EPSP; the time to peak (T) was taken as the the time required from the start of the artifact to the peak of the field potential; the area within 40 ms is shadowed and represented by A.

B: In intracellualr recordings the peak amplitude (P) and time to peak were taken in the same way as in the field recording. The time to half peak amplitude (HT) was taken as the time required for the amplitude to reach 50% of the peak; the area of the response within 40 ms is shadowed.

III. RESULTS

III.I.Immunocytochemistry

At the LM level, the immunofluorescent staining showed that aspartate immunoreactivity was only found in a few medially located anterior lateral line nerve ganglion cells (Figure 6A: arrows). Axons emanating from these cells ran through the deep fiber layer of the electrosensory lateral line lobe into the adjacent lateral line region, the nucleus medialis (Maler et al., 1974). Aspartate-immunoreactivity was not seen in the electrosensory lateral line lobe or other brainstem electrosensory structures. I conclude that aspartate is unlikely to be a transmitter within brainstem electrosensory nuclei although it may, together with glutamate (see below), be a transmitter of mechanoreceptive afferents.

Preabsorption of the primary antibody against glutamate with glutamate completely blocked all immunoreactivity (Figure 2).

Intense glutamate-immunoreactivity was found within all anterior lateral line nerve ganglion cells and their axons in the deep fiber layer and deep neuropil layer; the profiles of their somata were round or bipolar (Figure 6B: arrowhead).

Pyramidal cells of the electrosensory lateral line lobe (Figure 7A: white arrow), including the small deep basilar pyramidal cells within the granular cell layer (Bastian and Courtright, 1991) (Figure 7A: white arrowhead) were also intensely labelled by glutamate antibody as were their efferent axons within the plexiform layer (Figure 7B: white arrow and curved white arrow).

Proximal apical and basilar dendrites of pyramidal cells were intensely stained (Figure 7B: black arrow). A few medium-sized cells with triangular shaped somata within the deep fiber layer were also intensely glutamate-immunoreactive, but I could not associate these with previously described neurons of the electrosensory lateral line

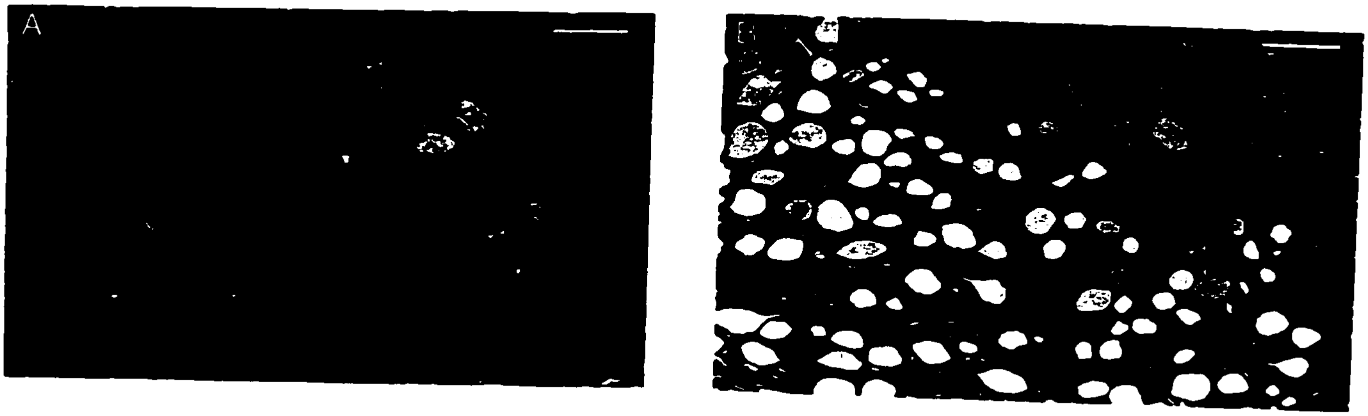


Figure 6: Immunofluorescent staining of anterior lateral line ganglion cells

Figure 6. Immunofluorescent staining of anterior lateral line ganglion cells.

A: Four aspartate immunoreactive cells (arrows) are seen at the medial edge of the anterior lateral line ganglion. Scale bar=200 μm.

B: Glutamate-immunoreactive cells in a section adjacent to A. All the ganglion cells and fibers are glutamate immunoreactive. Arrows point to the 4 medially located aspartate-immunoreactive cells (see A). Note that these 4 cells are less intensely stained than the rest which are not aspartate immunoreactive. The arrowhead points to a bipolar cell. Scale bar=200 μm.

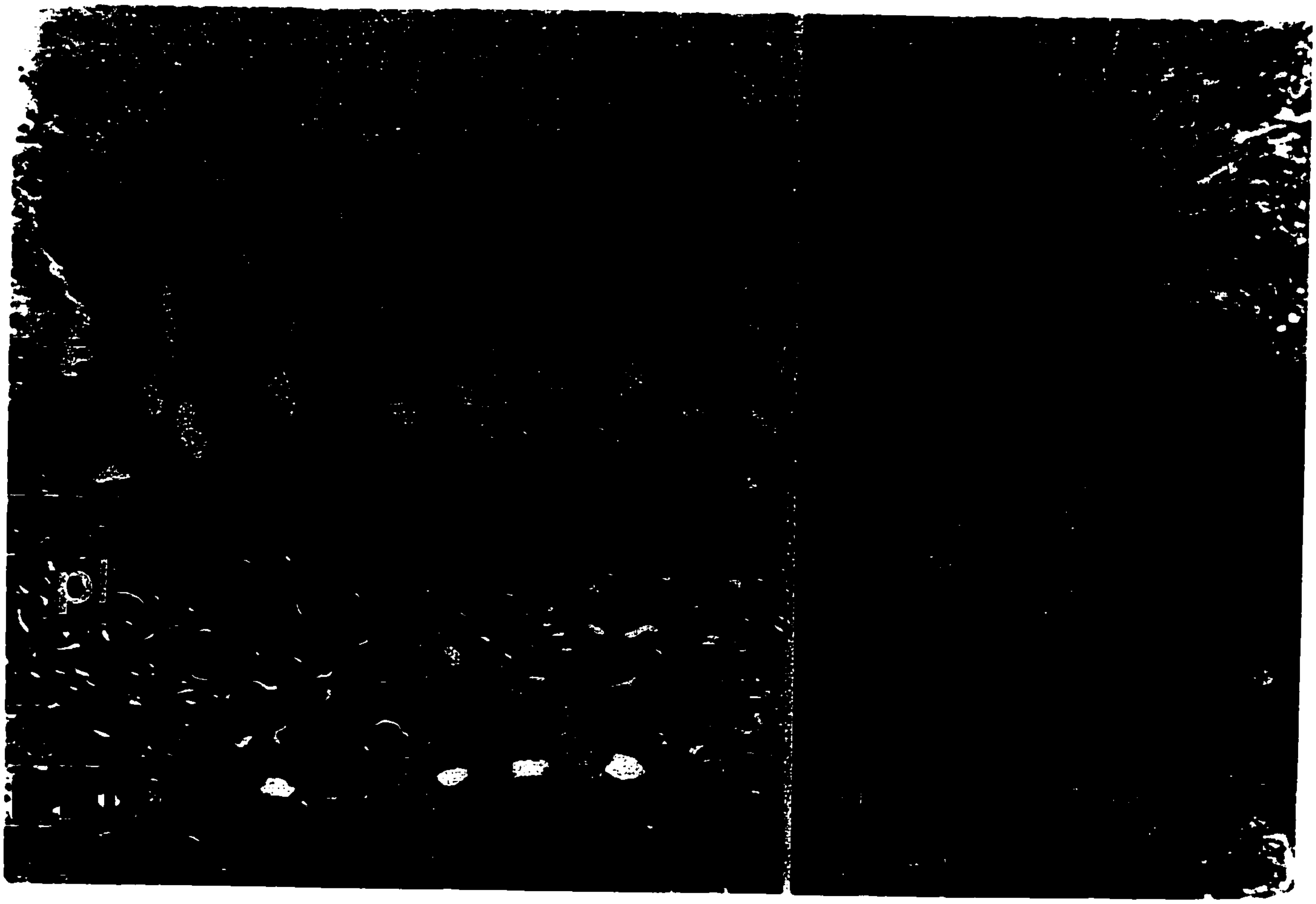


Figure 7: Immunofluorescent staining of electrosensory lateral line lobe

Figure 7. Immunofluorescent staining of electrosensory lateral line lobe.

A: Glutamate immunoreactivity is seen within electrosensory afferents in the deep fiber layer (**DFL**) and ELL efferent axons in the plexiform layer (**pl**). Granule cells in the granule cell layer (**gr**) are lightly labeled (black arrow). Spherical cells in the deep neuropil layer (**DNL**; curved white arrow) and pyramidal cells (white arrow) in the pyramidal cell layer (**pyr**) are intensely labeled; a deep basilar pyramidal cell (white arrowhead) is seen in the granule cell layer. Note that the apical dendrites of pyramidal cells within the tractus stratum fibrosum (**TSF**) are also intensely glutamate immunoreactive. The molecular layer (**ML**) is strongly but diffusely labeled. Scale bar =15 μm.

B: At higher magnification the axon hillock (white curved arrow) and proximal apical dendrites (black arrow) of a pyramidal cell (**pc**) are strongly glutamate immunoreactive. Axons in the TSF are strongly labeled, as are pyramidal cell axons (white arrow) in the plexiform layer (pl). Scale bar = 3.3 μm.

lobe (Maler, 1979).

Spherical cells (Maler, 1979) within the deep neuropil layer were also intensely stained (Figure 7A: curved white arrow). These cells receive electrosensory input from T-units (Maler et al., 1981; Mathieson, Heiligenberg and Maler, 1986) and project to the torus semicircularis (Carr et al.,1981), where they form mixed synapses (electrical and chemical) with cells in lamina 6 (Carr, Maler and Taylor, 1986).

Granular interneurons of the electrosensory lateral line lobe were lightly labelled (Figure 7A: black arrow) in comparison to pyramidal and spherical cells.

The fibers in the TSF were positively stained (Figure 7B: TSF) and their termination zone in the ventral molecular layer was intensely but diffusely stained at the light microscopic level (Figure 7A: ML). Based on their size and distribution, the glutamate-immunoreactive cells in the nucleus preeminentialis include small stellate cells which give rise to the tractus stratum fibrosum, and large cells including at least boundary cells (Sas and Maler, 1983). The dorsal molecular layer of the electrosensory lateral line lobe was also intensely but diffusely stained at LM level (Figure 7A: ML).

The distribution and density of glutamate-immunoreactive particles was compared across (a) synapses of the primary afferents in deep neuropil layer (N=38); (b) synapses in the pyramidal cell layer (N=40); (c) synapses in ventral molecular layer (terminals of tractus stratum fibrosum; N=104) and (d) synapses of the parallel fibers in dorsal molecular layer (N=128); I also estimated the density of glutamate-immunoreactive particles within somata and dendrites of pyramidal cells (N=29) and in glial cells (N=20). The immunogold particles were distributed throughout the terminals of primary afferents in the deep neuropil layer (mean and standard error: $22 \pm 1.4 / \mu m^2$), with a greater density over the area where synaptic vesicles were located (Figure 8C). In boutons within the pyramidal cell layer, gold particles are relatively sparse

($11\pm1.3/\mu m^2$; Figure 9). Glutamate-immunoreactive particles were most prominent in boutons within the ventral molecular layer (27 ± 0.8 / μm^2; Figure 8B) and were found at a lower density in parallel fiber boutons of the dorsal molecular layer (17 ± 0.8 / μm^2; Figure 8A). Glial cells had a very low density of immunogold particles (4 ± 1.9 / μm^2; Figure 8A, B, C: G). As expected from the LM, pyramidal cell dendrites had numerous immunogold particles (19 ± 1.6 / μm^2; Figure 9: PC). Statistical analysis (ANOVA) showed that the density of particles in all neuronal elements were significantly higher than those in glial cells ($p<0.001$) (Figure 10). The terminal boutons of all 3 excitatory inputs to the electrosensory lateral line lobe had a significantly higher density of immunogold particles than terminal boutons in the pyramidal cell layer ($p<0.001$). The particle density of ventral molecular layer, in turn, was significantly higher than the other 2 excitatory inputs to the electrosensory lateral line lobe ($p<0.001$).

III.II. Electrophysiology

III.II.I. Induction of posttetanic potentiation

Field potential recordings

Stimulation of the TSF produced a characteristic biphasic field potential in the VML (Figure 11 A trace 1): an initial positivity followed by a negativity. The negativity was typically 0.5-2 mV in amplitude (depending on stimulus intensity) and peaked with a latency of about 6 ms (6.17 ± 0.38 ms, N=30 recordings). Current source density analysis and pharmacological manipulations (Berman, et al., in press) have demonstrated that this negativity primarily reflects TSF evoked EPSPs in pyramidal cells. The field EPSP remained stable (<5% drift) for the 6 pretrials (Figure 11B). In control experiments (without tetanic stimulation) the field EPSP remained stable for at least 90 minutes (Figure 11B-2).

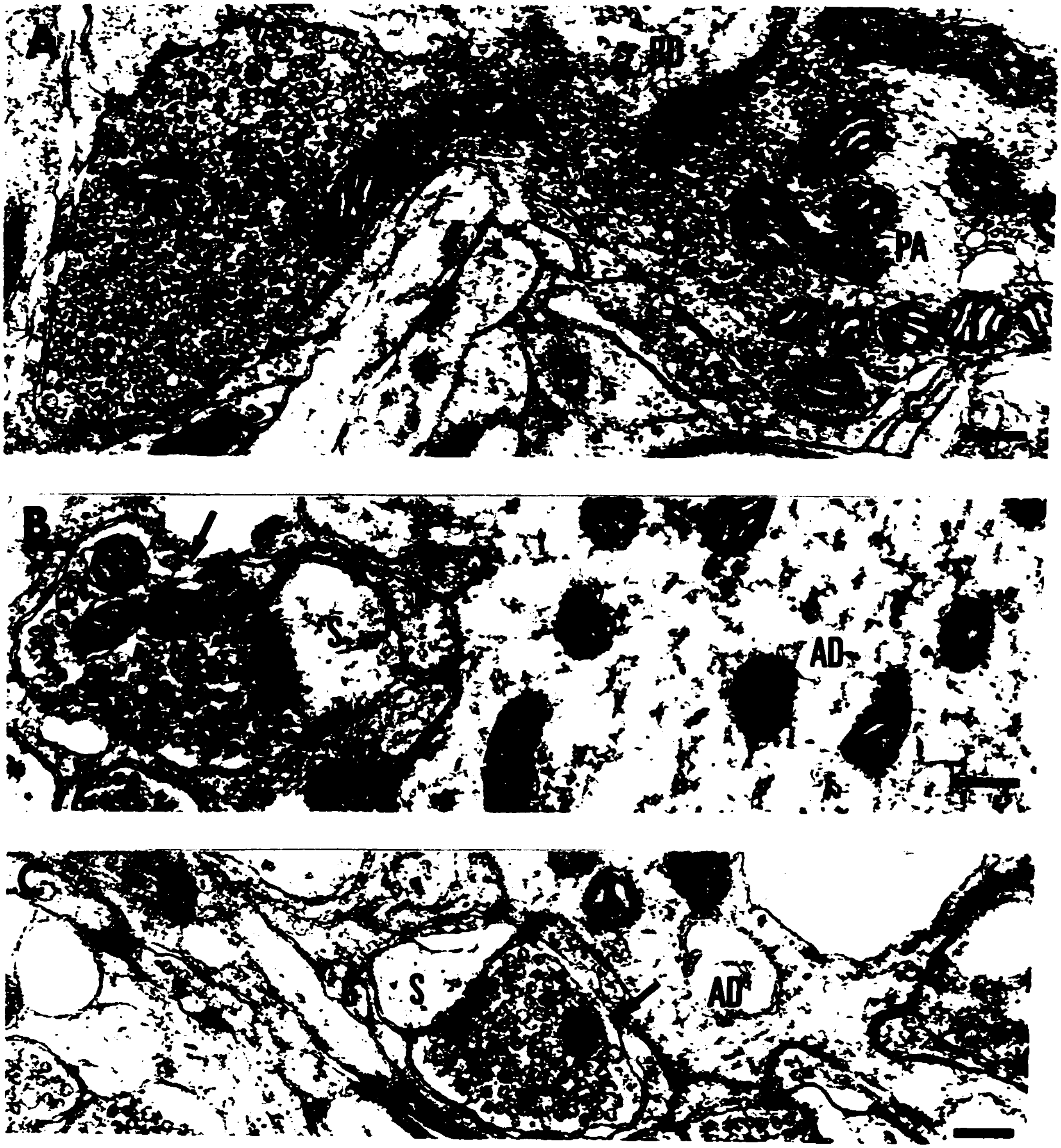


Figure 8: Immunogold particles in excitatory afferents in the deep neuropil layer

Figure 8. Immunogold particles in excitatory afferents in deep neuropil and molecular layers.

A: In the deep neuropil layer (DNL) the primary afferent terminal (**PA**, Maler et al.. 1981) has numerous particles associated with round synaptic vesicles and forms an asymmetric synapse with a basilar dendrite (**BD**) of a pyramidal cell. Note that no particles are seen in the glial processes (**G**). Scale bar=0.22 μm.

B: In the ventral molecular layer (VML). the TSF bouton (arrow. Maler et al.. 1981) has many particles associated with round vesicles; it makes an asymmetric synapse with a spine (**S**) of a pyramidal apical dendritic (**AD**). The enveloping glial process (**G**) is devoid of particles. Scale bar=0.21 μm.

C: In the dorsal molecular layer (DML) an immunoreactive parallel fiber bouton (arrow. Maler et al., 1981) forms an excitatory synapse with a spine (**S**) of a pyramidal apical dendrite (**AD**); no particles are seen in the nearby glia (**G**). Scale bar=0.21 μm.

Figure 9: Immunogold particles in inhibitory boutons in the pyramidal cell layer

Figure 9. Immunogold particles in inhibitory boutons in pyramidal cell layer.

A bouton derived from an inhibitory interneuron (**INT**, GABAergic, Maler and Mugaini, 1994) has few particles; note pleomorphic vesicles and synaptic contact with the somata of a pyramidal cell (**PC**). The somata of the pyramidal cell contains many gold particles, consistent with the LM results (see figure 7). Scale bar= 0.25 μm.

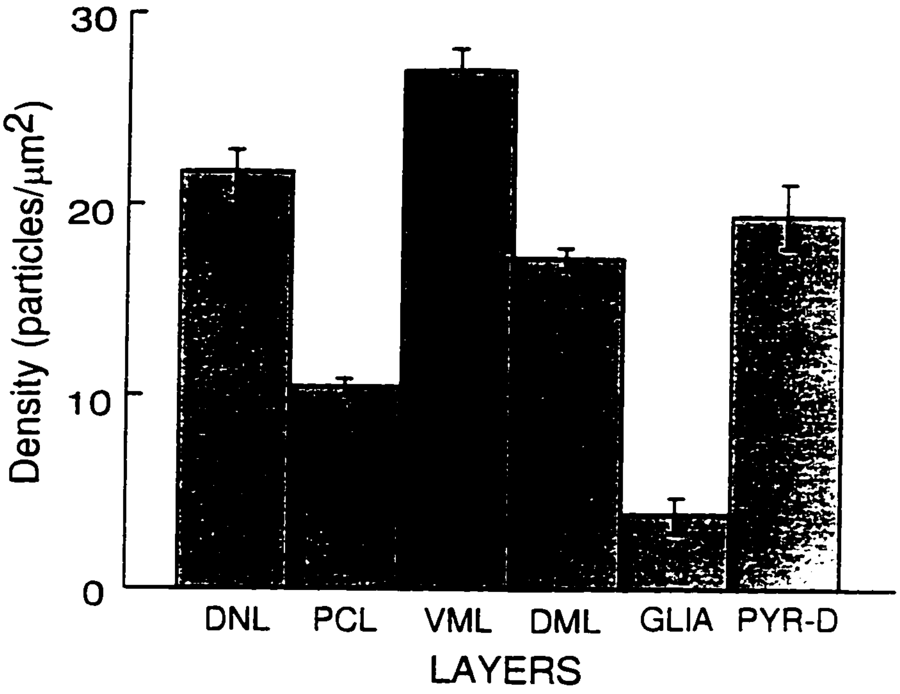


Figure 10: Mean densities of gold particles in terminal boutons of various layers of ELL.

Figure10. Mean densities of gold particles in terminal boutons of various layers of ELL. The density of particles in DNL, PCL, VML, DML and PYR-D was significantly higher than those in GLIA ($p<0.001$). The terminal boutons of all 3 excitatory inputs to the electrosensory lateral line lobe (DNL, VML and DML) had a significantly higher density of immunogold particles than terminal boutons in the pyramidal cell layer (PCL) ($p<0.001$). DNL= deep neuropil layer; PYR= pyramidal cell layer; VML= ventral molecular layer; DML= dorsal molecular layer; GLIA= glial cells; PYR-D= pyramidal dendrites. (Error bars: standard error)

Tetanic stimulation was designed to mimic the *in vivo* firing pattern of cells which give rise to the TSF (Bratton and Bastian,1990); stimulation was 100 Hz for 100 ms repeated 3-5 times at 1-3 sec intervals. This stimulation produced a rapid increase in the field EPSP (Figure 11A trace 2) which peaked at 5 sec after the tetanus (maximal increase: 44.79% ±9.93, N=11 slices; Figure 11B.); this was significantly greater than the time-matched control (4.91% ± 1.13, N=13 slices, $P<.01$). The potentiation decayed rapidly and the EPSP was not significantly different from that of the pretetanus by 2-5 minutes post-tetanus. Tetanic stimulation frequencies ranging from 100-300 Hz all produced equivalent PTP (Figure 11C). I chose the 100 Hz tetani for subsequent experiments because it is similar to the frequencies expected to occur *in vivo* in TSF fibers (100-250 Hz; Bratton and Bastian, 1990) and still permitted visualization of individual EPSPs during intracellular recordings. Tetanic frequencies of 1, 10, 50 and 350 Hz did not increase the field EPSP; notably the 1 Hz stimulation did not produce any short or long term synaptic depression (LTD) at these synapses.

In the presence of 4 mM Mn^{2+} the field amplitude decresed by about 60% (Figure 12A.); since Mn^{2+} blocks Ca^{2+} currents and synaptic transmission at these synapses, I interpret the remaining potential as the TSF fiber volley (Berman, et al., 1997, in press). The same 100 Hz tetanic stimulation parameters did not produce any change in the fiber volley (Figure 12B, C). It is therefore likely that the PTP was due to the potentiation of the EPSP rather than the fiber volley.

Intracellular recordings

The pyramidal cells had similar electrophysiological properties (input resistance: 34.13±1.06 MΩ; resting membrane potential: -61.3±-0.83 mV, N=10 cells) and current evoked action potentials (peak amplitude: 73.02±4.04 mV; time to half peak: 0.30±0. 02 ms, N=10 cells) as reported previously (Turner et al; 1994).

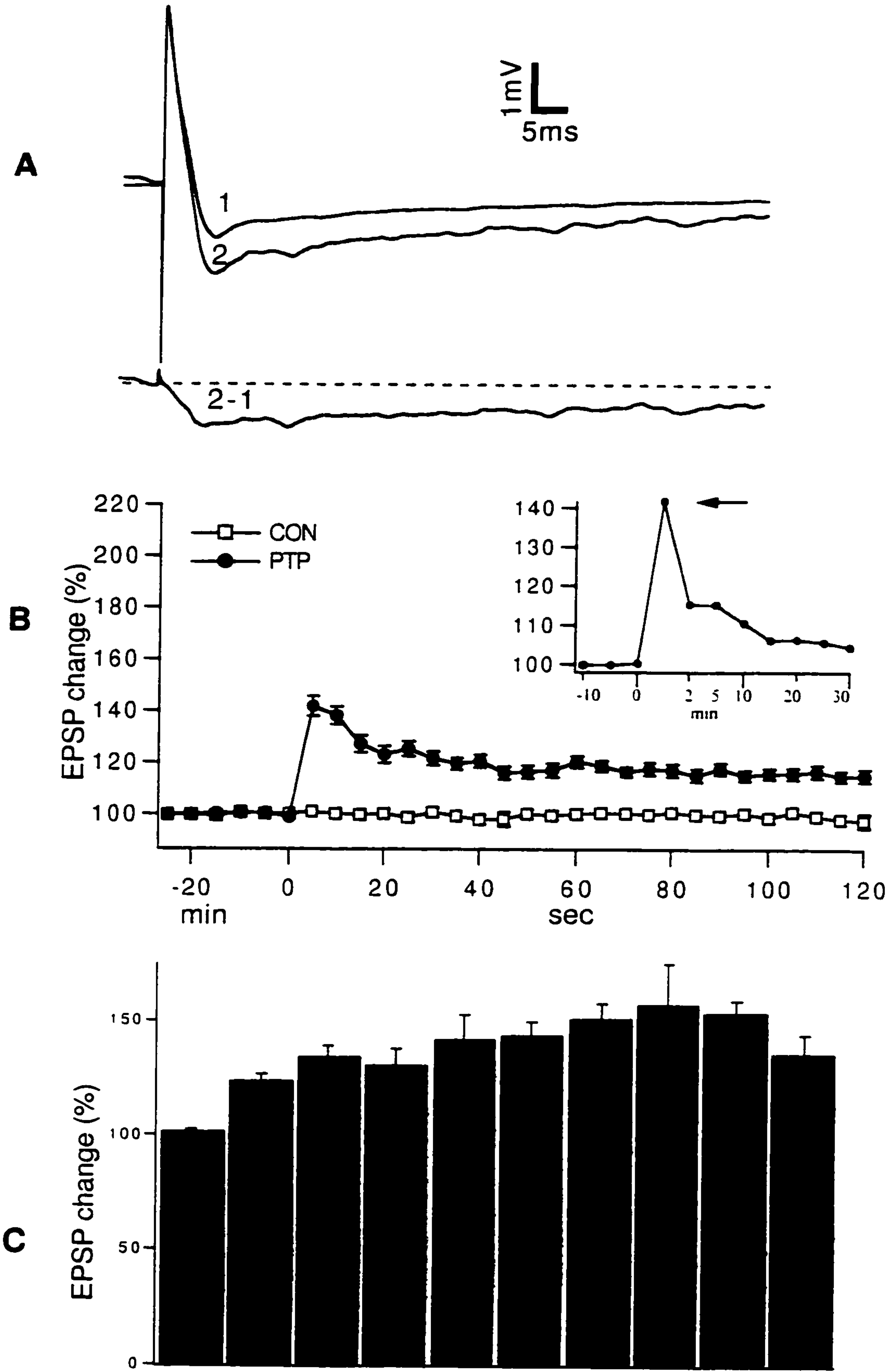


Figure 11: Field recordings showing PTP

Figure11. Field recordings showing PTP.

A: top: Superimposed TSF-evoked field potentials before (1) and 5 sec after (2) tetanic stimulation ; bottom: subtracted reponse (2-1) reveals potentiated field response.

B: Averaged response induced by 100 Hz stimulation (PTP: N=10 slices) and control: control (CON: N=13 slices). Note that pretetanus time scale is in minutes (30 minutes of baseline EPSPs were collected), and the posttetanus time scale is in seconds. Inset: averaged response of tetanically stimulated TSF-evoked EPSPs (error bars omitted for clarity; the arrow points at 5 sec) over a longer baseline; note decline of potentiated EPSPs to baseline during 10 minutes.

C: Averaged PTP change induced by different stimulation frequencies.

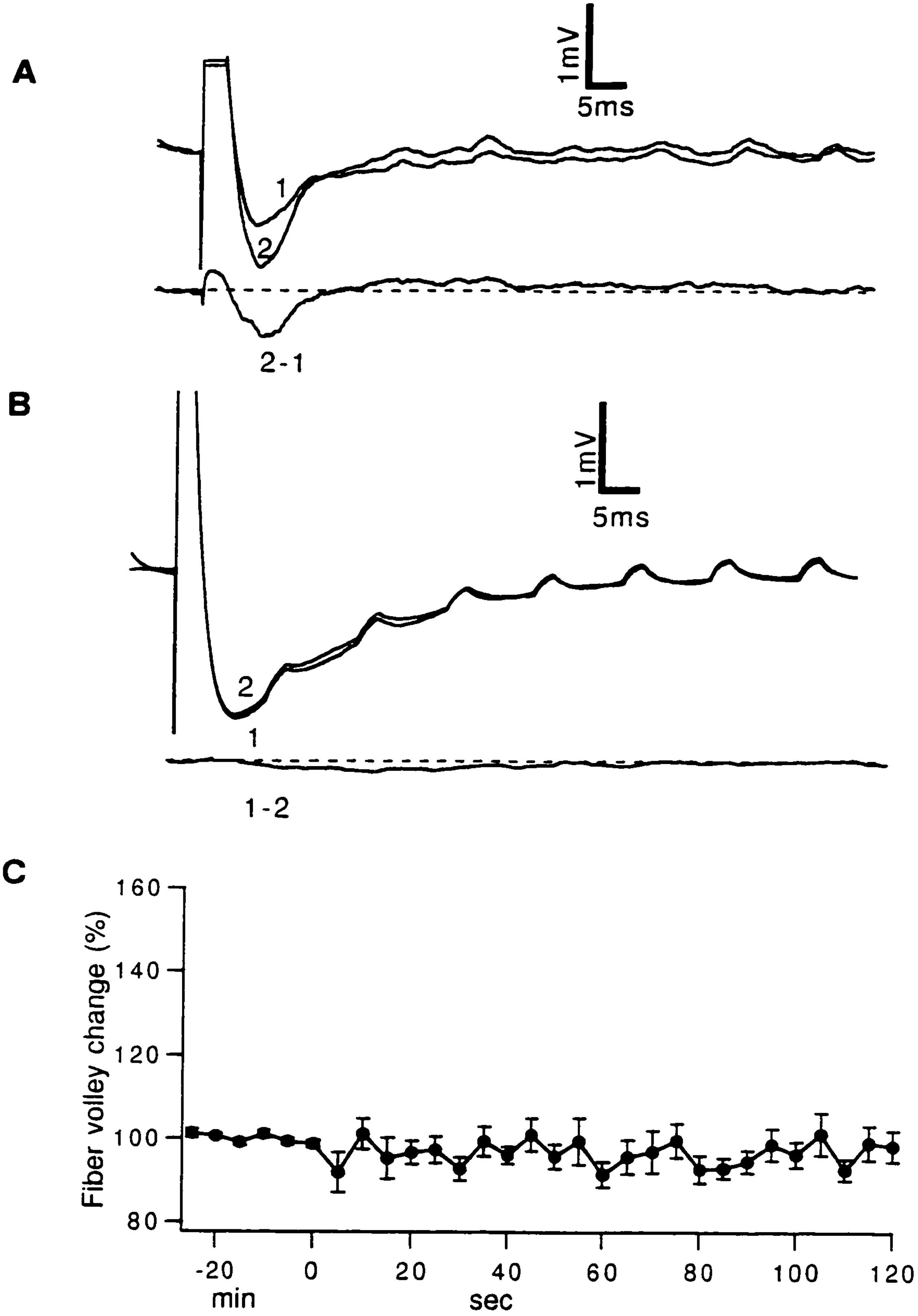


Figure 12: Field recordings showing manganese blocked synaptic transmission

Figure 12: Mn^{2+} blocked synaptic transmission.

A: A single test pulse evoked an EPSP in normal ACSF (2) while the EPSP in 4 mM Mn^{2+} containing ACSF (1) was reduced by 60% (2-1).

B: In the presence of Mn^{2+} there was no significant change (1-2) in the fiber volley before (2) and after (1) tetanization. Note that 60 Hz noise contaminates the recordings.

C: Averaged TSF-evoked response over 2 min posttetanus time showed no change with Mn^{2+} containing ACSF.

The stimulation of TSF evoked an EPSP from the pyramidal cells with a peak amplitude of 3-5 mV at a latency of 5 ms (5.38±.09 ms; Figure 13B). It took about 50 ms for the EPSPs to decay to baseline. My stimulation site was chosen to reduce both stimulation of the direct inhibitory feedback fibers (Maler and Mugnaini, 1994) and the antidromic stimulation of pyramidal cells. Only small IPSPs were evoked in some cases. During the tetanization, a gradual EPSP summation was seen (Figure 13A). Tetanic stimulation produced a maximum EPSP enhancement by 91% (91.39% ± 15.31, N=10 cells) at 5 sec; on average over the 120 sec the EPSP increase was by 40% (40.62% ± 9.43). These changes were significantly different from the baseline EPSP (for maximum EPSP: $P<0.01$, for average: $P<0.05$). The EPSP then decayed back to baseline by 2-5 min (Figure 13 C). At 150 sec the EPSP was not significantly different from the baseline (98.27% ± 7.38, $P>0.5$). The intracellular results therefore confirmed my conclusion from the field potential experiments: TSF-evoked EPSPs show a prominent PTP when activated by physiologically appropriate tetani.

The effects of KN-62 on PTP in VML

Recent immunocytochemical results (Maler, personal communication) showed a rich distribution of α–CaMKII in the VML of ELL. Therefore an attempt was made to investigate the role of this protein kinase on the PTP.

KN-62's effects on EPSP and PPF

Firstly, KN-62's effects on the pyramidal EPSP without tetanization was examined. The baseline EPSPs were collected first and then 3.5 μM KN–62 was puffed onto a focal site in VML. The post-KN-62 EPSPs were cellected at least 20 min after the drug application, which lasted throughout the recording. The EPSPs before and after KN-62 application were compared. The EPSP amplitude before the KN-62 was used as baseline (0.82±0.07 mV, N=24 cells), those after incubation in KN-62 for more than 20 min were 1.17 ± 0.15 mV (N=36 cells/slices, Figure 14 P1). This difference (+42%) was not statistically significant (P>0.09, Tukey).

As an index to transmitter release, the paired pulse results showed that at 50 ms interval the ratio P2/P1 (the peak amplitude of pulse 2 divided by that of the pulse 1) in pre-KN62 recordings was 146% (146.56% ± 9.96, N=14 cells/slices), while in post-KN62 recordings it was 110% (110% ± 9.65, N=18 cells/slices, Figure 14). This was a small but statistically significant difference (P<0.05), indicating that KN-62 reduces paired pulse facilitation.

KN-62's effects on PTP

In the presence of KN-62 applied focally in the VML (see above), the tetanization produced a maximum EPSP enhancement by 6% (6.77% ± 17.06, N=9 cells/slices) at 5 sec (Figure 15A, B,C-1) and on average over the 2 min posttetanus period the EPSP was reduced by 2% (98.28% ± 6.35 of the baseline, N=9 cells/slices, Figure 15B, 15C-2). Statistical analysis showed that the overall EPSP change in the presence of KN-62 was significantly smaller than the control (maximum increase=75.02% ± 10.59, average increase=27.69% ± 13.07, N=6 cells, P<0.001, Figure 15B, C) and an ANOVA indicated that the difference was attributed to the 5 sec and 10 sec time groups.

DMSO control on PTP in VML

In the presence of DMSO, a solvent used to dissolve KN-62, the tetanization produced a maximum increase by 137% (137.36% ± 4 1.87, N=6 cells, Figure 15C-1) and average increase by 44% (44.43% ± 19.01, N=6 cells, Figure 15C-2), which was not statistically different from the control (maximum increase=162.13% ± 36.10, average increase =53.72% ± 11.48, N=6 cells) collected at same time.

KN-04 control on PTP

KN-04, a structural analog of KN-62 (Ishikawa, et al., 1990), was used to test the

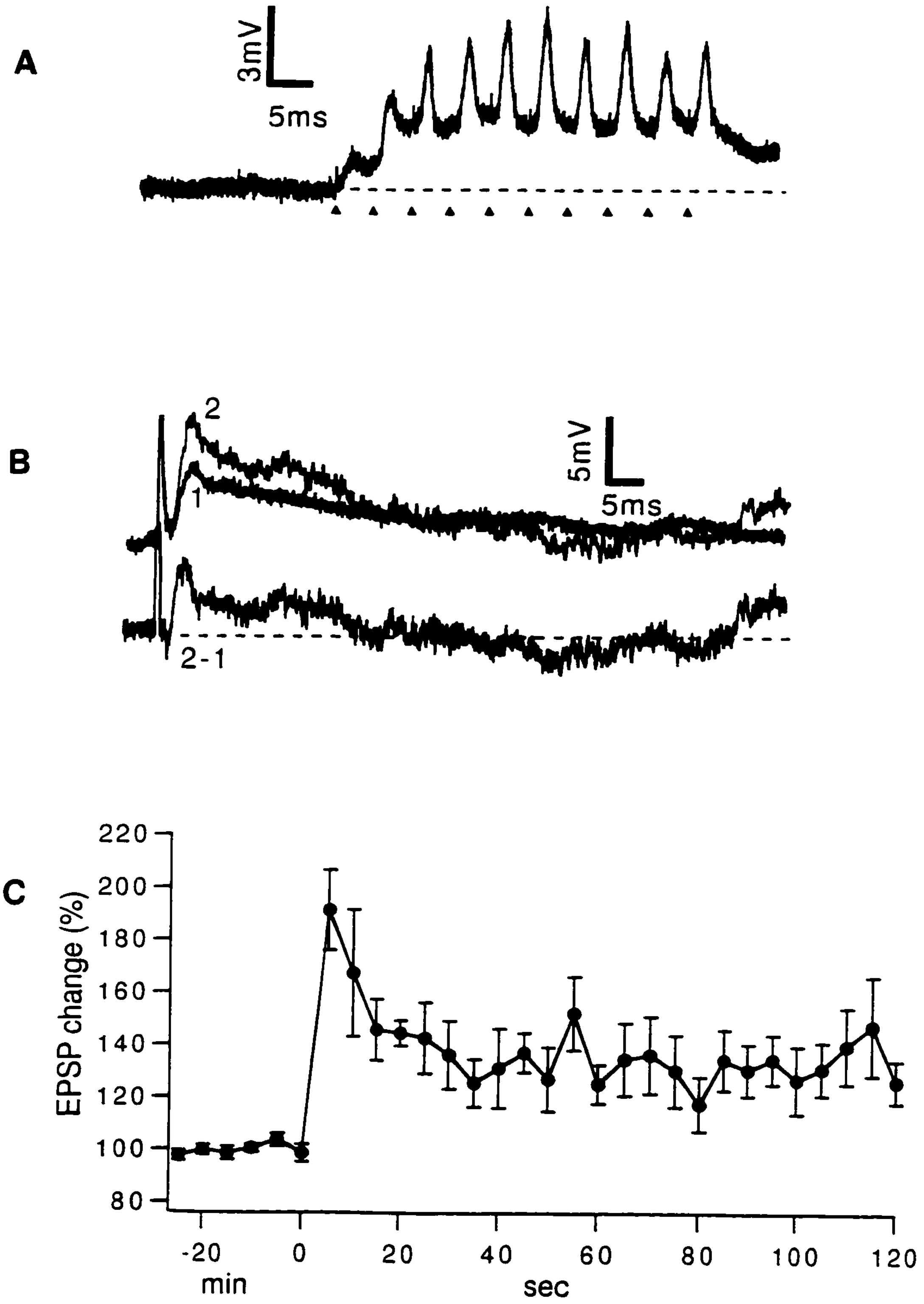


Figure 13: Intracellular recordings showing PTP

Figure 13: Intracellular recordings showing PTP.

A: During tetanization (10 pulses given at 100 Hz in 100 ms repeated 3-5 times) a compound EPSP was seen (the triangles indicate stimuli).

B: A single recording (1) showed an EPSP of about 3 mV in amplitude and 5 ms in latency. The long tail indicates an NMDA component. After tetanization (2) the EPSP peak amplitude almost doubled at 5 ms (2-1); no obvious amplitude change was seen at 40 ms (2-1).

C: In an average of 10 cases, the peak amplitude increased initially by 91% and fell to 40% over 2 min after tetanus. Note that the time scale is in minutes before tetanization and in seconds after tetanization (the same scales are used in the following PTP graphs.

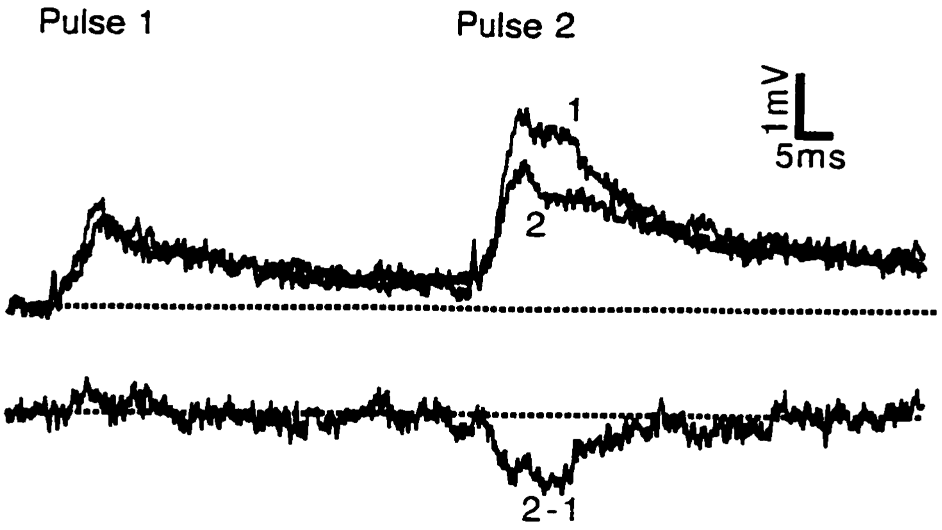


Figure 14: KN-62's effects on the pyramidal EPSP and paired pulse facilitation.

Figure 14: KN-62's effects on the pyramidal EPSP and paired pulse facilitation (PPF). A single recording (pulse 1) collected in the presence of 3.5 μM KN-62 (trace2) had the similar EPSP amplitude as that before KN-62 (trace 1), which means KN-62 had no effect on the baseline EPSP amplitude. However, the PPF expressed as pulse2/pulse1 in KN-62 (trace2) is significantly smaller ($P<0.05$) than before KN-62 (1) which means that KN-62 reduced PPF.

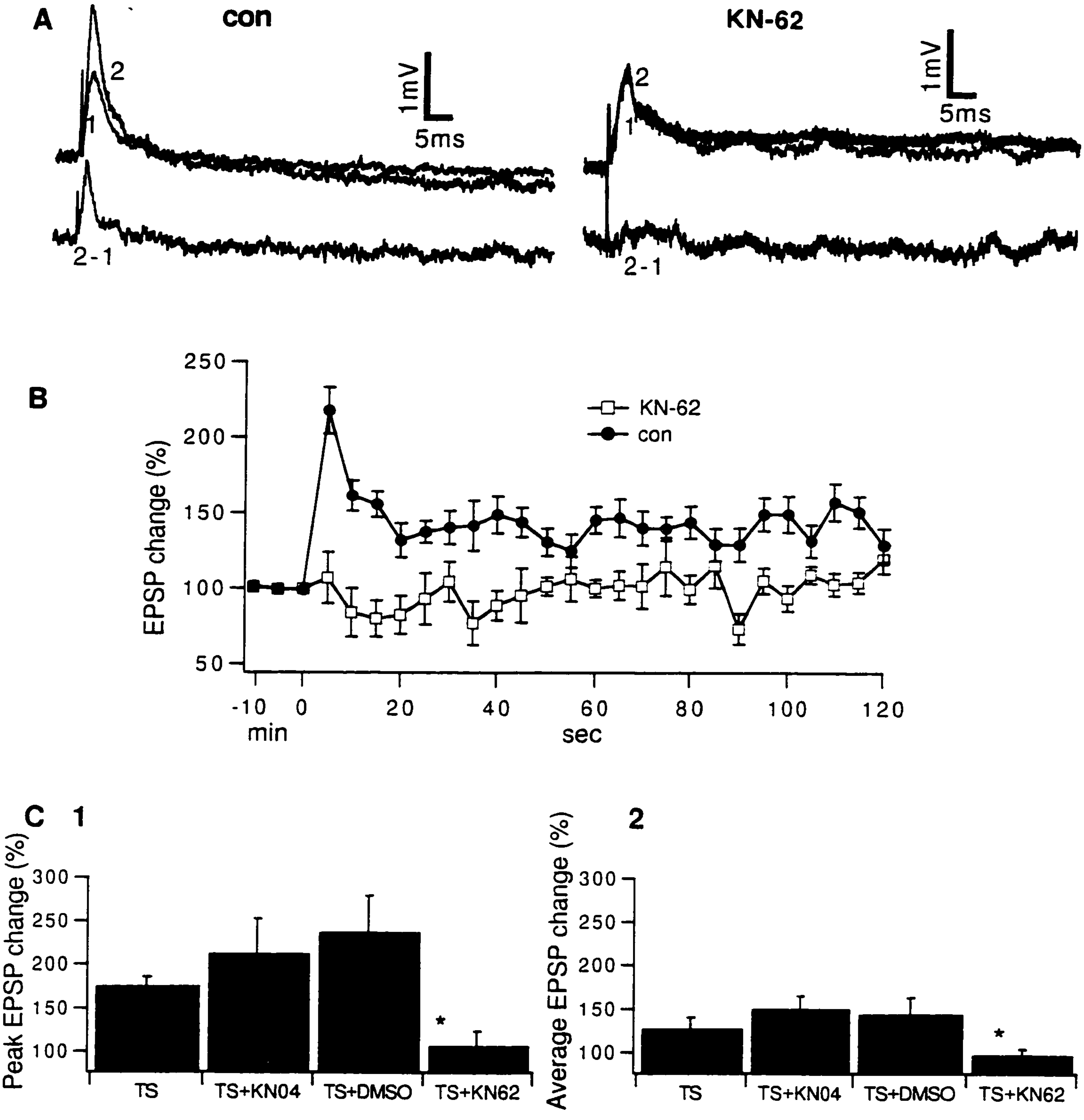


Figure 15: The PTP in VML was blocked by KN-62

Figure 15: The VML PTP was blocked by KN-62 .

A: con: In control experiment tetanization of TSF produced an EPSP (2) that had a higher amplitude than pretest baseline (1) by the amount indicated in the different trace (2-1). KN-62: In the presence of 3.5μM KN-62 tetanization induced an EPSP that had a similar amplitude (2) to the baseline EPSP (1). The net change is indicated by the difference (2-1).

B: In an average of 9 cases, tetanization in the presence of KN-62 did not increase the EPSP peak amplitude (KN-62). As compared to control (con), it was significantly smaller ($P<0.001$).

C: Bar graphs show the peak (C1, at 5 sec) and average (C2, over 2 min) TSF-evoked EPSP changes in the presence of different pharmacological agents. KN-62 group (indicated by *) has significant difference from the others. TS: tetanization alone; TS+KN04: tetanization in the presence of KN-04; TS+DMSO: tetanization in the presence of DMSO; TS+KN62: tetanization in the presence of KN-62.

specificity of KN-62 on the blockade of PTP. In the presence of 3.3 μM KN-04 the tetanization yielded 111% (111.90% ± 40.33) and 51% (51.58% ± 15.77) for maximum and average PTP increases respectively (Figure 15C); neither of them were significantly different from those without any drugs (maximum increase=75.02% ± 10.59, average increase=27.69% ± 13.07, P>0.05).

The effects of KN-62 on PTP in VML when postsynaptically applied

KN-62 intracellular application has been shown to be reliable in blocking CaMKII (Stanton and Gage, 1996) in other preparations. When 10.5 μM KN-62 was applied intracellularly into the pyramidal cells, the tetanization induced a maximum EPSP increase by 80% (80.56% ± 26.60, N=6 cells/slices, Figure 16 B) at 5 sec, and an average increase by 38% (38.54% ± 7.45) over the 2 min. It decayed to baseline in 2 min (Figure 16C: KN-62). The control had a maximum increase by 85% (85.71% ± 27.80, N=6 cells) at 5 sec and by 48% (48.15% ± 9.53) on average over 2 min with a similar time course (Figure 16C: CON). No significant difference was found between the 2 groups (P>0.7).

The effects of CaMKII inhibitory peptide on PTP in VML

The CaMKII inhibitory peptide can inhibit CaMKII activity (Smith et al., 1992). Its function was tested by intracellular application of 1.1 mM into pyramidal cells. Results are shown in Figure 17. Thirty to 45 minutes were given for the peptide to diffuse into the cells and tetanization of TSF evoked a maximum EPSP increase (84.85% ± 35.35, N=4 cells/slices, Figure 17-A) at 5 sec posttetanus time, it decayed to the baseline level in less than 2 min and on average over the 2 min period the EPSP increased by 38% (38.40% ± 18.67). In the control group collected during the same period of time the tetanization produced a maximum EPSP increase by 96% (96.66% ± 20.46, N=4 cells, Figure 17-B) and an average increase by 40% (40.57% ± 9.98). There were no statistical differences between the 2 groups (P>0.05).

KN-62's effects on PTP in DML

When 3.5 μM KN-62 was applied in DML, stimulation of the input fibers in the same layer evoked an EPSP which peaked at 9 ms (8.88 ± 0.31 ms, N=135 recordings, Figure 18-B) with a decay time of more than 50 ms. Tetanization of the input fibers produced a maximum PTP increase by 100% (102.35% ± 18.07, N=6 cells/slices) that peaked at 5 sec and on average over the 2 min posttetanus, the EPSP amplitude increased by 30% (30.19% ± 6.74, N=6 cells/slices, Figure 18-B, C: KN-62). It decayed to baseline in 2 minutes. In the control group collected during the same period of time tetanization produced a maximum increase by 111% (111.10% ± 30.36, N=5 cells, Figure 18 A) at 5 sec posttetanus time followed by a fast decay. The PTP went back to baseline in less than 2 min and on average over the 2 min posttetanus period, the EPSPs increased by 35% (35.47% ± 13.76, N=5 cells. Figure 18-C:CONTROL). There was no significant difference between the 2 groups (P>0.9).

The effects of protein phosphatase inhibitors on PTP

The protein phosphatase 1, 2A and 2B inhibitors were tested for their effects on the PTP. Since there is a rich distribution of α–CaMKII in the ventral molecular layer of the ELL, It was expected that the protein phosphatase inhibitors, particularly the inhibitor for calcineurin, a Ca^{2+}-calmodulin dependent protein phosphatase, would greatly enhance the peak amplitude and duration of the PTP.

Calyculin A is a putative PP1 and PP2A blocker and its effects on PTP in the VML is shown in Figure 19. After ejecting the drug (at 0.5μM concentration) onto a focal site in the VML, the test pulse produced an EPSP that was not different from the control. The tetanization of TSF induced a maximum PTP increase by 105% (105.19% ± 33.60, N=3 cells/slices) at 5 sec posttetanus time and an average increase by 65% (65.65% ±

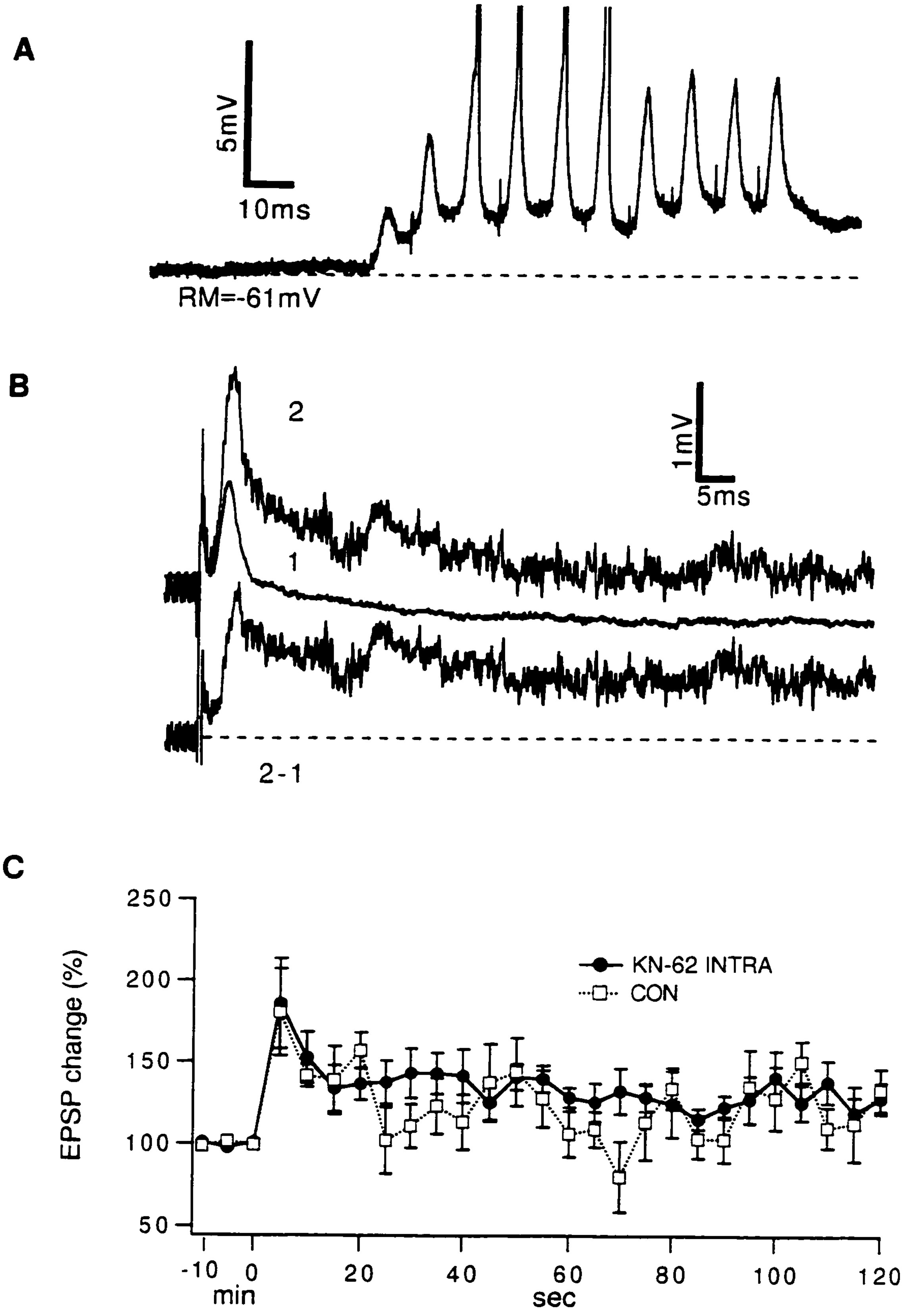


Figure 16: KN-62 did not block PTP in VML when applied intracellularly.

Figure 16: KN-62 did not block PTP in VML when applied intracellularly.

A: In the presence of 10.5 μM KN-62, the tetanization produced an EPSP Summation which triggered action potentials (truncated at -50 mV).

B: In the presence of intracellular KN-62, pretest (1) and posttetanization (2) EPSPs were recorded and the difference (2-1) showed an increase.

C: In an average of 6 cases, the peak increase was 86% at 5 sec and 48% over the 2 min period (KN-62 INTRA). The control group collected at same period of time showed similar results (CON) (P>0.7).

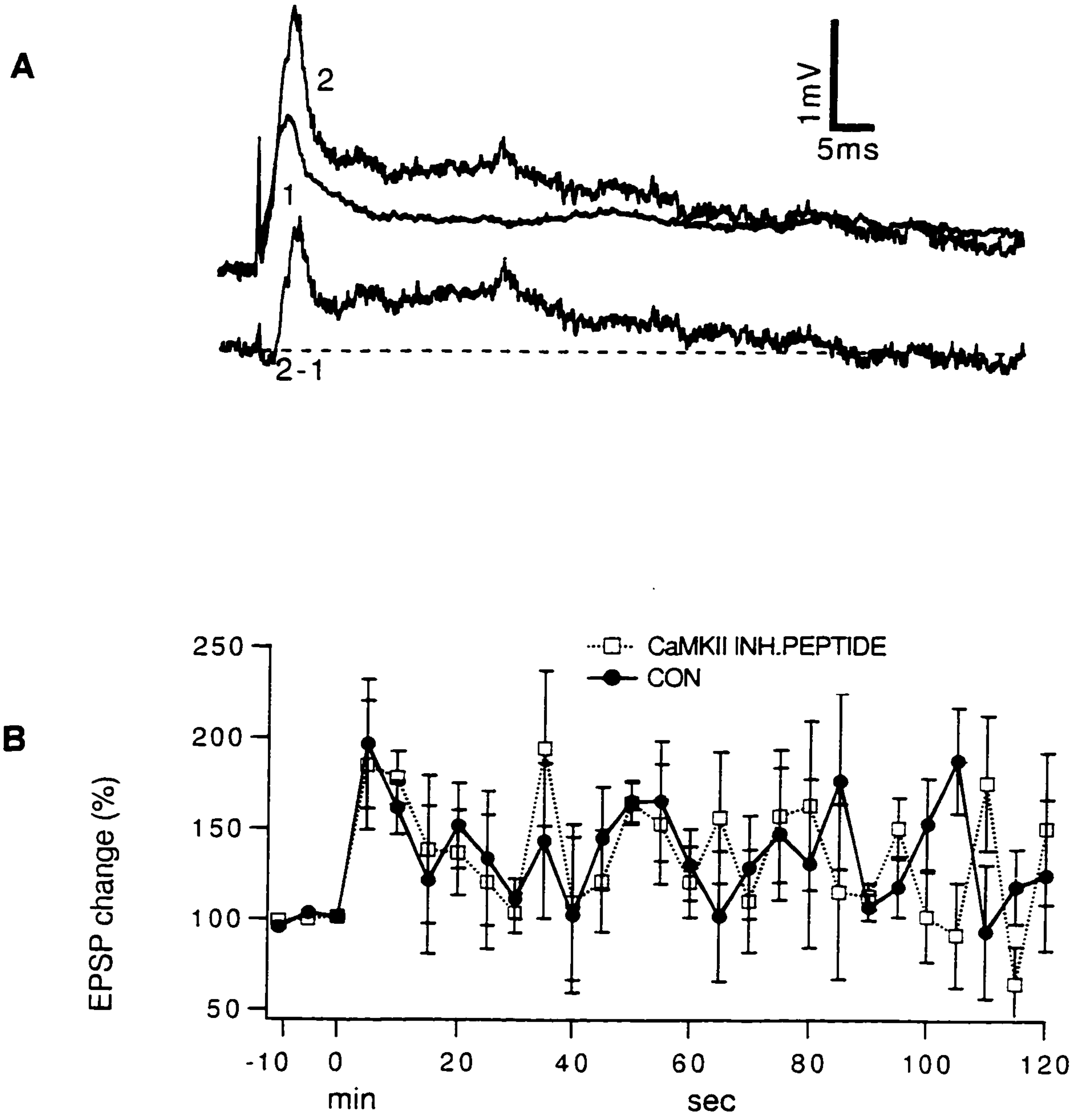


Figure 17: Intracellular application of CaMKII inhibitory peptide did not block PTP in VML

Figure 17: Intracellular application of CaMKII inhibitory peptide did not block PTP in VML.

A: A sample recording showing that in the presence of 1.1 μM CaMKII inhibitory peptide, tetanization produced an EPSP (2) which was larger than the baseline EPSP (1). The net increase in show by 2-1.

B: In an average of 4 cases, the PTP produced with the CaMKII inhibitory peptide had a peak increase by 85% (at 5 s) and 38% over 2 min posttetanus period (CaMKII INH.PEPTIDE). The PTP in the control group had a similar change (peak increase=97%, 2 min average=40%, CON). No statistical difference was found between these 2 groups (P>0.05).

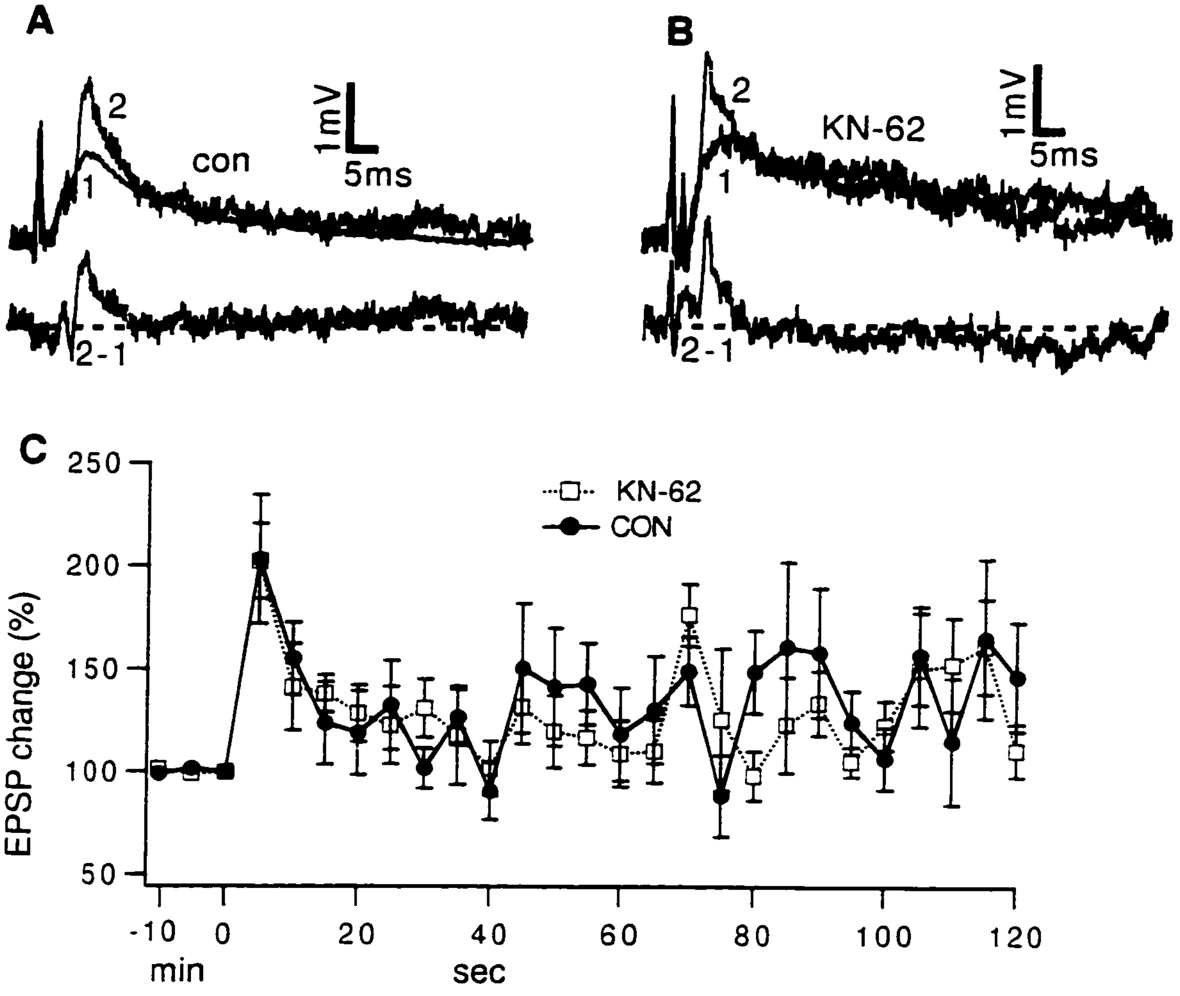


Figure 18: PTP in DML was not blocked by KN-62

Figure 18: PTP in DML was not blocked by KN-62.

A: A sample test pulse evoked a baseline EPSP in DML (trace 1) and tetanization produced an EPSP (trace 2) larger than the baseline EPSP. The difference was showed by 2-1.

B: The same cell as in A. In the presence of 3.5 μM KN-62 tetanization produced an EPSP amplitude (at 5 sec, trace 2) similar to that in the pretest (trace 1). The net increase is showed by the difference (2-1)

C: In an average of 6 cases collected in the presence of KN-62, the tetanization doubled the EPSP peak amplitude (at 5 sec) and over the 2 min posttetanus period the EPSP increase was by 30% (KN-62), which was not statistical different from the control (P>0.9).

25.81, Figure 19A: CALYCULIN A). This PTP had a fast decay phase in the first minute followed by a 2nd slow decay which oscillated above the baseline for more than 1 min. Compared to the control (the maximum increase=95.76% ± 30.12, the average increase=58.33% ± 18.28, N=3 cells, Figure 19 A: CON), this increase was not significant (P>0.8). It is possible that the lasting oscillation of the slow phase might indicate a need for a higher concentration or more incubation time, or the inclusion of other blockers to show an effect. FK-520 is a specific calcineurin blocker. In the presence of 1 μM FK-520 (low concentration), TSF stimulation evoked EPSPs from the pyramidal cells that were similar to the control EPSPs. Tetanization of the TSF produced a maximum increase upto 75% (74.54% ± 19.87, N=13 cells/slices) at 5 seconds after the tetanus; it decayed to about 25% in 30 sec and remained steady for more than 90 sec; on average, for the 2 minute period the EPSPs increased by 50% (49.49% ± 13.77, Figure 19B: FK-520; 19C: FK520 LOW). Compared to the control (maximum increase=69.36 ± 20.13%, average increase=43.91% ± 10.04, N=7 cells, Figure 19B: CON) this increase was not significant (P>0.7). When FK-520 was used at 10 μM (high concentration) with double incubation time (30 min), the tetanization produced a maximum PTP of 79% (79.16% ± 21.93, N=7 cells/slices) and an average of 50% (149.96% ± 12.05, Figure 19C: FK520-HIGH) over the 2 minute period, which was still not significantly different from control (P>0.7). I conclude that calcineurin-mediated protein dephosphorylation does not play an important role in PTP.

FK-520 (1 μm) and calyculin A (0.5 μm) were utilized together in an attempt to see any synergistic effects on the PTP. A single test pulse of TSF evoked an EPSP similar to that in control; tetanization produced a maximum PTP of 71% (71.76% ± 18.95, N=5 cells/slices, Figure 19C: FK520+CAL); on average, over the 2 min posttetanus period, the PTP increased by 50% (50.97% ± 12.61). The PTP seemed to have only one phase, a slow decay phase and it took more than 2 min to return to the baseline. The increase

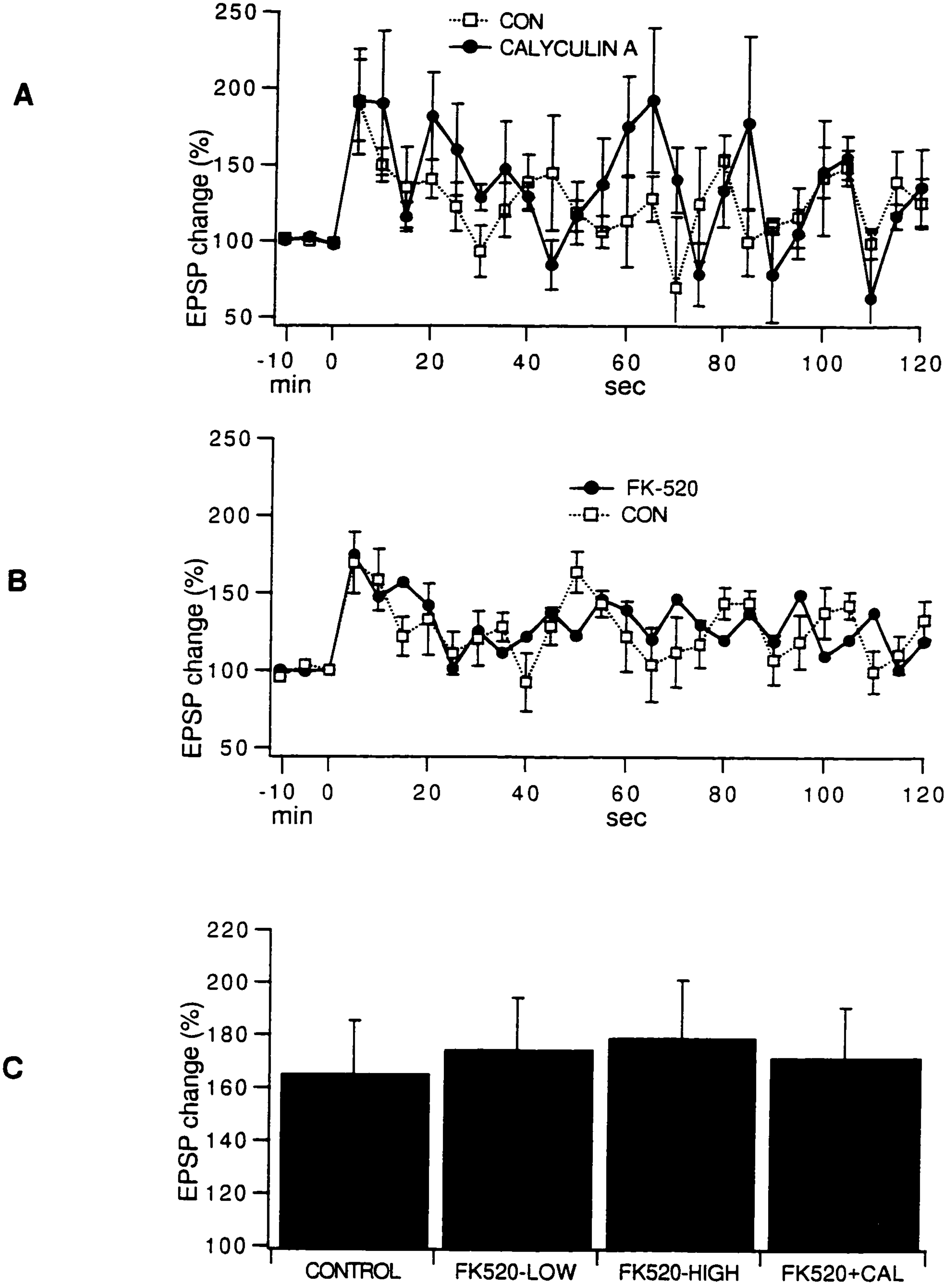


Figure 19: Calyculin A and FK-520 did not potentiate PTP in VML

Figure 19: Calyculin A and FK-520 did not potentiate PTP in VML.

A: In an average of 3 cases in the presence of 0.5 μM calyculin A in the VML (CALYCULIN A), tetanization produced a peak increase upto 105% at 5 sec and 65% over the 2 min period, which was not statistically different from control (CON, P>0.8).

B: In an average of 13 cases in the presence of 1 μM FK-520 (FK-520) tetanization produced a peak increase by 75% at 5 sec and 50% over 2 min, which was not significantly different from control (CON, P>0.7).

C: Bar graph summarizing the effects of phosphatase inhibitors on the PTP. FK-520, a calcineurin antagonist, did not potentiate PTP at low (1 μM) or high (10 μM) concentrations; when calyculin A, a PP1 and PP2A blocker, and FK-520 was used together, they failed to potentiated the PTP.

was not significantly different from control (PTP induced by tetanization without drugs), which had a maximum PTP of 81% (81.67% ± 22.30, N=5 cells) and an average increase by 51% (51.25% ± 13.64) over the 2 minute period.

III.II.II. Long-term plasticity was not inducible

Field recording:

Tetanization at 100 Hz (Figure 20A) as well as at other frequencies (Figure 20B) did not produce any long term changes (P>0.05 for 100 Hz, N=25 slices). Although it has been reported that under some conditions, low frequency stimulations could induce long term depression (LTD), the present results with low frequency stimulation failed to show any LTD. In other preparations the inability to show LTP has been due to excessive GABA-A inhibition (Steward, et al., 1990). Bath application of 70 μM bicuculline increased the pre-tetanic field EPSP by 30% in VML (Figure 21A-1) and PYR (Figure 21A-2), as expected given the presence of GABAergic interneurons which receive input from TSF (Maler and Mugnaini, 1994). In the presence of bicuculline, the tetanic stimulation increased the EPSP amplitude (averaged over 60 min) by 15% in VML (14.6% ± 4.0, N=9 slices, Figure 21B-1) and by 5% in the pyramidal cell layer (4.6% ± 2.2, N=9 slices, Figure 21B-2). These were not significantly different from either the baseline EPSPs or those produced by 100 Hz tetanic stimulation without bicuculline (Figure 21 B: CONs, P>0.5).

Intracellular recording

Tetanization alone at 100 Hz did not produce any potentiation of EPSPs over the 20 minute period that were significantly different from control (see below) collected at the same time (Figure 22A, C). In other preparations LTP was induced when the tetanus was paired with postsynaptic depolarization (Yoshimura and Tsumoto, 1994; Yoshioka and Sakurai, 1995). The same paradigm in this preparation induced a small change in

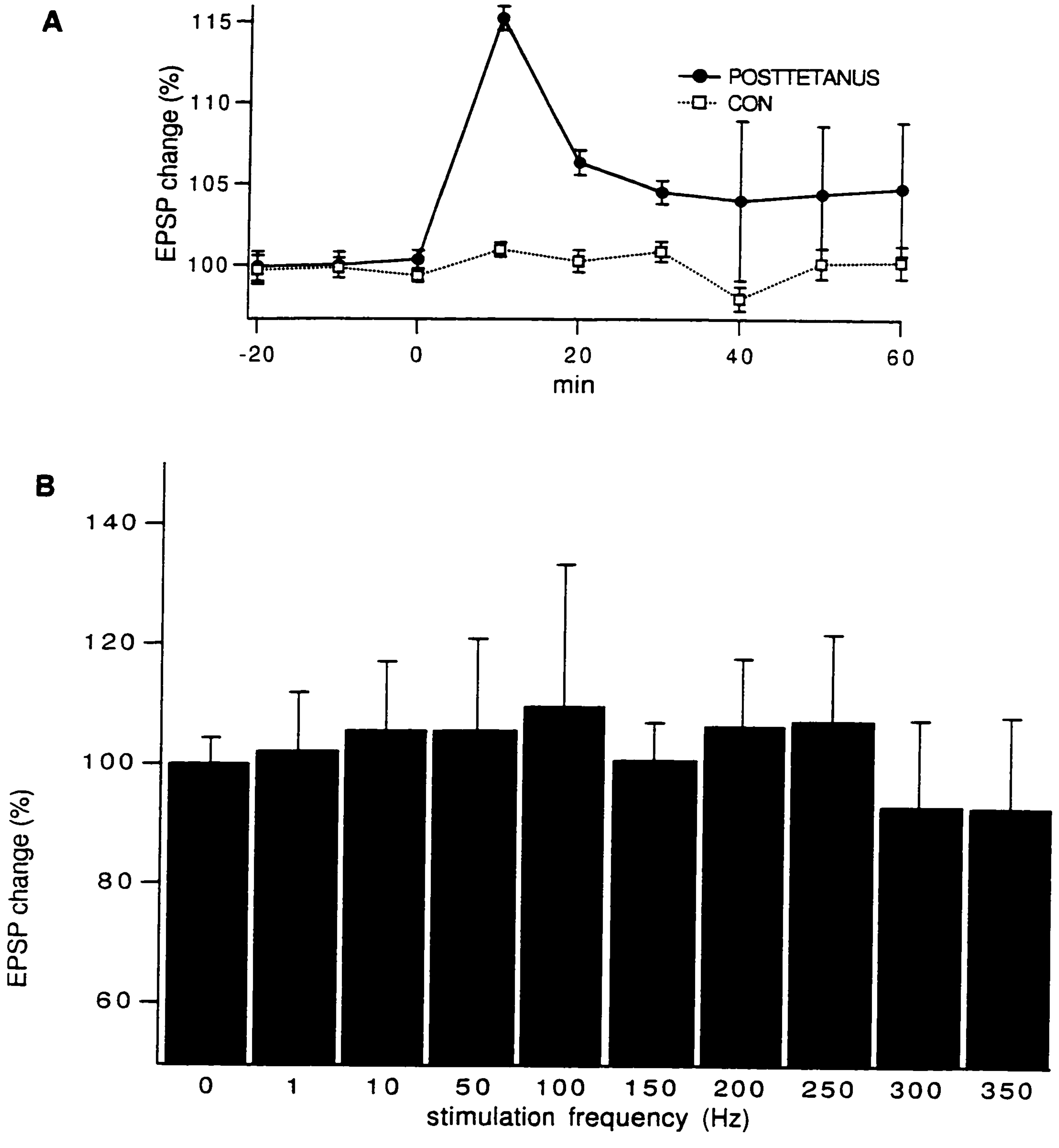


Figure 20: Long-term changes in response were not induced in field recordings

Figure 20: Long-term changes in response were not induced in field recordings.

A: In an average of 25 cases, the 100 Hz tetanization (POSTTETANUS) produced a maximum EPSP increase by 15% at 5 min posttetanus which averaged to 6% over 60 minute period. This EPSP change was not statistically different from control (CON, P>0.05).

B: Bar graph showing the mean EPSP changes over 60 min induced by different tetanization frequencies. Overall, none of the frequencies produced significant long-term changes.

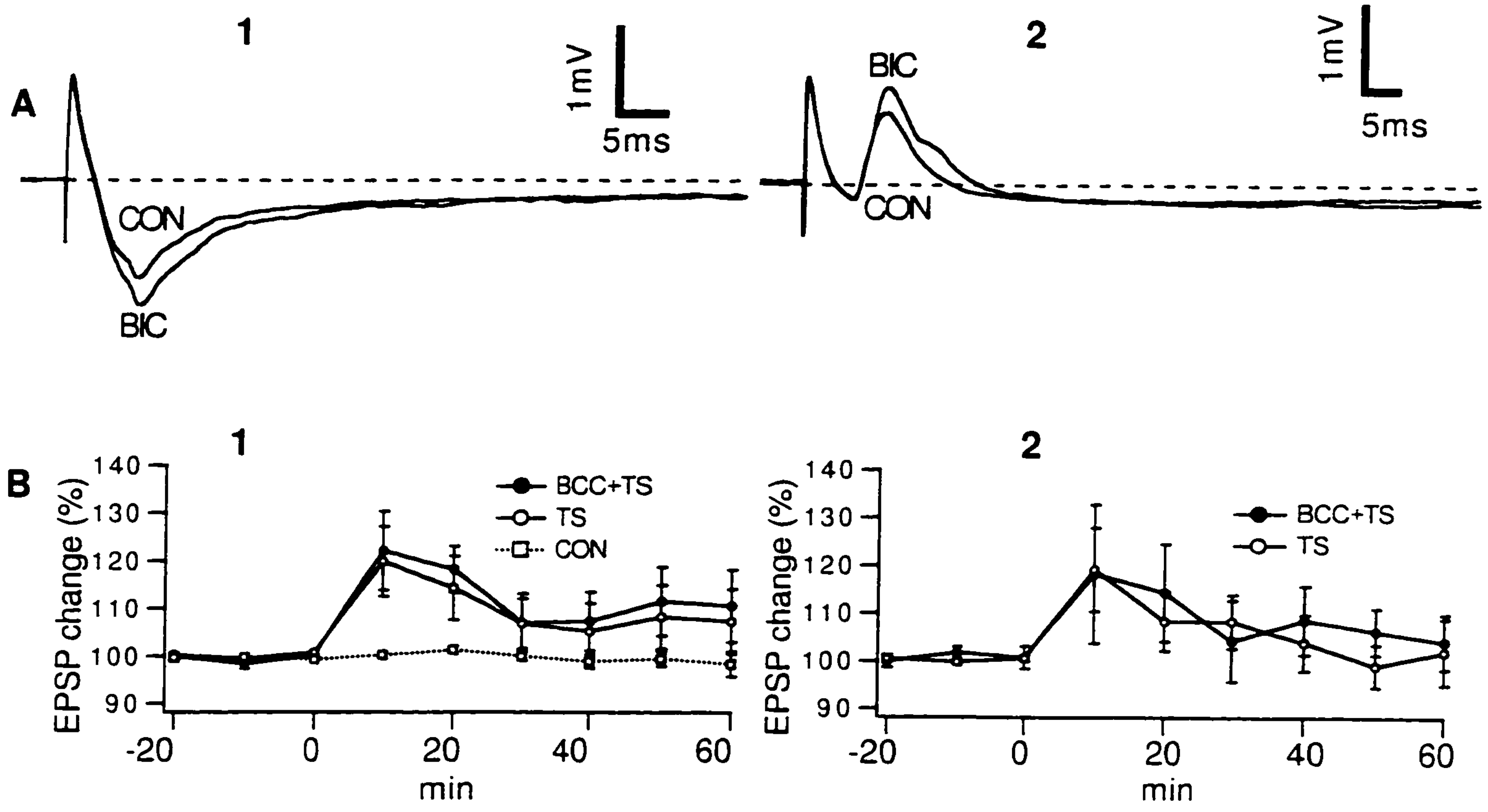


Figure 21: GABA disinhibition did not induce long-term changes in TSF-evoked EPSP.

Figure 21: GABA disinhibition did not induce long-term changes in TSF-evoked EPSP.

A. Seventy μM Bicuculline (BIC) enhanced the TSF-evoked field potentials in both VML (A1) and PYR (A2) over control values (CON).

B: Tetanic stimulation of TSF in normal ACSF (TS) or in bicuculline-containing ACSF (BCC+TS) did not cause long-term potentiation of TSF-evoked field potentials in either VML (B1) or PYR (B2) ($P>0.5$). Baseline EPSP is showed by CON.

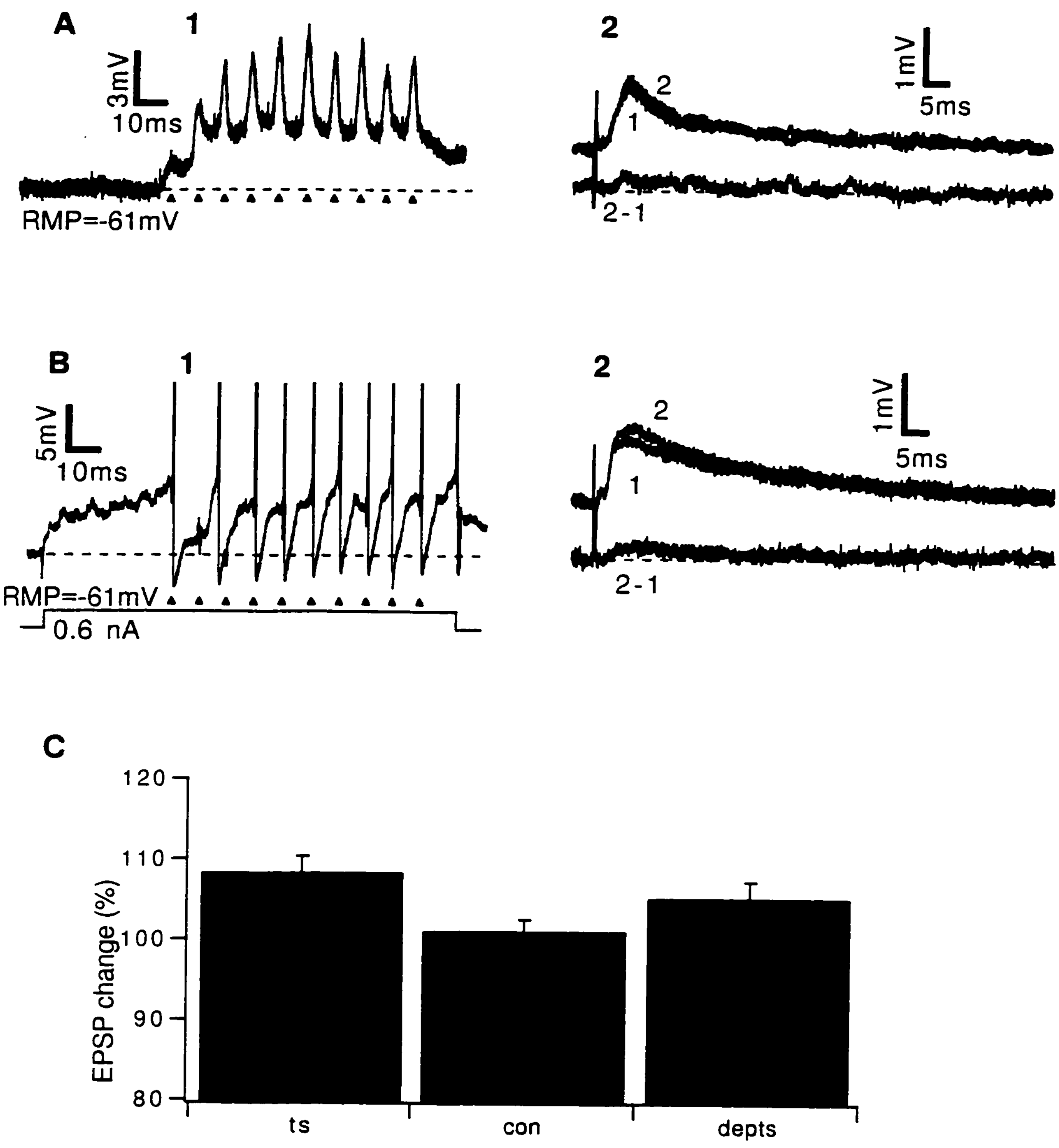


Figure 22: Long-term changes were not induced in intracellular recordings

Figure 22: Long-term changes were not induced in intracellular recordings.

A: Tetanization produced a compound EPSP (A1) but no significant EPSP amplitude change was induced at 5 minutes (A2).

B: When tetanization was paired with membrane depolarization there was a similar EPSP summation (B1) and EPSP amplitude change (B2) as shown in A.

C: Bar graph summarizing the EPSP changes over 20 minutes with different stimulation paradigms. (ts: tetanization alone, con: control, depts: tetanization paired with depolarization) the TSF-evoked EPSP (5.87% ± 3.85, N=15 cells, Figure 22B, C) over 15 minute of recording but this change was not different from the tetanization alone (8.65% ± 4.06, N=15 cells, P=0.99) or depolarization alone (6.51% ± 2.85, N=15 cells, P=0.99). The EPSP changes produced by different stimulation paradigms are shown in Figure 21C. It is concluded that tetanic stimulation of TSF does not induce LTP *in vitro*.

III.III.III. Anti-Hebbian plasticity

Intracellular recording

Tetanization alone at 100 Hz to the TSF induced no significant change in the EPSPs evoked from pyramidal cells examined 15 minutes after posttetanus (8.65% ± 4.06, N=15 cells/slices) (Time-matched control: 9.38% ± 10.76, N=10 cells, P>0.9, Figure 23 C).

When hyperpolarization (-0.6 nA moved the membrane potential from -61 mV down to -70.2 ± 3.86 mV, N=16 cells) was paired with 100 Hz tetanization, the pyramidal cell EPSP increased by 46% (45.56% ± 12.35, N=16 cells, Figure 23A) on average over the 15 minutes of recording and this change was significantly different from the tetanization (P<0.01) or the hyperpolarization alone (2.39% ± 2.57, N=15 cells, P<0.01, Figure 23C). This increase decayed to 15% at 20 min. In 7 cases, I recorded the fiber volley from the TSF concomitantly with the intracellular recording. The fiber volley remained stable or decreased by about 13% (87.07% ± 5.37 of control, N=7 cells/slices) as the EPSP was potentiated (30.12% ± 10.90).

When paired with depolarization, the 100 Hz tetanization produced an increase by 6% in the EPSP averaged over 15 minutes (5.87% ± 3.85, N=15 cells, Figure 23 B , C), which was not statistical different from the EPSP measured after tetanization (P>0.9) or depolarization alone (6.51% ± 2.85, N=15 cells, P>0.9, Figure 23C).

During all the intracellular recordings, no changes in input resistance or resting membrane potential was observed.

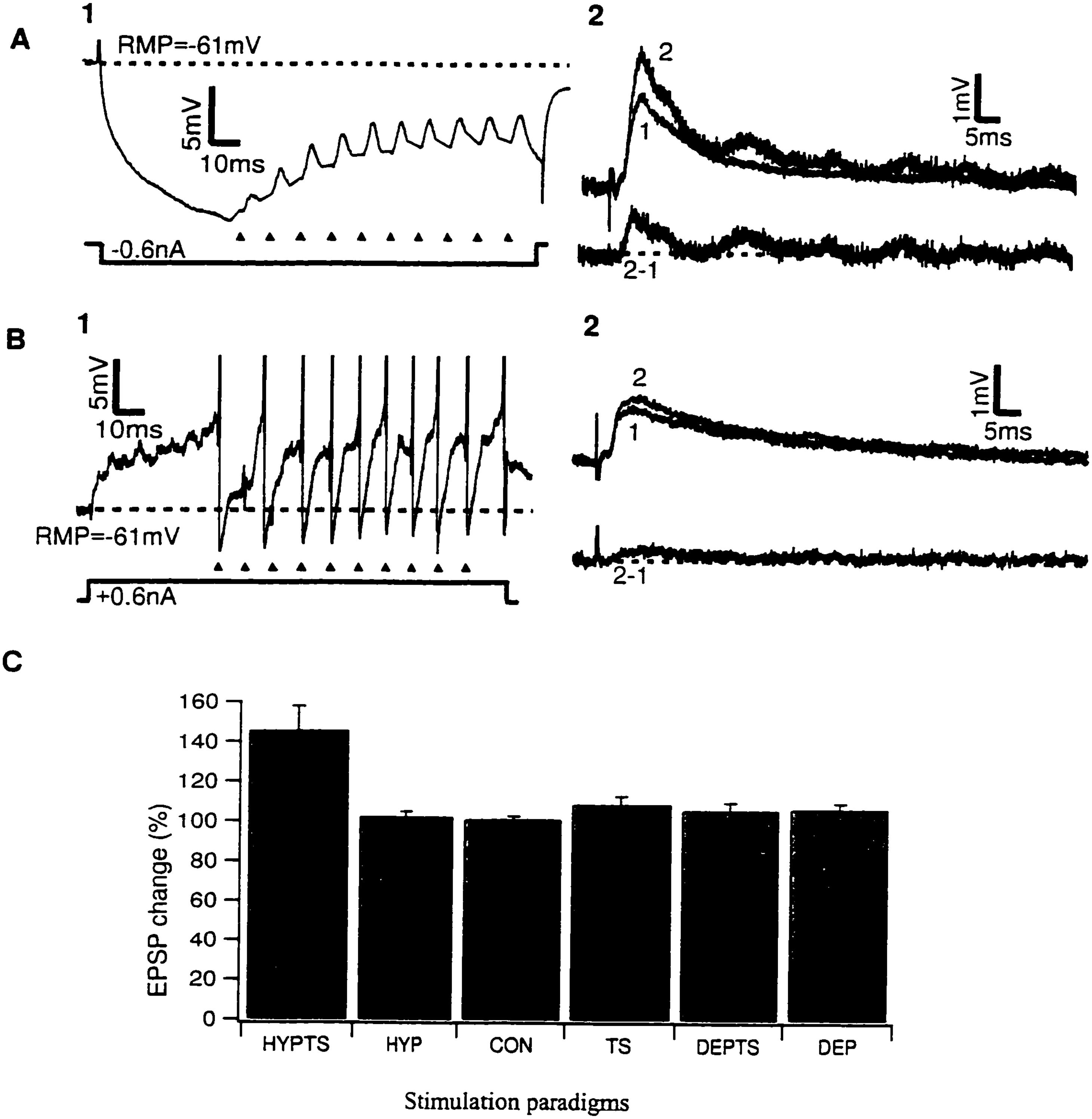


Figure 23: Tetanization paired with hyperpolarization induced anti-Hebbian plasticity

Figure 23: Tetanization paired with hyperpolarization induced anti-Hebbian plasticity.

A: When pairing tetanization with hyperpolarization the membrane potential was hyperpolarized -70 mV while the EPSP build up was still seen (A1). This pairing produced an EPSP (A2:2, not averaged) that had much higher amplitude than the baseline EPSP (A2:1, averaged) by about 45% (A2:2-1). Note that for illustrative purposes the trace of EPSPs superimposed on the hyperpolarization was also selected for figures 24 A and 25 A.

B: While pairing tetanization with depolarization, the membrane potential depolarized and spikes were triggered (B1, spikes were cut off at -40 mV). The EPSP produced by the pairing (B2:2) had a higher amplitude (2-1) than that of the baseline (1).

C: Bar graph showing the percentage change produced by different stimulation protocols. (HYPTS: hyperpolarization paired with tetanization, HYP: hyperpolarization alone, CON: control, TS: tetanization alone, DEPTS: depoalrization paired with tetanization, DEP: depolarization alone.)

The effects of APV on the anti-Hebbian plasticity

In the presence of 100 nM D,L-2-amino-5-phosphonovaleric acid (APV), an NMDA receptor antagonist (Berman et al., in press), the EPSP amplitude was reduced by about 20% and the tetanization of TSF paired with hyperpolarization of pyramidal cells (Figure 24A) produced an increase by pyramidal EPSP by 46% (46.49% ± 10.25, N=4 cells/slices, Figure 24B, C) over 15 minute post-tetanus period and decayed back to baseline at 20 min. This potentiation was not significantly different from the tetanization-hyperpolarization group (Figure 24C, P>0.99). I conclude that anti-Hebbian potentiation does not depend on NMDA receptors.

The effects of KN-62 on the anti-Hebbian plasticity

After KN-62 was puffed onto the focal VML site a single test pulse produced an EPSP simialr to that of control; tetanization of TSF paired with pyramidal cell hyperpolarization induced a 25% increase in EPSP amplitude (25.66% ± 10.22, N=9 cells/slices) over the 15 minute posttetanus period. This increase was not significantly different from the control (37.69% ± 6.83, N=4 cells, P>0.4). This suggests that CaMKII may not play an important role in the anti-Hebbian plasticity.

The effects of calyculin A (CAL-A) on the anti-Hebbian plasticity

The effects of CAL-A on anti-Hebbian plasticity is summarized in Figure 25. In the presence of 0.5 μM CAL-A, TSF stimulation evoked an EPSP similar to that of control; when tetanization was paired with hyperpolarization (Figure 25 A) a 36% increase in EPSP amplitude over the 15 minute posttetanus period was recorded (36.90% ± 11.16, N=6 cells/slices, Figure 25B, C-CAL-A), which was not significantly different from the 24% increase (24.64% ± 7.03, N=4 cells, Figure 25C-CON) observed in the control group (P>0.4). I conclude that the anti-Hebbian plasticity in the direct feedback system does not depend on the protein dephosphorylation mediated by PP1 and PP2A.

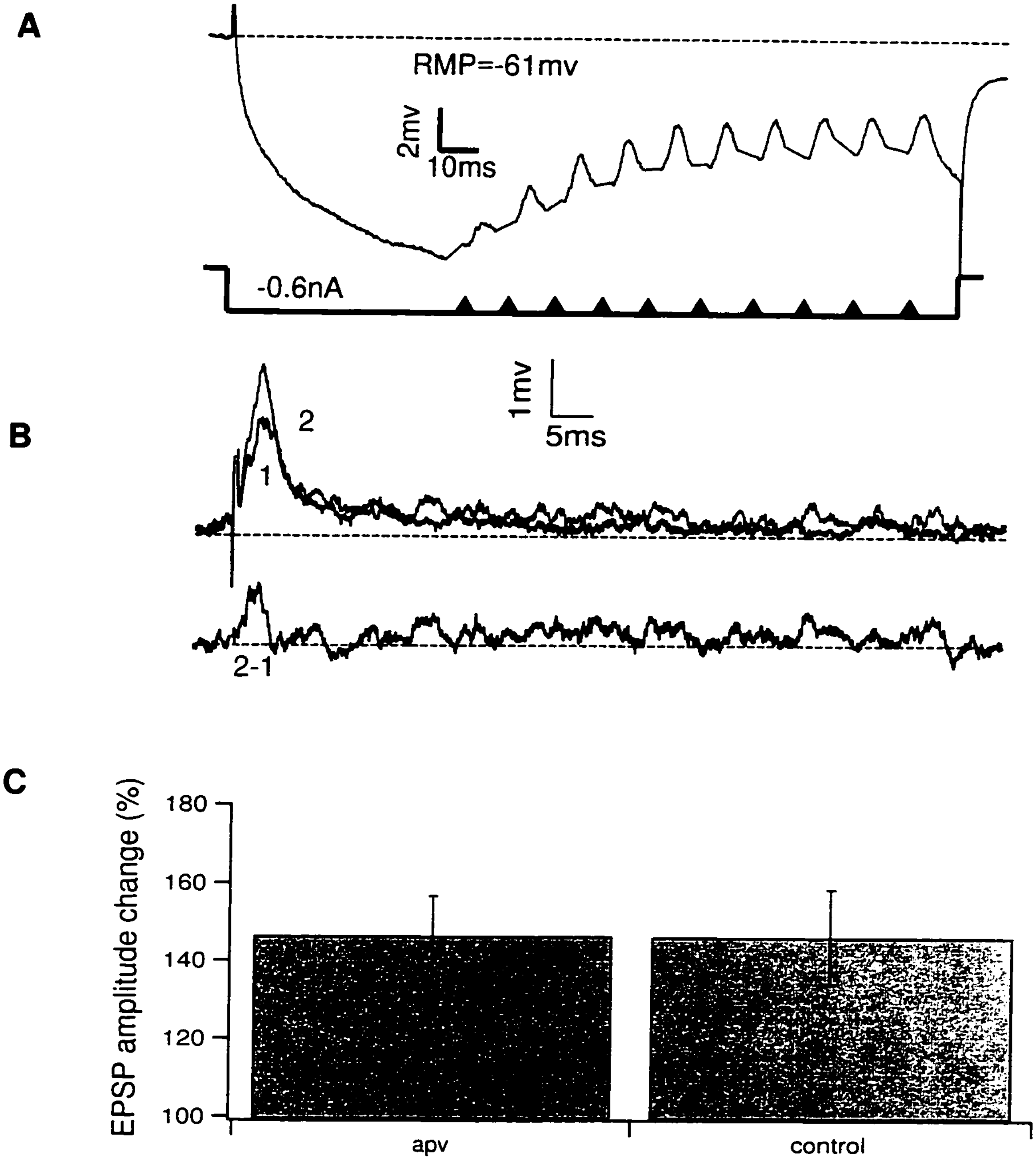


Figure 24: APV had no effects on anti-Hebbian plasticity

Figure 24: APV had no effects on anti-Hebbian plasticity.

A: the tetanization in conjunction with hyperpolarization brought down the membrane potential to -70 mV while the compound EPSP was still seen.

B: The pairing produced an EPSP that was higher (2) than the baseline EPSP (1). The net increase is indicated by the difference (2-1).

C: In an average of 4 cases collected in the presence of 100 nM APV, the pairing of tetanization with hyperpolarization produced an increase of EPSP by 46% over 15 min (apv) and this was not significantly different from control (control).

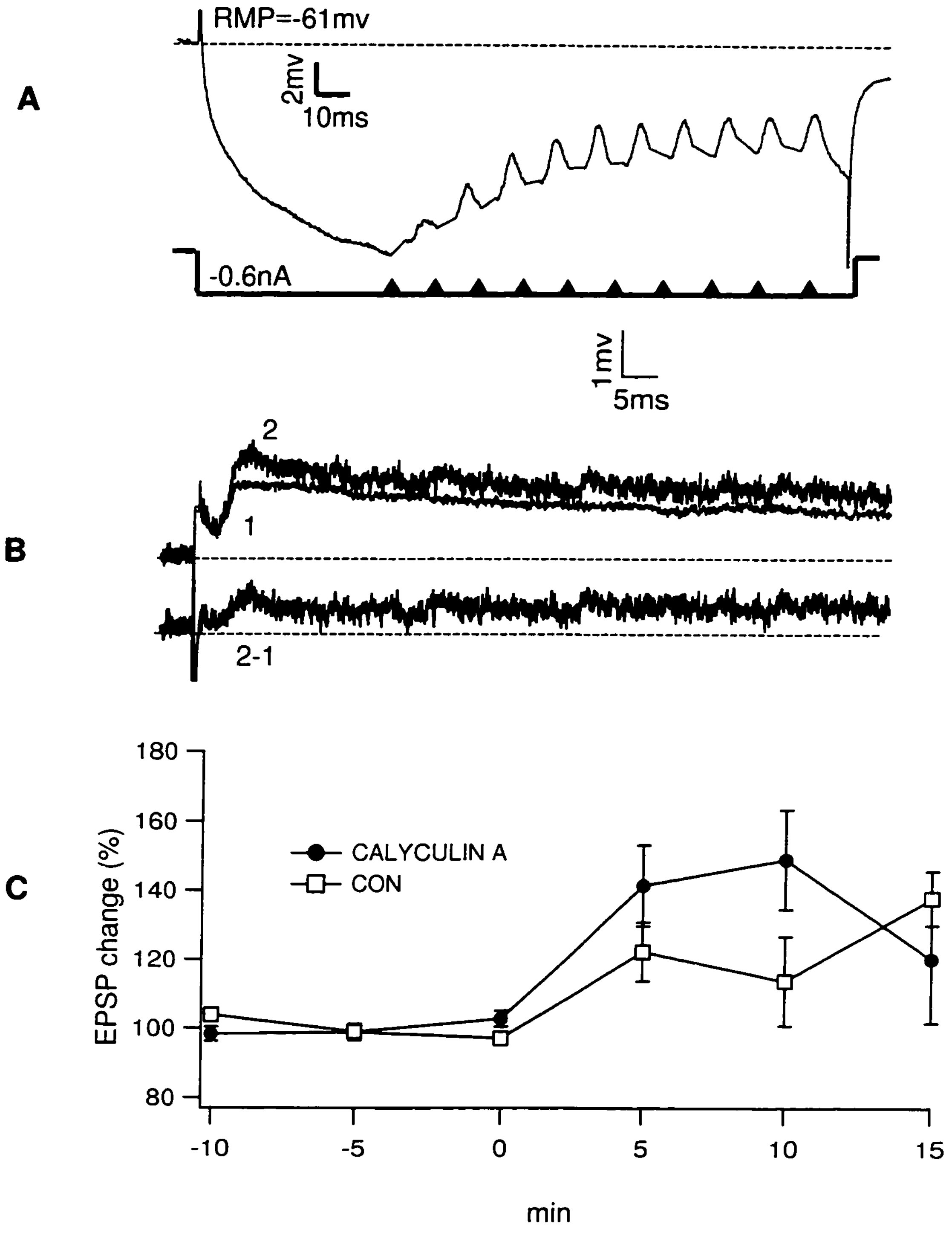


Figure 25: Calyculin A had no effects on anti-Hebbian plasticity.

Figure 25: Calyculin A had no effects on anti-Hebbian plasticity.

A: In the presence of calyculin A, pairing tetanization with hyperpolarization still produced a compound EPSP.

B: The pairing produced a higher EPSP amplitude (2) than the baseline EPSP (1). The net increase is indicated by the difference (2-1).

C: In an average of 6 cases collected in the presence of 0.5 μM calyculin A, the EPSP increased by 37% (CAL-A) , which was not statistically different from the control (CON).

IV. DISCUSSION

IV.I. Introduction

In the past decade neurobiology has developed to a field based on neural network models and molecular biology. The emergence and application of recombinant DNA, monoclonal antibody, computer and imaging techniques in a variety of disciplines (e.g. computer science, biochemistry, genetics) have greatly advanced research in neurobiology. These developments make it possible now for neurobiologists to explain the behaviour of a model system at cellular, synaptic and molecular levels. The hippocampus and cerebellum are model systems that have been used in the analysis of learning and memory and the neuromuscular junction has been used as a model system to study transmitter release.

The electrosensory lateral line lobe (ELL) of the *apteronotus leptorhynchus* has proven to be a good model system for the study of sensory processing. The ELL has 3 segments that process high frequency P-type tuberous receptor inputs, each of them with a similar structure. Each segment receives 3 anatomically distinct excitatory inputs: primary afferents from electroreceptors, the direct feedback fibers from n. preeminentialis and indirect feedback from n. preeminentialis via EGP.

IV.II. Morphology: Identification of glutamate as a neurotransmitter in the input pathways to ELL

Morphological (Maler et al., 1981) and physiological (Bastian, 1981b: Plant, Turner and Maler, 1992; Saunders and Bastian, 1984; Turner and Maler, 1989) studies have demonstrated that both electrosensory afferents (deep neuropil layer) and feedback inputs (ventral molecular layer, dorsal molecular layer) to the electrosensory lateral line lobe are excitatory, but their transmitter(s) were unknown. Since biochemical (Nadi and Maler,1987), binding (Maler and Monaghan, 1991) and electrophysiological (Bastian, 1993) studies have suggested that excitatory amino acids are likely transmitter candidates in the electrosensory lateral line lobe. I first hypothesized that glutamate and/or aspartate are selectively associated with the excitatory inputs to the electrosensory lateral line lobe. The results showed that glutamate is most likely the transmitter.

Several laboratories have conjugated aspartate or glutamate to proteins to increase their antigenicity. I utilized antibodies raised against glutamate or aspartate conjugated to keyhole limpet hemocyanin with glutaraldehyde (Hepler et al., 1988). These antibodies were demonstrated to have a high specificity for aspartate or glutamate conjugates, and no apparent cross reactivity with free amino acids or any glutamate and or aspartate containing compounds. My preabsorption control results support this conclusion.

In the nervous system, glutamate has multiple roles: in cell metabolism, as a precursor for the synthesis of GABA and as a transmitter. Some evidence indicates that glutamate immunoreactivity labels mainly the transmitter pool of glutamate. In the cerebellar and cerebral cortices, physiological and biochemical evidence has independently defined excitatory and inhibitory neurons utilizing glutamate and GABA respectively. Glutamate immunoreactivity has been found to be much higher in the excitatory neurons than in either the putative GABAergic neurons in which glutamate is used for synthesizing GABA (Carder and Hendry, 1994; Ottersen, Zhang and Walberg, 1992; Somogyi, Halasz, Somogyi, Storm-Mathisen and Ottersen, 1986) or other non-glutaminergic neurons where it is used for metabolic purposes (Storm-Mathisen and Ottersen, 1986). A recent study has argued that even though cortical GABAergic neurons may be more metabolically active than excitatory ones, they are not glutamate immunoreactive (Carder and Hendry, 1994). Thus previous work has clearly shown that the intensity of glutamate staining is correlated with the use of glutamate as a transmitter, and not with its role in metabolism or as a GABA precursor. The reason for this is not known, but it has been suggested that the transmitter pool of glutamate may be far larger that the metabolic or precursor pool (Storm-Mathisen and Ottersen, 1986). The findings in this paper provides another line of evidence for the selectivity of glutamate antibodies for the transmitter pool rather than for the metabolic or GABA precursor pool.

At the electron microscope (EM) level the nature of a synapse can be differentiated by the shape of vesicles and the packing density. An excitatory synapse has round vesicles which are densely packed while an inhibitory synapse has elongate pvesicles that are loosely packed.

Our immunocytochemical results at both LM and EM levels suggest that the primary afferents and the direct and indirect feedback inputs to the electrosensory lateral line lobe are glutamatergic. Morphological data (Maler et al.,1981) suggest that the boutons in the pyramidal cell layer are inhibitory and the majority of these boutons appear to utilize GABA as a transmitter (Maler and Mugnaini, 1994). This hypothesis is further supported by *in vitro* experiments in which stimulation of the granular interneurons of the electrosensory lateral line lobe resulted in IPSPs within pyramidal cells (Berman and Maler, 1993).

To demonstrate the role of glutamate as a transmitter the ratio of particle densities in excitatory versus inhibitory boutons is more important than its absolute density per se, since the latter is dependent on slight variations in technique (Buijs, Nunes-Cardoso, Hou and Shinn, 1993; Ottersen, 1989). The significantly higher density of glutamate-immunoreactivity in the excitatory afferents to the electrosensory lateral line lobe and in the pyramidal cells of the electrosensory lateral line lobe compared to that in the inhibitory boutons of the pyramidal cell layer suggests that glutamate is indeed the transmitter of all major excitatory inputs to the electrosensory lateral line lobe as well as of its output cells. In a quantitative study (Ottersen, 1989) the ratio of immunogold particle density in putatively excitatory boutons (based on morphological criteria) to that in putatively inhibitory boutons was 3.6. The ratio of gold particles in excitatory boutons versus inhibitory ones (boutons in the pyramidal cell layer) in the electrosensory lateral line lobe of my study is 2.1 (deep neuropil layer), 2.7 (ventral molecular layer) and 1.7 (dorsal molecular layer) (Figure 7). The lowest ratio (1.7) was for the parallel fibers of the dorsal molecular layer, which, in mammals, are considered to be glutamatergic (Sandoval and Cotman, 1978). These relatively low ratios may be explained by the very high continuous firing rate of electrosensory afferents (from 143-465 Hz, the average is 294 Hz) (Bastian, 1981a). Since the granular interneurons are monosynaptically driven by electrosensory afferents (Maler et al., 1981), they presumably fire tonically at high rates. Their relatively high (in comparison to the inhibitory boutons studied by Otterson) (Ottersen, 1989) levels of glutamate may be required as a precursor for the synthesis of GABA, which are presumably released at a high rate. Purkinje cells in the same sections were nearly devoid of glutamate-immunoreactivity presumably because their lower rate of activity may require a lower level of GABA synthesis and therefore less glutamate as a precursor.

A puzzling feature of my results is the greater density of glutamate-immunoreactivity in the boutons of the ventral molecular layer versus those of electrosensory afferents in the deep neuropil layer. Physiological studies (Bastian, 1981a,b ; Bratton and Bastian, 1990) have demonstrated that the average firing rate of electrosensory afferents is probably much greater than that of the stellate cells which give rise to the tractus stratum fibrosum and thus the terminal boutons of the ventral molecular layer. Therefore it had been expected that electrosensory afferent terminal boutons would have the highest density of glutamate- immunoreactivity. Perhaps the high levels of glutamate in ventral molecular layer boutons is correlated with the dense packing of vesicles within boutons of this layer (Maler et al.,1981). The physiological relevance of this observation is unknown.

Our results suggest that glutamate is the transmitter of afferents in the ventral molecular layer and dorsal molecular layer, consistent with an earlier biochemical study of Nadi and Maler (1987). These authors found a relatively low concentration of glutamate in the deep neuropil layer, contrary to my observation of a high density of glutamate in electrosensory afferents within this layer. This apparent contradiction may be caused by the relative sparsity of electrosensory afferents (Maler et al., 1981) and the presence of many inhibitory cells and fibers within the deep neuropil layer (Maler and Mugnaini, 1994).

I conclude that the major excitatory inputs to the electrosensory lateral line lobe, electrosensory afferents and the direct (ventral molecular layer) and indirect (dorsal molecular layer) feedback pathways, all use glutamate as their neurotransmitter. Projection neurons of the electrosensory lateral line lobe are also glutamatergic. This result supports the view that glutamate may be the transmitter of most excitatory synapses in the vertebrate nervous system (Orrego and Villanueva, 1993).

IV.III. Electrophysiology and pharmacology of TSF-evoked synaptic plasticity

Behavioral and electrophysiological studies have indicated that there is a gain control mechanism in the ELL which involves feedback pathways (Bastian, 1986a, b) and uses glutamate as transmitter. Electrophysiological studies in other preparations have established a good correlation between excitatory amino acid transmitter and plasticity. I proposed that some forms of plasticity exist in the ELL pathways that carry out the gain control mechanism. Since relatively complete studies have been done in the centromedial segment and in the direct feedback projection to ELL, the CMS has become the target for this investigation.

The present study demonstrates that tetanization of the TSF pathway produces 2 types of plasticity of ELL pyramidal cell EPSPs: firstly, a short-lived (<30 s) post-tetanic potentiation, and secondly, a longer-lasting (10 minutes) anti-Hebbian potentiation when the tetanization is paired with postsynaptic hyperpolarization. However, my stimulation paradigms did not induce any long-term change (LTP or LTD) in synaptic efficacy.

IV.III.I. Induction of posttetanic potentiation and its mechanisms

PTP is a commonly observed enhancement of synaptic transmission which follows tetanic stimulation and lasts for about 5 min (Magleby, 1979; Regehr, Delaney and Tank, 1994). PTP has been induced in hippocampus (Langdon et al., 1995; Salin, Scanziani, Malenka and Nicoll, 1996), invertebrate synapses and vertebrate neuromuscular junctions (NMJ, Atwood and Wojtowicz, 1986; Magleby, 1987). My induction of PTP from the ELL of weakly electric fish *in vitro* and by others *in vivo* (Bastian, 1996a, b) provide another model system for the study of plasticity.

PTP has been thoroughly characterized in invertebrates and in the neuromuscular junctions (NMJ, Atwood and Wojtowicz, 1986; Magleby, 1987) and currently it is intensely investigated in other preparations such as the hippocampus. Although the frequency range for inducing PTP depends on the preparation (Salin et al., 1996) and although PTP duration varies slightly, similarities in induction and time course suggest that PTP might be governed by the same mechanism.

A hypothesis based on quantal transmitter release (Katz, 1969) has been used to elucidate the PTP mechanism. According to the quantal theory, transmitters such as GABA, glutamate and acetylcholine are released as multimolecular packets called quanta. While at the NMJ each quantum has about 5000-10000 transmitter molecules the quantal size at the central synapses varies. A quantum is packaged in a single synaptic vesicle and released by exocytosis from release sites in a specific active zone in the presynaptic terminal. At rest, there is spontaneous release resulting in spontaneous miniature synaptic potentials (Stevens, 1993). The release of a quantum is probabilistic and independent of the release of other quanta. Each release site releases 0 or 1 quantum in response to an action potential and synapses have different quantities of release sites. Therefore, there is a variation in the number of released quanta, and the size of postsynaptic potentials. When an action potential propagates to the terminals voltage-gated Ca^{2+} channels (Jackson, 1995; Verhage, Ghijsen, Lopes and Silva, 1994; Roberts, Jacobs and Hudspeth, 1990; Robitaille and Tremblay, 1991; Cohen, Jones and Angelides, 1991) and Na^{+} channels open. The resultant Ca^{2+} influx produces a local Ca^{2+} accumulation in the active zone. The higher local Ca^{2+} concentration triggers vesicle fusion. During release the transmitter molecules get into the synaptic cleft and the synaptic vesicles are recycled by replenishing the transmitter content from cytoplasmic stores. In the present study Mn^{2+} bath application blocked the field potential by 60%, which supports the role of Ca^{2+} in synaptic transmission within the VML. Mn^{2+} is a divalent ion that binds with voltage-gated Ca^{2+} channels in a competitive manner with Ca^{2+}. At low concentration of Ca^{2+} in ACSF, Mn^{2+} completely blocks Ca^{2+} entry into the presynaptic terminals and terminate the synaptic transmission (Anderson, 1983; Tan, Barnett, Hehn, Quay and Galper, 1991).

It has been observed that if the terminals are excited by 2 consecutive action potentials, the second one produces a bigger response presumably by adding Ca^{2+} to the residual Ca^{2+} that remains from the first action potential and consequently yielding a greater transmitter release (Katz and Miledi, 1968; Robitaille and Tremblay, 1991; Zucker, 1989). This residual Ca^{2+} theory has also been used to explain PTP.

The effects of protein kinases

Recently, relations between the intraterminal Ca^{2+} buildup and action potential frequencies has been successfully simulated (Tank, Regehr and Delaney, 1995). While a few experiments show a direct linear relationship between the presynaptic Ca^{2+} and enhancement (Delaney, Zucker and Tank, 1989; Delaney and Tank, 1994), others demonstrated the maximal PTP occurs after Ca^{2+} reaches its peak (Regehr et al. 1994; Salin et al; 1996). The mismatch in the time course of PTP and Ca^{2+} suggests that the residual Ca^{2+}, in addition to triggering the vesicle fusion, also modulates the probability of transmitter release through a Ca^{2+}-sensitive biochemical process. The biochemical target of Ca^{2+} remains controversial. Some evidence shows that this target is protein phosphorylation mediated by CaMKII since CaMKII inhibitor blocks or reduces the PTP (Griffith, Wang, Zhong, Wu and Greenspan; 1994; Salin et al., 1996; Wang, Renger, Griffith, Greenspan and Wu, 1994), a finding which agrees with my KN-62 results in VML. However, in other preparations, presynaptic injections of the calmodulin binding domain and the calmodulin inhibitor calmidazolium, bath application of KN-62 and calmidazolium did not have effects on PTP or other transmitter-mediated processes (Kamiya and Zucker, 1994). This is in agreement with my DML recordings where KN-62 did not affect PTP. The results of KN-62 in VML and DML can be explained by proposing that the Ca^{2+} target in the terminals of VML and DML are different. In VML synapses it is CaMKII while in DML synapses it may be some other protein kinase.

CaMKII is a member of Ca^{2+} calmodulin-dependent protein kinase superfamily, which is phylogenetically conserved during evolution. The α-subunit, which is neuron specific (Tobimatsu and Fujisawa, 1989), has been identified in the VML of the ELL of the electric fish (Maler, personal communication). In the presynaptic terminals, several vesicle proteins are found to be the substrates for CaMKII. Synapsin I is found to be located on synaptic vesicles (Huttner, Schleiber, Greengard and De Camilli, 1983; De Camilli, Cameron and Greengard, 1983; De Camilli, Harris, Huttner and Greengard, 1983) and acts as a cross-linker between the vesicles and cytoskeleton. Phosphorylation of synapsin I is believed to dissociate synapsin I from the skeleton, allowing the synaptic vesicles to move to the active zone, and therefore increase the probability of vesicle release (Llinás, MaGuinness, Leonard, Sugimori and Greengard, 1985; Llinás et al., 1991). The postsynaptic potentials increase in response to the increased transmitter release. Synaptotagmin, synaptophysin and VAMP/SBV are integral membrane proteins and they participate in vesicle docking, fusion and formation of the channel pore. The phosphorylation of synaptotagmin (Popoli, 1993), synaptophysin (Rubenstein, Greengard and Czernik, 1993) and VAMP/SBV (Nielander et al., 1995) by CaMKII might also increase the availability of transmiter to be released. Recently, another vesicle protein, rabphilin-3A, has been proposed to be a substrate for CaMKII (Fykes, Li and Südhof, 1995) for regulation of the transmitter release.

The present study further shows that KN-62 acts on presynaptic terminals rather than postsynaptic ones. The TSF fiber terminals in VML and parallel fiber terminals in DML contact the same pyramidal cells. PTP is inducible from both VML (Bastian, 1996b) and DML synapses. When applied focally, KN-62 blocked TSF-evoked PTP in VML and had no effects on the parallel fiber-evoked PTP in DML. If KN-62 acted postsynaptically, PTP evoked from both layers would be blocked, otherwise this blockade would be presynaptic. A presynaptic site for this effect is further supported by the following 2 lines of evidence: (1) the intracellualr injection of the CaMKII inhibitory peptide into the pyramidal cells left the PTP in VML unaffected. (2) when the KN-62 was applied intracellularly into the postsynaptic neuron (pyramidal cells), the TSF-evoked PTP in VML was not affected. Although KN-62 could diffuse out to the VML its concentration would decrease dramatically at high volume.

Finally, the PTP blockade by KN-62 in VML is specific since KN-04, a structural analogue of KN-62, had no effects; nor did DMSO.

Althought it has been reported that KN-62 blocks Ca2+ channels in other preparations (Hajimohammadreza, Probert, Coughenour, Borosky, Marcoux, Boxer, Wang, 1995; Sitges, Dunkley, Chiu, 1995), it does not seem to be the case in my preparation since the baseline EPSP was not reduced by KN-62.

Gutamate presynaptic metabotropic receptors might play a role in regulating transmitter release and therefore PTP, but their possible contribution has not been explored.

In summary, this part of the study shows that PTP in TSF-pyramidal synapses is presynaptically mediated by CaMKII while the biochemical basis of PTP in parallel fiber-pyramidal synapses is not known. Given the presence of forskolin and phorbol ester binding in DML (Maler, personal communication) it is possible that PKA and PKC might be involved. It is concluded that what appears to be a similar physiological phenomena (PTP) may have different molecular mechanisms.

The effects of protein phosphatases:

The PTP time course is also controlled by protein dephosphorylation processes mediated by phosphatases. The phosphatases consist of a superfamily and 5 members of the family, PP1, PP2A, PP2B, PP2C (Fukunaga et al., 1993; Wang, Orser, Brautigan and MacDonald, 1995) and PP3 (Honkanen et al., 1994), have been characterized. In non-nervous systems, antagonism to the phosphatases potentiates the CaMKII or PKC-mediated phosphorylation, resulting in hyperphosphorylation (Takuma, Ichida, Okumura and Kanazawa, 1993; Hwang, Bragado, Duan and Williams, 1996). In the nervous system, phosphatase antagonists have been shown to increase transmitter release (Herron and Malenka, 1994; Marantz, Reiss, Przedecki Naor, 1995; Nichols, RA., et al., 1994; Vickroy, Malphurs and Carriger, 1995; Wang, Chen, Kolaj and Randic, 1995;). However, in the ELL, calyculin A did not enhance the PTP.

Since calcineurin (PP2B) is Ca^{2+}-dependent and shows a dense distribution in VML (Maler, personal communication), FK-520, a calcineurin antagonist, was also tested. FK-520 inhibits the calcineurin by competing for the Ca^{2+}-calmodulin binding sites. Its blocking of calcineurin in non-nervous systems has been confirmed while its effects in the nervous system remains to be elucidated. One report indicated that FK-520 increases transmitter release in synaptosomes (Nichols et al., 1994). Surprisingly, FK-520 only showed an insignificant effect on the PTP, even at a high concentration. More surprisingly, when calyculin A and FK-520 were applied together, even after being incubated for a longer time, PTP was unchanged. It seems to be that protein phosphatases PP1, PP2A and PP2B may not be important for PTP of the TSF pathway. Alternatively, the antagonists tested may not work in the fish.

The inability of classic phosphatase blockers to antagonize protein phosphatases was reported before (Fukunaga et al., 1989). This seems to suggest the involvement of other phosphatases. Recently, 2 new types of phosphatases, namely PP2C (Fukunaga et al., 1993; Wang et al., 1995) and PP3 (Honkanen et al., 1991; Shenolikar, 1994), have been identified. PP2C is Mg^{2+}-dependent and is not blocked by the PP1 and PP2A inhibitors (okadaic acid and calyculin A). Ca^{2+} at concentrations above the physiological range (20-90mM) inhibits its activity. PP2C was found to specifically block the CaMKII-mediated phosphorylation (Fukunaga et al., 1993). The development of new specific PP2C antagonists will be helpful in identifying the dephosphorylation mechanism in VML. The possibility of PP3 involvement is unlikely since it is also blocked by calyculin A (Honkanen et al., 1994).

Functional aspects:

Functionally, the TSF feedback pathway has been suggested to be the basis of a "searchlight" mechanism which aids the animal in the electro-detection of small moving objects (Bratton and Bastian, 1990), a hypothesis supported by the anatomy (Maler and Mugnaini, 1994) and physiology of the system (Berman et al., in press). Like in thalamus, the feedback pathway enhances the pyramidal response to a strong focal ascending input through its excitatory pathway while it depresses the weak inputs through its inhibitory pathway. This results in focusing attention to a specific ascending input. The presence of PTP at TSF synapses in VML further suggests that the putative searchlight mechanism can be modified by the recent experience of the fish. If the fish scans an object which causes a strong activation of the TSF, then any input to the same electroreceptors will cause a potentiated response of TSF synapses for the next 30 sec: this will presumably summate with the direct electroreceptor input caused by that object and cause it to be more readily detected by the fish. Since *A. leptorhynchus* scans its environment in a stereotyped manner (Lannoo and Lannoo, 1993), it is possible that the same object will stimulate the same sequence of receptors on the body of the fish, leading to PTP of the TSF pathway and an improved ability to locate that object. Objects encountered during a different scanning motion will activate a different sequence of electroreceptors and will presumably not cause PTP of the same TSF fibers (Berman et al., in press). A presynaptic location for PTP is important in this regard, since it implies that there is no generalized postsynaptic enhancement (of TSF synapses) of the pyramidal cell response to TSF input. Thus there will be no change in the response to objects scanned from a different direction; in another words, a randomly encountered object is not likely to activate the potentiated TSF fibers and will not produce an enhanced response. The direct electrosensory feedback system may therefore selectively enhance the detectability of objects along the fish's scanning path versus randomly encountered objects. Clearly, given the complex dynamics of this feedback pathway, these ideas will have to be modeled and tested by *in vivo* experiments.

IV.III.II. The lack of long-term plasticity

Tetanic stimulation of certain fiber systems in the hippocampus produces a long term potentiation of EPSPs (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973); stimulation of these same fibers at low rates produces a long term depression (Dunwiddie and Lynch, 1978; Dudek and Bear, 1992). Both the LTP and LTD at these sites depend on NMDA receptors and the Ca^{2+} influx which results from their activation (Reyes and Stanton, 1996; Mulkey and Malenka, 1992). Similar, NMDA receptor mediated, long term forms of synaptic plasticity are found in mammalian cerebral cortex (Artola and Singer,1987; Artola, Bröcher and Singer, 1990; Berry, Teyler and Han, 1989; Hirsch and Crepel, 1990; Tsumoto, Hagihara, Sato and Hata, 1987). TSF fibers utilize glutamate as a transmitter (Wang and Maler, 1994) and autoradiographic binding studies (Maler and Monaghan, 1991) and in situ hybridization (Bottai, Ellis, Maler and Dunn, 1996; Bottai et al; in press) suggest that TSF evoked EPSPs in ELL pyramidal cells are mediated in part by NMDA receptors. This has now been directly confirmed by electrophysiological recordings (Berman et al., in press). It was therefore surprising that I could not elicit either LTP or LTD with any stimulation protocol of TSF. The inability to elicit LTP was found even when I enhanced excitatory transmission by pharmacological blockade of GABA. The Hebbian protocol (tetanization paired with postsynaptic depolarization), which has been successfully tested in other preparations (Durand, Kovalchuk and Konnerth; 1996; Jaffe and Johnston, 1990; Otsu, Kimura and Tsumoto, 1995;) also failed to induce LTP. LTP and LTD depend on a complex cascade of second messenger interactions (O'Dell, Kandel and Grant, 1991; Malenka et al., 1989; Malinow et al., 1989; Linden and

Routtenberg, 1989). A number of studies have demonstrated that the initial steps essential for LTP involves activation of postsynaptic α–CaMKII (Glazewski, Chen, Silve and Fox, 1996; Silva et al., 1992 a, b) and protein kinase C (Akers et al., 1986; Hu et al., 1987; Malenka et al., 1986). Although CaMKII is present in ELL, the α-CaMKII isoform is confined to TSF terminals in VML, while pyramidal cells contain only the β–CaMKII isoform (Maler and Hincke, unpublished observations). I therefore hypothesized that it would not be possible to elicit LTP from ELL pyramidal cells (via TSF) because they lack critical second messenger systems. The lack of LTP in the direct feedback system is consistent with the idea that the NMDA receptors at this site are important for non-linear enhancement of electrosensory input (Berman et al., in press) rather than LTP. This is also reasonable, from the standpoint of sensory processing, since LTP or LTD in the ELL would prevent the electrosensory system from tracking rapidly changing inputs.

IV.III.III. Anti-Hebbian plasticity

Anti-Hebbian plasticity is a common feature of electrosensory systems. It was first discovered by Bell (1981) in the mormyrid fish. The electrosensory lobe neurons in the mormyrid receive input fibers from electroreceptors that are activated by electric organ discharges (EOD). In addition they also receive fibers from higher centers that carry the signals associated with the EOD motor command (corollary discharge). With each EOD the electrosensory lobe receives both electrosensory afferent inputs and corollary discharges from higher centers. *In vivo* recordings in mormyrid show that by pairing an electrosensory stimulus (local excitatory or inhibitory) with an EOD motor command produces a corollary discharge response that has the opposite sign to the electrosensory stimulus; that is, pairing with an excitatory electrosensory stimulus leads to a reduction in the excitatory or inhibitory corollary discharges while pairing an inhibitory electrosensory stimulus results in a potentiation of excitatory corollary discharges. This plasticity also has spatial specificity: the pairing is effective only if the electrosensory stimulus is within the neuron's receptive field (Bell, 1981; 1982; Bell and Grant, 1992; Bell, Caputi, Grant and Serrier, 1993).

This anti-Hebbian plasticity has since been described in elasmobranchs as the mechanism used to cancel repetitive signals produced by the fish's own movement, for example, the ventilation movement (Montgomery and Bodznick, 1994). A recent neural network modelling study based on the circuitry of elasmobranch's dorsal octavolateral nucleus (DON) suggests that removing the repetitive signal from the electrosensory input requires a descending feedback pathway that carries the corollary input related to the repetitive signals.

Bastian (1995; 1996a) has recently reported that, in *A. leptorhynchus*, bending the tail causes EOD amplitude modulation which is received over the entire body surface, as well as proprioceptive input. While the electroreceptors respond well to this EOD amplitude modulation, the ELL pyramidal cells do not. When the tail bending is paired with an additional local electrosensory stimulus of the same frequency as tail bending (which is also in phase with tail bending: increase of EOD amplitude; or out of phase with tail bending: decrease of EOD amplitude) the basilar pyramidal cell fires preferentially as the EOD amplitude increases and is silent as the EOD amplitude decreases. However, the modulation of pyramidal cell firing rate decreases over time as the local electrosensory stimulus is applied. If tail bending is again applied alone following the pairing of the tail bending with the local electrosensory stimulus, it becomes an effective stimulation to the pyramidal cell, which responds in a pattern that is in antiphase relative to the pattern caused by pairing the tail bending with the local electric stimuli, that is, if the pairing causes the basilar pyramidal cell to discharge the subsequent tail bending alone silences it; if the pairing silences the cell the following bending causes it to fire. The pyramidal cell response decays quickly and stops responding in 2-3 min with continuing tail bending. Finally, when presented alone to the cell's receptive field, the local electrosensory stimulus produces a smaller EOD amplitude modulation but the pyramidal cell responds strongly to the local stimulus. The tail bending alone presented before and after the local electric stimulus does not produce significantly different responses in the pyramidal cells. Further experiments show that global electrosensory or proprioceptive inputs paired with local electric stimuli produce the same results. These results suggest that the declining response of the pyramidal cell to the increased electrosensory input caused by pairing local electric stimulus with tail bending involves an active cancellation mechanism and these experiments also show that this mechanism depends on electrosensory input or proprioceptive input.

In mormyrids, Bell et al. (Bell et al; 1993) have shown that this active cancellation is due to anti-Hebbian alterations of synaptic strength in the feedback inputs to the molecular layer of the ELL. Bastian (1996a) has directly shown that anti-Hebbian plasticity in *A. leptorhynchus* can be prevented by pharmacological blockade of AMPA receptors in the molecular layer of the ELL. Since drug application *in vivo* might affect both ventral and dorsal molecular layers of the ELL (direct and indirect feedback systems, respectively), the latter study could not specify the precise cellular site of the plasticity.

In an *in vivo* intracellular recording, Bastian (1996b) also utilized electrical stimulation of the TSF in conjunction with either (1) physiological activation of ELL pyramidal cells via input to their receptive fields, or (2) current injection into these cells to hyper- or depolarize them. The latter experiments are directly comparable to those I are reporting and my results are in close agreement with those of Bastian. Bastian reports that pairing tetanic stimulation of TSF with hyperpolarization of pyramidal cells leads to a significant increase in the amplitude of TSF evoked EPSPs by 43.3 % (our result is 45.6%). It is interesting that the increase in spike number is substantially greater (70%) than that of the EPSP; the reason for this disparity is not known, but it suggests that a non-linear step, such as Na^+ currents, intervenes between synaptic potentials and spike generation. Pairing tetanic stimulation of TSF with depolarization of pyramidal cells leads to a small increase in the evoked EPSP, which might be due to PTP; this is comparable to my results in that I also find that TSF tetani paired with depolarization does not lead to a decrease in evoked EPSPs. Although Bastian attempted to limit his stimulation to TSF, this is difficult to prove *in vivo*; furthermore, it is not possible to rule out a lowered threshold for stimulated fibers *in vivo* (due, for example, to pathological changes at the site of stimulation). In the slice preparation I can selectively stimulate TSF (Berman et al., in press) and I have shown that the TSF fiber volley is not enhanced by tetanic stimulation. Thus my replication of Bastian's results *in vitro* confirms that they are due to non-pathological stimulation of TSF fibers.

There are important differences between my results and those of Bastian. Bastian (1996b) reports that pairing tetanus of TSF with depolarization of pyramidal cells causes TSF stimulation to evoke a hyperpolarization of pyramidal cells; this is the cause of the decreased spike discharge to TSF stimulation and the other side of the anti-Hebbian plasticity. Equivalent pairings in my slice preparation did not produce such hyperpolarizations. Bastian has proposed that the hyperpolarization in his experiments is due to an enhancement of TSF evoked inhibition. It is therefore possible that, in my *in vitro* preparation, I are not effectively stimulating inhibitory pathways. For example my stimulating electrode is placed so as to prevent activation of the direct inhibitory feedback projection (Maler and Mugnaini, 1993; 1994). Furthermore, the main interneuron which would be activated by TSF stimulation (the vml cell; Maler and

Mugnaini, 1994) also receives extensive input from the dorsal molecular layer (Maler. 1979) and might therefore be difficult to bring to threshold by TSF stimulation *in vitro*. given the lack of background input to most of its dendritic tree. Bastian reports that anti-Hebbian plasticity last far longer *in vivo* (>20 min) than it does *in vitro* (<20 min). It should be noted that this was not tested in a directly comparable way using TSF stimulation paired with intracellular hyperpolarization, but rather with the use of natural stimulation to hyper- or depolarize pyramidal cells. Natural stimulation will cause activation of dorsal plus ventral molecular layer inputs and will cause hyperpolarization of pyramidal cells via various interneurons. It is therefore likely that the additional circuits and processes activated by natural stimulation are responsible for the differences between my results and those of Bastian. This idea is supported by Bastian's (1996b) demonstration that pairing TSF stimulation with physiologically evoked hyperpolarization (via IPSPs) causes far greater anti-Hebbian enhancement (>300%) than pairing stimulation with hyperpolarization. It will be an important focus of future work to examine in great details the *in vivo* results using *in vitro* techniques in order to understand more completely the cellular mechanisms responsible for anti-Hebbian plasticity.

The anti-Hebbian potentiation of TSF evoked EPSPs is likely to be one of the cellular bases of the sensory anti-Hebbian plasticity described by Bastian (1995. 1996a). Its role is presumably to reduce the responsiveness of the electrosensory system to expected reafferent input (Bastian, 1996b; Bell et al., in press). The molecular layer of the ELL is similar to that of the cerebellum (Maler and Mugnaini. 1994) and it is interesting that there have been reports of anti-Hebbian plasticity in the mammalian cerebellum (Ito et al, 1982; Ekerot and Kano, 1985; Sakurai. 1987). This suggests that a similar cancellation mechanism may exist in cerebellum. A good example for human being would be like this: A person wears clothes that have different quality in terms of weight from the previous one. Initially he feels the heavier clothes and in a while this feeling disappears. Physiologically, the new clothes activate receptors (Meissner and Pacini corpuscles in particular) and the touch and pressure are transmitted to the cortex. After a period of time the receptors become adapted. At higher level (the thalamus or the sensory cortex) stimulated repeteadly by the same pattern of touch and pressure inputs the CNS may produce a negative image of the inputs to cancel the sensations produced by the clothes and the person may no longer feel the heaviness of the clothes.

The molecular bases of the anti-Hebbian plasticity in this system is still completely obscure; my only information is negative: this type of plasticity appears not to depend on NMDA receptors or on protein phosphorylation/dephosphorylation. This seems to be predictable since NMDA receptors are activated at -30 mV or higher (Alford, Renguelli, Schofield and Collingridge, 1993; Burnashev et al., 1995; Kataoka and Ohmori, 1994) and at the membrane potential (-75 mV) produced by the anti-Hebbian protocol, NMDA receptors may not be open (Zidichouski and Jhamandas, 1993) due to a Mg^{2+} blockade. Therefore, the anti-Hebbian plasticity may not depend on NMDA- mediated Ca^{2+}.

Glutamate postsynaptic metabotropic receptors might play a role in anti-Hebbian plasticity, but their contribution has not been explored.

It has been observed that PKC/PTK (protein tyrosine kinases, PTK) mediated protein phosphorylation (Boxall, Lancaster and Garthwaite, 1996) is responsible for LTD in the cerebellum (another version of anti-Hebbian plasticity induced by tetanization paired with depolarization) and dephosphorylation (Mulkey, Endo, Shenolikar and Malenka, et al., 1994) is responsible for the LTD in hippocampus. The possible involvement of these 2 kinases-mediated biochemical processes in the anti-Hebbian plasticity in ELL requires more work in the future.

Functional aspects

Anti-Hebbian plasticity might more generally be considered as a mechanism for normalizing synaptic transmission. Sustained changes in EOD amplitude lead to a slowly adapting response of *A. leptorhynchus* tuberous electroreceptors (Xu, Payne and Nelson, 1996). ELL pyramidal cells may hyperpolarize if there is a tonic decrease in their input (basilar pyramidal cells or E cells; the opposite would be expected for non-basilar or I cells; Saunders and Bastian, 1984) and this would reduce their response to a local increase in EOD amplitude. If anti-Hebbian plasticity increased the response of pyramidal cells to the TSF input, it would compensate for the hyperpolarization; the net effect would be to permit the pyramidal cell to give the same response to the local input independent of the cell's resting membrane potential. The TSF might then be considered to operate at 3 time scales. Anti-Hebbian plasticity normalizes TSF-evoked EPSPs over minutes, allowing the system to cancel out slow changes in EOD amplitude. PTP, superimposed on the anti-Hebbian plasticity, enhances the response to TSF input over approximately 30 seconds. The voltage dependent augmenting response of pyramidal cells to TSF input (Berman et al., in press) is superimposed on PTP and increases the effectiveness of the putative "searchlight" mechanism over a millisecond time scale.

Several recent studies have emphasized the importance of normalizing synaptic strength (Abbott, Varela, Sen Nelson, 1997; Bell and Sejnowski, 1995; De Schutter, 1995). Although the underlying cellular mechanisms are probably different, in each case normalization permits the output cell to respond in an appropriate fashion over a wide range of afferent input. In particular, Abbott et al. (1997) have emphasized the role of synaptic depression in normalizing gain control of afferent inputs discharging tonically at different rates. Synaptic depression would be inappropriate for the TSF, a system which discharges phasically (Bratton and Bastian, 1990) and may require an augmenting synaptic response (Berman et al., in press). A comparative analysis of synaptic normalization may better reveal how biophysical mechanisms are adapted to the requirements of different neural systems.

V. REFERENCES:

Abbott L F, Varela J A, Sen K, Nelson S B:Synaptic depression and cortical gain control. Science 275: 220, 1997.

Akers R F, Lovinger D M, Colley P A, Linden D J, Routtenberg A. Translocation of protein kinase C activity may mediate hippocampal long-term potentiation. Science 231: 587, 1986.

Alder J, Xie Z, Valtorta F, Greengard P, Poo M: Antibodies to synaptophysin interfere with transmitter secretion at neuromuscular synapse. Neuron 9: 759, 1992.

Alesiani M, Galli A, Mori F, Pecorari R, Carla V, Cherici G, Pellicciari R: Thiokynurenates: a new group of antagonists of the glycine modulatory site of the NMDA receptor. European J Pharmacol 199: 227, 1991.

Alford S, Renguelli B G, Schofield J G, Collingridge G L: Characterization of Ca^{2+} signals induced in hippocampal CA1 neurones by the synaptic activation of NMDA receptors. J Physiol 469: 693, 1993.

Alger B E, Teyler T J: Long-term and short-term plasticity in the CA1, CA3 and dentate regions of the rat hippocampal slice. Brain Res 110: 463, 1976.

Andersen P (1975): Organization of hippocampal neurons and their interconnections. In Isaacson RL,and Pribram,eds.The Hippocampus.New York:Plenum Publishing Corp, 155.

Andersen P, Sundberg S H, Sveen O, Wigstrom H: Specific long-lasting potentiation of synaptic transmission in hippocampal slices. Nature 266:736, 1977.

Anderson M: Mn^{2+} ions pass through calcium channels. A possible explanation. J Gen Physiol 81:805, 1983.

Aram J A, Michelson H B, Wong R K: Synchronized GABAergic IPSPs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. J Neurophysiol 65:1034,1991.

Artola A, Bröcher S, Singer W: Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. Nature 347:69, 1990.

Artola A, Singer W: Long-term potentiation and NMDA receptors in rat visual cortex. Nature 330:649, 1987.

Atwood H L, Wojtowicz J M: Short-term and long-term plasticity and physiological differentiation of crustacean motor synapses. Intl Rev Neurobiol 28:275, 1986.

Bagust J, Kerkut G A: The use of the transition elements manganese, cobalt, and nickel as synaptic blocking agents on isolated , hemisected, mouse spinal cord. Brain Res 182: 474, 1980.

Barnard E A, Darlison M G, Seeberg P: Molecular biology of the GABAa receptor: the receptor/channel superfamily. TINS 10: 502, 1987.

Bashir Z I, Alford S, Davies S N, Randall A D, Collingridge G L: Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature 349:156, 1991.

Bashir Z I, Bortolotto Z A, Davies C H, Berretta N, Irving A J, Seal A J, Henley J M, Jane D E, Watkins J C, Collingridge G L: Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature 363:347, 1993.

Baskys A: Metabotropic receptors and 'slow' excitatory actions of glutamate agonists in the hippocampus. Trends in Neurosci. 15: 92, 1992.

Bass A H (1986): Electric organ revisited. In Bullock T H, Heiligenberg W, eds. Electroreception. New York, Jonh Wiley and Sons, 13.

Bastian J: Frequency response characteristics of electroreceptors in weakly electric fish (Gynmotoidei) with a pulse discharge. J Comp Physiol 112: 165, 1976.

Bastian J: Electrolocation I: How the electroreceptors of *Apteronotus albifrons* code for moving objects and other electrical stimuli. J Comp Physiol 144: 465, 1981a.

Bastian J: Electrolocation II: The effects of moving objects and other electrical stimuli on the activities of two categories of posterior lateral line lobe cells in *Apteronotus albiforns*. J Comp Physiol 144: 481, 1981b.

Bastian J: Gain control in the electrosensory system mediated by descending inputs to the electrosensory lateral line lobe. J Neurosci 6:553, 1986a.

Bastian J: Gain control in the electrosensory system: a role for the descending projections to the electrosensory lateral line lobe. J Comp Physiol A-Sensory Neural & Behav Physiol 158:505, 1986b.

Bastian J: Electrolocation (1986 c): behavior, anatomy, and physiology. In Bullock T H, Heiligenberg W, eds. Electroreception. New York, Jonh Wiley and Sons, 577.

Bastian J: The role of amino acid neurotransmitters in the descending control of electroreception. J Comp Physiol A 172: 409, 1993.

Bastian J: Pyramidal-cell plasticity in weakly electric fish: a mechanism for attenuating responses to reafferent electrosensory inputs. J Comp Physiol A-Sensory Neural & Behav Physiol 176:63, 1995.

Bastian J: Plasticity in an electrosensory system.1.General features of a dynamic sensory filter. J Neurophysiol 76:2483, 1996a.

Bastian J: Plasticity in an electrosensory system.2. Postsynaptic events associated with a dynamic sensory filter. J Neurophysiol 76:2497, 1996b.

Bastian J, Bratton B: Descending control of electroreception. I. properties of nucleus praeminentialis neurons projecting indirectly to the electrosensory lateral line lobe. J

Neurosci 10 : 1226, 1990.

Bastian J, Courtright J: Morphological correlates of pyramidal cell adaptation rate in the electrosensory lateral line lobe of weakly electric fish. J Comp Physiol A-Sensory Neural & Behav Physiol 168:393, 1991.

Bell A J, Sejnowski T J: An information-maximization approach to blind separation and blind deconvolution. Neural Comp 7: 1129, 1995.

Bell C C: An efference copy which is modified by reafferent input. Science 214: 450, 1981.

Bell C C: Properties of a modifiable efference copy in an electric fish. J Neurophysiol 47: 1043, 1982.

Bell C C, Grant K: Sensory processing and corollary discharge effects in the mormyromast regions of the mormyrid electrosensory lobe II.Cell types and corollary discharge plasticity. J Neurophysiol 58: 859, 1992.

Bell C C, Caputi A, Grant K, Serrier J: Storage of a sensory pattern by anti-Hebbian synaptic plasticity in anelectric fish. Proc Natl Acad Sci USA 90:4650, 1993.

Ben-Ari Y, Aniksztejn L, Bregestovski P: Protein kinase C modulation of NMDA currents: an important link for LTP induction. Trends Neurosci 15:333, 1992.

Berman N J, Maler L: Characteristics of IPSPs in ELL pyramidal cells *in vitro* in the weakly electric fish *apteronotus leptorhynchus*. J Comp Physiol A 173: 749, 1993.

Berry R L, Teyler T J, Han T Z: Induction of LTP in rat primary visual cortex: tetanus parameters. Brain Res 481:221, 1989.

Blahos J, Wenthold R J: Relationship between N-methyl-D-aspartate receptor NR1 splice variants and NR2 subunits. J Biol Chem 271:15669,1996.

Bliss T V P, Lømo T: Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol Lond 232:331, 1973.

Bliss T V P, Gardner-Medwin A R: Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol Lond 232:357, 1973.

Bliss T V P: long-term potentiation and release of endogenous amino acids from dentate gyrus of anaesthetized rats. J Physiol 377:391, 1986.

Bliss T V P , Errington M L, Lynch M A: Long-term potentiation in the dentate gyrus *in vivo* is associated with a sustained increase in extracellular glutamate. Adv Exp Med Biol 268:269, 1990.

Bliss T V P, Collingridge G L: A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361:31, 1993.

Block F, Schwarz M, Sontag K H: Non-NMDA-mediated transmission of somatosensory-evoked potentials in the rat thalamus. Brain Res Bulletin 31:449, 1993

Bockaert J: G proteins and G-protein-coupled receptors: structure, function and interactions. Current Opinion in Neurobiol. 1: 32, 1991.

Bolshakov V Y, Siegelbaum S A: Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264: 1148, 1994

Bortolotto Z A, Collingridge G L, Edgbaston U K: Activation of glutamate metabotropic receptors induces long-term potentiation. European J Pharmacol 214: 297, 1992.

Bottai D, Ellis B, Maler L, Dunn R: NMDA receptor 1 splice variants: differential cellular expression in the central nervous system of the electric fish apteronotus leptorhnchus. Soc Neurosci Abstr 22:1996, 1996.

Boxall A R, Lancaster B, Garthwaite J: Tyrosine kinase is required for long-term depression in the cerebellum. Neuron 16 :805, 1996.

Bratton B, Bastian J: Descending control of electroreception II: properites of nucleus praeminentialis neurons projecting directly to the electrosensory lateral line lobe, J Neurosci 10: 1241, 1990.

Braun W, Kallen J, Mikol V, Walkinshaw M00 D, Wuthrich K: Three-dimensional structure and actions of immunosuppressants and their immunophilins. FASEB 9:63, 1995.

Buijs R M, Nunes-Cardoso B, Hou Y-X, Shinn S, Postembedding GABA and glutamate immunogold electron microscopy; a combination with anterograde PHAL tracing. Neurosci Prot 050-10: 1, 1993.

Bullock T H (1986): Significance of findings on electroreception for general neurology. In Bullock T H, Heiligenberg W, eds. Electroreception. New York, Jonh Wiley and Sons, 651.

Burnashev N, Schoepfer R, Monyer H, Ruppersberg J P, Gunther W, Seeberg P, Sakman B: Control by asparagine residues of calcium permeability and Mangnesium blockade in the NMDA receptor. Science 257: 1415, 1992.

Burnashev N, Zhou Z, Neher E, Sakmann B: Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. J Physiol 485:403, 1995.

Carder R K, Hendry H C: Neuronal characterization, compartmental distribution, and activity-dependent regulation of glutamate immunoreactivity in adult monkey striate cortex. J Neurosci 14: 242, 1994.

Carew T J, Sahley C L: Invertebrate learning and memory: from behavior to molecules. Ann Rev Neurosci 9:435, 1986.

Carr C E, Maler L (1986): Electroreception in Gymnotiform fish. In Bullock T H, Heiligenberg W, eds. Electroreception. New York, Jonh Wiley and Sons. 319.

Carr C E, Maler L, Heiligenberg W, Sas E: Laminar organization of the afferent and efferent systems of the torus semicircularis of gymnotiform fish: morphological substrates for parallel processing in the electrosensory system. J Comp Neurol 203:649, 1981.

Carr C E, Maler L, Sas E: Peripheral organization and central projections of the electrosensory nerve in gymnotiform fish. J Comp Neurol 211: 139, 1982.

Carr C E, Maler L, Taylor B: A time-comparison circuit in the electric fish midbrain.II function morphology. J Neurosci 6: 1372,1986.

Charlton M P, Smith S J, Zucker R S: Role of presynaptic calcium ions and channels in synaptic facilitation and depression at the squid giant synapse. J Physiol Lond 323: 173, 1982.

Clements J D, Westbrook G L: Activation kinetics reveal the number of glutamate and glycine binding sites on the N-methyl-D-aspartate receptor. Neuron 7: 605,1991.

Cline H T, Constantine-Paton M: NMDA receptor antagonists disrupt the retinotectal topographic map. Neuron 3:413,1989.

Cohen P, Holmes C F, Tsukitani Y: Okadaic acid: a new probe for the study of cellular regulation. Trends in Biochem Sci 15: 98,1990.

Cohen M W, Jones O T, Angelides K J: Distribution of Ca^{2+} channels on frog motor nerve terminals revealed by fluorescent omega-conotoxin. J Neurosci 11:1032,1991.

Collingridge G L, Kehl S J, McLennan H: Excitatory amino acid in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol Lond 334: 33, 1983.

Collingridge G L, Herron C E, Lester R A: Frequency-dependent N-methyl-D-aspartate receptor-mediated synaptic transmission in rat hippocampus. J Physiol 399:301,1988.

Crepel F, Krupa M: Activation of protein kinase C induces a long-term depression of glutamate sensitivity of cerebellar Purkinje cells. An *in vitro* study. Brain Res 458: 397,1988.

Crepel F, Jaillard D: Protein kinases, nitric oxide and long-term depression of synapses in the cerebellum. Neuroreport 1: 133,1990.

Crick F: Function of the thalamic reticular complex: the searchlight hypothesis. Proc.Natl.Acad.Sci.USA. 81:4586,1984.

Davies C H, Starkey S J, Pozza M F, Collingridge G L: GABA autoreceptors regulate the induction of LTP. Nature. 349: 609, 1991.

Davies J D, Watkins J C: Actions of D- and L- forms of 2-amino-5 phosphonovalerate and 2-amino-4 phosphonobutyrate in the cat spinal cord. Brain Res 235: 378, 1982.

De Camilli P, Benfenati F, Valtorta F, Greengard P: The synapsins. Ann Rev Cell Biol 6: 433, 1990.

De Camilli P, Cameron R, Greengard P: Synapsin I (protein I) a nerve terminal specific phosphoprotein. I. Its general distribution in synapses of the central and peripheral nervous system demonstrated by immunofluorescence in frozen and plastic sections. J Cell Biol 96: 1337, 1983.

De Camilli P, Harris S M, Huttner WB, Greengard P: Synapsin I (protein I) a nerve terminal specific phosphoprotein. II. Its specific association with synaptic vesicles demonstrated by immunocytochemistry in agarose-embedded synaptosome. J Cell Biol 96: 1355,1983.

Delaney K R, Tank D W: A quantitative measurements of the dependence of short term synaptic enhancement on presynaptic residual calcium. J Neurosci 14: 5885, 1994.

Delaney K R, Zucker R S, Tank D W: Calcium in motor nerve terminals associated with posttetanic potentiation.J Neurosci 9: 3558, 1989.

Deniel H, Hemart N, Jaillard D, Crepel F. Long-term depression requires nitric oxide and guanosine-3',-5'-cyclic monophosphate production in cerebellar Purkinje cells. European J Neurosci 5: 1079, 1993.

De Schutter E: Cerebellar lon-term depression might normalize excitation of Purkinje cells: a hypothesis. TINS 18: 291,1995.

Dolphin A C, Errington M L, Bliss T V: Long-term potentiation of the perforant path *in vivo* is associated with increased glutamate release. Nature 297: 496, 1982.

Dudek S M, Bear M F: 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, 1992.

Dunwiddie T, Lynch G: Long-term potentiation and depression of synaptic responses in the rat hippocampus: localization and frequency dependency. J Physiol Lond 276:353, 1978.

Durand G M, Kovalchuk Y, Konnerth A: Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381:71, 1996.

Ebert B, Madsen U, Johansen T N, Krogsgaard-Larsen P: NMDA receptor agonists: relationships between structure and biological activity. Adv Exp Med Biol 87:483, 1991.

Eccles J C , Ito M, Széntagothai J (1967): The cerebellum as a neural machine. Berlin, Heidelberg and New york, Springer-Verlage.

Edwards F: LTP is a long term problem. Nature 350:271, 1991.

Ekerot CF, Kano M: Long-term depression of parallel fiber synapses following stimulation of clibming fibers. Brain Res 342: 357, 1985.

Erondu N E, Kennedy M B: Regional distribution of type II Ca^{2+} calmodulin-dependent protein kinase in rat brain. J Neurosci 5:3270, 1985.

Exton J H, Taylor S J, Augert G and Bocckino S B: Cell signalling through phospholipid breakdown. Mole. Cell. Biochem. 104: 81, 1991.

Figurov A, Boddeke H, Muller D: Enhancement of AMPA-mediated synaptic transmission by the protein phosphatase inhibitor calyculin A in rat hippocampal slices. European J Neurosci 5:1035, 1993.

Fukunaga K, Kobayashi T, Tamura S, Miyamoto E: dephosphorylation of autophosphorylated Ca^{2+}/calmoduline dependent protein kinase II by protein phosphotase 2C. J Biol Chem 268: 133,1993.

Fukunaga K, Rich D P, Soderling T R: Generation of the Ca^{2+}-independent form of Ca^{2+}/calmodulin-dependent protein kinase II in cerebellar granule cells. J Biol Chem 264: 21830, 1989.

Fukunaga K, Soderling T R, Miyamoto E: Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. J Biol Chem 267: 22527,1992.

Fykes E M, Li C, Südhof C: Phosphorylation of rabphilin-3A by Ca^{2+}/calmodulin- and cAMP-dependent protein kinases *in vitro*. J Neurosci 15: 2385, 1995.

Garver T D, Oyler G A, Harris K A, Polavarapu R, Damuni Z, Lehman R A, Billingsley M L: Tau phosphorylation in brain slices: pharmacological evidence for convergent effects of protein phosphatases on tau and mitogen activated protein kinase. Mol Pharmacol 47: 745, 1995.

Geppert M, Goda Y, Hammer R E, Li C, Rosahl T W, Stevens C F, Südhof C: Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. Cell 79: 717, 1994.

Gerber G, Kangrga I, Ryu P D, Larew J S, Randic M: Multiple effects of phorbol esters in the rat spinal dorsal horn. J Neurosci 93: 606, 1989.

Glaum S R, Slater N T, Rossi D J, Miller R J: The role of metabotropic glutamate receptor at the parallel fiber-Purkinje cell synapse. J Neurophysiol 68: 1453, 1992.

Glazewski S, Chen C M, Silva A, Fox K: Requirement for alpha-CaMKII in experience-dependent plasticity of the barrel cortex. Science 272:421, 1996.

Goda Y, Stevens C F: Long-term depression properties in a single system. Neuron 16: 103, 1996.

Greengard P, Valtorta F, Czernik A J, Benfenati F: Synaptic vesicle phosphoproteins and regulation of synaptic function. Science 259: 780, 1993.

Griffith L C, Wang J, Zhong Y, Wu C F, Greenspan R J: Calcium/calmodulin-dependent protein kinase II and potassium channel subunit eag similarly affect plasticity in Drosophila. Proc Natl Acad Sci USA 91:10044, 1994.

Haavik J, Schelling D L, Campbell D G, Andersson K K, Flatmark T, Cohen P: Identification of protein phosphatase 2A as the major tyrosinehydroxylase phosphatase in adrenal medulla and corpus striatum: evidence from the effects of okadaic acid. FEBS Lett 251:36,1989.

Hahm J O, Langdon R B, Sur M: Disruption of retinogeniculate afferent segregation by antagonists to NMDA receptors. Nature 351: 568, 1991.

Hajimohammadreza I, Probert A W, Coughenour L L, Borosky, Marcoux F W, Boxer P A, Wang K K: A specific inhibitor of calcium/calmodulin-dependent protein kinase-II provides neuroprotection against NMDA- and hypoxia/hypoglycemia-induced cell death. J Neurosci 15: 4093, 1995.

Harris E W, Cotman, C W: Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. Neurosci Lett 70: 132, 1986.

Harris E W, Ganong A H, Cotman C W: Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res 323:132, 1984.

Hatta K, Yamamato T, Hori T, Okuwa M, Moroji T: 3-((+/-)2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) more potently antagonizes the high-affinity Mg2+

binding site on the N-methyl-D-aspartate/phencyclidine receptor ion channel complex than the L-glutamate recognition site. Neurosci Lett 124: 229, 1991.

Hawkins R D, Zhuo M, Arancio O: Nitric oxide and carbon monoxide as possible retrograde messengers in hippocampal long-term potentiation. J Neurobiol 25: 652, 1994.

Hebb D O (1949): The organization of behavior: A neuropsychological theory. New York, John Wiley & Sons.

Heiligenberg W (1986): Jamming avoidance responses: model system for neuroethology. In Bullock T H, Heiligenberg W, eds. Electroreception New York, John Wiley and Sons, 613.

Heiligenberg W: Electrosensory systems in fish. Synapse 6: 196,1990.

Heiligenberg W, Dye J: Labelling of electroreceptive afferents in a gymnotoid fish by intracellular injection of HRP: the mystery of mutiple maps. J Comp Physiol 148:287, 1982.

Heinemann U, Stabel J, Rausche G: Activity-dependent ionic changes and neuronal plasticity in rat hippocampus. Prog Brain Res 83: 197,1990.

Hepler J R, Toomim C S, McCarthy K D, Conti F, Battaglia G, Rustioni A, Petrusz P: Characterization of antisera to glutamate and aspartate. J Histochem and Cytochem 36: 13, 1988.

Herron C E, Malenka R C: Activity-dependent enhancement of synaptic transmission in hippocampal slices treated with the phosphatase inhibitor calyculin A. J Neurosci 14: 6013,1994.

Hirano T: Effects of postsynaptic depolarization in the induction of synaptic depression between a granule cell and a Purkinje cell in rat cerebellar culture. Neurosci Lett 119:145, 1990.

Hirsch J C, Crepel F: Use-dependent changes in synaptic efficacy in rat prefrontal neurons *in vitro*. J Physiol 427:31, 1990.

Hollmann M (1997): The topology of glutamate receptors: sorting through the domains. In Monaghan D, Wenthold R, eds. The ionotropic glutamate receptors. Humana Press, Totowa, New Jersey, 39.

Hollmann M, Maron C, Heinemann S: N-glycosylation site tagging suggests a 3-transmembrane domain topology for the glutamate receptor GluR1. Neuron 13: 1331, 1994.

Honkanen R E, Codispoti B A, Tse K, Boynton A L, Honkanen R E: Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases. Toxicon 32: 339, 1994.

Honkanen, R E, Zwiller J, Daily S L, Khatra B S, Dukelow M, Boynton A L: Identification, purification, and characterization of a novel serine/threonine protein phosphatase from bovine brain. J Biol Chem 266: 6614,1991.

Hu G Y, Hvalby O, Walaas S I, Albert K A, Skjeflo P, Andersen P, Greengard P: Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. Nature 328: 426, 1987.

Huffman R F, Henson O W Jr: The descending auditory pathway and acousticomotor systems: connections with the inferior colliculus. Brain Res. 15:295, 1990.

Hume R I, Dingledine, R, Heinemann S F: Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253:1028, 1991.

Huttner W B, Schleiber W, Greengard P, De Camilli P: Synapsin I (protein I) a nerve terminal specific phosphoprotein . III. Its association with synaptic vesicles studied in a highly purified synaptic vesicle preparation. J Cell Biol 96:1374,1983.

Hwang J, Bragado M J, Duan R D, Williams J A: Protein phosphatase inhibitors potentiate Ca^{2+}/calmodulin-dependent protein kinase II activity in rat pancreatic acinar cells. Biochem Biophys Res Communications 225:520,1996.

Ingebritsen T S, Cohen P: The protein phosphatases involved in cell. regulation. 1. Classification and substrate specificities. European J Biochem 132:255,1983.

Isaac J T, Nicoll R A, Malenka R C: Evidence for silent synapses: implications for the expression of LTP. Neuron 15:427, 1995.

Isaacson J S, Walmsley B: Receptors underlying excitatory synaptic transmission in slices of the rat anteroventral cochlear nucleus. J Neurophysiol 73:964,1995.

Ishihara H, Martin B L, Brautigan D L, Karaki H, Ozaki H, Kato Y, Fusetani N, Watabe S, Hashimoto K, Uemura D, et al: Calyculin A and okadaic acid: inhibitors of protein phosphatase activity. Biochem Biophys Res Com 159:871,1989.

Ishikawa N, Hashiba Y, Hidaka H: Effects of a new Ca^{2+}-Calmodulin dependent protein kinase II inhibitor on GABA release in cerebrospinal fluid of the rat. J. Pharmacol. Exp Ther 254: 598, 1990.

Ito M, Sakurai M, Tongroach P: Climbing fibers induced depression of mossy fiber responsiveness and glutamate sensitivity of cerebellar Purkinje cells. J Physiol Lond 324:113. 1982.

Jaarsma D, Sebens J B, Korf J: Localization of NMDA and AMPA receptors in rat barrel field. Neurosci Lett 133:233,1991.

Jackson M B: Presynaptic excitability. Intl Rev Neurobiol 38:201, 1995.

Jackson T. Structure and function of G protein coupled receptors. Pharmacol. Therap. 50: 425, 1991.

Jaffe D, Johnston D: Induction of long-term potentiation at hippocampal mossy-fiber synapses follows a Hebbian rule. J Neurophysiol 64:948,1990

Jessell T M, Kandel E R: Synaptic transmission: a bidirectional and self-modifiable form of cell-cell communication. Cell 72/Neuron 10 (Suppl) 1, 1993

Johnson T D: Modulation of channel function by polyamines. Trends in Pharmacol Sci 17:22,1996

Johnston D, Williams S, Jaffe D, Gray R: NMDA-receptor-independent long-term potentiation. Ann Rev Physiol 54: 489, 1992.

Kamiya H, Zucker R: Residual Ca^{2+} and short-term synaptic plasticity. Nature 371: 603, 1994.

Kandel E R, Schwartz J H: Molecular biology of learning: modulation of transmitter release. Science 218: 433, 1982.

Kataoka Y, Ohmori H: Activation of glutamate receptors in response to membrane depolarization of hair cells isolated from chick cochlea. J Physiol 477 (Pt 3):403, 1994.

Katsuki H, Kaneko S, Tajima A, Satoh M: Separate mechanisms of long-term potentiation in two input systems to CA3 pyramidal neurons of rat hippocampal slice as revealed by the whole-cell patch clamp technique. Neurosci Res 12: 393, 1991.

Katz B, Miledi R T: The role of calcium in the neuromuscular facilitation. J Physiol

Lond 195:481,1968.

Katz B (1969): The release of natural transmitter substances. Thomas C C, eds. Springfield, Illinois.

Kauer J A, Malenka R C, Nicoll R A: A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. Neuron 1:911, 1988.

Keinanen K, Wisden W, Sommer B, Werner P, Herb A, Verdoorn,T A, Sakman B, Seeberg P H: A family of AMPA -selective glutamate receptors. Science 249: 56, 1990.

Kelly P T, McGuiness T L, Greengard P: Evidence that the major postsynaptic density protein is a component of a Ca^{2+}/calmodulin-dependent protein kinase. Proc Natl Acad Sci USA 81: 945, 1984.

King A E, Lopez-Garcia J A: Excitatory amino acid receptor-mediated neurotransmission from cutaneous afferents in rat dorsal horn *in vitro*. J Physiol 472:443,1993.

Kinney G A, Peterson B W, Slater N T: The synaptic activation of N-methyl-D-aspartate receptors in the rat medial vestibular nucleus. J Neurophysiol 2:1588,1994.

Kissinger C R, Parge H E, Knighton D R, Lewis C T, Pelletier L A, Tempczyk A, Kalish V J, Tucker K D, Showalter R E, Moomaw E W, Gastinel L N, Habuka N, Chen X, Maldonado F, Barker J E, Bacquet R, Villafranca J E: Crystal structures of human calcineurin and the human FKBP12-FK506-calcineurin complex. Nature Lond 378: 641, 1995.

Kitamura Y, Miyazaki A, Yamanaka Y, Nomura Y: Stimulatory effects of protein kinase C and calmodulin kinase II onN-methyl-D-aspartate receptor/channels in the postsynaptic density of rat brain. J Neurochem 61:100,1993.

Klann E, Chen S J, Sweatt J D: Persistent protein kinase activation in the maintenance phase of long-term potentiation. J Biol Chem 266: 24253, 1991.

Kogan J H and Aghajanian G K: Long-term glutamate desensitization in locus coeruleus neurons and its role in opiate withdrawal. Brain Res 689: 111, 1995.

Kolaj M, Cerne R, Chen G, Brickey D A, Randic M: Alpha subunit of calcium/calmodulin-dependent protein kinase enhances excitatory amino acid and synaptic responses of rat spinal dorsal horn neurons. J Neurophysiol 72: 2525,1994.

Konnerth A, Dressen J, Augustine G J: Brief dendritic calcium signals initiate long-lasting synaptic depression in cerebellar Purkinje cells. Proc Natl Acad Sci USA 89: 7051, 1992

Kullmann D M: Amplitude fluctuations of dual-component EPSCs in hippocampal pyramidal cells: implications for long-term potentiation. Neuron 12:1111, 1994.

Kullmann D M, Siegelbaum S A: The site of expression of NMDA receptor-dependent LTP: New fuel for an old fire. Neuron 15: 997, 1995.

Lai Y, Nairn A C, Corelick F, Greengard P: Ca^{2+}/calmodulin dependent protein kibase II : identification of autophosphorylation sites responsible for generation of Ca^{2+}/ calmodulin-independence. Proc Natl Acad Sci USA 84:5710, 1987.

Lambert J D, Jones R S: A reevaluation of excitatory amino acid-mediated synaptic transmission in rat dentate gyrus. J Neurophysiol 64:119,1990.

Langdon R B, Johnson J W, Barrionuevo G: Posttetanic potentiation and presynaptically induced long-term potentiation at the mossy fiber synapse in rat hippocampus. J Neurobiol 26:370, 1995.

Lannoo M J, Lannoo S J: Why do electric fishes swim backwards. An hypothesis based on gymnotiform foaging behavior interpreted through sensory constraints. Environmental Biology of Fishes. 36: 157, 1993.

Lannoo M J, Maler L, Tinner B: Ganglion cell arrangement and axonal trajectories in the anterior lateral line nerve of the weakly electric fish Apteronotus leptorhynchus (Gymnotiformes). J Comp Neurol 280:331, 1989.

Larson J, Wong D, Lynch G: Patterned stimulation at the theta frequency is optimal for induction of long-term potentiation. Brain Res 368: 347, 1986.

Larson-Prior L J, Slater N T: Excitatory amino acid receptors mediate slow synaptic transmission in turtle cerebellum. Neurosci Lett 104:286,1989.

Lau L F, Huganir R L: Differential tyrosine phosphorylation of N-methyl-D-aspartate receptor subunits. J Biol Chem 270:20036, 1995.

Lauritzen M, Rice M E, Okada Y, Nicholson C: Quisqualate, kainate and NMDA can initiate spreading depression in the turtle cerebellum. Brain Res 475:317,1988.

Lehmann J, Colpaert F, Canton H: Glutamate and glycine co-activate while polyamines merely modulate the NMDA receptor complex. Prog Neuro-Psychopharmacol Biol Psychiatry 5: 183,1991.

Leone C, Gordon F J: Is L-glutamate a neurotransmitter of baroreceptor information in the nucleus of the tractus solitarius? J Pharmacol Exp Ther 250:953, 1989.

Liao D, Hessler N A, Malinow R: Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. Nature 375: 400, 1995.

Lieberman D N and Mody I: Regulation of NMDA channel function by endogenous Ca(2+)-dependent phosphatase. Nature 369: 235, 1994.

Lin J W, Sugimori M, Llinás R R, McGuinness T L, Greengard P: Effects of synapsin I and calcium-calmodulin-dependent protein kinase II on spontaneous transmitter release in the squid giant synapse. Proc Natl Acad SciU SA 87:8257, 1990.

Linden D J, Connor J A: Participation of postsynaptic PKC in cerebellar long-term depression in culture. Science 254:1656,1991.

Linden D J, Connor J A: Long-term depression of glutamate currents in cultured cerebellar Purkinje neurons does not require nitric oxide signalling. European J Neurosci 4:10, 1992.

Linden D J, Conner J A: Long-term synaptic depression. Ann Rev Neurosci 18: 319, 1995.

Linden D J, Dickinson M H, Smeyne M, Connor, J A: A long-term depression of AMPA currents in cultured cerebellar Purkinje neurons. Neuron 7:81, 1991.

Linden D J, Routtenberg A: The role of protein kinase C in long-term potentiation: a testable model. Brain Res 14:279, 1989.

Linden D J, Smeyne M, Connor J A: Induvction of cerebellar long-tem depression in culture requires postsynaptic action of sodium ions. Neuron 11: 1093, 1993.

Lisman J E, Goldring M A: Feasibility of long-term storage of graded information by the Ca^{2+}/calmodulin-dependent protein kinase molecules of the postsynaptic density. Proc Natl Acad Sci USA 85: 5320, 1988.

Llinás R, Gruner J A, Sugimori M, McGuinness T L, Greengard P: Regulation by synapsin I and Ca^{2+}-calmodulin-dependent protwin kinase II of transmitter release in squid giant synapse. J Physiol 436:257, 1991.

Llinás R , MaGuinness T L , Leonard C S , Sugimori M, Greengard P: Intraterminal injection of synapsin I or calcium / calmodulin-dependent protein kinase II alters neurotransmitter release at the squid giant synaps. Proc Natl Acad Sci USA 82:3035, 1985.

López-García J C Arancio O , Kandel E R, Baranes D: A presynaptic locus for long-term potentiation of elementary synaptic transmission at mossy fiber synapses in culture. Proc Natl Acad Sci USA 93:4712, 1996.

Lou L L , Lloyd S J, Schulman H: Activation of the multifunctional Ca^{2+}/calmodulin-dependent protein kinase by autophosphorya;tion: ATP modulates production of an autonomous enzyme. Proc Natl Acad Sci USA 83:9497,1986.

Lynch G S , Dunwiddie T, Gribkoff V: Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. Nature 266: 737,1977.

Lynch G, Larson J, Kelso S, Barrionuevo G, Schottler F: Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305:719, 1983.

MacDonald J F , Bartlett M C , Mody I , Reynolds J N, Salter M W: The PCP site of the NMDA receptor complex. Adv Exp Med Biol 268:27,1990.

MacDonald J F, Nowak L M: Mechanisms of blockade of excitatory amino acid receptor channels. Trends in Pharmacol Sci 11: 167, 1990.

Magleby K L: Facilitation, augmentation, and potentiation of transmitter release. Prog. Brain Res 49:175, 1979.

Magleby K L (1987): Short term change in syanptic efficacy. In Edelman G M, Gall W E, Cowan W M, eds. Synaptic function. New York, Wiley, 21.

Malenka R C: Synaptic plasticity in the hippocampus: LTP and LTD. Cell 78: 535, 1994.

Malenka R C, Kauer J A, Perkel D J, Mauk M D, Kelly P T, Nicoll R A, Waxham M N: An essential role for postsynaptic calmodulin and protein kinase activity in long-term potentiation. Nature 340:554, 1989.

Malenka R C, Kauer J A, Zucker R S, Nicoll R A: Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. Science 242:81, 1988.

Malenka R C Madison D V, Nicoll R A: Potentiation of synaptic transmission in the hippocampus by phorbol esters. Nature 321:175, 1986.

Maler L, The posterior lateral line lobe of certain gymnotoid fish: quantitative light microscopy J Comp Neurol 183: 323, 1979.

Maler L, Finger T, Karten J H: Differential projections of ordinary lateral line receptors and electroreceptors in the gymnotid fish, *Apteronotus (Sternarchus) albifrons*. J Comp Neurol 158: 363, 1974.

Maler L, Monaghan D: The distribution of excitatory amino acid binding sites in the brain of an electric fish, *apteronotus leptorhynchus*. J Chem Neuroanat 4: 39, 1991.

Maler L, Muganaini E: Organization and function of feedback to the electrosensory lateral line lobe of gymnotiform fish, with emphasis on a searchlight mechanism. J Comp Physiol A 173:667, 1993.

Maler L, Mugnaini E: Correlating GABAergic circuits and sensory function in the electrosensory lateral line lobe of a Gymnotiform fish. J Comp Neurol 345: 224, 1994.

Maler L, Sas E, Carr C E, Matsubara J: Efferent projections of the posterior lateral line lobe in gymnotiform fish. J Comp Neurol 211:154, 1982.

Maler L, Sas E, Rogers J: The cytology of the posterior lateral line lobe of high frequency electric fish (*Gymnotidae*): dendritic differentiation and synaptic specificity in a simple cortex. J Comp Neurol 158: 87, 1981.

Malinow R, Madison D V, Tsien R W: Persistent protein kinase activity underlying long-term potentiation. Nature 335:820, 1988.

Malinow R, Schulman H, Tsien R W: Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245:862, 1989.

Manabe T, Wyllie D J, Perkel D J, Nicoll R A: 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, 1993.

Marantz Y , Reiss N , Przedecki F, Naor Z: Involvement of protein phosphatases in gonadotropin releasing hormone regulated gonadotropin secretion. Mol Cell Endocrinol 111: 7,1995.

Masu M , Tanabe Y , Tsuchida K Shigemoto R, Nakanishi S: Sequence and expression of a metabotropic glutamate receptor. Nature 349: 760, 1991.

Mathieson W B, Heiligenberg W, Maler L, Ultrastructure studies of physiologically identified electrosensory afferent synapses in the gymnotiform fish, *Eigenmannia.* J Comp Neurol 255: 526,1986

Mathieson W B, Maler L: Morphological and electrophysiological properties of a novel *in vitro* preparation: the electrosensory lateral line lobe brain slice. J Comp Physiol A-Sensory Neural & Behav Physiol 163:489, 1988.

Mayer M L, Westbrook G L: Permeation and block of N-methyl D-aspartic acid receptor channels by divalent cation in mouse cultured central neurons. J Physiol 394: 501, 1987.

McNaughton B L: Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. J Physiol 324:249, 1982.

McNaughton B L, Douglas R M, Goddard G V: Synaptic enhancement of fascia dentate: Coperativity among coactive afferents. Brain Res 157: 277, 1978.

Monaghan D T: Differential stimulation of [3H]MK-801 binding to subpopulations of NMDA receptors. Neurosci Lett 122: 21,1991.

Montgomery J C, Bodznick D: An adaptive filter that cancels self-induced noise in the electrosensory and lateral line mechanosensory systems of fish. Neurosci Lett 174: 145,1994.

Moriyoshi K, Masu M, Ishii T, Shigemoto R, Mizuno N, Nakanishi S: Molecular cloning and characterization of the rat NMDA receptor. Nature 354:31, 1991.

Mulkey R M, Endo S, Shenolikar S, Malenka R C: Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486,1994.

Mulkey R M, Malenka R C: Mechanism underlying homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9: 967, 1992.

Muller D, Lynch G: Long-term potentiation differentially affects two components of synapticresponses in hippocampus. Proc Natl Acad Sci USA 85: 9346, 1988.

Murakami N, Sakai N, Nei K, Matsuyama S, Saito N and Tanaka C: Potassium and calcium channel involvement in induction of long-lasting synaptic enhancement by calyculin A, a protein phosphatase inhibitor, in rat hippocampal CA1 region. Neurosci Lett 176: 181, 1994.

Nadi S, Maler L. The laminar distribution of amino acids in the caudal cerebellum and electrosensory lateral line lobe of weakly electric fish (*Gymnotidae*). Brain Res 425: 218, 1987.

Nakanishi S: Molecular diversity of glutamate receptors and implications for brain function. Science 258: 597, 1992.

Nakanishi S, Masu M, Bessho Y, Nakajima Y, Hayashi Y, Shigemoto R: Molecular diversity of glutamate receptors and their physiological functions. EXS 71:71,1994.

Nelson M E, Paulin M G: Neural stimulation of adaptive reafference suppression in the elasmobranch electrosensory system. J Comp Physiol A 177: 723,1995.

Nemeth E F, Jackson H, Parks T N: Pharmacologic evidence for synaptic transmission mediated by non-N-methyl-D-aspartate receptors in the avian cochlear nucleus. Neurosci Lett 40: 39,1983.

Nichols R A, Suplick G R, Brown J M: Calcineurin-mediated protein dephosphorylation in brain nerve terminals regulates the release of glutamate. J Biol Chem 269: 23817, 1994.

Niedzielski A S, Wenthold R J: Expression of AMPA, kainate, and NMDA receptor subunits in cochlear and vestibular ganglia. J Neurosci 15 (Pt 2): 2338,1995.

Nielander H B, Onofri F, Valtorta F, Schiavo G, Montecucco C, Greengard P, Benfenati F: Phosphorylation of VAMP/synaptobrevin in synaptic vesicles by endogenous protein kinases. J Neurochem 65:1712, 1995.

O'Dell T J, Kandel E R, Grant S G: Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. Nature 353: 558, 1991.

Orrego F, Villanueva S: The chemical nature of the main central excitatory transmitter: a critical appraisal based upon release studies and synaptic vesicle localization. Neurosci 56: 539,1993.

Otani S, Ben-Ari Y, Roisin-Lallemand M P: Metabotropic receptor stimulation coupled to weak tetanus leads to long-term potentiation and a rapid elevation of cytosolic protein kinase C activity. Brain Res 613: 1, 1993.

Otsu Y , Kimura F, Tsumoto T: Hebbian induction of LTP in visual cortex: perforated patch-clamp study in cultured neurons. J Neurophysiol 74: 2437, 1995.

Ottersen O P: Quantitative electron microscopic immunocytochemistry of neuroactive amino acids. Anat Embryol 180: 1, 1989.

Ottersen O P Zhang N , Walberg F: Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. Neurosci 46: 519, 1992.

Otto T, Eichenbaum H , Wiener S I, Wible C G: Learning-related patterns of CA1 spike trains parallel stimulation parameters optimal for inducing hippocampal long-term potentiation. Hippocampus 1:181,1991.

Peters H, Koh J, Choi D W: Zinc selectively blocks the action of N-methyl D-aspartate on cortical neurons. Science 236: 589, 1987.

Pin J P and Duvoisin R: The metabotropic glutamate receptors: structure and functions. Neuropharmacol. 34: 1, 1995.

Pisano P, Samuel D, Nieoullon A, Kerkerian-Le Goff L: Activation of the adenylate cyclase-dependent protein kinase pathway increases high affinity glutamate uptake into rat striatal synaptosomes. Neuropharmacol 35: 541,1996.

Plant J R, Turner R W, Maler, L: Fast NMDA transmission in a sensory feedback pathway. Neurosci Abstr 18: 348, 1992.

Popoli M: Synaptotagmin is endogenously phosphorylated by Ca^{2+}/calmodulin protein kinase II in synaptic vesicles. FEBS Lett 317: 85, 1993.

Raymond L A, Blackstone C D, Huganir R L: Phosphorylation and modulation of recombinant GluR6 glutamate receptors by cAMP-dependent protein kinase. Nature 361: 637, 1993.

Raymond L A, Tingley W G, Blackston C D, Roche K W, Huganir R L: Glutamate receptor modulation by protein phosphorylation. J Physiol Paris 88: 181,1994.

Regehr W G, Delaney K R, Tank D W: The role of Presynaptic calcium in short term enhancement at the hippocampal mossy fiber synapse. J Neurosci 14: 523, 1994.

Reyes M, Stanton P K: Induction of hippocampal long-term depression required release of Ca+ from separate presynaptic and postsynaptic intracellular stores. J Neurosci 16:5951, 1996.

Roberts W M, Jacobs R A, Hudspeth A J: Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. J Neurosci 10: 3664, 1990.

Robitaille R, Tremblay J P: Non-uniform responses to Ca^{2+} along the frog neuromuscular junction: effects on the probability of spontaneous and evoked transmitter release. Neurosci 40: 571, 1991.

Roche K W, O'Brien R J, Mammen A L, Bernhardt J, Huganir R L: Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16: 1179, 1996.

Rosahl T W, Spillane S D, Missler M, Herz J, Selig D, Wolff J R, Hammer R E, Malenka R C, Südhof T C: Essential functions of synapsin I and II in synaptic vesicle regulation. Nature 375: 488, 1995.

Rose G M, Dunwiddie T V: Induction of hippocampal long-term potentiation using physiologically patterned stimulation. Neurosci Lett 69: 244, 1986.

Rosen, A.S. and Morris, M.E. Depolarizing effects of anoxia on pyramidal cells of rat neocortex. Neurosci. Lett. 124:169-73,1991.

Rubenstein J L, Greengard P, Czernik A J: Calcium-dependent serine phosphorylation of synaptophysin. Synapse 13: 161, 1993.

Sacaan A I, Johnson K M: Regulation of polyamine and magnesium inhibition of the N-methyl-D-aspartate (NMDA) receptor ionophore complex. Neurosci Lett 121: 219, 1991.

Saitoh T, Schwartz J H: Phosphorylation-dependent subcellular translocation of a Ca^{2+}/calmodulin-dependent protein kinase produces an autonomous enzyme in Aplysia neruons. J Cell Biol 100: 835, 1985.

Sakurai M: Synaptic modification of parallel fibre-Purkinje cell transmission in *in vitro* guinea-pig cerebellar slices. J Physiol Lond 394: 463, 1987.

Sakurai M: Calcium is an intracellular mediator of the climbing fiber in induction of cerebellar long-term depression. Proc Natl Acad Sci USA 87: 3383, 1990.

Salin P A, Malenka R C, Nicoll R A: Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron 16: 797, 1996.

Salin P A, Scanziani M, Malenka R C, Nicoll R A: Distinct short term plasticity at two excitatory synapses in the hippocampus. Proc Natl Acad Sci USA 93: 13304, 1996

Sandoval M E, Cotman C W: Evolution of glutamate as a transmitter of cerebellar parallel fibers. Neurosci 3: 199, 1978.

Sas E, Maler L: The nucleus praeeminentialis: a Golgi study of a feedback center in the electrosensory system of gymnotid fish. J Comp Neurol 221: 127, 1983.

Sas E, Maler L: Retinofugal projections in a weakly electric gymnotid fish (*Apteronotus leptorhynchus*). Neurosci 18: 247, 1986.

Sas E, Maler L: The organization of afferent input to the caudal lobe of the cerebellum of the gymnotid fish *Apteronotus leptorhynchus*. Anat Embryol 177: 55,1987.

Saunders J, Bastian J: The physiology and morphology of two types of electrosensory neurons in the weakly electric fish *Apteronotus leptorhynchus*. J Comp Physiol A 154: 199, 1984.

Scheich H, Bullock T H, Hamstra R H Jr: Coding properties of two classes of afferent nerve fibers: high-frequency electroreceptors in the electric fish, Eigenmannia. J Neurophysiol 36: 39, 1973.

Schiavo G, Benfenati F, Poulain B, Rossetto O, Polvorino de Laurito P, DasGupta B R, Montecucco O: Tetanus and botulinum-B neurotoxin block transmitter release by proteolytic cleavage of synaptobrevin. Nature 359: 832, 1992.

Schoepfer R, Monyer H, Sommer B, Wisden W, Sprengel R, Kuner T, Lomeli H, Herb A, Kohler M, Burnashev N, et al: Molecular biology of glutamate receptors. Prog Neurobiol 42: 353, 1994.

Schoepp D D, Conn P J: Metabotropic glutamate receptors in brain function and pathology. Trends Pharmacol Sci 14: 13, 1993.

Schulman H: The multifunctional Ca^{2+}/calmodulin-dependent protein kinases. Curr Opin Cell Biol 5: 247, 1993.

Sheng M, Cummings J, Roldan L A, Jan Y N, Jan L Y: Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368: 144, 1994.

Shenolikar S: Protein serine/threonine phosphatases -new avenues for cell regulation. Ann Rev Cell Biol 10: 55, 1994.

Shibuki K, Okada D: Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature 24: 349, 1991.

Shigemoto R, Nakanishi S, Hirano T: Antibodies against a metabotropic glutamate receptor, mGluR1, inhibit long-term depression in cerebellar culture. Neurosci Res (Suppl. 18): 329, 1993.

Shumway C A: Multiple electrosensory maps in the medulla of weakly electric gymnotiform fish. I. Physiological differences. J Neurosci 9: 4388, 1989a.

Shumway C A: Multiple electrosensory maps in the medulla of weakly electric gymnotiform fish. II. Anatomical differences. J Neurosci 9: 4400, 1989b.

Sihra T S, Nairn A C, Kloppenburg P, Lin Z and Pouzat C: A role for calcineurin (protein phosphatase-2B) in the regulation of glutamate release. Biochem Biophy Res Com 212: 609,1995.

Silva A J, Paylor R, Wehner J M, Tonegawa S: Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257: 206, 1992a.

Silva A J, Stevens C F, Tonegawa S, Wang Y: Deficient hippocampal long-term potentiation in alpha-calcium calmodulin kinase II mutant mice. Science 257: 201, 1992b.

Silverstein F S, Naik B: Effect of depolarization on striatal amino acid efflux in perinatal rats: an *in vivo* microdialysis study. Neurosci Lett 128: 133, 1991.

Sim A T, Lloyd H G, Jarvie P E, Morrison M, Rostas J A and Dunkley P R: Synaptosomal amino acid release: effect of inhibiting protein phosphatases with okadaic acid. Neurosci Lett 60: 181, 1993.

Sitges M, Dunkley P R, Chiu L M: A role for calcium/calmodulin kinase(s) in the regulation of GABA exocytosis. Neurochem Res 20: 245, 1995.

Skrede K K, Malthe-Sorenssen D: Increased resting and evoked release of transmitter following repetitive electrical tetanization in hippocampus: a biochemical correlate to long-lasting synaptic potentiation. Brain Res 208: 436, 1981.

Smith M K, Colbran R J, Brickey D A, Soderling T R: Functional determinants in the autoinhibitory domain of calcium/calmodulin-dependent protein kinase II. J Biol Chem 2 67: 1761, 1992.

Somogyi P, Halasz K, Somogyi K, Storm-Mathisen J, Ottersen O P: Quantification of immunogold labelling reveals enrichment of glutamate in mossy and parallel terminals in cat cerebellum. Neurosci 197: 1045,1986.

Stanton P K, Gage A T: Distinct synaptic loci of Ca^{2+}/ calmodulin-dependent protein kinase II necessary for long-term potentiation and depression. J Neurophysiol 76: 2097, 1996.

Stevens C F: Quantal release of neurotransmitter and long term potentiation. Cell 72(Suppl.): 55, 1993.

Stevens C F, Wang Y: Changes in reliability of synaptic functions as a mechanism for plasticity. Nature 371: 704, 1994.

Storm-Mathisen J, Ottersen O P (1986): Antibodies against amino acid neurotransmitters. In Panula P, Päivärinta H, Soinila S, eds. Neurohistochemistry: modern methods and applications. New York, Liss, 107.

Südhof T, Czernik A J, Kao H T, Takei K, Johston P A, Horiuchi A, Kanazir S D, Wagner M A, Perin M S, De Camilli P, Greengard P: Synapsin: mosaics of shared domains in a family of synaptic vesicle phosphoproteins. Science 245: 1474, 1989.

Südhof T, Jahn R: Proteins of syanptic vesicles involved in exocytosis and membrane recycling. Neuron 6: 665, 1991.

Sumi M, Kiuchi K, Ishikawa T, Ishii A, Hagiwara M, Nagatsu T, Hidaka H: The newly synthesized selective Ca^{2+}/calmodulin dependent protein kinase II inhibitor KN-93 reduces dopamine contents in PC12h cells. Biochem Biophys Res Communication 181: 968, 1991.

Takuma T, Ichida T, Okumura K, Kanazawa M: Protein phosphatase inhibitor calyculin A induces hyperphosphorylation of cytokeratins and inhibits amylase exocytosis in the rat parotid acini. FEBS Lett 323: 145, 1993.

Tan W, Barnett J V, Hehn G M, Quay S C, Galper J B: Effect of manganese (II) bis(glycinate)dichloride on Ca^{2+} channel function in cultured chick atrial cells. Toxicol 68: 63-73, 1991.

Tank D W, Regehr W G, Delaney K R: A quantitative analysis of presynaptic calcium dynamics that contribute to short-term enhancement. J Neurosci 15: 7940, 1995.

Tobimatsu T, Fujisawa H: Tissue specific expression of four types of rat calmodulin dependent protein kinase II mRNAs. J Biol Chem 264: 17907, 1989.

Tong G, Shepherd D, Jahr CE: Synaptic desensitization of NMDA receptors by calcineurin. Science 267:1510,1995.

Trimble W S, Linial M, Scheller R H: Cellular and molecular biology of the presynaptic nerve terminal. Ann Rev Neurosci 14: 93, 1991.

Tsumoto T: Excitatory amino acid transmitters and their receptors in neural circuits of the cerebral neocortex. Neurosci Res 9: 79-102, 1990.

Tsumoto T, Hagihara K, Sato H, Hata Y: NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats. Nature 327: 513, 1987.

Tukumitsu H, Chijiwa T, Hagiwara M, Mizutani A, Terasawa M, Hidaka H: KN-62, 1-[N,O-bis(5-isoquinolinesulphonyl)-N-methyl-L-tyrosyl]-4-phenylpiperazine, a specific inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II. J Biol Chem 265: 4315, 1990.

Turner R, Maler L: Synaptic plasticity in the cerebellar parallel fiber projection to the electrosensory lateral line lobe of gymnotiform fish. Neurosci Soc Abstr 15: 1135, 1989.

Turner R W, Maler L, Deerinck T, Levinson S R, Ellisman M H: TTX-sensitive dendritic sodium channels underlie oscillatory discharge in a vertebrate sensory neuron.

J Neurosci 14: 6453, 1994.

Turner R W, Plant J R, Maler L: Oscillatory and burst discharge across electrosensory topographic maps. J Neurophysiol 76:2364, 1996.

van Deusen E B, Meyer R L: Pharmacologic evidence for NMDA, APB and kainate/quisqualate retinotectal transmission in the isolated whole tectum of goldfish. Brain Res 536: 86, 1990.

Verhage M, Ghijsen W E, Lopes D A, Silva F H: Presynaptic plasticity: the regulation of Ca(2+)-dependent transmitter release. Prog Neurobiol 42: 539, 1994.

Vickroy T W, Malphurs W L, Carriger M L: Regulation of stimulus-dependent hippocampal acetylcholine release by okadaic acid-sensitive phosphoprotein phosphatases. Neurosci Lett 191: 200, 1995.

Walch-Solimena C, Jahn R, Südhof T C: Synaptic vesicle protein in exocytosis: what do we know? Curr Opin Neurobiol 3: 329, 1993.

Wang D, Maler L: The immunocytochemical localization of glutamate in the electrosensory system of the gymnotiform fish, Apteronotus leptorhynchus. Brain Res 653: 215, 1994.

Wang J, Renger J J, Griffith L C, Greenspan R J, Wu C F: Concomitant alterations of physiological and developmental plasticity in Drosophila CaM kinase II-inhibited synapses. Neuron 13: 1373,1994.

Wang L Y, Orser B A, Brautigan D L, MacDonald J F: Regulation of NMDA receptors in cultured hippocampal neurons by protein phosphatases 1 and 2A. Nature 369: 230, 1994.

Wang R A, Cheng G, Kolaj M, Randic M: Alpha-subunit of calcium/calmodulin-dependent protein kinase II enhances gamma-aminobutyric acid and inhibitory synaptic responses of rat neurons *in vitro*. J Neurophysiol 3: 2099, 1995.

Wang Y, Santini F, Qin K, Huang C Y: A Mg(2+)-dependent, Ca(2+)-inhibitable serine/threonine protein phosphatase from bovine brain. J Biol Chem 270: 25607, 1995.

Weisskopf M G, Castillo P E, Zalutsky R A, Nicoll R A: Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. Science 265: 1878, 1994.

Wenzel A, Scheurer L, Kunzi R, Fritschy J M, Mohler H, Benke D: Distribution of NMDA receptor subunit proteins NR2A, 2B, 2C and 2D in rat brain. Neuroreport 7: 45, 1995.

Westbrook G L: Glutamate receptor update. Current Opinion in Neurobiol. 4:337-346, 1994.

Wisden W, Seeburg P H: Mammalian ionotropic glutamate receptors. Curr Opin in Neurobiol 3: 291, 1993.

Wo Z G, Oswald R E: A topological analysis of goldfish kainate receptors predicts three transmembrane segments. J Biol Chem 270: 2000, 1995

Wood P L, Rao T S, Iyengar S, Lanthorn T, Monahan J, Cordi A, Sun E, Vazquez M, Gray N, Contreras P: A review of the *in vitro* and *in vivo* neurochemical characterization of the NMDA/PCP/glycine/ion channel receptor macrocomplex. Neurochem Res 15: 217,1990.

Wright M S, Sefland I, Walaas S I: Cloning of the long intracellular loop of the AMPA-selective glutamate receptor for phosphorylation studies. J Receptor Res 13: 653, 1993.

Wyllie D J, Nicoll R A: A role for protein kinases and phosphatases in the Ca(2+)-induced enhancement of hippocampal AMPA receptor-mediated synaptic responses. Neuron 13: 635, 1994.

Xiang Z, Greenwood A C, Kairiss E W, Brown T H: Quantal mechanism of long-term potentiation in hippocampal mossy-fiber synapses. J Neurophysiol 71: 2552, 1994.

Xiao M Y, Karpefors M, Gustafsson B, Wigström H: On the linkage between AMPA and NMDA receptor-mediated EPSPs in homosynaptic long-term depression in the hippocampal CA1 region of young rats. J Neuroscience 15: 4496, 1995.

Xiao M Y, Wigström H, Gustaffson B: Long-term depression in hippocampal CA1 region is associated with equal changes in AMPA and NMDA-receptor-mediated synaptic potentials. European J Neurosci 6: 1055, 1994.

Xie X, Berger T W, Barrionuevo G: Isolated NMDA receptor-mediated synaptic responses express both LTP and LTD. J Neurophysiol 67: 1009, 1992.

Xu Z, Payne J R, Nelson M: Logarithmic time course of sensory adaptation in electrosensory afferent nerve fibers in a weakly electric fish. J Neurophysiol 76: 2020, 1996.

Yakel J L, Vissavajjhala P, Derkach V A, Brickey D A, Soderling T R: Identification of a Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in non-N-methyl-D-aspartate glutamate receptors. Proc Natl Acad Sci USA 92: 1376, 1995.

Yoshimura Y, Tsumoto T: Dependence of LTP induction on postsynaptic depolarization: a perforated patch-clamp study in visual cortical slices of young rats. J Neurophysiol 71: 1638, 1994.

Yoshioka N, Sakurai M: Post-synaptic depolarization in induction of long-term potentiation in the CA1 hippocampus. Neuroreport 6:3 33, 1995.

Young A B, Dauth G W, Hollingsworth Z, Penney J B, Kaatz K, Gilman S: Quisqualate- and NMDA-sensitive [3H]glutamate binding in primate brain. J Neurosci Res 27: 512, 1990.

Zakon H H (1986): The electroreceptor periphery. In Bullock T H, Heiligenberg W, eds. Electroreception. New York, Jonh Wiley and Sons, 103.

Zalutsky R A, Nicoll R A: Comparison of two forms long-term potentiation in single hippocampal neurons. Science 248: 1619, 1990.

Zhao W, Bennett P, Sedman GL, NG KT: The impairment of long-term memory formation by the phosphatase inhibitor okadaic acid. Brain Res Bulletin 36: 557, 1995.

Zidichouski J A, Jhamandas J H: Electrophysiological characterization of excitatory amino acid responses in rat lateral parabrachial neurons *in vitro*. Brain Res 611: 313 1993.

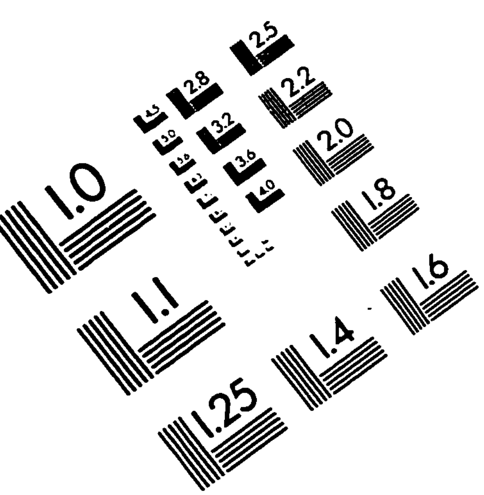
Zucker R S: Short-term synaptic plasticity. Ann Rev Neurosci 12: 13, 1989.

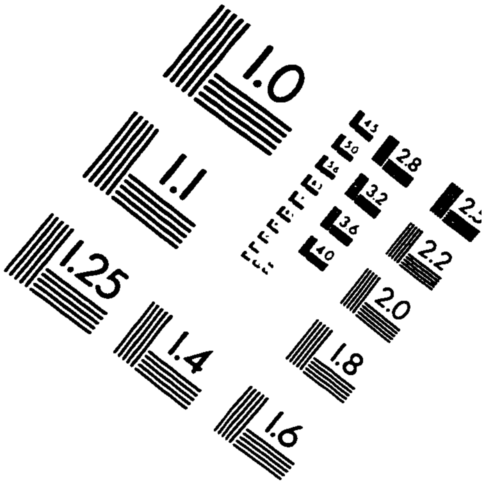
Zucker R S: Exocytosis: a molecular and physiological perspective. Neuron 17: 1049, 1996.

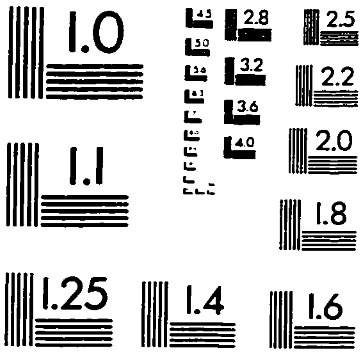
Zucker R S, Delaney K R, Mulkey R, Tank D W (1991): Presynaptic calcium in transmitter release and posttetanic potentiation. In Stanley E F, Nowycky M C, Triggle P D, eds. Calcium entry and action at the presynaptic terminal. New York, New York Academy of Sciences, 191.

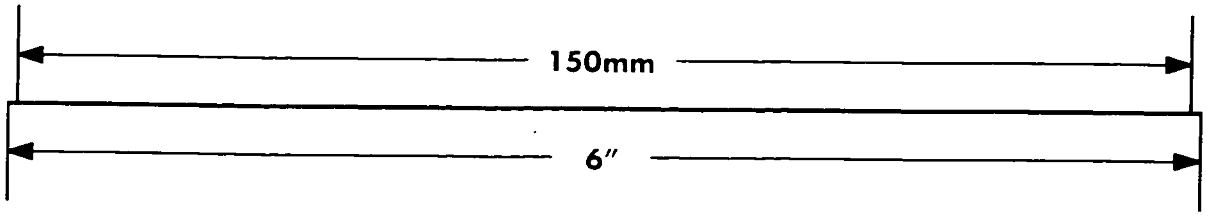
Zukin R S, Bennett M V: Alternatively spliced isoforms of the NMDARI receptor subunit. Trends Neurosci 18: 306, 1995.

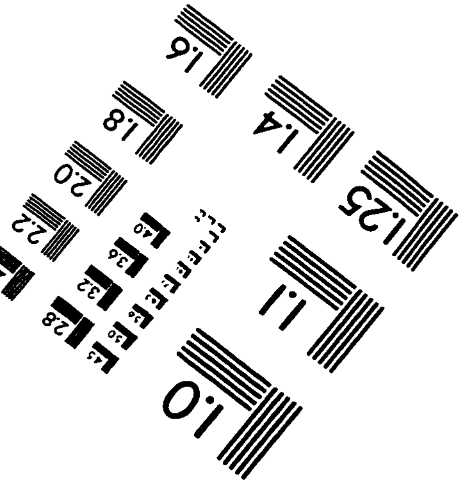
IMAGE EVALUATION
TEST TARGET (QA-3)











APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989